An Australian Government Initiative

THE NATIONAL SYSTEM FOR THE PREVENTION AND MANAGEMENT OF MARINE PEST INCURSIONS

Australian marine pest monitoring manual

Version 2.0

Keeping marine pests out of Australian waters

Important

The Australian Government Department of Agriculture Fisheries and Forestry advises that the information contained within the *Australian marine pest monitoring manual* is designed as a guide for marine pest monitoring and is intended to provide the rationale for specific inputs and decisions in the monitoring process.

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Contact points

Questions about ongoing monitoring or this manual?

For further information about the ongoing monitoring approach, this monitoring manual, or assistance please contact DAFF or the relevant state or territory. Contact details of National System partners can be found at www.marinepests.gov.au/national_system/partners.

If you find a new or suspected incursion of a marine pest, contact the relevant state/territory or the Monitoring Coordination Point within 48 hours. A written report must be submitted within four weeks.

Contents

Executive summary

The Australian governments (Commonwealth, state and Northern Territory) recognise the importance of ongoing monitoring or surveillance in managing marine pest risks. They have agreed to a species targeted ongoing National Monitoring Strategy (NMS).

This strategy forms an integral part of Australia's National System for the Prevention and Management of Marine Pest Incursions (the National System) and provides for standardised monitoring to detect high risk species at priority locations around Australia.

In the context of the NMS, monitoring means regular ongoing sampling of the marine environment to collect information on the presence and absence of target species and to detect species that exhibit invasive characteristics.

Monitoring data will help guide marine pest management actions that:

- trigger and inform emergency response arrangements
- make decisions on the ongoing management and control of established marine pest populations, including informing National System risk assessments
- review and improve other measures that form part of the National System
- inform broader policy decisions.

The *Australian marine pest monitoring manual* is a 'how to guide' for monitoring in the context of the National System. It will assist governments, monitoring designers and those carrying out the monitoring programs to meet consistent national standards.

The manual describes the procedures to be used in designing and implementing a monitoring program that will meet agreed minimum principles, including:

- design – selecting the species to be monitored, the observation systems and calculating the sample size using standard templates and tools
- implementation – field guides for sampling techniques and processes for sample collection

- sample handling, preservation and analysis
- reporting – includes standard datasheets and reporting instructions to maintain consistency in results.

A Monitoring design package (MDP) has been developed as a companion to the manual. The MDP includes a number of design templates, user guides and tools that will assist survey designers to standardise and improve monitoring design. Use of the MDP will also facilitate the assessment and review of designs and the data collected.

The *Australian marine pest monitoring manual* complements the *Australian marine pest monitoring guidelines* which outlines Australia's policy approach for marine pest monitoring. The guidelines provide the rationale for the routine collection of monitoring data, governance arrangements and how the data collected will be used to inform decision making.

Introduction

Australia has a coastline of approximately 60 000 km and a marine jurisdiction of some 16 million km^2. These environments are susceptible to invasion by marine pests with the potential to seriously impact the marine environment, marine industries and coastal communities.

To minimise the risks posed by marine pests, Australian governments (Commonwealth, state, and territory) have agreed to a comprehensive national approach known as the National System for the Prevention and Management of Marine Pest Incursions (National System).

The National System includes a national monitoring strategy (NMS) that provides for targeted monitoring of species most likely to have a significant impact and at locations most likely to be invaded.

The NMS focuses on ongoing standardised monitoring to detect high risk species at priority locations around Australia. Minimum quality principles for monitoring have been agreed that ensure that monitoring data is collected using rigorous, consistent methods that allow for informed and scientifically-sound decision making and enable nationwide comparison over time.

The *Australian marine pest monitoring manual* is a 'how to guide' for monitoring in the context of the National System. It describes the procedures to be used in designing and implementing a monitoring program to meet agreed minimum quality standards.

The manual is supported by a Monitoring design package (MDP) which includes design templates, user guides and tools to assist survey designers meet the quality standards. Survey designers should contact the Monitoring Coordination Point at DAFF for a copy of the latest version of the MDP.

Results from the monitoring program will support the prevention and emergency preparedness and response elements of the National System. In particular, monitoring data will help guide marine pest management actions to:

- inform the risk assessment of vectors to inform National System prevention measures (pre-border controls)
- provide earliest detection possible to inform emergency response arrangements in the event of an incursion

- inform decision making for the ongoing management and control of established marine pest populations, including informing risk assessments
- inform broader policy decisions on marine pest management.

Evaluation and review of the NMS will provide an adaptive management framework for continuous improvement. An ongoing review cycle for the NMS will encompass a review of the monitoring locations, the monitoring program designs, the manual and guidelines and a review of the NMS as a whole in meeting the needs of the National System.

Australia's policy approach to monitoring marine pests, the rationale for data collection, governance arrangements and how the data collected will be used to inform decision making are outlined in the *Australian marine pest monitoring guidelines*.

Purpose of the monitoring manual

This manual describes a minimum set of processes and procedures for marine pest monitoring and the collection of monitoring data from marine environments. The aim is to ensure that data is collected using rigorous, consistent methods and meets agreed Quality Assurance/ Quality Control (QAQC) principles.

In this manual, monitoring is defined as regular ongoing sampling of the marine environment with a view to collecting data on the presence/ absence of target species and to detect species exhibiting invasive characteristics.

Monitoring may be undertaken for various purposes as outlined in Box 1:

Box 1. Monitoring objectives

Primary Monitoring Objectives

- to detect new incursions of established target species at a given location i.e. species already established elsewhere in Australia but not recorded at that location
- to detect target species not previously recorded in Australia that are known to be pests elsewhere.

Secondary Monitoring Objective

- to detect introduced species that appear to have clear impacts or invasive characteristics.

A species and location targeted approach has been adopted that focuses effort on species most likely to have a significant impact and locations most likely to be invaded. This approach is intended to collect presence/ absence data but not abundance data, and in this respect the approach differs from the baseline surveys carried out in ports around Australia (protocols for baseline surveys are described in Hewitt & Martin 1996, 2001). These baseline surveys provided comprehensive data on the presence/absence of a large number of both native and exotic species sampled in each surveyed location. In many cases this information will provide the starting point for ongoing monitoring programs.

This manual provides instructions to:

- identify and engage all relevant stakeholders (section 1.2)
- define the monitoring location (section 2.1)
- collect information required about the monitoring location (sections 2.2, 2.3)
- produce a monitoring survey design incorporating:
  - target list of species to monitor based on environmental conditions and species tolerances (section 2.4)
  - survey timing, list of observation systems for each monitoring location and the minimum sample size for a set detection sensitivity (section 2.5)
- analyse and verify sampling results (section 4)
- report on monitoring results (section 5)
- review monitoring strategy (section 6).

Each stage in the process has a set of principles that outline the minimum information that needs to be collected. Meeting these principles will ensure that monitoring programs meet QAQC requirements. These principles also provide an indication of the qualifications required by monitoring personnel to meet the minimum requirements.

Australian jurisdictions have agreed to undertake ongoing monitoring at an agreed number of locations. This manual has been developed to be used by any person involved in this ongoing monitoring. The manual can also be used for designing and implementing ongoing monitoring programs in other locations.

Overview of the monitoring process for a given location

The monitoring process for each NMN involves six phases including:

1. analysis
2. design
3. planning
4. implementation
5. monitoring reporting
6. evaluation and review.

Table 1 summarises the key steps for each of these discrete design phases, while Table 2 provides timelines relevant to the reporting aspects of the process.

1. Analysis

Careful analysis ensures access to key data, documents and resources at the start of the project limiting the risk that participants might move forward without critical information.

The analysis phase involves:

- determining the monitoring goals and objectives
- identifying participants and stakeholders and defining their roles and responsibilities (governance)
- identifying available data i.e. maps, habitat information, oceanographic conditions, occupational health and safety issues.

2. Design

For each location, the overall monitoring design will be documented and reported with a clear view to achieving identified monitoring program objectives. This process includes collating existing data and analysing it to allow:

- identification of target species, associated habitats and vector nodes, observation systems and analysis methods and the appropriate sample size
- assessment of the cost-effectiveness of sampling and analysis methods
- selection of the spatial and temporal elements of sampling.

Table 1. Overarching guidance for development of monitoring programs

SECTION	PROCESSES	KEY OUTPUTS	REPORTING REQUIREMENTS
Analysis	1.1 Determine monitoring objectives 1.2 Identify relevant jurisdictions & responsibilities, along with necessary permits 1.3 Compile inventory of existing information	1. Stakeholder agreement on monitoring objectives 2. Relevant permits secured 3. Checklist of existing information	
Design	2.1 Define the monitoring location 2.2 Collate existing data 2.3 Delineate habitats & choose sub-locations 2.4 Identify target species 2.5 Choose timing of sampling, observation systems & sample size 2.6 Select monitoring sites within locations 2.7 Produce monitoring design report	1. Monitoring area map(s), including habitat & sub-location delineation 2. Boxplots for temperature & salinity (annual cycle) 3. Hazard analysis table 4. Target species list 5. Sample timing, list of observation systems, level of replication & costs 6. List of sampling sites	1. Monitoring Design Report and Implementation Plan
Planning	3.1 Identify appropriate field & taxonomic personnel 3.2 Check equipment & train staff 3.3 Develop work plan & confirm security clearances 3.4 Develop draft implementation plan	1. Appropriate museum & taxonomic staff engaged 2. Work plan & confirmation of security clearances 3. Submit monitoring design report & implementation plan for approval	
Implementation	4.1 Sample collection 4.2 Develop post-sample collection report 4.3-4.5 Process, analyse & identify samples 4.6 Transport & transfer samples to storage 4.7 Verify & interpret results 4.8 Report suspected incursions	1. Post sample collection report submitted 2. Presence/absence status for the target species list 3. Voucher collection prepared & stored 4. Submit suspected incursion report (if applicable)	2. Interim report - post sample collection 3. Suspected incursion report (if applicable)
Monitoring reporting	5.1 Produce monitoring report	1. Monitoring report submitted	4. Monitoring report
Evaluation & review	6.1 Compile list of proposed changes to monitoring design 6.2 Consider improvements to manual	1. Summary of changes to monitoring design & suggested improvements to manual for inclusion in monitoring report	

The 'Processes' column outlines the steps that need to be sequentially addressed for monitoring programs at each NMN location, with numbering corresponding to sections within the relevant sections of the manual. Survey designers should refer to the individual sections for detailed instructions for each process.

Table 2. Required reports and due dates

REPORT	TIMING
Monitoring design report#	At completion of design phase
Implementation plan#	Before commencing monitoring
Interim report - post sample collection	Within 48 hrs of completion of all field trips to collect samples
Suspected marine incursion - verbal report	Within 48 hrs of verifying new incursion
Suspected marine incursion - written report	Within 1 month of verbal report
Monitoring report	Within 1 month of completing monitoring program

To facilitate the assessment process, it is recommended that the implementation plan is submitted as an attachment with the monitoring design report.

Note: for planning purposes, at least 3 weeks should be allowed for assessment of monitoring design reports, along with additional time to address any concerns raised as part of the assessment process.

3. Planning

Development of a monitoring implementation plan ensures that the logistical components of the monitoring design, such as equipment and personnel, are considered, documented and in place ready for commencement of the monitoring program.

4. Implementation

Once samples have been collected there are a number of sample processing and analysis steps required:

In the field –

- preliminary sorting and identification of samples
- fixation and preservation to ensure sample integrity and correct storage of specimens.

In the laboratory –

- comprehensive sample processing and analysis
- permanent preservation and cataloguing of samples
- compilation of results and recording them in a standard electronic format for each location
- target species identifications will also be verified by taxonomic experts

- data will be quality-controlled, and once verified, will be interpreted in terms of their accuracy and precision, but also in the context of existing knowledge (e.g. current understanding of marine pest distributions)
- suspected incursion reporting will take place when an incursion or suspected incursion has been detected.

5. Monitoring reporting

Results will be reported in a standard format for each location. The required elements for the monitoring report will be provided to help ensure consistency of reporting.

6. Evaluation and review

Information provided through the reporting processes will be used to:

- review the monitoring program design and implementation at each location
- review the manual
- review the overall approach to ongoing monitoring for Australia.

The review processes and timeframes can be found in the companion *Australian marine pest monitoring guidelines*.

Quality assurance and quality control

At each step in the monitoring process, QAQC principles are provided. Box 2 provides brief definitions of QAQC. Meeting these principles ensures data is of a suitable quality for informed and scientifically sound decision making. These principles provide a means for evaluation and review allowing continuous improvement and ensuring that the monitoring approach is an adaptive management system.

Box 2. QAQC definitions

Quality assurance (QA) is an integrated system of activities to ensure data (and its use) meets pre-defined standards of quality with a stated level of confidence.

In the context of marine pest monitoring this means that the activities set out in this monitoring manual will yield data of an acceptable level to be used in management decisions under the National System.

Quality control (QC) is the system of technical activities whose purpose is to measure and control the quality of data.

Quality control activities included in this manual include the approval of monitoring designs and implementation plans, and mandatory submission of reports with compulsory sections. The methods and procedures outlined are based on current best practice so will need to be periodically updated as field and laboratory techniques advance.

Marine pest monitoring data that meets these QAQC principles will be deemed of suitable quality for use in national decision making processes.

This manual can be used to design and implement monitoring programs in any location. Data from these monitoring programs can also be used in national decision making if the QAQC principles are met.

1. Analysis process

1.1 Determining objectives

Principle: The monitoring program at a minimum must meet the objectives stated in Box 1 and be agreed through stakeholder consultation.

For locations that are being monitored to meet national pest management requirements (i.e. NMN locations), the relevant government authority will approve the monitoring objectives.

The relevant management authority and/or monitoring program coordinator should agree on any additional monitoring objectives for the location. This should be done considering resource constraints (i.e. time and money) and in consultation with:

- the program's owner/coordinator
- stakeholders providing funding support to the project
- other stakeholders involved in the project.

The monitoring area within a location should also be delineated at this stage.

1.2 Stakeholder engagement and governance

Principles:

1. **All groups that have jurisdiction or responsibility for the different areas of the monitoring location must be identified and consulted. Governance arrangements must also be established.**
2. **Permits must be obtained from the relevant groups before monitoring commences.**

Identifying and engaging all relevant stakeholders is critical. The responsibility or jurisdiction for different areas of a monitoring location is likely to rest with a number of different groups i.e. different levels of government; the Port Authority; marina operators; aquaculture operators; those with responsibility for boat ramps or navigation buoys; recreational users and slipway or drydock operators (see Figure 1).

All relevant groups will need to be involved in deciding how the monitoring is to be implemented. For monitoring programs to meet national requirements the Monitoring Coordination Point may assist with this process. For other monitoring programs the jurisdictional representative and/or the Monitoring Coordination Point are available to provide advice and conflict intervention if required (view contact list at www.marinepests.gov.au/national_system/partners).

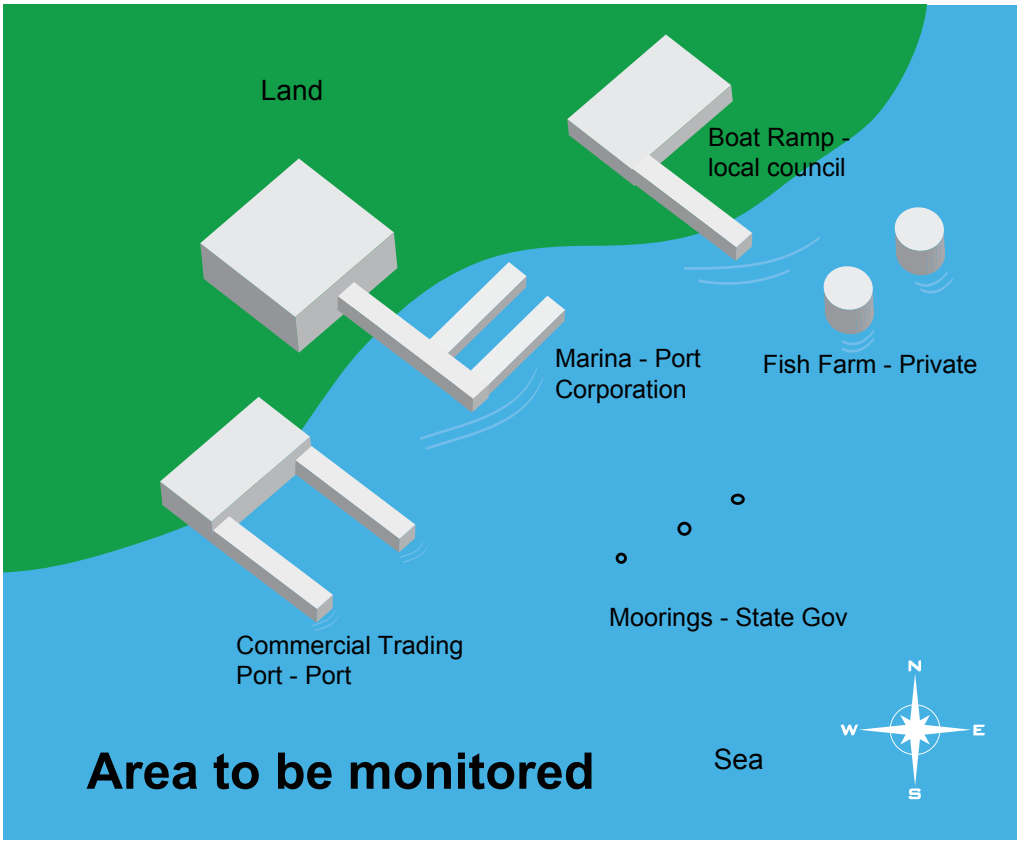


Figure 1. Example diagram of a monitoring location showing facilities and the groups with management responsibility

1.2.1 How to identify jurisdictional responsibilities and consultation requirements within a location.

1. Identify all nodes or facilities within the location. A node is an area where any potential vectors of marine pests (e.g. vessels) coincide with a suitable habitat for pest establishment (e.g. marina, boat ramp)
2. Identify the group with responsibility for each node
3. Initiate contact with each group, explaining the monitoring program and identifying roles and responsibilities for different parties (e.g. provided with outcomes, provision of funding, input to design etc.).

The communication method and frequency of contact with each stakeholder group should match the operation and needs of each specific group.

Table 3 has been provided below to assist with identification of responsibilities.

Table 3. Identification of nodes and their jurisdictional responsibility

NODE/FACILITY	DETAILS (E.G. NUMBER OF BERTHS, NUMBER OF LEASES ETC.)	RESPONSIBLE GROUP (E.G. PORT AUTHORITY, STATE OR LOCAL GOVERNMENT ETC.)
Commercial trading port		
Marina		
Waterways		
Boat ramp		
Navigation buoys		
Markers and other structures		
Jetty		
Slipway		
Drydock		
Aquaculture lease		
Recreational users		
Other		

1.2.2 Obtaining permits to conduct monitoring fieldwork

It is likely that a permit will be required to carry out monitoring fieldwork or enter privately owned property in some parts of the monitoring location. Contact should be made with each jurisdictional group to establish the requirements and obtain agreement that permits will be made available for the purposes of monitoring when required. In addition, granting of permits may require samplers to undergo specific OH&S training prior to allowing access in certain locations.

1.3 Inventory of existing information

Principle: The information needed to design and implement a monitoring program must be available.

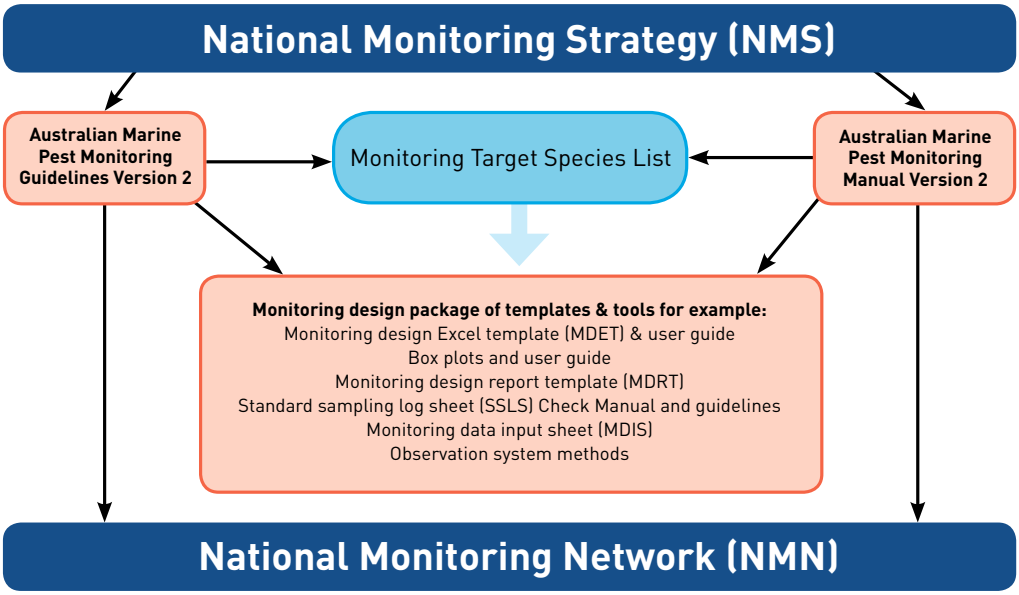
Complete the checklist in Table 4 to identify information gaps that may hinder design and implementation of a monitoring program. If a location is missing some of the information, steps may need to be taken to compile that information, such as an independent research and development project or similar before monitoring can commence. Note: Communicate any significant delays in commencing the monitoring program caused through the collation of existing information to the monitoring program coordinator and, if necessary, the relevant jurisdictional representative and/or the Monitoring Coordination Point. Examples are provided in *italics*.

Table 4. Checklist of information needed to design and implement an ongoing monitoring program

INFORMATION ITEM	AVAILABLE (YES/NO)	IF NOT AVAILABLE, ACTION REQUIRED
Map(s) of monitoring area	*No*	*Map to be developed – new project*
Previous marine pest monitoring data and map (e.g. port baseline survey reports)		
Summary of likely effects of oceanographic conditions on monitoring (may be provided by local experts)		
Marine pest suitable habitat types and position within monitoring location		
Magnitude (size, frequency and timing) of introduction/ translocation vectors		
Local constraining factors, including health and safety		
Permit and authorisation requirements for monitoring location (e.g. port security requirements)		

QAQC Check: Monitoring program coordinators must provide evidence to indicate the permit requirements can be met and that design information is available.

2. Design process



Designing a monitoring program requires an understanding of the environmental, biological, governance and budgetary circumstances that apply at a particular location. This section considers all of these factors in describing the necessary procedures and principles to meet minimum QAQC requirements for monitoring.

The manual cannot provide instructions for all scenarios and certain QAQC requirements may not be achievable for some locations. Under these circumstances, it is important that any deviation from minimum requirements is explained in the monitoring design report.

Principle: The following steps must be completed in designing the monitoring program:

- **define the monitoring location**
- **collate existing data**
- **delineate habitats and choose sub-locations**
- **identify target species**
- **choose timing of sampling, observation systems and sample size**
- **select sites to monitor within a location**
- **submit the monitoring design report for assessment and approval by the Monitoring Design Assessment Panel (MDAP) before commencing monitoring.**

The Monitoring design package (MDP) has been developed to assist the design process and includes a range of templates, tools and user guides. Survey designers should contact the Monitoring Coordination Point at DAFF for a copy of the latest version of the MDP.

The Monitoring design Excel template (MDET), a tool in the MDP, provides calculations and choices for some key aspects of the survey design. MDET spreadsheets can be used to help determine:

- the target species list
- observation systems and sample sizes
- estimates of survey costs.

The MDET is not prescriptive, but a guidance tool to be used by specialists with experience in designing marine biological surveys. Monitoring designers need to make judgements about their monitoring approach and make fine adjustments to customise the design to the unique requirements of individual locations. General instructions on using the MDET are provided in this manual, with more detailed directions on customising design calculation provided in the *Monitoring design Excel template user guide* in the MDP.

For each location the overall monitoring design must be documented in a formal report and submitted for approval before monitoring commences. Within the MDP, the Monitoring design report template (MDRT) has been developed to assist in the designing of marine pest monitoring surveys as outlined in this manual. It is complementary to and utilises the output from the MDET. The use of the MRDT in completing monitoring survey designs is not mandatory but is highly recommended to facilitate the design process, design approvals and for comparing designs among different locations. The MDAP has been formed to independently evaluate monitoring program designs and will help to ensure consistency in the assessment approach across Australia (see section 2.7.2). In addition to its role in assessing monitoring designs MDAP can also be engaged early during the design process to provide initial feedback and advice on survey design issues.

2.1 Define the monitoring location

Principle: The boundary of the monitoring area must be defined for each location.

It is the responsibility of the jurisdictions to define the boundary of the area to be monitored in each NMN location within their jurisdiction. In defining this area, the jurisdictional representatives should consider the following factors:

- stakeholders
- vector nodes
- vector movements
- environmental factors, particularly oceanographic, such as water movement patterns and/or habitats more likely to be colonised by pest species than native species (e.g. treated surfaces, immersed infrastructure etc.)
- monitoring survey budget and logistical constraints
- previously recorded marine pest incursions.

Consultations with all potential stakeholders should be conducted when defining the extent of the monitoring location. The outer boundary of the monitoring location should:

- encompass all potential vector nodes, for example: marinas, commercial ports/wharves/berths, aquaculture facilities, fishing vessel moorings/berth/etc, tug bases, pontoons, boat ramps and slipways and drydocks
- encompass all vector routes for example, where deballasting may occur
- consider extending out into the open water, especially if vectors or suitable habitats occur outside the location e.g. offshore anchorages for commercial vessels, areas where ballast water uptake and discharge takes place, and adjacent rocky reef systems
- consider environmental factors, such as water movements, to encompass any areas where water may remain for a period of time for example because of the presence of an eddy or tidal pooling
- encompass all habitat types including bridge pylons, breakwaters etc.

2.2 Collate existing data

Principle: The following information must be collated for each location:

1. **local knowledge and skills audit**
2. **existing marine pest data**
3. **oceanographic data**
4. **vector parameters**
5. **monitoring area map(s)**
6. **hazard analysis and occupational health and safety issues**
7. **port security requirements (if required).**

This process will assist monitoring designers, with each of these required elements described below.

2.2.1 Local knowledge and skills audit

Principle: Carry out an audit of the local knowledge and skills available in the monitoring location.

Undertake an audit of people with an understanding of the monitoring location (oceanographic or ecological) and with relevant local knowledge and experience.

This information will be useful in identifying personnel who could contribute to the monitoring design process or be employed in the implementation processes. Identifying these people will also help build monitoring capacity in the monitoring location and may be able to provide site specific information that can assist in the on-site survey process.

2.2.2 Previous marine pest data

Principle: Collate previous data for the location from previous activities.

Collate any data on previous marine pest monitoring activities or relevant studies. Provide a list of exotic species previously found and the corresponding locations (including GPS position if available) where they were detected.

This information will be useful when identifying target species to monitor and the location of monitoring sites. Ensure any available baseline port survey data has been included. If the available information is suitable, consider including a map showing the locations of all marine pests previously detected at the monitoring location.

2.2.3 Oceanographic data

Principle: Monthly maximum and minimum water temperature (°C) and salinity (ppt) data must be provided in graph form for the monitoring location.

This data will assist in identifying species that can survive in the location. An Excel file has been developed as part of the MDP ('Boxplots.xls') that produces boxplots of temperature and salinity.

Boxplots allow managers to see the range of temperature and salinity values at a location throughout and between years. Monthly resolution is required. A minimum of five years of data is necessary for the boxplots to be of assistance.

The environmental range chosen must reflect appropriate minimum and maximum values and must be representative of the dataset. Design assessors may question the range selected if values appear to be unusual events. For detailed instructions, refer to the *Documentation guide for use of boxplots* in the MDP.

If detailed, multi-year temperature or salinity data are not available for a monitoring location, designers should plot the best available data in order to infer these values. All data sources must be clearly cited in the monitoring design report.

2.2.4 Identification of vectors

Principles:

1. **All vector nodes (such as ports, slipways, buoys, berths) must be marked on a map (section 2.2.5).**
2. **All vector pathways (such as shipping channels, small vessel/ferry routes) must be marked on a map (section 2.2.5).**

This information is required to help determine the prioritisation of sampling sites within a location. Complete Table 5 and include in the monitoring design report (if this section has not been sufficiently covered in stakeholder engagement and governance).

Table 5. Identification of vectors

VECTORS	STAKEHOLDERS	NODES AND PATHWAYS	SOURCE
Sailing vessels	*General public and marina operators*	*Marinas and slipways in the location*	*Domestic and international*

2.2.5 Monitoring area map(s)

Principle: Create required map(s) in GIS and electronic format (e.g. JPG file format).

Maps are to include the following:

- title
- scale – a minimum resolution of 1:25000
- north point
- axes coordinates of latitude and longitude in decimal degrees to a minimum of 5 decimal places (~ 1 metre accuracy), using the relevant national datum standard[1])
- artificial structures such as commercial vessel berths, marinas, slipways, breakwaters, bridges, channel markers, etc.
- natural features including beaches, underwater reefs etc.
- anchorage points and dredge disposal sites
- legend (key to the above features, but also a description of the source of the information used in creating the map)
- date of production.

GPS information must be provided by reference and datum (coordinate system). The national datum standard should be used (the Australian Standard is GDA94). The MDRT provides more information about mapping.

2.2.6 Hazard analysis and occupational health and safety issues

Principle: Provide a hazard analysis (Table 6) with a list of constraining factors that may affect monitoring success and relevant management actions in the monitoring design report.

Monitoring success may be constrained by a range of factors including:

- environmental conditions e.g. turbidity, winds, wave exposure, tidal currents and range
- OH&S issues (e.g. temperature conditions and dangerous animals).

1 The Australian Standard is GDA 94 (http://www.ga.gov.au/geodesy/datums/gda.jsp).

There may be specific OH&S requirements or processes that need to be completed before monitoring can commence. For example monitoring in locations containing dangerous substances, such as petroleum, may require staff to undertake safety training before sampling.

A formal risk analysis may need to be undertaken for certain constraining factors. Designers are advised to check with their OH&S representative or local legislation and management authorities (e.g. port corporations, marina owners, and/or slipway operators etc.) for assistance.

These factors may affect the suitability of monitoring for particular target species or the use of certain observation systems.

Complete Table 6 to carry out the hazard analysis for the monitoring location using the following steps (some examples are provided in *italics*):

1. Identify the hazards or constraining factors for the location. A list of possible hazards and occupational health and safety issues has been included in the table; additional points can be added as necessary
2. Identify the possible effects of each factor on monitoring activities or results
3. Consider the period of occurrence of the hazard, which may help with mitigation
4. Determine possible management actions to mitigate the effects of each factor. Where it is not possible to mitigate each factor, alternative action may be required. Designers are advised to check with their OH&S representative or local legislation authority for assistance
5. Include the hazard assessment in the monitoring design and retain a copy for reference by survey teams during the actual survey.

Table 6. Hazard analysis for oceanographic conditions of monitoring locations

HAZARD/CONSTRAINING FACTOR	EFFECT AT MONITORING LOCATION	PRESENT (Y, N OR INTERMITTENT [I])	MANAGEMENT ACTIONS
Turbidity/visibility (Secchi disk depth)	*Relatively high (Secchi depth range of 0-2m) inside estuary but low elsewhere*	*Y*	*Change to non-visual observation systems in estuary as underwater visibility poor#*
Wind direction (16 compass points)	*Highly seasonal, prevailing NNW in spring*	*Y*	*Need to work on lee-side*
Wind speed (knots)	*Highly variable but strong sea breeze in afternoon year round*		*Need to work on lee-side or complete field work before afternoon sea breeze picks up*
Tidal currents (ms^{-1})	*Strong in estuary*		*Need to dive during slack tide*
Tidal range and times	*Will influence time of day for sampling in areas with large tidal ranges*		*Boat access, standard depth for pylon sampling, depth estimates when sampling*
Water residence time (# of days before body of water is completely refreshed)	*Water time short (2 days)*		*Timing of samples adjusted to coordinate with spawning/growth activity*
Rainfall patterns			*Timing of samples adjusted to match season/time of year*
Temperature extremes			
Harbour configuration ('hot spots', stratification)			
Dangerous animals Sharks Stinging jelly fish Crocodiles Other marine taxa (e.g. sea snakes)		*Y* *I (Nov – Apr)*	*No diving* *Divers to wear stinger suits*
Vessel traffic	*Shipping channel with regular traffic*	*Y*	*Schedule sampling around vessel timetables*
Dredging & construction activities			
Pollution (e.g. sewer outfall)			

Table 6. continued

HAZARD/CONSTRAINING FACTOR	EFFECT AT MONITORING LOCATION	PRESENT (Y, N OR INTERMITTENT [I])	MANAGEMENT ACTIONS
Pressure gradients (e.g. water pump inlets)			
Charged metal protectors (cathodic protection)			
Visibility			
Diving related Decompression sickness Entanglement			
Other			

#Note that the MDET takes turbidity into account and penalises visual observation systems in turbid water.

2.2.7 Port security issues (if applicable)

Principle: Appropriate authorisation from the relevant authority is required before sampling commences in a commercial trading port.

Relevant authorities may have specific security requirements for collecting samples in monitoring locations. Identify all security requirements in a location and, if necessary, obtain the appropriate security clearances.

2.3 Delineate habitats and sub-locations

Principle: Delineate the area to be monitored into habitat types and sub-locations (where necessary) and show on a map (section 2.2.5). Document and report on the source and resolution of the information used in habitat and sub-location delineation.

Delineating habitats in the monitoring location allows stratification of the area to be sampled to:

- identify areas where target species are likely to establish based on habitat preference
- assist in determining and delineating sub-locations
- calculate the habitat area or volume to determine the sample size.

Use the categories outlined in Table 7 to identify detailed habitat types in the location. Source resources for this purpose such as habitat maps of the location if required. If not available, deduce approximate boundaries of habitats using the best available resources e.g. marine charts, aerial photographs and consultation with local experts. Record details on how the habitats were delineated in the monitoring design report.

The MDET only includes provision for five coarse habitat types (as labelled 1 to 5 in Table 7). As such, aggregate detailed habitat types up into the appropriate category (see Table 7) when using this tool.

Table 7. Habitat types and areas

HABITATS (DETAILED LEVEL)	HABITAT AREA OR VOLUME (M2 OR M3)	HABITAT (COARSE LEVEL - AGGREGATED FOR MDET)		TOTAL SURVEY AREA OR VOLUME (M2 OR M3)	INTERTIDAL %
Mud		Soft-sediment	1. Epifauna		
Sand					
Silt					
Seagrass/algal bed			2. Infauna		
Mangrove[2]					
Other (specify)					
Pylons (concrete & timber)		3. Hard horizontal/ vertical			
Other artificial surfaces (e.g. moorings, slipways, boat ramps etc.)					
Reef (e.g. rock, coral)					
Other (specify)					
Pelagic (e.g. fish)		4. Pelagic horizontal/ vertical			
Planktonic (e.g. larvae, phytoplankton)		5. Plankton horizontal/ vertical			

Due to the large area of many NMN locations it may not be feasible to sample the entire location, in which case it will be necessary to split it into smaller sub-locations. Guidance for determining appropriate sub-locations is detailed below.

Guidance for determining sub-locations

Determine sub-locations on the basis of *homogenous environmental* conditions (turbidity, temperature, salinity, pollution level e.g. enclosed marina, commercial port, open coastal environment) and may include a number of habitat types. When determining sub-locations, ensure that:

- each of the identified habitat types are included in at least one sub-location
- each of the identified vector nodes are included in at least one sub-location

2 Refer to SARDI report on mangrove sampling techniques (Wiltshire & Rowling 2009)

- consideration has been given to areas of previous incursions
- local factors such as prevailing currents, larval pooling areas and vector pathways are considered.

Mark the position of the sub-locations on a map and record GPS coordinates of sub-locations (e.g. all the vertices) in the relevant tables in the monitoring design report. More than one map may be required depending on the size of the monitoring location. Present a separate map for each sub-location if there is too much information to present on one map. The MDRT provides more information about the mapping.

In order to use the MDET, complete Table 7 for each sub-location, showing calculated areas and/or volumes of each detailed habitat category. Aggregate these values up into each of the five coarse level habitat types used in the MDET and insert them into the 'Location_data' worksheet in the MDET. Note: the monitoring design report should also include an additional table showing the total reduction in areas for each habitat type between the entire location and the sub-locations used in the final design.

2.4 Identify target species

Principle: Determine target species that can survive in the location based on temperature and salinity tolerances.

The list of the monitoring target species (Attachment A) has been endorsed by the National Introduced Marine Pests Coordination Group (NIMPCG). The *Australian marine pest monitoring manual* contains further information about this list. These target species were identified as high risk for Australia as a whole, based on their invasion and impact potential, and human health impacts.

The purpose of the monitoring program, however, is to target the species most likely to be introduced and become established at a particular location, based on temperature and salinity tolerance data. In addition, monitoring designs should have the capacity to detect and identify non-target species that have not previously been found at the location but that have invasive characteristics. Section 2.5.7 describes possible invasive characteristics to assist in detecting unknown non-target species.

Survival is defined as the successful completion of a species' life cycle through the year within the temperature and salinity ranges of the monitoring location. MDET automates the determination of species survival.

Once all the appropriate information is entered into the template and the 'calculate sample size' button has been activated in the 'Method_ data' worksheet, species will be excluded based on intolerances. The exclusion process occurs at the life stage level, potentially resulting in the exclusion of one life stage while the remaining life stage is still included. Because the tolerance data for many species may be based on limited data the designs should target all life stages that are able to survive within the location's temperature and salinity range.

If survey designers wish to exclude a life stage based on the fact that the species is unable to complete its lifecycle, sufficient justification must be provided to demonstrate the tolerance of the species is significantly divergent from the local temperature and salinity conditions to prevent establishment. Species previously detected in the monitoring location should be included in the monitoring design process. If designers want to exclude any species based on additional information, appropriate reasoning must be provided in the monitoring design report.

2.5 Choose timing of sampling, observation systems and sample size

Overarching Principle:

Suitable observation systems should be used to target locations and times of year that provide the highest likelihood of detecting the target species.

Guiding Principles:

1. **Target monitoring to ensure all suitable habitat types for each target species are sampled. Where there is more detailed habitat information available than the habitats defined by the MDET, distribute sampling units to ensure that each suitable habitat type is appropriately sampled**
2. **Time monitoring to target periods of aggregation and/or likely high density of each life stage for all species to maximise the likelihood of detection**
3. **Sample larvae[3] in addition to juveniles/adults where genetic and/or morphological techniques allow accurate identification of these life stages.**

3 In this context larval sampling also applies to the planktonic life stages of macroalgae.

2.5.1 Timing of sampling

Time the survey so that the highest possible proportion of species and life stages can be sampled jointly. Table 8 provides an example of how to select appropriate survey times (extracted from the MDET) for a number of species and life stages. In most instances, such as those outlined in Table 8, a spring or summer sampling event will include most adult and larval life stages in temperate waters. Consider a secondary sampling event if this allows observers to target the larval life stages of multiple species present in winter or autumn.

2.5.2 Larval sampling

The preferred approach (i.e. to sample adult and larval life stages) is only practical for those species for which larval probes have been developed [see Attachment A and Doblin & Bolch 2008], or for which morphological identification is practical. This information is provided in the MDET. Furthermore, the feasibility of larval sampling may be influenced by the timing of sampling. The period when larval life stages are present in the water column varies between species and can vary according to unpredictable local oceanographic events. An estimate of the larval period for the target species during an annual seasonal cycle is found in the MDET. Larval period estimates in the MDET are a guide only and can vary both intra- and inter-annually and from location to location.

Table 8. Optimal periods for sampling target species and life stages in temperate waters

LIFE STAGE	SPECIES	SEASON			
		SPRING	SUMMER	AUTUMN	WINTER
Adult stages	*Asterias amurensis*	■	■	■	■
	Musculista senhousia	■	■	■	■
	Sabella spallanzanii	■	■	■	■
	Undaria pinnatifida	■	■		■
	Varicorbula gibba	■	■	■	■
Larval stages	*Asterias amurensis*	■			■
	Musculista senhousia	■	■	■	
	Sabella spallanzanii			■	■
	Undaria pinnatifida	■	■		
	Varicorbula gibba	■	■		

Shaded areas indicate the likely presence of each species, while unshaded areas indicate likely absence of each species at different times of the year

2.5.3 Using the MDET to choose observation systems and sample size

The MDET user guide provided in the MDP contains a detailed description on using the MDET to select observation systems and the appropriate sample size. Produce a summary (e.g. Table 9) of observation systems, sample sizes and costs for each location (or sub-locations) and include it in the relevant section of the MDRT. Field guides are available to explain how and when to use each method and the specific QAQC requirements (see *Observation system methods* in the MDP). Note: also refer to SARDI report on mangrove sampling techniques (Wiltshire & Rowling 2009).

For each suitable habitat a selected species need only be sampled once on each survey occasion. Therefore, the maximum frequency chosen for each life stage should not normally exceed '1' in the MDET 'Choices' worksheet. Designers may consider increasing this frequency to '2' for species where only one identifiable life stage occurs in one suitable habitat, if there are large variations in seasonal abundance that coincides with the seasonal availability of other species' life stages (e.g. larvae). Targeting multiple species in this manner ensures that secondary sampling events are cost effective.

Video surveys and photographs can provide valuable information for identification purposes (e.g. colouration and form), especially for highly motile species that can't be easily collected during diver or snorkel visual surveys. Therefore, it is strongly recommended to use photographs and video in planned surveys that rely heavily on visual sampling methods. *In-situ* photographic records of target species, suspected target species, or other potential marine pests should be used as a verification aid until a specimen is collected and positively identified. Refer to sections 2.5.3, 4.7.2 and Attachment B for more information about video survey requirements.

2.5.4 Manual calculations outside of the MDET template

Manual calculation is required for observation systems that are not included in the MDET. Use information provided in the MDET where possible to ensure consistency within and between designs. MDAP will scrutinise any calculations made outside of the MDET. The following formula (adapted from Hayes et al. 2005) provides the basis for automated calculations in the MDET and must be used to calculate sample sizes independent of the MDET.

$$I = \frac{-\log_e(1-s)}{\left(\frac{p}{a}\right) \times e \times A}$$

Where: I = sample size

p = population size to be detected of the target species (must be sourced directly from the 'Species_data' worksheet in the MDET)

a = area (m^2) of appropriate habitat of location or sub-location (must be consistent with values entered in the 'Location_data' worksheet in the MDET)

s = survey sensitivity, set to 0.8 (i.e. 80 per cent)

e = sample method efficiency (to be determined by the designers)

A = sample method area (to be determined by the designers).

The survey designer needs to determine the sample efficiency and sample method area. Rationale for choosing these values must be included in the monitoring design report. Note: see the MDET 'Design_calcs' worksheet and the *MDET user guide* for examples and more information about these parameters.

Table 9. Example summary table detailing observation systems, sample sizes and cost

SUB-LOCATION	HABITAT TYPE	OBSERVATION METHOD	SAMPLE SIZE	TOTAL COST ($)
Sub-location X	Soft epifauna	Beam trawl		
		Diver visual		
	Soft infauna	Core		
		Grab		
		Dredge		
	Hard horizontal/ vertical	Diver visual		
		Quadrat scrape		
		Fish trap		
	Pelagic horizontal/ vertical	Phytoplankton trawl		
		Zooplankton trawl		
	Pelagic horizontal/ vertical	Phytoplankton trawl		
		Zooplankton trawl		
Sub-location Y	Soft epifauna	Beam trawl		
		Diver visual		
	Soft infauna	Core		
		Grab		
	Pelagic horizontal/ vertical	Phytoplankton trawl		
		Zooplankton trawl		
	Pelagic horizontal/ vertical	Phytoplankton trawl		
		Zooplankton trawl		
Sub-location Z	Hard horizontal/ vertical	Diver visual		
		Quadrat scrape		

2.5.5 Modification of sample sizes

For certain 'species-method' combinations, sample sizes derived from the template (or from manual calculations) may be too high to be practical to implement. Under these circumstances, modification of sample sizes may provide a way of achieving realistic sample sizes for particular 'species-method' combinations. Make these adjustments using the 'Adjust_sample_size' worksheet in MDET. The *MDET user guide* in the MDP should be referred to for detailed instructions on how to use this feature of the MDET.

Base sample size alterations on local knowledge and previous experience in marine pest monitoring. The 'Adjust_sample_size' worksheet in the MDET recalculates sensitivity values and revised costs following reduction in sample sizes.

Designers must include justifications for reducing samples sizes as well as recording the associated decrease in survey sensitivity. Any adjusted sample sizes should be included in the summary table outlined in Table 9. MDAP will consider the justifications for changes to sample sizes.

2.5.6 Modification of survey area for sub-locations

If sample sizes remain too high to be practical after the above modifications (i.e. 2.5.5) have been considered, the area of individual sub-locations may need to be reduced. Under these circumstances, adhere to the guidelines outlined in section 2.3 and provide justification for altering sub-location areas in the design report.

2.5.7 Monitoring for unknown invasive species

Principle: During monitoring and sampling analysis, look for species that appear to have clear impacts or exhibit invasive characteristics (see list below).

Species that appear to have clear impacts or invasive characteristics may be detected through this monitoring program (see secondary objectives for monitoring Box 1).

During monitoring activities or sampling analysis, consider species that exhibit the following characteristics as potential invasive species:

- tendency towards monoculture or high local abundance
- association with degraded habitats
- sudden appearance in this monitoring location^
- strong association with artificial substrate#
- rapid increase in abundance^.

^ assumes prior knowledge of taxa in monitoring location.
# assumes comparable sampling of artificial and natural substrata has occurred.

Any one of these triggers may immediately indicate an unknown invasive species. Others, such as abundance or distribution, may only become apparent after further monitoring. If a suspected new invasive species is detected it is recommended further investigation of the species is initiated in the form of additional monitoring and/or taxonomic verification.

2.6 Select sites to monitor within a location

Principle: Sites should be distributed uniformly (not randomly) within suitable habitats.

Perform all spatial sampling using systematic approaches rather than random. Determine the sites to be sampled by placing a grid over each habitat type and distributing uniformly the required sample size within the survey area. To select the sampling sites within the location, the following steps should be considered:

1. Place a grid over the habitat map using an appropriate scale. The scale should be chosen so that there are at least as many points on the grid as the sample size (l)
2. Select notional sample sites using the grid in a systematic manner

Example:

If each grid cell is approximately 0.1 km x 0.1 km the distribution of samples should be as follows.

HABITAT TYPE	AREA	NUMBER OF SAMPLES	NUMBER OF GRID CELLS	DISTRIBUTION OF SAMPLES
Mud-intertidal	5 km^2	55	500	1 in 9 cells
Sand-intertidal	25 km^2	72	2500	1 in 35 cells
Rock-sub tidal	45 km^2	30	4500	1 in 150 cells

Note: Grids should be used to uniformly distribute a large number of sites over an expansive area of habitat (e.g. soft sediment), but alternative approaches may be required for systematic sampling of certain habitat types (e.g. wharf pylons, rocky intertidal habitats, small patchy habitats). For these habitats, the method used to systematically distribute samples should be explained in the monitoring design report. For example, systematic sampling of wharf pylons may involve sampling at regular distances along a wharf structure.

3. Choose exact sample sites by considering factors such as location of vector nodes, areas of likely habitat, oceanographic features and potential hazards. If a notional site cannot be accessed (e.g. port activities prevent safe access), the site should be moved to a nearby area that is more accessible. Descriptions and justifications should be provided for any sites moved in the above manner. See 'Further guidelines for site selection' below

4. Record the GPS coordinates of final selected sample sites in the relevant section of the MDRT. Sample sites should also be marked within the spatial grid on the monitoring sub-location map(s). If the monitoring area is complex, survey designers may consider presenting a separate map for each sub-location. The MDRT provides more information about the mapping.

Further guidelines for site selection:

It is important to note that the surveys being performed are not for the purpose of calculating unbiased estimates of abundance but rather to detect presence. Thus, it is not necessary to sample at the exact location of the grid, provided the location of the sample point is documented with latitude and longitude coordinates (see relevant standard). Therefore, modify the position of actual survey points to nearby areas that contain likely habitat, rather than sampling areas with a known low likelihood of containing the target species.

However the extent to which a survey point can be moved is restricted to being within the area that the sample represents i.e. for a 1 in 9 cell sample (see example above), the sample location must remain within the boundary of the particular cell in which it has been allocated. Where deviation from this approach is deemed necessary to target suitable habitats, justification must be provided. It is important that two adjacent sample sites are not modified to such a degree that they are very close, thus duplicating the effort in a particular area and leaving large gaps in the survey of suitable habitat that is never examined.

2.7 Monitoring design reporting & approval process

Principle: A monitoring design report must be submitted and assessed by the MDAP. Approval of the design is required before monitoring can commence.

2.7.1 Submission of monitoring design report

For each location, the overall monitoring design will be assessed encompassing all aspects of the design. The monitoring design must follow the QAQC specifications, as outlined in each section above, and be documented and submitted for approval before commencing monitoring (i.e. before the implementation phase). Survey designers are encouraged to use the MDRT when preparing design reports to ensure the necessary specifications are met (outlined in Table 10). Where the MDET has been used to develop survey designs, the relevant MDET file(s) should be submitted electronically to accompany the monitoring design report. If survey designs include sub-locations or modification(s) to survey area, designers should submit the MDET file for the entire monitoring location, along with MDET files relevant to successive stages of design development. The final monitoring design report need only include output from the sub-locations used in the final design (i.e. Tables 7 and 9). MDAP will scrutinise the justifications for modifications to the survey area. Note: the monitoring design report should also include an additional table showing the total reduction in areas for each habitat type between the entire location and the sub-locations used in the final design.

Include the implementation plan (see section 3) as an attachment to the monitoring design report for checking by MDAP.

For approval of the monitoring design for all locations in Australia, submit the report in electronic or hard copy to the relevant jurisdictional body and the Monitoring Coordination Point (see www.marinepests.gov.au/national_system/partners for contact details).

2.7.2 Assessment of monitoring design

The MDAP will assess the design against predetermined criteria (Table 10). MDAP's main purpose is to assess applications for marine pest monitoring designs in Australia under the National System. The MDAP consists of four people representing the Commonwealth and jurisdictions (with technical expertise as necessary) nominated to facilitate timely and effective assessments of monitoring designs. A second person should also be nominated by each group as a backup. Other specific expertise and advice may be sought elsewhere, and the jurisdictions and Monitoring Working Group (MWG) consulted as required. DAFF will chair the MDAP and provide a secretariat.

Allow at least three weeks notice for the assessment of monitoring designs before proceeding with survey implementation (pending approval). For planning purposes, provide sufficient time to address any issues raised by the MDAP. In addition to its role in assessing monitoring designs, MDAP (through the Monitoring Coordination Point) can also be engaged during the design process to provide advice on survey design issues.

Note that once a design has been approved for a NMN location, re-assessment of the monitoring design report to the MDAP is not required unless there are significant changes to the monitoring design or if there have been subsequent changes to the manual and guidelines following formal review of design processes (as outlined in section 6 - Evaluation and review process).

MDAP will prioritise assessment of NMN location designs. However, other monitoring designs may be considered for assessment by MDAP on a case by case basis. Under these circumstances, any related assessment costs may need to be determined and recovered.

Table 10. Required elements and assessment criteria for monitoring design reports

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#	MDAP COMMENTS AND SUGGESTIONS
Objectives (1.1)	Objectives are clearly stated and in line with the primary and secondary objectives in the manual.	Are the objectives clearly stated and achievable?	
Design authors	Identify primary survey design contact person and monitoring designers for any questions regarding design.	Are details for the primary contact person and monitoring designers provided and up to date?	
Stakeholder engagement & governance (1.2)	Identify relevant stakeholders, management bodies and governance arrangements.	Are all likely stakeholders identified? Have all marine pest vectors been included? Have details of monitoring design contacts and governance arrangements been provided?	
Define the monitoring location (2.1)	Identify outer boundary of monitoring location.	Are all marine pest vector routes encompassed in the location? Have environmental factors been considered? Are all likely habitats encompassed?	
Collating existing data including a local knowledge and skills audit (2.2.1)	List appropriate individuals, groups or organisations with local knowledge of the monitoring location.	Is a list of individuals, groups, or organisations with local knowledge and skills provided? Have these skills and knowledge contributed to the design?	

Table 10. continued

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#	MDAP COMMENTS AND SUGGESTIONS
Previous marine pest data (2.2.2)	List any previous monitoring activities or studies including other connected locations, exotic species detected and their GPS positions.	Have results from previous baseline surveys been considered in the design? Are the data presented correctly in table? Are the locations of any detected species included?	
Oceanographic data (2.2.3)	Provide plots of temperature and salinity (boxplots preferred), including sources and quality of data using a monthly resolution.	Do the plots provide the required details and have they been entered correctly into MDET? Have attempts to locate data been documented?	
Identification of vector parameters (2.2.4)	Describe vector nodes, pathways and origins, including sources of data.	Are all vector parameters identified? Do they match the stakeholders and vectors identified above?	
Monitoring area map(s) (2.2.5)	Provide an A4 and electronic version of map(s) including all information required, map/data sources, habitat overlays, vector nodes and pathways, and any hazards. Note. more than one map may be required.	Is the map easy to read and useful? Does the map present all information required and use GDA94 datum? Does the monitoring area and sub-locations target all likely incursion sites?	
Hazard analysis and occupational health and safety issues (2.2.6)	Identify all potential hazards, their effect on monitoring activities, critical times of the year and management actions to minimise hazards.	Is the hazard analysis completed? Can the management options be implemented to minimise risks?	

Table 10. continued

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#	MDAP COMMENTS AND SUGGESTIONS
Habitat and sub-location delineation (2.3)	Identify habitats and include on monitoring area map(s). Identify including sub-locations, and determine habitat coverage, survey area dimensions and % intertidal area. Additional habitat types not listed in the manual must be justified.	Are the habitats well defined? Are area and % presented in the table and MDET correctly? Have all areas been allocated a habitat type? Has the nature of the data used been described?	
Select target species (2.4)	Identify target species to be monitored based on temperature and salinity tolerances.	Has the MDET been used accurately? Are adequate justifications provided for rejecting species/life stages that could survive?	
Choose timing of sampling, observation systems and sample size (2.5)	Select the most efficient observational methods based on cost and ecological considerations. Identify the sample size required to detect each target species. Sampling should target adult and larval life stages where feasible and may necessitate a secondary sampling event.	Has the correct output of sample sizes and observational methods been included in the survey as estimated by the MDET? Are the method choices practical, cost effective and will they provide a reasonable probability of detecting pest species? Have any modifications to sample sizes been justified? Are changes to observational methods (alternative systems) adequately justified?	

Table 10. continued

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#	MDAP COMMENTS AND SUGGESTIONS
Modification of sample sizes (2.5.5)	MDET provides a tool for designers to back calculate changes in detection sensitivity, sample sizes and survey costs.	Have changes to the MDET sampling regime been justified? Are estimates of any resulting parameters provided? Is the proposed sampling efficiency adequately estimated? Are any manual calculations inline with the MDET (refer sections 2.5.4 & 2.5.5)?	
Selecting sites to monitor within a location (2.6)	Sampling sites are selected and spaced appropriately to sample each identified habitat type. Sample locations are plotted accurately on the monitoring area map(s).	Have samples been appropriately distributed? Are the GPS locations of each sample site provided?	
Other	Provide any additional comments and identify any other problems or difficulties encountered during the design phase.	Will any problems or difficulties identified delay implementation or reduce the effectiveness of the monitoring design?	

* Section numbers correspond with the monitoring manual.
# Quality Assurance and Quality Control (QAQC) principles in the manual should be applied.

MDET = Monitoring design Excel template

3. Planning process

Principle: an implementation plan should be submitted for approval before commencing the monitoring program (i.e. commencing sample collection).

The purpose of the implementation plan is to ensure that the logistical components of the monitoring design (such as equipment and personnel) are considered, documented and in place ready for commencement of the monitoring program. All sections in the implementation plan must be completed (an example of the contents are provided in Table 11) and forwarded to the Monitoring Coordination Point (section 2.7.1). To assist the assessment process, include the implementation plan as an attachment and submit it at the same time as the monitoring design report (Note. the required elements of the implementation plan are also included in the MDRT).

3.1 Personnel

Personnel engaged in monitoring activities are required to have suitable qualifications (that meet statutory regulations, if required) and experience. For example personnel engaged in sample collection should have suitable qualifications and experience for collecting samples in aquatic environments and ensuring their integrity for subsequent sample processing and analysis.

Suitable qualifications in this context means formal education, training or experience in taxonomy of marine organisms (in particular marine pests), the competent handling, maintenance and use of sampling equipment (e.g. dredges, grabs, cores, nets), suitable diver qualifications, access to equipment (e.g. boats), an ability to store and preserve samples appropriately, demonstrated knowledge of OH&S procedures and appropriate first aid and CPR qualification.

The design and implementation sections of this manual (sections 2 and 4 respectively) outline the steps that must be undertaken during the monitoring program. Refer to these sections to identify the appropriate personnel (e.g. local experts and/or stakeholders) to utilise in the monitoring process.

Appropriate taxonomic expertise

Principle:

1. **Before implementation, taxonomists that will identify specimens derived from monitoring activities must be identified and contact initiated**
2. **Before implementation, arrangements for storing voucher collections must be confirmed with the appropriate institution.**

Many of the sample analysis techniques require taxonomic expertise to meet QAQC requirements. If a target species is found, verify its identification by a taxonomic expert. Lists of taxonomic expertise, identification tools and curators and collection managers are provided at Attachment C and D. Taxonomists must be engaged at the outset to ensure timely processing of samples.

Monitoring program coordinators need to identify the most appropriate taxonomic personnel using resources provided in this document (Attachment C and D). Monitoring program coordinators should also check with their jurisdictional authority or the Monitoring Coordination Point to establish if there are any pre-existing arrangements with taxonomists that can be extended to their monitoring program. While there is no formal accreditation process for taxonomists (an Australian Standard is currently being developed through the Australian Biological Resources Study), the appropriateness of engaged personnel, based on qualifications and/or relevant experience, will be checked by the MDAP through the implementation plan approval process (see section 3.5, Table 11).

Before monitoring commences, make arrangements with the appropriate institution for the receipt and storage of the voucher collection for the monitoring location (see Attachment D). Voucher collections (section 4.5.6) include one or preferably a few representatives of each target or suspected invasive species collected during the monitoring survey, which are verified by a taxonomic expert and set aside to form reference specimens against which new material is compared. Pre-existing voucher collections also represent a valuable resource for identifying and comparing new specimens with accurately identified ones.

Voucher collections must be stored in the relevant museum or herbarium.

3.2 Standard sampling log sheet

Principle: All monitoring data and results should be recorded in the standard sampling log sheet.

The Standard sampling log sheet is included in the MDP. Use this to ensure that field monitoring results are recorded in a consistent manner to allow comparison between years for a location and if required, comparison between locations.

3.3 Equipment check

Check equipment needed for sampling to ensure it is available and is ready for use during the monitoring times. Some equipment is quite specialised and may need to be built and affect the time the first monitoring activity can commence. Similarly time may be needed to train staff to use specialised equipment.

3.4 Timing, permits and security clearances

Use the implementation plan to set out the work schedule including the date and timing of sampling. Provide confirmation of permits, security clearance(s) or authorisation to undertake sampling (i.e. signed letter from Port Authority with permission to sample).

3.5 Implementation plan approval process

It is preferable to submit the implementation plan to the Monitoring Coordination Point at the same time as the monitoring design. MDAP will assess the implementation plan against predetermined criteria. Following approval of the plan, monitoring can commence.

Table 11. Required elements and assessment criteria for monitoring implementation plans

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#
Personnel (3.1) Note – 3.2 Standard sampling log sheet – does not need to be submitted with the implementation plan	Identifies person responsible for reporting to jurisdictional body and/or Monitoring Coordination Point, and their contact information.	Minimum details to be included: name, phone and fax numbers and email address (including after hours contact phone number)
	Identifies personnel responsible for collecting samples and their qualifications/experience; personnel must meet criteria set out in manual.	Are the qualifications and/ or experience of personnel appropriate for their sample collection responsibilities? Do they have a proven reputation for delivery?
	Identifies personnel responsible for sorting, analysing and identifying samples and their qualifications/experience; demonstrated proof that taxonomists and relevant museum/sample archive facility has been engaged (e.g. signed letter/email).	Are qualifications and/ or expertise of personnel appropriate for their sample processing and analysis responsibilities? Do they have a proven reputation for delivery? Have taxonomists been engaged to verify identifications?
	Organisation chart that shows lines of authority and responsibilities for collecting and analysing samples.	
Equipment check (3.3)	Identifies sampling equipment to be used and relevant support facilities (e.g. nearby berths for sampling vessels, storage locations for equipment/ samples, regional laboratories/museums).	Is the equipment suitable to undertake the sampling? Are support facilities appropriate and practical?
	Identifies where samples will be analysed.	Are analytical facilities appropriate?
	States how and where samples will be stored.	Are the sample storage conditions and facilities appropriate?
	Provides list of sample labels.	Do the labels contain the correct number of elements and correct codes?

Table 11. continued

ASSESSMENT CRITERIA*	REQUIRED ELEMENTS	QUALITY CONTROL CHECKS#
Timing, permits and security clearances (3.4)	Provides work schedule including date and timing of sampling.	Is it realistic? Does the work schedule match the timing for sampling identified in the monitoring design?
	Confirmation of permits, security clearance/ authorisation	
Other	Describes hazard mitigation/ contingency planning (if appropriate) during sample collection; Must include all elements identified in the hazard table (see 2.2.6)	Are all the environmental and OH&S issues the monitoring design identified discussed? Are the hazard mitigation/ contingency planning strategies appropriate and practical
	Provides proposed budget with justification of each budgetary component	Is the budget appropriate for the tasks at hand?

* Section numbers correspond with the monitoring manual.
# The quality control checks will be used by the parties approving the implementation plan.

4. Implementation process

During implementation, undertake the necessary field work at each location to gather the required number of samples for laboratory analysis. This section explains the common steps that need to be undertaken during collection, processing and analysis of samples.

Field and sample processing guides are available to assist monitoring teams with the implementation process. The *Observation system methods* guide in the MDP provides detailed instructions on sample collection for each observation system, while sample processing guides (Attachment B) provide instructions for specific observation systems.

4.1 Sample collection

Sample collection will take place according to the monitoring design approved by MDAP.

4.1.1 Position fixing

The location of each sampling site should be recorded so it can easily be relocated in subsequent monitoring surveys.

Principle: The minimum required information includes:

1. **date (required format: dd/mm/yyyy)**
2. **time of day (24hr clock in local time but should indicate whether daylight savings time or not)**
3. **unique site specific identification code/number**
4. **GPS location (latitude, longitude in decimal degrees to a minimum of 6 decimal places, using the relevant national datum standard[4])**
5. **relevant navigational markers (e.g. St. Kilda pier)**
6. **site description (i.e. habitat).**

4 The Australian Standard is GDA 94 (www.ga.gov.au/geodesy/datums/gda.jsp).

4.1.2 Labelling samples and specimens

Principles: Samples must be labelled with a unique identifier as per the standard below on appropriate label paper.

Label samples and specimens to ensure that pest species can be accurately identified and their precise location recorded. This is to avoid misidentification or incorrect geographic location. Label samples and specimens with unique identifiers. The labels outlined below relate to specific information that will allow individuality.

Labelling Standard

SURVEY ID	SITE ID	METHOD ID	SPECIMEN NUMBER
XXXXXXX	XXXX	XX	XX

The first 13 characters identify the unique sample (the sampling strategy assumes a single replicate sample per observation method per site), while the entire 15 character code identifies individual specimens. For some observation systems individual specimens may be labelled in the field, however, for most observation systems the sample will need to be transported back to the laboratory for sorting. In this instance, samples should be labelled with the 13 character code.

Example

The unique identifying code for the second specimen taken by a beam trawl at channel 10 marker in Adelaide in March 2008 will be coded as:

SURVEY ID	SITE ID	METHOD ID	SPECIMEN NUMBER
ADL0803	CH10	TR	02

Leading to a unique specimen code of ADL0803CH10TR02.

Key to unique specimen code

LABEL	DESCRIPTION	CODE DETAILS
Survey ID	Code to identify the survey location and iteration (based on date). For standard codes for the monitoring locations see Table 12	Use three characters to describe the location, with two numbers for year and two numbers for the month the survey was conducted e.g. ADL0803; for a survey in Adelaide in March 2008
Site ID	Code to identify the site location where the sample was taken. Note: these must be unique[5]	Four symbols to describe the site location e.g. CH10; for channel marker # 10
Method ID	Code to identify the survey method used to collect the sample. See Table 13 for a full list of sample method codes[6]	Two character code e.g. TR; beam trawl
Specimen Number	Number representing the specimen number	Two numbers e.g. 02; for the second specimen taken.

Where the labelling standards specify month or specimen number, any single digit must be prefixed with a zero. For example March should be represented as 03 and the first sample as 01. Year should be recorded by the last two digits, for example 2008 should be coded as 08. Site identifying codes must be four symbols long. If less than four symbols are required, it must be suffixed with an underscore(s) to fill four spaces, for example W1__

The following are guidelines for labelling marine pest specimens.

1. Use the labelling standard above and record all details onto the label.
2. Take special care with labels placed in alcohol. Use paper of a high quality rag or linen. Write on acid-free labels in pencil or inks that contain vegetable gum (such as India inks). Computer generated (laser printed) labels are acceptable, provided that labels are baked to an appropriate quality paper (See Attachment D).
3. Put labels inside the specimen bag or jar. For specimens that are likely to chafe, discolour or cause physical damage to the label, place the label inside a small zip-lock plastic bag that protects damage from specimens during transport and storage. If an outside label is needed, use it in addition to that inside the jar and cover with clear plastic tape. With very large specimens, attach the label directly to the specimen as well as on the outside of the bag.

5 It is important to ensure these identifiers are unique. If a survey design is submitted with duplicates in this field it will not be accepted by the MDAP.

6 If a method is used that is not in Table 13, the code used needs to be explained and recorded in the survey design and be unique from existing codes. One letter codes must be suffixed by an underscore, for example A_.

4. When labelling specimens during field sampling, be aware that some animals will eat or otherwise destroy paper labels.

Table 12. NMN location codes

LOCATION	CODE
Adelaide	ADL
Botany Bay	BTB
Bowen	ZBO
Brisbane	BNE
Bundaberg	BDB
Burnie	BWT
Cairns	CNS
Dalrymple Bay	HPT
Dampier	DAM
Darwin	DRW
Fremantle	FRE
Gladstone	GLT
Hobart	HBA
Melbourne	MEL
Newcastle	NTL
Portland	PTJ
Port Hedland	PHE
Port Kembla	PKL
Sydney	SYD
Townsville	TSV

Table 13. Observation system codes

OBSERVATION SYSTEM	CODE
Quadrat scrape	P_
Diver visual	DV
Snorkel visual	SV
Zooplankton trawl	ZT
Phytoplankton trawl	PT
Beam trawl	TR
Grab	VG
Core	C_
Trap	T_
Walk visual	BW

Note: Codes are consistent with the codes used in the National Port Survey Database. Location codes are international standard UN/LOCODE codes (United Nations Code for Trade and Transport Locations).

4.2 Interim reporting – post sample collection

Principle: a post sample collection report should be submitted after each monitoring activity.

After each sampling period a report is required to indicate whether monitoring is on track. The post sample collection report should contain all the elements listed in Table 14 and include any new marine pests detected (refer to sections 7.3 and 7.4 in the monitoring guidelines). Submit the report in electronic form to the relevant jurisdictional body and the Monitoring Coordination Point (see www.marinepests.gov.au/national_system/partners for contact details).

Table 14. Required elements of the interim report following sample collection

QUALITY ASSURANCE ACTIVITY	QUALITY CONTROL CHECK
Identifies contact person for questions regarding the post sample collection report.	Details include at a minimum: name, phone and fax numbers and email address.
Includes sample collection summary and an indication as to whether all samples were collected as planned.	Includes the number of samples collected and the percentage of the total required and if sampling is on track.
If not all samples were collected, includes management action to keep monitoring program on track.	Includes contingency plan to collect more samples or to get monitoring program on track.
Indicates whether any target species (or unknowns) were detected.	If found, includes species names and action taken.
Identifies person(s) responsible for future reporting including the monitoring report.	Details to include at a minimum: name, phone and fax numbers and email address.

4.3 Sample processing and analysis

Principles:

1. **Collected samples must be processed using approved procedures**
2. **Identification of sample must be undertaken by personnel with demonstrated expertise in identifying target species.**

Process samples collected from each location using approved procedures to ensure the integrity of the sample. Identification of samples must be undertaken by personnel who have a demonstrated expertise in identifying target species.

The following sections describe the procedures for processing and analysing samples collected during monitoring. QAQC issues have been highlighted by indicating:

1. the level of expertise required for separate sample processing and sample identification steps
2. specific quality control checks.

QAQC has identified three levels of expertise that may be required to undertake sample processing and analysis:

- level 1: anyone can do it, no training required
- level 2: some training required
- level 3: formal training or formal qualifications required (i.e. marine taxonomists or marine biologists).

Note: These people will be identified in the implementation plan (section 3.1).

Sample processing has been divided into two parts: sample processing in the field (section 4.4) and sample processing in the laboratory (section 4.5).

A summary of sample processing methods appropriate for each sample collection method is shown in Table 15.

During sample processing and analysis, identification should be targeted to the organisms on the target species list for this monitoring location (section 2.4) and other species that show invasive characteristics (section 2.5.7).

Full lists of natives and exotic species are NOT required.

Table 15. Sample processing methods appropriate for each observation system

OBSERVATION SYSTEM	SAMPLE TYPE	PROCESS ENTIRE SAMPLE (Y/N)	FIELD PROCESSING (INCLUDING SUB-SAMPLING REQUIREMENTS)	LAB PROCESSING AND ANALYSIS
Small cores (hand collected)	Sediment (small volume)	N	Examine core for bioturbation (benthic mixing); store upright cold and in the dark.	Section top 2cm of core. Sieve, concentrate and examine aliquots under a compound microscope and identify dinoflagellate cysts.
Harpoon core				

Table 15. continued

OBSERVATION SYSTEM	SAMPLE TYPE	PROCESS ENTIRE SAMPLE (Y/N)	FIELD PROCESSING (INCLUDING SUB-SAMPLING REQUIREMENTS)	LAB PROCESSING AND ANALYSIS
Large cores (e.g. box cores)	Sediment (large volume)	Y	Visually observe entire sample removing any incompatible specimens; sieve sample (1 mm), fix. Store samples in cool, dark place.	Wash and preserve specimens according to taxon. Sort further and identify.
Grab sampler		Y		
Settlement trays		Y		
Benthic sled/ dredge	Sediment (large volume)	N	Visually observe entire sample removing any incompatible specimens; homogenise and subsample to obtain a representative sample and elutriate. Fix and store samples in cool, dark place.	Sieve sample, wash and preserve specimens according to taxon. Sort further and identify.
Beam trawls		N		
Phytoplankton & zooplankton net tows	Plankton	Y	Wash entire sample into cod-end; pour sample into screw-capped jar, fix (if appropriate); store upright in cool, dark place.	Split sample - use 1 portion for culturing live individuals; preserve other portion; examine under a compound or dissecting microscope and identify. For molecular probe analyses, extract DNA, apply primers and probes to identify.
		Y		For molecular probe analyses, extract DNA, apply primers and probes to identify.
Bucket/ sampling bottle		Y or N		

Table 15. continued

OBSERVATION SYSTEM	SAMPLE TYPE	PROCESS ENTIRE SAMPLE (Y/N)	FIELD PROCESSING (INCLUDING SUB-SAMPLING REQUIREMENTS)	LAB PROCESSING AND ANALYSIS
Scrapings by divers	Fouling organisms	Y	Collect material in mesh bag, place in zip-lock bag or sealable box, fix and store in cool, dark place.	Rinse bag, sieve material then preserve specimens according to taxon. Identify using dissecting microscope.
Spat bags		Y		
Settlement plates		Y	Photograph settlement plates, fix and store submerged in fixative.	Preserve organisms intact on plate. Identify using dissecting microscope.
Beach seines	Fish and large invertebrates	Y	Separate incompatible specimens. Sort sample into broad taxonomic groups (i.e. fish, seaweed, invertebrates), place into separate sample containers, fix and store in cool dark place.	Further sort samples preserve according to taxon and identify.
Traps		Y		
Poison stations		Y		
Visual transect with video/diver	Images with individual specimens	N	Annotate field log with image names and download images onto computer; backup video footage; sort specimens and place in individual jars/ zip-lock bags; fix and store in cool dark place.	Narcotise, preserve according to taxon and identify, cross-checking with images or video footage.
Beach walk/ shore searches	Images with individual specimens	Y		

4.4 Sample processing in the field

The extent of sample processing in the field is dependent on the type of sample collected. There is minimal processing of small volume sediment cores, plankton samples, fouling organisms on plates and images/video collected during visual transects (see Table 15 above). However, large volume sediment samples, material from beach seines, traps, and poison stations must be processed before being transported back to the laboratory. In addition, samples for DNA analysis need to be separated or sub-sampled before a narcotising agent or fixative is applied to the rest of the sample (see section 4.4.3).

Detailed sample processing guides containing instructions for field processing samples collected using specific observation systems are provided at Attachment B. Procedures used to process more than one sample type are described below.

It is critical that sample labels (see section 4.1.2) are maintained throughout the processing and analysis steps.

4.4.1 Separation of incompatible specimens

Sample processing principle: Level 2 expertise

Once samples have been removed from the collection device, examine and select organisms to be separated before transporting back to the laboratory. Separation of incompatible parts of the sample is a quality assurance process that provides intact samples for easier identification. Organisms to be removed and handled separately include:

- omnivorous animals (that may eat or damage other organisms in the sample)
- organisms that produce slime or mucus (e.g. sponges)
- hard organisms such as bryozoans, coral or large molluscs (that will damage delicate organisms)
- organisms that require different fixing and preserving treatment (see Table 16 and section 4.4.3).

4.4.2 Sub-sampling for DNA analysis

Sample processing principle: Level 3 expertise

Principle: All samples should be labelled with a unique identifier in the field.

DNA sub-samples may be required under two circumstances:

1. for molecular probe analyses of phytoplankton and zooplankton samples[7]

7 Doblin, MA & Bolch, CJS 2008 'Review of genetic methods as a tool for identification of introduced marine pests', Access: UTS Report for the Department of Environment, Water, Heritage and the Arts, pp 43

2. to verify the taxonomic identity of species whose taxonomy is difficult (e.g. *Caulerpa taxifolia*).

For any species that requires DNA analysis, suitably fixed and preserved material must be available. Fixing by formalin is unsuitable for DNA analysis as it is an aldehyde which will quickly dissolve delicate calcareous structures. If DNA analysis is required, sub-sample the original sample to keep part of it for DNA analysis and fix the rest of the sample according to the guidelines outlined in section 4.4.3.

Keep material for DNA analysis cold and freeze at -80 ºC within 1 hour of collection (liquid nitrogen can be used for freezing samples in the field). If freezing is not practical, some samples (e.g. invertebrates, macroalgae, plankton) can be fixed and preserved in SET buffered (0.375 M NaCl, 2.5 mM EDTA, 40 mM Tris HCl, pH 7.8) 90 per cent ethanol or dried using a desiccant (e.g. macroalgae). Refer to individual sample processing guides for further instruction (see Attachment B).

It essential that samples are processed correctly for DNA analysis as deterioration of DNA material affects the reliability of planned analyses. It is recommended that personnel undertaking field surveys consult with those expert DNA taxonomists engaged in sample analysis prior to sample collection. This will ensure the most effective preservation technique is used for particular sample types, taking environmental factors (e.g. temperature) and logistical features of the monitoring location (e.g. proximity of laboratory facilities to field site) into account.

4.4.3 Narcotisation and fixation

Sample processing principle: Level 2 expertise

Principles:

1. **Immediately after collection, samples are required to be stored in one of the following ways if a delay is anticipated before sorting and preservation (if necessary):**
    - **a. insulated containers at ambient temperature in the dark, or**
    - **b. stored on ice, which will act to narcotise the species**
2. **Species that require narcotisation must be processed within three hours (see Table 16).**

Narcotisation

Target species that require narcotisation are identified in Table 16. These specimens need to be narcotised ('relaxed') to ensure they do not distort on contact with fixative, making them unidentifiable. Species can be narcotised using a 25 per cent w/v (weight/volume) solution of magnesium sulphate ($MgSO_4$) or magnesium chloride ($MgCl_2$):

Step 1: Weigh out 25 g of the inorganic salt (magnesium sulphate/ magnesium chloride ($MgSO_4$/$MgCl_{2)}$ and dissolve it in 75 ml of deionised distilled water.

Step 2: Add the solution to the specimen in seawater over a period of an hour. Narcotisation is complete when the specimen does not react to touch (or reacts slowly or sluggishly).

Note: For specimens of Tunicata, individuals should be narcotised using menthol crystals, for up to three hours for colonies and five or more hours for large solitary specimens (Kott 2007).

Step 3: Fix the samples immediately. (see below)

Alternatively, specimens can be frozen:

Step 1: In a small volume of seawater (enough to cover the specimen) store the specimen at -20 °C until the water freezes.

Step 2: Upon thawing the specimen must be fixed immediately.

Freezing of bulk samples of unprocessed specimens and sediment/substrate should be avoided since some specimens – those that thaw first – will deteriorate before the entire sample thaws.

Fixation

Fixation coagulates and stabilises proteins in specimens so that they do not distort or deteriorate during preservation, analysis, identification and storage. In most cases specimens need to be fixed immediately after sample collection and after narcotising if appropriate. Table 16 contains a list of the preferred fixation methods for marine invertebrates.

Formalin is used to fix many specimens. It is purchased as a solution of 40 per cent formaldehyde in water which is equivalent to 100 per cent formalin. The 100 per cent formalin solution is then diluted 1:9 with seawater to achieve its final concentration (typically 4-10 per cent). For large soft bodied organisms (e.g. solitary ascidians), it may be necessary to inject fixative into the body of the organism.

Fish are fixed in a final concentration of 10 per cent formalin. Prior to fixation, anaesthetise fish using a suitable anaesthetic (e.g. clove oil). Place the specimens into an appropriately-sized empty sample jar (ensuring they are not damaged) and add 10 per cent formalin to cover specimens. Note: There should be no seawater present in the jar because it will dilute the preservative.

Invertebrates are typically fixed in 70 per cent ethanol or 4 per cent formalin.

Macroalgae are typically fixed in 4 per cent formalin.

When fixing using formalin, add the appropriate volume of 10 per cent formalin to the volume of the specimen in seawater to get the right concentration. Apply the following formula to determine how much 10 per cent formalin to add:

volume of 10 per cent formalin to add = 0.667 x (volume of seawater with specimen)

Note: Formalin is an aldehyde which will quickly dissolve delicate calcareous structures (this is why echinoderms and many other taxa are fixed in ethanol). Mixing formalin in seawater is a partially effective buffer against acidity and this should be applied in all cases. Addition of a small quantity of sodium tetraborate ('borax') crystals is even better (use a level teaspoonful of borax per litre of 10 per cent formalin).

Table 16. Preferred narcotising, fixation and preservation methods for major groups of marine taxa

This information is also available and updated via the Taxonomy Research and Information Network (TRIN) at www.taxonomy.org.au and TRIN Wiki at http://wiki.trin.org.

TAXON	NARCOTISATION*	FIXATION	PRESERVATION	COMMENTS
Annelida (Leeches, Oligochaetes, Polychaetes)	M or F	4% formalin	70% ethanol	Leeches and some polychaete families are easier to identify if anaesthetised, but this is generally impractical in large benthic studies.
Crustacea	F	4% formalin	70% ethanol	
Brachiopoda	No or F	70% ethanol	70% ethanol	
Bryozoa (=Ectoprocta)		70% ethanol	70% ethanol	

Table 16. continued

TAXON	NARCOTISATION*	FIXATION	PRESERVATION	COMMENTS
Cnidaria Octocorallia	M or F	70% ethanol	70% ethanol	Formalin will dissolve spicules and render many octocorals unidentifiable.
Cnidaria – Scyphozoa	M or No	4% formalin	4% formalin	
Cnidaria (others)	M or F or No	70% ethanol	70% ethanol	
Ctenophora	No or M	4% formalin	4% formalin	
Echinodermata	No or F or M	70% ethanol	70% ethanol	Formalin will render many echinoderms unidentifiable, especially holothurians.
Echiura	M	4% formalin	70% ethanol	Narcotise (freezing or propylene phenoxytol or $MgCl_2$) if at all possible
Kamptozoa	No or M	4% formalin	70% ethanol	
Mollusca Opisthobranchia (=nudibranchs)	M or F	4% formalin	70% ethanol	Narcotise (freezing or propylene phenoxytol or $MgCl_2$) if at all possible; photographs also very useful
Mollusca	No or F or M	4% formalin	70% ethanol	
Nemertea	M	4% formalin	70% ethanol	Probably unidentifiable unless narcotised (freezing or propylene phenoxytol or $MgCl_2$)
Phoronida	No or F or M	4% formalin	70% ethanol	

Table 16. continued

TAXON	NARCOTISATION*	FIXATION	PRESERVATION	COMMENTS
Platyhelminthes	F or M	4% formalin	70% ethanol	Fix living specimens on frozen 4% formalin [see safety notes above] or narcotise (freezing or propylene phenoxytol or $MgCl_2$). Otherwise probably unidentifiable.
Porifera	No	70% ethanol	70% ethanol	Formalin will render most sponges unidentifiable
Pycnogonida		70% ethanol	70% ethanol	
Sipuncula	M	4% formalin	70% ethanol	Very difficult to identify unless first narcotised (freezing or propylene phenoxytol or $MgCl_2$)
Tunicata	MC	4% formalin	70% ethanol	
Zooplankton	No	4% formalin	70% ethanol	
Phytoplankton	No	1% glutaraldehyde	1% glutaraldehyde	1% Lugol's solution can also be used
Macroalgae		4% formalin	Glycerol-ethanol solution;	Preservation may also be achieved by air-drying
All others		4% formalin	70% ethanol	"default method"

* where M = $MgSO_4$ or $MgCl_2$, MC = menthol crystals, F = freezing and No = narcotisation not needed, source: Hewitt, C.L. and Martin, R.B. (2001)

4.5 Sample processing in the laboratory

Sample processing principle: Level 2 expertise (some training required).

Principles: Sample integrity should be maintained during sample processing so that fixed samples are stored for at least 72 hours.

Once samples have been transported back to the laboratory, fix samples for at least 72 hours before washing, sorting and transferring to preservative. General procedures for how to process samples in the laboratory are described below.

Sample processing guides (see Attachment B) provide detailed instructions for laboratory processing of samples collected using specific observation systems. More general procedures that can be used to process more than one sample type are described below.

4.5.1 Washing samples

Sample processing principle: Level 1 expertise (no training required).

Principle: The samples should be washed with seawater if un-fixed and freshwater if fixed, until the water runs clear.

Washing has two purposes: (a) to remove fixative from the sample, and (b) to remove mud and other fine material from the sample to facilitate sorting.

- washing of samples in the laboratory must continue to use the same or smaller aperture size sieve as was used during field processing. Depending on the nature of the sample, a nest of sieves with finest aperture on the bottom may assist in these processes
- tap water is used to wash fixed samples
- unfixed or freshly collected marine samples should be washed in seawater (Note: They must not be washed with freshwater or osmosis will damage many soft-bodied invertebrates)
- water should be applied gently so as to minimise damage to specimens (best if the sieve can be partly submerged)
- washing of fixed samples should take place in a fume hood using appropriate protective gloves and safety equipment for the fixative (see relevant Material Safety Data Sheets).

Step 1: Place the sample in an appropriately sized sieve.

Step 2: Gently wash (with seawater/freshwater as appropriate) until the water runs clear.

4.5.2 Initial sorting and transferring to preservative

Sorting samples

Sample processing principle: Level 2 expertise (some training required).

Principle: Sorting should be accurate and relevant for the target species of interest.

Sorting involves separating specimens into groups based on morphology. The level of initial sorting depends in part on the knowledge and experience of the sorter, and on the species richness of the sample. Some target species are conspicuous and unique and are easily separable from the remainder of a sample. Other target species may require a greater level of sorting before identification.

Step 1: Once clean water runs from the sieve the sample can remain submerged in water (sea or fresh). Transfer the sample into manageable portions to trays for sorting.

Step 2: Wash samples one at a time so that initial sorting can take place while the sample is submerged in water.

Identification should target species on the monitoring target species list for the monitoring location (see section 2.4) and other species that show invasive characteristics (see section 2.5.7). It is not necessary to identify all native and exotic species.

Note: If the sample remains unsorted for longer than about 24 hours, drain it of water and transfer it to preservative solution. This process may need to be reversed when sorting resumes.

Preserving samples

Sample processing principles: Level 2 expertise (some training required).

Principles: Once samples have been fixed for the appropriate period and sorted, they should be preserved.

Following sorting, samples are preserved. Preservation is necessary for all specimens except those for DNA analysis (see section 4.4.3), as it allows long-term storage in a fluid which protects the specimen (as much as possible) from deterioration.

Note: Fixation and preservation are often confused as some solutions can be used as both fixative and preservative.

The preferred fixation and preservation methods for major groups of marine taxa are presented in Table 16. The recommendations in Table 16 are a compromise between what is ideal and what is practical when treating large unsorted samples. Specific methods, especially narcotisation procedures, are preferred when possible for various taxa. Further information in relation to taxa specific fixation and preservation methods may be found in the sample processing guides (Attachments B, C, D and E).

In order to preserve specimens place the fixed and washed specimen in a sample jar with its label (see 4.1.2) and add the appropriate volume of preservative to cover the specimen plus an additional 5 cm in case of evaporation (see Table 16).

Note: Analytical grade ethanol is available as a 95 per cent solution. Use deionised distilled water to dilute to 70 per cent.

4.5.3 Organising samples

Sample processing principle: Level 2 expertise (some training required).

Principles:

1. **Specimens should be organised into taxonomic lots**
2. **All components of a sample must be retained as separate labelled lots, maintained and stored.**

Organising specimens into similar taxonomic lots enables easy comparison between specimens during further sorting and identification. It is therefore important to sort material into taxonomic lots as soon as possible.

It is essential to organise material into taxonomic lots before transferring to museums or herbariums. Material that is poorly sorted will be expensive for museums to label and process, and could be rejected.

All components of a sample must be retained as separate labelled lots, maintained and stored in a way that allows rapid access. Budgets, tender and contract documents will need to reflect this commitment.

Separating, identifying and recording species or other taxa present in a sample and then recombining all material into one container is unacceptable. A significant consequence is that the data is effectively unverifiable since re-examination of the sample by any other person would first require that they provide the time, money and resources required to sort the sample again. Recombining of samples makes it impossible to meet the QAQC standards applied in this document.

4.5.4 Engaging taxonomic expertise

Sample processing principle: Level 3 expertise (formal training or qualifications required)

Principle: The identified taxonomic experts (section 3.1) should be engaged in the remaining sample analysis steps.

The remaining sample analysis steps require taxonomic expertise to meet the QAQC requirements. Use the appropriate taxonomic experts that were identified and contacted before monitoring commenced (section 3.1). A listing of taxonomic expertise and identification tools is provided in Attachment C. Taxonomic experts may be engaged to provide advice on sample processing to the other Level 3 experts involved in the survey (i.e. generic marine taxonomists or marine biologists).

4.5.5 Finer sorting

Sample processing principles:

1. **Level 3 expertise for sorting or training and supervision of sorting staff**
2. **Level 2 expertise for sorting.**

Principles:

1. **A taxonomist should train and then work with the sorter(s) to check accuracy of species units**
2. **A taxonomist should check sorting accuracy of the first 10 samples, and every 1 in 10 samples thereafter.**

Sorting and identification of species in a sample involves two distinct processes:

1. recognising species units and distinguishing them accurately (recognising "look-alikes")
2. if necessary, identifying species units (i.e. giving each species a genus and species name, or best level possible), and determining the presence of target species.

The primary goal for sorting of samples is to achieve reproducible results. This means the sample must be sorted into taxonomic groupings that can be recognised by all sorters without error.

Depending on the sample type, many target taxa may be recognised and separated during the initial sorting phase. This may involve immediate recognition and separation of, for example: large species such as *Asterias amurensis*; or of organisms requiring detailed examination to detect likely target species (e.g. barnacles). Abundant specimens that cannot be readily identified as native species (i.e. potential 'outbreaks' of 'unknown' species) should also be separated at this stage for subsequent identification.

Failure to keep the recognition of like-organisms and identification steps separate is common and a major source of error, especially among inexperienced workers. If 'look-alikes' are recognised accurately then a small voucher collection (see below) can be assembled and a taxonomist can provide names which can be applied with some confidence to all other material. However, if 'look-alikes' confuse several species then all material will need to be resorted. To avoid error and resorting of samples, an experienced taxonomist must be involved with training and/or quality control of identifications early in the sorting process.

Following sorting into look-alikes, provide relevant taxonomists with suspected target species for verification (section 4.7).

Step 1: Separate out any immediately recognisable target species specimens or abundant specimens of 'unknown' species.

Step 2: Carefully sort out target species units and 'look-alikes'.

Step 3: Retain a few representatives of each target species for a voucher collection (section 4.5.6).

Step 4: Retain a further few representatives of each target species for a reference collection while the voucher collection is in transit.

4.5.6 Voucher collections

Sample processing principle: Level 3 expertise (formal training or qualifications required).

Principle: A voucher collection including target species, or suspected invasive species collected during the survey, should be produced and lodged with the appropriate institution (see Attachment D).

Voucher collections include one or preferably a few representatives of each target or suspected introduced species collected during the survey, which are set aside to form reference specimens against which new material is compared. Algal vouchers should be primarily prepared as herbarium sheets (see Attachment B), however, permanent microscope mounts may be suitable for small specimens. If the herbarium has a wet stack, liquid preserved sub-samples should accompany the herbarium sheets. If DNA verification is undertaken, there should also be a corresponding herbarium sheet voucher of the plant. During implementation, arrangements should have been made for storing voucher collections for the monitoring location at the appropriate institution (section 3.1).

Voucher collections must be stored in the relevant museum or herbarium.

Voucher collections should be maintained and documented with illustrations and descriptions of key characters, reference to published keys and descriptions, differences from similar taxa, and other notes helpful to current and future identifiers (e.g. 'antennae often lost'). Computer-based media are the most efficient for storing this data, including digital images and digitised drawings.

It is important for the group undertaking the monitoring to retain a reference collection. A reference collection is a copy of the voucher collection, i.e. includes a specimen of every species in the voucher collection. The reference collection should be kept while the voucher collection is in transit to its final holding location in case it is lost.

Note: About Quality Assurance - A voucher collection can only be considered representative of target species identifications if a taxonomist has been involved in its development and met the quality control requirements.

The quality control requirements for voucher collections include all of the following:

- random checks of at least 10 per cent of all target species "look-alikes" specimens by a taxonomist
- random verification of at least 10 per cent of all voucher and non-voucher specimens by a taxonomist
- notes or other documentation demonstrating on what criteria identifications were made
- clearly labelled specimens (see section 4.1.2).

4.6 Sample transport and transfer to storage facilities

Sample processing principle: Level 2 expertise (some training required).

Principle: samples must be packed appropriately before being transported to storage facilities.

Transport and transfer of samples and data to a museum or an equivalent institution can only take place by prior agreement with staff of the receiving institution. Arrangements for receiving and holding specimens must be made prior to sample collection and should be organised during the planning stage (sections 3.1 and 4.5.6).

Packing and mailing

Sample processing principle: Level 2 expertise (some training required).

Principle: keep a record of shipping details, in case tracking is required.

Collection managers in each museum or herbarium (see Attachment D) can provide advice on packaging and mailing samples. Expert DNA taxonomists should also be consulted prior to packaging and sending DNA samples to ensure that sample quality is maintained.

Formalin (Corrosive Fluid Class 8), and ethanol exceeding 24 per cent (Flammable Liquid Class 3), are dangerous goods. Dangerous goods must be packed by appropriately trained and accredited personnel, or by approved couriers. For up-to-date advice on dangerous goods and approved commercial couriers refer to the Australian Government Civil Aviation Safety Authority website (www.casa.gov.au/dg/).

It may be preferable to send samples in a form not considered as dangerous goods since there are fewer restrictions, making the process faster and cheaper. There are a number of options to avoid issues surrounding transportation of dangerous goods:

- temporarily transfer specimens from 70 per cent ethanol to 20 per cent ethanol for transport. Most invertebrates will not deteriorate if previously well-fixed and held in 20 per cent for a week or two. However, this method is impractical when numerous samples or minute specimens are involved. Samples in 20 per cent ethanol must be carefully labelled or mail authorities will assume they are dangerous goods
- DNA tissue can be removed from 95 per cent ethanol and transported in vials empty of fluid
- material that has been fixed 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 remain moist with a little liquid in a well-sealed container
- if possible it is important to maintain storage conditions while in transit e.g. place frozen samples or samples usually stored at 4 °C in an insulated container with a dry ice pack or in a temperature controlled container. Note: If dry ice packs are used, they need to comply with the appropriate regulations (see www.casa.gov.au/dg/).

It is important for the monitoring agent to retain a reference collection of specimens in the voucher collection while the voucher collection is in transit to its final holding location in case it is lost. However reference collections will be useful for future monitoring activities and therefore it is suggested that if possible it be retained indefinitely.

4.7 Verification and interpretation of results

During sample analysis (previous sections) target species identifications will be confirmed by taxonomic experts and results will be compiled in a standard electronic format for each location (see Monitoring data input sheet in the MDP).

If a new or suspected incursion of a marine pest is found contact the relevant state/territory or the Monitoring Coordination Point within 48 hours**.** A written report must be submitted within four weeks.

4.7.1 Checking sample and specimen labelling accuracy

Sample processing principle: Level 2 expertise (some training required).

Principles:

1. **Labels must match field log information**
2. **All samples and specimens should be accounted for (including all parts that were separated during sample processing and analysis)**
3. **Samples and specimens should be stored with the appropriate custodian.**

Cross-check sampling logs with samples, specimens and their labels. Note: Labelling requirements vary depending on the observation system. For most observation systems (e.g. visual census, quadrat scrapes) labelling applies to samples and individual specimens, however labelling of specimens is not feasible for observation systems where the entire sample is stored (e.g. zooplankton and phytoplankton trawls). All samples and specimens that were collected should be accounted for to determine: (1) whether labels match field log information; and (2) their whereabouts. To do this, use Table 17 as a guide. This information should be sufficient for anyone to identify where the sample or specimen is and retrieve it. Note: Refer to section 4.1.2 for explanation of labelling codes.

Table 17. Example of table used to verify labelling of samples is accurate

SAMPLE LABEL	SAMPLE TYPE	SPECIMEN NO.	SPECIMEN LABEL	STORAGE LOCATION
ADL0803CH01TR	Beam trawl	1	ADL0803CH01TR01	SA Museum
ADL0803CH01TR	Beam trawl	2	ADL0803CH01TR02	SA Museum
ADL0803CH01DV	Diver visual	1	ADL0803CH01DV01	SA Museum
ADL0803CH01DV	Diver visual	2	ADL0803CH01DV02	SA Museum
ADL0803CH01ZT	Zooplankton trawl	-	NA#	SA Museum

#Note: specimen labels are not applicable because zooplankton trawls are stored as entire samples.

4.7.2 Verifying target species presence

Sample processing principle: Level 3 expertise (formal training or qualifications required).

Principles:

1. **Identification of target species must be verified by a taxonomic expert**
2. **The identified taxonomic experts (section 3.1) engaged in identifying specimens derived from monitoring activities should be identified and contacted before monitoring commences**
3. **A target species is considered present if a minimum of one individual (alive or dead), or part of a shell/exuviae is found in a sample**
4. **Target species presence must be substantiated with three lines of supporting evidence:**
   - **(i) a suitably preserved and stored specimen of the species from the collection site**
   - **(ii) reliable taxonomic verification**
   - **(iii) date and location of the observation.**

Target species specimens should be sent to the identified taxonomic expert (section 3.1) for verification.

A minimum of one individual (alive or dead) or part of a shell/exuviae must be found in a sample to record a positive detection of a target species. The evidence required to substantiate this is:

1. a suitably preserved and stored specimen of the species
2. reliable taxonomic verification
3. date and location of the observation.

Taxonomists verifying target species should document the evidence for pest presence i.e. by noting the presence of:

- a live individual (n=1)
- a live group of individuals (n>1), if possible[8]
- crab exuvia(e) or mollusc shells
- empty/full cysts
- molecular sequence or Restriction Fragment Length Polymorphism
- toxin profile etc.

Note: New introduced marine pest incursions or range expansions cannot be confirmed from photographs alone (Sutton and Hewitt; 2004). Video graphic records and photographic records are not sufficient evidence to determine target species presence; images must be matched with physical specimens.

4.7.3 Collating sample analysis results and checking data entry accuracy

Sample processing principle: Level 1 expertise (no training required).

Principle: Monitoring results should be checked for data entry errors.

Collate the sample analysis results and enter the data into the standard electronic formatted data sheet ('Monitoring data input sheet' in the MDP).

Enter the specimen verification results into the results table to indicate whether target species were detected (1) or not detected (0).

Verify the accuracy of data entry by having an independent person go through the table and cross-check with the analysis reports.

8 While the aim of the monitoring program is to detect presence/absence and not abundance, an indication of abundance can be useful additional information.

4.7.4 Data interpretation

Sample processing principle: Level 3 expertise (formal training or qualifications required).

Principle: monitoring data and results should be interpreted to indicate whether monitoring objectives have been met.

Monitoring data should be considered in light of the monitoring objectives and previous survey results. Data interpreters should report their results in a monitoring report, including the required elements outlined in section 6.1. The monitoring report is designed to answer the following questions:

1. What target or 'unknown' species were detected during monitoring?
2. Where were they located within the monitoring location (i.e. latitude, longitude, site name, habitat type)?
3. What is the evidence verifying the species (e.g. live individual, live group of individuals, dead individual(s), DNA, dinoflagellate cyst, shell etc.)?
4. Which observation systems method was used?
5. Was this a new record of the target species in the location (i.e. were they previously detected in the location)?
6. Was this a new record of the target species in Australia?
7. Were there any other reported sightings, outside of the monitoring program, of target species or suspected pest species in the location? If yes, please provide details.

In conclusion, data interpreters should indicate whether the monitoring objectives have been met.

4.8 Reporting of suspected marine pest incursions

Principles:

1. **If a new incursion of a target species or suspected invasive species (i.e. 'unknown' species, section 2.5.7) is detected a verbal report must be made to the relevant jurisdictional body or the Monitoring Coordination Point within 48 hours**
2. **A written report must be received by the relevant jurisdictional body or Monitoring Coordination Point within four weeks of the initial verbal report (see above).**

Any new incursion detected at a location during routine monitoring must be verbally reported by the monitoring program coordinator to the relevant authority within 48 hours. The suspected incursion report requires the following information:

- contact details for the person who found the species and for the person who provided taxonomic verification
- the species identified and where the specimen is lodged
- condition of the specimen (i.e. alive, damaged, dead)
- the date and time that the species was detected
- where the species was found (lat/long (GPS location)), site name, and a description of locality (e.g. marina, wharf, boat ramp; height on shore or depth of water; substrate type (rock, shells, sand, mud etc.); etc.)
- estimation of abundance (n=1, 1<n<10, 11<n<100, n>100) to inform immediacy of response
- method used to detect the species.

Within four weeks of the initial verbal report, a written report must be submitted to the relevant jurisdictional authority and the Monitoring Coordination Point. Include all the elements listed in Table 18.

Reports will be reviewed by the relevant jurisdictional body who will determine the action required.

Table 18. Elements to be included in the suspected incursion report[9]

FINDER'S DETAILS		VERIFIER'S (TAXONOMIST) DETAILS	
Name:		Name:	
Address:		Organisation:	
Phone:		Phone:	
Fax:		Fax:	
Email:		Email:	
1. On what day (include date) and time was the organism found/observed?			
2. Where was the organism found/observed? If possible, provide the site code or closest landmark or navigation marker or GPS location.			
3. What type of organism was found/observed? Provide species name following taxonomic verification.			
4. What did the organism look/feel like? (include approximate size, colour, texture)			
5. Where is the specimen currently held?			
6. When the organism was found/observed and what condition was it in? (alive, dead, damaged)			
7. Was the organism found/observed on the shore or in the water? If on the shore, at what tide level - high tide, mid tide, or low tide? If in the water, at what depth?			
8. If it was a crab, seastar, mollusc or worm:			
a. How many individuals were there? Few (1-10) Moderate (11-100) Many (over 100)			
b. Over what area were the animals distributed? Small (< 10 m^2) Moderate (in between) Large (> 10 000 m^2)			

9 Based on New Zealand Ministry of Fisheries Surveillance Network in the Marine Environment Reporting Sheet (Form B).

Table 18. continued

9. If it was an alga/seaweed:
a. How much area did it cover? Small (← 10 m2) Moderate (in between) Large (→ 10 000 m2)
b. For spreading growth forms, how dense was the growth? Light (a few scattered plants) Moderate (in between) Large (dense growth)
c. For large macroalgae, how many individuals were there? Few (1-10) Moderate (11-100) Many (over 100)
10. Describe the location where the organism was found/observed. On what kind of surface (substrate) was the organism found/observed? Sand, mud, cobble, rocks, boulders, wharf piles, boat, marina, buoy/ropes/, equipment, marine infrastructure (pontoon etc.), etc.
11. Other comments.

5. Monitoring reporting process

5.1 Monitoring report

Principle: a monitoring report describing monitoring results for each monitoring location must be submitted to the Monitoring Coordination Point.

The required elements of the monitoring report have been provided to ensure consistency across all locations and to assist in ensuring that all required information is included.

Table 19 provides the contents list for the monitoring report. Ensure that all the required elements are included and submit the report within one month of completion of the monitoring activities. MDAP will review monitoring reports.

Note: Reports for new incursions (suspected incursion reporting) must be submitted within 48 hours of verification rather than waiting for inclusion in the monitoring report. Section 4.8 provides the details for suspected incursion reporting. The monitoring report must be submitted in electronic or hard copy form to the relevant jurisdictional body and the Monitoring Coordination Point (see www.marinepests.gov.au/national_system/partners for contact details).

5.2 Reporting for other special circumstances

5.2.1 Additional Monitoring

Principle: results from monitoring undertaken over and above the minimum requirements should be reported using the same format as the monitoring report.

This manual can be used to design and implement ongoing monitoring programs over and above the minimum requirements. If results from additional monitoring are to be considered for national decision making processes then they should be reported as part of the monitoring report. To meet the QAQC requirements the same elements will need to be reported on as for a standard monitoring program. This will ensure that the data can be used to inform decisions as well as making sure that the data can be stored in a uniform format. If the results are not to be used in national decision making processes then meeting the reporting requirements is not critical. Additional monitoring reports will be reviewed by the relevant jurisdictional body and the Monitoring Coordination Point who will determine any action required.

Table 19. Required elements of the monitoring report for each location

ELEMENT	QUALITY ASSURANCE ACTIVITY	REQUIRED DETAILS	QUALITY CONTROL CHECK#
General	Includes author and contributors to the report	Includes at a minimum: names, affiliation, phone and fax numbers and email address	
	Includes contact person for questions regarding the monitoring report	Includes at a minimum: name, affiliation, phone and fax numbers and email address	
	Defines the monitoring location	Includes name, jurisdiction and latitude. May include map(s) of location	
	Includes details of stakeholder partners	Includes at minimum: names, organisation, phone and fax numbers and email address	
	Includes details of those who designed the monitoring program	Includes at a minimum: names, organisation, phone and fax numbers and email address	
	Includes details of those who implemented the monitoring program	Includes at a minimum: names, organisation, phone and fax numbers and email address	
Results	Details of sample periods	Includes date(s) of sample collection	Do these dates match with prior reports? Are the periods appropriate for the selected target species?
	Data from sample sites	Includes site name, location (GPS), habitat type, temperature and salinity	Is all the information provided?
	Details on samples collected	Includes type of sample (habitat), number of samples collected, observation system used, sample size determined in monitoring program design	Does this match the sample design and implementation plans? Are any deviations explained and management actions provided?

Table 19. continued

	Details on samples analysed	Includes number of samples analysed, number of target/ unknown species detected and action taken	Does the number of samples analysed match the design? Are any deviations explained? Were the appropriate suspected incursion reports provided?
	Details on target species detected	Includes species name, evidence of presence, observation system used, new record status and taxonomist used for verification	Is all the evidence provided? Was a qualified taxonomist used?
	Conclusion on success of monitoring activities	Statement addressing if objectives have been met	Does this match the monitoring design?
	Describe difficulties or problems encountered during sampling		
	Describes management actions to reduce problems		
	Describes difficulties or problems encountered in meeting minimum monitoring requirements		
	Describes management actions to reduce problems		
	Describes difficulties or problems encountered in reporting on monitoring results		
Review	Feedback on manual	Includes comments on whether instructions and the layout were clear and identifies ways of improvement	
	Recommendations for changes to the monitoring program, manual and/or National Monitoring Strategy	Includes recommendation and justification	

[#] The Quality Control checks will be used by MDAP when reviewing the monitoring report.

6. Evaluation and review process

Principle: the review section of the monitoring report must be submitted to the relevant jurisdictional body and the Monitoring Coordination Point.

The evaluation and review processes will be undertaken by the Monitoring Coordination Point, jurisdictional bodies and the appropriate government departments or agencies. The following section explains this process to outline how feedback from biennial monitoring (every two years) will be used to evaluate and review the monitoring programs. For a description of the overall strategic review of the National Monitoring Strategy, refer to the guidelines.

6.1 Evaluation and review of the monitoring program design for each location

Evaluation and review of the monitoring program design will occur after each biennial survey and is designed to ensure continuous improvement of the monitoring program design at each location.

Complete the review section of the monitoring report (see Table 19) and submit as part of the monitoring report. Identify and clearly explain any proposed changes to the monitoring program Changes might include an adjustment in the monitoring area, inclusion of a new monitoring habitat (previously unidentified), application of a different observation systems method, or a change in the location and frequency of sampling. Proposed changes will be reviewed by the Monitoring Coordination Point before sampling commences in subsequent surveys.

More significant changes may require resubmission of the monitoring design report (section 2.7).

6.2 Evaluation and review of the monitoring manual

To ensure continuous improvement of the monitoring process, the manual will be reviewed every two years for minor amendments. Minor amendments might include correction of errata and editorial changes, as well as minor changes to the approach (e.g. calculation of sample size). Feedback on the manual should be submitted as part of the monitoring report (see Table 19).

A more comprehensive review will be undertaken every four years to identify more substantial changes to the monitoring process such as the incorporation of new technologies.

The most up to date version of the manual will be maintained on the marine pest website (www.marinepests.gov.au).

Monitoring program designs may need to be updated following manual revisions.

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Glossary

TERM	DEFINITION
Agreed pest of concern	Any marine pest that is agreed, through formal processes established under the Marine IGA and the National System, to pose a significant potential or actual threat to any part of Australia's marine environment or industry, if introduced, established or translocated.
National System	The National System for the Prevention and Management of Marine Pest Incursions.
NIMPIS	National Introduced Marine Pest Information System.
	'Ballast water' means water (including sediment that is or has been contained in water) held in tanks and cargo holds of ships to increase stability and manoeuvrability during transit.
Biofouling	The attachment of marine organisms to any part of a vessel, or any equipment attached to or on board the vessel, aquaculture equipment, mooring devices and the like.
BRS	Bureau of Rural Sciences.
CCIMPE	Consultative Committee on Introduced Marine Pest Emergencies.
CSIRO	Commonwealth Scientific and Industrial Research Organisation.
DAFF	Department of Agriculture, Fisheries and Forestry.
Detection	The interception of a suspected pest species or its identification in a location following incursion.
Established population	A self sustaining population of an introduced species.
Exotic marine species	Any species not normally considered to occur and that may or may not be present in Australia marine environment.
Incursion	Unauthorised entrance or movement of a suspected pest species into a region where it is not already established.
Interception	Identification of a suspected pest species on a vessel or other vector prior to transfer to another vessel, vector or local habitat.
Introduction	The transport of an exotic marine species to a location within Australia's marine environment from a source beyond Australia's marine environment.
Jurisdictions	All the relevant states and territories of Australia.
Marine pest	Any exotic marine species, that may pose a threat to Australia's marine environment or industry, if introduced, established or translocated.
Measure	Action undertaken to prevent or limit damage to Australia marine environment or industry.
MDET	Monitoring design Excel template.
MDRT	Monitoring design report template.

TERM	DEFINITION
MDAP	Monitoring Design Assessment Panel.
MDP	Monitoring design package.
Monitoring coordination point	The central contact point for information about national monitoring arrangements and requirements.
Monitoring program coordinator	The person/party charged with coordinating the design and implementation of the ongoing monitoring program in each jurisdiction or location including the contracting processes and financial management.
MWG	Monitoring Working Group.
NIMPCG	National Introduced Marine Pest Coordination Group.
NMN	National Monitoring Network.
Node	An area where vessels and other potential marine pest vectors coincide (e.g. marina, boat ramp).
OH&S	Occupational Health and Safety.
Quality assurance	The integrated system to ensure data (and its use) meets pre-defined standards of quality with a stated level of confidence.
Quality control	The system of technical activities whose purpose is to measure and control the quality of the data.
Target species	A species that is an established pest in Australia and is targeted for translocation risks. Or a pest species known from other parts of the world and not yet introduced into Australia and targeted for new incursion risks.
Translocation	The transport of an exotic marine species from one area of Australia's marine environment to another.
Vector	Anything capable of introducing or translocating an exotic marine species.
Vessel	Any ship, boat or other description of vessel used in navigation by sea.

Attachment A Monitoring target species

The target species that must be considered for a monitoring program for a given location in Australia are listed in this table. This list (endorsed by NIMPCG) has been compiled from a number of reports that considered the invasion potential and impact potential of a large range of species. The target species classification and selection processes are detailed in the *Australian marine pest monitoring manual*. Note: this list will be incorporated into the MDP when reviewed.

Table A1. Monitoring target species list

	SPECIES NAME	COMMON NAME
1	*Acartia tonsa*	Calanoid copepod
2	*Alexandrium catenella* *	Toxic dinoflagellate
3	*Alexandrium minutum* *	Toxic dinoflagellate
4	*Alexandrium monilatum*	Toxic dinoflagellate
5	*Alexandrium tamarense*	Toxic dinoflagellate
6	*Asterias amurensis* *	Northern Pacific seastar
7	*Balanus eburneus*	Ivory barnacle
8	*Balanus improvisus* (marine/ estuarine incursions only)	Bay barnacle
9	*Beroe ovata*	Comb jelly
10	*Blackfordia virginica*	Black Sea jelly
11	*Bonnemaisonia hamifera*	Red macroalga
12	*Callinectes sapidus*	Blue crab
13	*Carcinus maenas* *	European shore crab
14	*Caulerpa racemosa* (possibly an Australian native)	Green macroalga
15	*Caulerpa taxifolia* (exotic strains only)	Green macroalga
16	*Chaetoceros concavicornis*	Centric diatom
17	*Chaetoceros convolutus*	Centric diatom
18	*Charybdis japonica* * barcoded	Asian paddle/lady crab
19	*Codium fragile* spp. *fragile*[1]	Green macroalga
20	*Corbula* (*Potamocorbula*) *amurensis*	Brackish-water/Asian clam
21	*Crassostrea gigas* *	Pacific oyster
22	*Crepidula fornicata*	American slipper limpet
23	*Didemnum* spp. (exotic invasive species only)	Tunicate – sea squirt

Table A1. continued

	SPECIES NAME	COMMON NAME
24	*Dinophysis norvegica*	Toxic dinoflagellate
25	*Ensis directus*	Jack-knife clam
26	*Eriocheir* spp.	Mitten crabs
27	*Grateloupia turuturu*	Red macroalga
28	*Gymnodinium catenatum* *	Toxic dinoflagellate
29	*Hemigrapsus sanguineus*	Japanese shore crab
30	*Hemigrapsus takanoi / penicillatus*	Pacific crab
31	*Hydroides dianthus*	Tube worm
32	*Limnoperna fortunei*	Golden mussel
33	*Marenzelleria* spp. (invasive species and marine/estuarine incursions only)	Red-gilled mud worm
34	*Mnemiopsis leidyi*	Comb jelly
35	*Musculista senhousia* *	Asian bag/date mussel
36	*Mya arenaria*	Soft shell clam
37	*Mytilopsis sallei*	Black-striped mussel
38	*Neogobius melanostomus* (marine/estuarine incursions only)	Round goby
39	*Perna perna*	South African brown mussel
40	*Perna viridis* *	Asian green mussel
41	*Pfiesteria piscicida* *	Dinoflagellate
42	*Pseudodiaptomus marinus*	Asian copepod
43	*Pseudo-nitzschia seriata*	Pennate diatom
44	*Rapana venosa*	Asian/veined rapa whelk
45	*Rhithropanopeus harrisii*	Harris mud crab
46	*Sabella spallanzanii* *	European/Mediterranean fan worm
47	*Sargassum muticum*	Asian seaweed
48	*Siganus luridus*	Dusky spinefoot
49	*Siganus rivulatus*	Marbled spine foot/rabbit fish
50	*Tortanus dextrilobatus*	Asian copepod
51	*Tridentiger bifasciatus*	Shimofuri goby
52	*Tridentiger barbatus*	Shokohazi goby
53	*Undaria pinnatifida* *	Japanese seaweed
54	*Varicorbula (Corbula) gibba* *	European clam
55	*Womersleyella setacea*	Red seaweed

1 Codium fragile spp. fragile is on the Interim CCIMPE trigger list. Noting that the CCIMPE criteria for removal requires that data indicates that impacts overseas/in Australia are likely to be less than previously thought or it becomes widely distributed in Australia, it does not seem likely at this time that justification could be provided to remove this species from the CCIMPE trigger list.

* = species with a genetic/molecular probe or barcoded (see Doblin & Bolch 2008)

Attachment B Sample processing guides

Note: these guides can be printed and laminated for easy use in the field and laboratory.

Sample processing method: Small cores or harpoon cores

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Cysts and resting stages of: *Alexandrium catenella* *Alexandrium minutum* *Alexandrium tamarense* *Alexandrium monilatum* *Gymnodinium catenatum* *Pfiesteria piscicida*	After collection, samples are stored upright in racks or in partitioned boxes in a darkened insulated container over ice or refrigerator at 4 °C (do not freeze).	• sonicator • compound microscope (minimum 400X magnification) • 1 ml counting chamber (e.g. Sedgwick-Rafter counting cells)

Sample processing (Level 2 expertise)

In the laboratory, the upper 60 mm of the core sample is carefully removed from the tube and stored in the dark at 4 °C in a sealed plastic container (large enough to contain sample) until further examination. For cyst / cell isolation, the upper 1-2 cm of the core is mixed with approximately 30 ml of filtered seawater to produce a watery slurry (Bolch 1997). Subsamples (5-10 ml) of the slurry are sonicated for 2 minutes (e.g., using an ultrasonic needle probe, 150-200 W; Bolch 1997) to dislodge detritus. The sample is then passed through a 90 µm sieve, collected onto a 20 µm sieve, and washed (with seawater of same salinity as sample) to remove sand grains and detritus. At this stage, samples could be separated using sodium polytungstate (Bolch 1997) and stored from 0–4 °C (i.e. on ice) in sealed plastic or glass containers before analysis.

Sample identification (Level 3 expertise)

Subsamples (1 ml) of the sonicated and sieved samples are examined on wet mounted slides using a compound light microscope (400x magnification or above). To confirm the identity of some species, isolated cysts are incubated in nutrient-enriched seawater and monitored periodically for germination. Germinated cells are then identified using a microscope.

Quality assurance

A minimum of 2 slides are examined per sample.

Parataxonomic / identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL www.padil.gov.au
- Matsuoka, K. and Fukuyo, Y. (2003) Taxonomy of cysts. In: Manual on Harmful Marine Microalgae; UNESCO, Paris, pp. 563-592
- Fukuyo, Y. Atlas of dinoflagellates. University of Tokyo, Japan. http://dinos.anesc.u-tokyo.ac.jp/atlas_ver1_5/main.htm
- Fukuyo, Y. Cyst identification guide. University of Tokyo, Japan. http://dinos.anesc.u-tokyo.ac.jp/

References

Bolch, CJS (1997) The use of sodium polytungstate for the separation and concentration of living dinoflagellates cysts from marine sediments. Phycologia 36(6): 472-478.

Sample processing method: Large cores or grab samples

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Adults and some juveniles of gregarious benthic fauna including: *Asterias amurensis* – Northern Pacific seastar *Callinectes sapidus* – Blue crab *Carcinus maenas* – European green crab *Caulerpa taxifolia* – Green macroalga *Caulerpa racemosa* – Green macroalga *Charybdis japonica* – Lady crab *Corbula amurensis* – Brackish water bivalve *Crassostrea gigas* – Feral Pacific oyster (juvenile and adults) *Crepidula fornicata* – American slipper limpet *Ensis directus* – Jack-knife clam *Eriocheir* spp. – Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Hemigrapsus takanoi/pencillatus* – Pacific crab *Limnoperna fortunei* – Golden mussel *Marenzelleria* spp. – Red gilled mud worm *Musculista senhousia* – Asian bag mussel (juvenile and adults) *Mya arenaria* – Soft shell clam *Mytilopsis sallei* – Black striped mussel *Perna perna* – Brown mussel *Perna viridis* – Asian green mussel *Rapana venosa* - Gastropod *Sabella spallanzanii* – Mediterranean fanworm *Sargassum muticum* – Asian seaweed *Varicorbula gibba* – European clam	Coarse sieve (1 mm) entire sample and sort into broad taxonomic groupings, removing any incompatible specimens. Within 8 hours, fix groups of organisms separately according to taxon (see Table 16) and store samples in a darkened insulated container over ice or refrigerator at 4 °C (do not freeze). Note: Samples for DNA analysis are frozen at -80°C, fixed in 90 % ethanol, or dried using a desiccant (macroalgae).	1 mm stainless steel or brass sieves dissecting microscope.

Sample processing (Level 2 expertise)

In the laboratory:

- wash light fraction in copious freshwater over 1 mm sieve to remove fixative
- decant sample from sieve to jar in freshwater
- within 12 hours sort sample (see section 4.5.2) under dissecting microscope into multiple vials of major taxa containing 70 per cent ethanol (or other preservative as Table 16)
- reduce water and transfer any remaining unsorted material to 70 per cent ethanol if sorting incomplete after 12 hours.

If missed earlier, abundant specimens that can not be readily identified as native species (i.e. potential 'unknown' species) should be separated for identification.

Sample identification (Level 3 expertise)

Identification must be carried out using identification guides and keys in consultation with a suitable taxonomist(s) who can verify sort accuracy and confirm the identity of suspected target species.

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects.

References

Bolch, CJS (1997) The use of sodium polytungstate for the separation and concentration of living dinoflagellates cysts from marine sediments. Phycologia 36(6): 472-478.

Sample processing method: Settlement plates

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Encrusting (fouling) species (juveniles and adults) including: *Balanus eburneus* – Ivory barnacle *Balanus improvisus* – Bay barnacle *Blackfordia virginica* – Black Sea jellyfish *Carcinus maenas* – European green crab *Charybdis japonica* – Lady crab *Codium fragile* spp. *fragile* – Green macroalga *Crassostrea gigas* – Feral Pacific oyster *Didemnum* spp. – Ascidian *Eriocheir* spp. – Mitten crab *Grateloupia turuturu* – Red macroalga *Hemigrapsus sanguineus* – Japanese shore crab *Hydroides dianthus* – Serpulid polychaete *Limnoperna fortunei* – Golden mussel *Musculista senhousia* – Asian bag mussel *Mytilopsis sallei* – black striped mussel *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Corbula amurensis* – Brackish-water bivalve *Sabella spallanzanii* – Mediterranean fanworm *Sargassum muticum* – Asian seaweed *Undaria pinnatifida* – Japanese kelp	Before retrieval, observe the plate(s) in situ and take note of anything unusual (e.g. signs of damage or tampering). This will help with the interpretation of laboratory observations. Place settlement plates into suitable containers and label (do not stack or allow plates to damage each other). Samples should be stored in the dark on ice and transported to the laboratory. Within 8 hours, fix all samples to achieve final concentration of 4 % seawater buffered formalin (i.e. formalin made up with seawater, or other treatment as specified in Table 16). Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	Dissecting microscope.

Sample processing (Level 2 expertise)

In the laboratory:

- plates should be processed one at a time, making sure there's enough time to rinse and observe the sample
- rinse the mesh bag containing a plate with freshwater to remove the formalin. Remove the plate from the mesh bag and place it in a shallow tray filled with freshwater
- wash material remaining in the mesh bag over a 1 mm sieve with freshwater and backwash specimens retained on the sieve into a Petri dish
- before specimen identification, photograph the plate using a digital camera. A photograph should be taken of the entire plate, and then each quarter individually (Sutton and Hewitt, 2004). Each photograph or image should include a scale (e.g. ruler) and sample label. The plates are photographed to document the sample and are later used to cross-check identifications made with a microscope.

Note: processing a settlement plate takes about 4 hrs depending on experience, so plates should not be removed unless they can be examined in one day.

Sample identification (Level 3 expertise)

Submerged plates are placed under a dissecting microscope and examined for target species. Identification must be carried out using identification guides and keys in consultation with a suitable taxonomist(s) who can confirm the identity of suspected target species. Abundant specimens that can not be readily identified as native species (i.e. potential 'unknown' species) should be identified.

After examination, plates should be fixed in 4 % seawater buffered formalin for 3 days before preserving in 70 % ethanol (Hewitt and Sutton, 2004).

Quality assurance

The entire plate is inspected and suspect target species are verified by an appropriate taxonomist.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects.

References

Sutton, C. and Hewitt, C. (2004) Detection kits for community-based monitoring of introduced marine pests. Revised final report to National Heritage Trust/Coast and Clean Seas, NHT 21247;

www.marine.csiro.au/crimp//reports/CDKreport.pdf.

Sample processing method: Settlement trays

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Settled larvae and juveniles and some adult stages of soft sediment species including: *Asterias amurensis* – Northern Pacific seastar *Carcinus maenas* – European shore crab *Charybdis japonica* – Lady crab *Codium fragile* spp. *fragile* – Green macroalga *Crassostrea gigas* – Feral Pacific oyster *Eriocheir* spp. – Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Hydroides dianthus* – Serpulid polychaete *Musculista senhousia* – Asian bag mussel *Mytilopsis sallei* – Black striped mussel *Perna viridis* – Asian green mussel *Perna perna* – South African brown mussel *Undaria pinnatifida* – Japanese kelp *Varicorbula gibba* – European clam	After retrieval, trays are either emptied into sample containers or contents are sieved (1 mm) and coarsely sorted. In both cases, incompatible specimens are removed. If contents are sorted, organisms are fixed according to taxon (see Table 16). Samples are stored in a darkened insulated container, over ice or in a refrigerator at 4 °C (do not freeze). Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	1mm stainless steel or brass sieves dissecting microscope.

Sample processing (Level 2 expertise)

In the laboratory:

- wash in copious freshwater over 1 mm sieve to remove fixative
- decant from sieve to jar in freshwater
- within 12 hours sort sample (see section 4.5) under dissecting microscope into multiple vials containing major taxa containing 70 % ethanol (or other preservative as specified in Table 16)
- reduce water and transfer any remaining unsorted material to 70 % ethanol if sorting incomplete after 12 hours
- carefully wash over 1 mm sieve any fragile specimens which had been fixed separately in the field, and transfer into the same set of vials.

Sample identification (Level 3 expertise)

Identification must be carried out using identification guides and taxonomic keys in consultation with a suitable taxonomist(s) who can verify sort accuracy and confirm the identity of suspected target species.

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

References

Sutton, C. and Hewitt, C. (2004) Detection kits for community-based monitoring of introduced marine pests. Revised final report to National Heritage Trust/Coast and Clean Seas, NHT 21247;

www.marine.csiro.au/crimp//reports/CDKreport.pdf.

Sample processing method: Benthic sled or beam trawls

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Sessile and motile epibenthic invertebrates and fishes including: *Asterias amurensis* – Northern Pacific seastar *Carcinus maenas* – European green crab *Caulerpa taxifolia* – Mediterranean seaweed *Caulerpa racemosa* – Green macroalga *Charybdis japonica* – Lady crab *Codium fragile* spp. *fragile* – Green macroalga *Corbula amurensis* – Brackish-water bivalve *Crassostrea gigas* – Feral Pacific oyster (juvenile and adults) *Eriocheir* spp. –Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Hemigrapsus takanoi/pencillatus* – Pacific crab *Hydroides dianthus* – Serpulid tube worm *Musculista senhousia* – Asian bag mussel (juvenile and adults) *Mytilopsis sallei* – Black striped mussel *Neogobius melanostomus* - Round goby *Perna perna* – Brown mussel *Perna viridis* – Asian green mussel *Rapana venosa* - Gastropod *Sabella spallanzanii* – Mediterranean fanworm *Siganus rivulatus* - Marbled spinefoot *Undaria pinnatifida* - Japanese kelp *Varicorbula gibba* – European clam	Sample is washed into a container and screened for any obvious specimens that are then stored in sample containers. Remainder of sample is then homogenised and representatively subsampled for further processing. Subsample is sieved (1 mm) and sorted into broad taxonomic groupings, removing any incompatible specimens. Groups of organisms are fixed separately according to taxon (see Table 16) and samples stored in a darkened insulated container, over ice or in a refrigerator at 4 °C (do not freeze). Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	• 1mm stainless steel or brass sieves; • dissecting microscope.

In the laboratory:

- wash light fraction in copious freshwater over 1 mm sieve to remove fixative
- decant sample from sieve to jar in freshwater
- within 12 hours sort sample (see section 4.5.2) under dissecting microscope into multiple vials of major taxa containing 70 % ethanol (or other preservative as Table 16)
- reduce water and transfer any remaining unsorted material to 70 % ethanol if sorting incomplete after 12 hours
- carefully wash over 1.0 mm sieve any fragile specimens which had been fixed separately in the field, and transfer into separate vials.

If missed earlier, abundant specimens that can not be readily identified as native species (i.e. potential 'unknown' species) should be separated for identification.

Sample identification (Level 3 expertise)

Identification must be carried out using identification guides and taxonomic keys in consultation with a suitable taxonomist(s) who can verify sort accuracy and confirm the identity of suspected target species.

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Phytoplankton / macroalgae net tow

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Vegetative cells of: *Pseudo-nitzschia seriata* – Pennate diatom *Dinophysis norvegica* – Toxic dinoflagellate *Chaetoceros concavicornis* – Centric diatom *Chaetoceros convolutes* – Centric diatom Vegetative cells (or cysts / resting stages) of: *Alexandrium catenella* - Toxic dinoflagellate *Alexandrium minutum* – Toxic dinoflagellate *Alexandrium monilatum* – Toxic dinoflagellate *Alexandrium tamarense* – Toxic dinoflagellate *Gymnodinium catenatum* - Toxic dinoflagellate *Pfiesteria piscicida* - Dinoflagellate Early life history stages of : *Undaria pinnatifida* – Japanese seaweed	If morphological and molecular approaches for phytoplankton identification are used, net samples should be sub-sampled and processed separately for microscopic observations and molecular probe analysis. Sub-samples for morphological identification: Samples are poured from cod-end jars into storage containers, topped up with seawater from the sampling site and kept in a darkened insulated container over ice (do not freeze). Samples can be fixed and preserved using Lugol's solution or glutaraldehyde (1 % final concentration). Phytoplankton sample incubation should be carried out within 2-4 hours of collection. Sub-samples for molecular probe analysis: Samples should be frozen at -80 °C, or rinsed and preserved in SET buffered 90 % ethanol.	• compound/TEM microscope • culture medium and incubator • thermal cycler for PCR plus electrophoresis equipment and associated chemicals and supplies.

There are two potential approaches for detecting target phytoplankton in marine waters: (1) direct observation with a high-powered microscope (minimum 400x magnification); and (2) species-specific DNA or RNA molecular probes. Molecular probes are currently available for a number of species [see Attachment A and Doblin and Bolch 2008] including Gymnodinium catenatum (Patil et al., 2005), members of the Alexandrium genus (Anderson et al., 2005; Bolch and de Salas, 2007; Hosoi-Tanabe and Sako, 2005; Galluzzi et al., 2004) and Pfiesteria piscicida (and P. shumwayae) (Park et al., 2007; Rublee et al., 2005). A probe is also available for early life history stages of Undaria pinnatifida (Hayes et al., 2007).

Identification of Pseudo-nitzschia seriata requires TEM (Lapworth et al., 2001). Note that for many species, identification relies on combined morphological and genetic approaches (e.g. Fehling et al. 2004 for Pseudo-nitzschia seriata) because of morphological variation between closely related genotypes (or vice versa).

Note: Molecular probes must be used with care because rDNA and rRNA levels in intact cells are variable and because the presence of target rDNA and rRNA in cell fragments, faecal pellets, or detritus introduces uncertainties in the results. Results are therefore best compared with direct observations of cells.

In some locations, phytoplankton identification may be linked to existing shellfish sanitation programs that measure microalgal toxins, but verification of target organism presence must be made using the methods specified here.

Sample processing (Level 2 expertise)

Specific instructions for detection of target species in environmental samples are found in the references below.

Sample identification (Level 3 expertise)

Sample identification requires the expertise of (1) a taxonomist or phytoplankton ecologist with experience in examining natural samples and (2) a molecular biologist to interpret molecular probe results or both (3).

Quality assurance

A minimum of 2 slides are examined per sample and a minimum of 3 replicates are required for molecular analysis. Taxonomists and molecular biologists are required to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects
- Fukuyo, Y. Atlas of dinoflagellates. University of Tokyo, Japan. http://dinos.anesc.u-tokyo.ac.jp/atlas_ver1_5/main.htm.

References

Anderson DM, Kulis DM, Keafer BA, Gribble KE, Marin R, Scholin CA (2005) Identification and enumeration of Alexandrium spp. from the Gulf of Maine using molecular probes. Deep-Sea Research Part II-Topical Studies in Oceanography 52 (19-21): pp. 2467-2490 .

Bolch, CJS. & de Salas, MF. (2007). Molecular evidence for ballast water introduction of the toxic dinoflagellates Gymnodinium catenatum and the Alexandrium "tamarense complex to Australasia". Harmful Algae 6(4): pp.465-485.

Camelo TR, Hasle GR (1997) Identifying Marine Phytoplankton. Academic Press, San Diego, CA, pp.858.

Daguin C, Voisin M, Engel C, Viard F (2005) Microsatellites isolation and polymorphism in introduced populations of the cultivated seaweed Undaria pinnatifida (Phaeophyceae, Laminariales). Conservation Genetics 6 (4): pp. 647-650.

Doblin MA, Bolch CJS (2008) Review of genetic methods as a tool for identification of introduced marine pests. Access:UTS Report for the Department of Environment, Water, Heritage and the Arts, pp.43 .

Fehling J, Green DH, Davidson K, Bolch CJ, Bates SS (2004) Domoic acid production by Pseudo-nitzschia seriata (Bacillariophyceae) in Scottish waters. J. Phycol. 40(4): pp. 622-630.

Galluzzi L, Penna A, Bertozzini E, Vila M, Garces E, Magnani M (2004) Development of a real-time PCR assay for rapid detection and quantification of Alexandrium minutum (a dinoflagellate). Appl. Environ. Microbiol. 70 (2): pp.1199-1206.

Hallegraeff GM (2002) Aquaculturists' guide to harmful Australian Microalgae. University of Tasmania, Hobart, pp.136 .

Hayes, KR, Gunasekera, R, Patil, J, Sliwa, C, Migus, S, McEnnulty, F, Dunstan, P, Green, M, Dunstan, P, Sutton, C (2007) Empirical validation: Small vessel translocation of key threatening species, stage II: Undaria pinnatifida. Final report to the Australian Government National Heritage Trust, pp.54 .

Hosoi-Tanabe S, Sako Y (2005) Species-specific detection and quantification of toxic marine dinoflagellates Alexandrium tamarense and A. catenella by real-time PCR assay. Marine Biotechnology 7 (5): pp.506-514.

Lapworth, C.J., Hallegraeff, G.M. and Ajani, P.A. (2001)
Identification of domoic-acid-producing Pseudo-nitzschia species in Australian waters. 9. International Conference on Harmful Algal Blooms, Hobart (Australia), 7 – 11 February, 2000. pp. 38-41.

Muraoka D, Saitoh K (2005) Identification of Undaria pinnatifida and Undaria undarioides Laminariales, Phaeophyceae using mitochondrial 23S ribosomal DNA sequences. Fisheries Science 71 (6): pp.1365-1369.

Park, TG, de Salas MF, Bolch, CJS. and Hallegraeff, GM (2007) Development of a real-time PCR for quantification of the heterotrophic dinoflagellate Cryptoperidiniopsis brodyi (Dinophyceae) in environmental samples. Applied and Environmental Microbiology 73(8): pp. 2552-2560.

Patil JG, Gunasekera RM, Deagle BE, Bax NJ, Blackburn SI (2005) Development and evaluation of a PCR based assay for detection of the toxic dinoflagellate, Gymnodinium catenatum (Graham) in ballast water and environmental samples. Biological Invasions 7 (6): pp.983-994.

Rublee, P.A., Remington, D.L., Schaeffer, E.F. and Marshall, M.M. (2005) Detection of the dinozoans Pfiesteria piscicida and P. shumwayae: A review of detection methods and geographic distribution. J. Eukaryot. Microbiol. 52(2), pp.83-89.

Scholin CA, Marin R, Miller PE, et al. DNA probes and a receptor-binding assay for detection of Pseudo-nitzschia (Bacillariophyceae) species and domoic acid activity in cultured and natural samples. J. Phycol. 35 (6): pp.1356-1367 Suppl. S.

Sample processing method: Zooplankton / invertebrate larvae net tow

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Invertebrate larvae and larval fishes including: *Acartia tonsa* – Calanoid copepod *Asterias amurensis* – Northern Pacific seastar *Balanus eburneus* – Ivory barnacle *Beroe ovata* – Comb jelly *Blackfordia virginica* – Black Sea jellyfish *Bugula nertina* – Bryozoan *Carcinus maenas* – European green crab *Chaetoceros concavicornis* – Centric diatom *Chaetoceros convolutus* – Centric diatom *Charybdis japonica* – Lady crab *Crassostrea gigas* – Feral Pacific oyster *Eriocheir* spp. –Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Hydroides dianthus* – Serpulid polychaete *Mnemiopsis leidyi* – Comb jelly *Musculista senhousia* – Asian bag mussel *Mytilopsis sallei* – Black striped mussel *Neogobius melanostomus* – Round goby *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Corbula amurensis* – Brackish-water bivalve *Pseudodiaptomus marinus* - Calanoid copepod *Rapana venosa* - Gastropod *Sabella spallanzanii* – Mediterranean fanworm *Varicorbula gibba* – European clam	Immediately after arrival on deck, the cod end should be removed from the net and the sample poured gently into a sample jar(s). A wash bottle of 0.2 µm filtered seawater is used to rinse all animals out of the cod end and into the sample jar. If morphological and molecular approaches for zooplankton identification are used, net samples should be sub-sampled and processed separately for microscopic observations and molecular probe analysis. Sub-sampling for morphological identification: A jar is filled to 3/4 of full capacity with 0.2 µm filtered seawater, and 50 ml of full-strength formalin added to achieve a final concentration (when the jar is filled) of 5 %. After addition of formalin, 20 ml of a saturated solution of sodium borate in seawater is added (to maintain neutral pH of the formalin-seawater solution) and the jar filled to the top with filtered seawater. After the jar is inverted several times to insure complete mixing of fixative and sample, it is returned to its storage box and kept in cool, dark conditions until analysis. Zooplankton sample incubation should be carried out within 2-4 hours of collection. Sub-samples for molecular probe analysis: Samples should be frozen at -80 °C, or rinsed and preserved in SET buffered 90 % ethanol.	• high-power dissecting microscope • thermal cycler for PCR plus electrophoresis equipment and associated chemicals and supplies.

Target species detected using this method include zooplankton, invertebrate larvae and juvenile fishes. Zooplankton and juvenile fishes are identified by direct observation with a high-powered dissecting microscope, however invertebrate larvae are identified by species-specific DNA or RNA molecular probes. Molecular probes are currently available for a number of pest species [see Attachment A and Doblin and Bolch 2008] including Asterias amurensis (Deagle et al. 2003; Patil et al. 2004) and Crassostrea gigas (Patil et al., 2005).

Note: Molecular probes must be used with care because rDNA and rRNA levels in intact cells are variable and because the presence of target rDNA and rRNA in dead or fragments of specimens introduces uncertainties in the results. Results are therefore best compared with direct observations of cells.

Sample processing (Level 2 expertise)

If morphological and molecular approaches for identification are used, plankton samples should be subsampled and processed separately for microscopic observations and molecular probe analysis. Specific instructions for detection of target species in environmental samples are found in the references.

Sample identification (Level 3 expertise)

Sample identification requires the expertise of (1) a taxonomist or ecologist with experience in examining natural samples and (2) a molecular biologist to interpret molecular probe results or both (3).

Quality assurance

A minimum of 3 replicates are required for molecular analysis. Taxonomists and molecular biologists are required to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

References

Deagle, BE, Bax, N, Patil, JG (2003) Development and evaluation of a PCR-based test for detection of Asterias (Echinodermata: Asteroidea) larvae in Australian plankton samples from ballast water. Marine and Freshwater Research 54(6): pp.709 – 719.

Doblin MA, Bolch CJS (2008) Review of genetic methods as a tool for identification of introduced marine pests. Access:UTS Report for the Department of Environment, Water, Heritage and the Arts, pp.43 .

Patil JG, Gunasekera RM, Deagle BE, Bax, NJ (2005) Specific detection of Pacific oyster (Crassostrea gigas) larvae in plankton samples using nested polymerase chain reaction. Marine Biotechnology 7 (1): pp.11-20.

Patil, JG, Hayes, KR, Gunasekera RM, Deagle BE, McEnnulty, FR, Bax, NJ, Hewitt, C.L. (2004) Port of Hastings National Demonstration Project – Verification of the Type II error rate of the Ballast Water Decision Support System (DSS). Final report prepared for the EPA;

www.marine.csiro.au/crimp//reports/HastingsFinalReport.pdf

Patti FP and Gambi MC (2001) Phylogeography of the invasive polychaete Sabella spallanzanii (Sabellidae) based on the nucleotide sequence of internal transcribed spacer 2 (ITS2) of nuclear rDNA. Mar. Ecol. Progr. Ser. 215: pp. 169-177.

Sample processing method: Bucket / sampling bottle

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
As for Phytoplankton / Macroalgae and Zooplankton / Invertebrate larvae net tows Vegetative cells of: *Pseudo-nitzschia seriata* - dinoflagellate *Dinophysis norvegica* - dinoflagellate *Chaetoceros concavicornis* – Centric diatom *Chaetoceros convolutes* – Centric diatom Vegetative cells (or cysts / resting stages) of: *Gymnodinium catenatum* *Alexandrium catenella* *Alexandrium minutum* *Alexandrium tamarense* *Pfiesteria piscicida* Early life history stages of : *Undaria pinnatifida* – Japanese seaweed Invertebrate larvae and larval fishes including: *Asterias amurensis* – Northern Pacific seastar *Balanus eburneus* – Ivory barnacle *Beroe ovata* – Comb jelly *Blackfordia virginica* – Black Sea jellyfish *Carcinus maenas* – European green crab *Charybdis japonica* – Lady crab *Crassostrea gigas* – Feral Pacific oyster *Eriocheir* spp. –Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Hydroides dianthus* – Serpulid polychaete *Limnoperna fortunei* – Golden mussel *Mnemiopsis leidyi* – Comb jelly *Musculista senhousia* – Asian bag mussel *Mytilopsis sallei* – Black striped mussel *Neogobius melanostomus* – Round goby *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Corbula amurensis* – Brackish-water bivalve *Pseudodiaptomus marinus* - Calanoid copepod *Rapana venosa* - Gastropod *Sabella spallanzanii* – Mediterranean fanworm *Varicorbula gibba* – European clam	After collection, samples are poured into storage containers and kept in a darkened insulated container over ice (do not freeze).	• high-power compound dissecting microscope • filtration apparatus • thermal cycler for PCR plus electrophoresis equipment and associated chemicals and supplies.

Sample processing (Level 2 expertise)

In the laboratory, the water sample is subsampled for live examination or for DNA analysis. Samples are examined live using a compound or dissecting microscope.

Samples for DNA analysis are filtered through a 5 µm pore-sized hydrophilic Durapore filter (Millipore). The filter is then transferred into a 1.5 ml tube and then DNA extracted using a commercial kit (Qiagen) following the suppliers instructions. The DNA is retrieved in elution buffer and stored at -80 deg C until analysis (Patil et al., 2005). Specific instructions for molecular detection of target species in environmental samples are found in the references.

Sample identification (Level 3 expertise)

Sample identification requires the expertise of (1) a taxonomist or ecologist with experience in examining natural samples and (2) a molecular biologist to interpret molecular probe results or both (3).

Quality assurance

A minimum of 3 replicates are required for molecular analysis. Taxonomists and molecular biologists are required to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

References

Anderson DM, Kulis DM, Keafer BA, Gribble KE, Marin R, Scholin CA (2005) Identification and enumeration of Alexandrium spp. from the Gulf of Maine using molecular probes. Deep-Sea Research Part II-Topical Studies in Oceanography 52 (19-21): pp.2467-2490.

Daguin C, Voisin M, Engel C, Viard F (2005) Microsatellites isolation and polymorphism in introduced populations of the cultivated seaweed Undaria pinnatifida (Phaeophyceae, Laminariales). Conservation Genetics 6 (4): pp.647-650.

Deagle, BE, Bax, N, Patil, JG (2003) Development and evaluation of a PCR-based test for detection of Asterias (Echinodermata : Asteroidea) larvae in Australian plankton samples from ballast water. Marine and Freshwater Research 54(6): pp.709 – 719.

Fehling J, Green DH, Davidson K, Bolch CJ, Bates SS (2004)
Domoic acid production by Pseudo-nitzschia seriata
(Bacillariophyceae) in Scottish waters. J. Phycol. 40(4): pp.622-630.

Galluzzi L, Penna A, Bertozzini E, Vila M, Garces E, Magnani M (2004) Development of a real-time PCR assay for rapid detection and quantification of Alexandrium minutum (a dinoflagellate). Appl. Environ. Microbiol. 70 (2): pp.1199-1206.

Hosoi-Tanabe S, Sako Y (2005) Species-specific detection and quantification of toxic marine dinoflagellates Alexandrium tamarense and A. catenella by real-time PCR assay. Marine Biotechnology 7 (5): pp.506-514.

Muraoka D, Saitoh K (2005) Identification of Undaria pinnatifida and Undaria undarioides Laminariales, Phaeophyceae using mitochondrial 23S ribosomal DNA sequences. Fisheries Science 71 (6): pp.1365-1369.

Park, TG, de Salas MF, Bolch, CJS. and Hallegraeff, GM (2007) Development of a real-time PCR for quantification of the heterotrophic dinoflagellate Cryptoperidiniopsis brodyi (Dinophyceae) in environmental samples. Applied and Environmental Microbiology 73(8): pp. 2552-2560.

Patil JG, Gunasekera RM, Deagle BE, Bax, NJ (2005) Specific detection of Pacific oyster (Crassostrea gigas) larvae in plankton samples using nested polymerase chain reaction. Marine Biotechnology 7 (1): pp. 11-20.

Patil JG, Gunasekera RM, Deagle BE, Bax NJ, Blackburn SI (2005) Development and evaluation of a PCR based assay for detection of the toxic dinoflagellate, Gymnodinium catenatum (Graham) in ballast water and environmental samples. Biological Invasions 7 (6): pp. 983-994.

Patil, JG, Hayes, KR, Gunasekera RM, Deagle BE, McEnnulty, FR, Bax, NJ, Hewitt, C.L. (2004) Port of Hastings National Demonstration Project – Verification of the Type II error rate of the Ballast Water Decision Support System (DSS). Final report prepared for the EPA;

www.marine.csiro.au/crimp//reports/HastingsFinalReport.pdf

Patti FP and Gambi MC (2001) Phylogeography of the invasive polychaete Sabella spallanzanii (Sabellidae) based on the nucleotide sequence of internal transcribed spacer 2 (ITS2) of nuclear rDNA. Mar. Ecol. Progr. Ser. 215: pp. 169-177.

Scholin CA, Marin R, Miller PE, et al. DNA probes and a receptor-binding assay for detection of Pseudo-nitzschia (Bacillariophyceae) species and domoic acid activity in cultured and natural samples. J. Phycol. 35 (6): 1356-1367 Suppl. S.

Sample processing method: Scrapings by divers

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Fouling (sedentary, encrusting) species (settled, juvenile and adult life stages) including: *Balanus eburneus* – Ivory barnacle *Balanus improvisus* – Bay barnacle *Bonnemaisonia hamifera* – Red macroalga *Caulerpa racemosa* – Green macroalga *Caulerpa taxifolia* – Green macroalga *Codium fragile* ssp. *fragile* – Green macroalga *Crassostrea gigas* – Feral Pacific oyster *Didemnum* spp. – Ascidian *Grateloupia turuturu* – Red macroalga *Hydroides dianthus* – Serpulid polychaete *Musculista senhousia* – Asian bag mussel *Mytilopsis sallei* – Black striped mussel *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Sabella spallanzanii* – Mediterranean fanworm *Sargassum muticum* – Asian seaweed *Undaria pinnatifida* – Japanese kelp *Womersleyella setacea* – Red macroalga	Decant entire sample from catch bag to fish-box or similar container and wash through 1 mm sieve using filtered seawater. Separate any fragile specimens and those requiring special treatment, and label (see section 4.1.2). Wash entire sample retained on sieve into labelled plastic bag/bags and within 8 hours, fix all samples to achieve final concentration of 4 % formalin (or other treatment as specified in Table 16). Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	• 1 mm stainless steel or brass sieves • dissecting microscope.

Sample processing (Level 2 expertise)

In the laboratory:

- wash in copious freshwater over 1 mm sieve to remove fixative
- decant from sieve to jar in freshwater
- within 12 hours sort sample (see section 4.5) under dissecting microscope into multiple vials containing major taxa containing 70 % ethanol (or other preservative as specified in Table 16)
- reduce water and transfer any remaining unsorted material to 70 % ethanol if sorting incomplete after 12 hours
- carefully wash over 1 mm sieve any fragile specimens which had been fixed separately in the field, and transfer into the same set of vials

Sample identification (Level 3 expertise)

Further sort preserved samples into lower taxonomic groups under a dissection microscope. Identification must be carried out in consultation with a suitable taxonomist(s) who can verify sort accuracy and confirm the identity of suspected target species.

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Spat bags

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Settled larvae and juveniles of species with planktonic larval stages including: Asterias amurensis – Northern Pacific seastar Balanus eburneus – Ivory barnacle Balanus improvisus – Bay barnacle Carcinus maenas – European shore crab Charybdis japonica – Lady crab Crassostrea gigas – Feral Pacific oyster Hydroides dianthus – serpulid polychaete Musculista senhousia – Asian bag mussel Perna perna – South African brown mussel Perna viridis – Asian green mussel Varicorbula gibba – European clam	Decant entire sample from mesh bag to fish-box or similar container and wash through 1 mm sieve using filtered seawater. Separate any fragile specimens and those requiring special treatment, and label (see section 4.1.2). Wash entire sample retained on sieve into labelled plastic bag/bags and within 8 hours, fix all samples to achieve final concentration of 4 % formalin (or other treatment as specified in Table 16). Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	Dissecting microscope.

Sample processing (Level 2 expertise)

In the laboratory:

- wash in copious freshwater over 1 mm sieve to remove fixative
- decant from sieve to jar in freshwater
- within 12 hours sort sample (see section 4.5) under dissecting microscope into multiple vials containing major taxa containing 70 % ethanol (or other preservative as specified in Table 16)
- reduce water and transfer any remaining unsorted material to 70 % ethanol if sorting incomplete after 12 hours
- carefully wash over 1 mm sieve any fragile specimens which had been fixed separately in the field, and transfer into the same set of vials

Sample identification (Level 3 expertise)

Further examine sample under a dissection microscope and identify the organisms using taxonomic keys or identification guides.

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

References

Sutton, C. and Hewitt, C. (2004) Detection kits for community-based monitoring of introduced marine pests. Revised final report to National Heritage Trust/Coast and Clean Seas, NHT 21247;

www.marine.csiro.au/crimp//reports/CDKreport.pdf.

Sample processing method: Beach seines

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Adult and juvenile fish and mobile epifauna, including: Neogobius melanostomus – Round goby Siganus luridus – Dusky spine foot Siganus rivulatus – Marbled spine foot Tridentiger barbatus – Shokohazi goby Tridentiger bifasciatus – Shimofuri goby	After collection, samples are coarsely sorted. Separate fragile specimens and those requiring special treatment, and label. Within 8 hours, fix all samples according to taxon (see Table 16). Very large specimens (>5 cm length or >12 g weight) may need to be injected with fixative to ensure sufficient fixative reaches internal tissues. Fish should be anaesthetised prior to fixation, using a suitable anaesthetic (e.g. clove oil) Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	Dissecting microscope.

Sample processing (Level 2 expertise)

Specimens are left in the fixative for 7-10 days, before they are thoroughly rinsed with freshwater and preserved in a solution of 70 % ethanol or other preservative (Table 16).

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Fish and crab traps

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Adult and juvenile fish and mobile epifauna, including: Asterias amurensis – Northern Pacific seastar Carcinus maenas - European green crab Charybdis japonica – Lady crab Eriocheir spp. – Mitten crab Hemigrapsus sanguineus – Japanese shore crab Neogobius melanostomus – Round goby Siganus luridus – Dusky spine foot Siganus rivulatus – Marbled spine foot Tridentiger bifasciatus – Shimofuri goby Tridentiger barbatus – Shokohazi goby	After collection, samples are coarsely sorted. Note that holding live, cheliped bearing specimens with other animals should be avoided as they may damage more fragile species. Separate fragile specimens and those requiring special treatment, and label. Within 8 hours, fix all samples according to taxon (see Table 16). Very large specimens (>5 cm length or >12 g weight) may need to be injected with fixative to ensure sufficient fixative reaches internal tissues. Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	Dissecting microscope.

Sample processing (Level 2 expertise)

Specimens are left in the fixative for 7-10 days, before they are thoroughly rinsed with freshwater and preserved in a solution of 70 % ethanol or other preservative (Table 16).

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Poison stations

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Adult and juvenile fish including: *Neogobius melanostomus* – Round goby *Siganus luridus* – Dusky spine foot *Siganus rivulatus* – Marbled spine foot *Tridentiger bifasciatus* – Shimofuri goby *Tridentiger barbatus* – Shokohazi goby	After collection, samples are coarsely sorted. Note that holding live, cheliped bearing specimens with other animals should be avoided as they may damage more fragile species. Separate fragile specimens and those requiring special treatment, and label. Within 8 hours, fix all samples according to taxon (see Table 16). Very large specimens (→5 cm length or →12 g weight) may need to be injected with fixative to ensure sufficient fixative reaches internal tissues. Note: Samples for DNA analysis are frozen at -80 °C or fixed in 90 % ethanol.	Dissecting microscope.

Sample processing (Level 2 expertise)

Specimens are left in the fixative for 7-10 days, before they are thoroughly rinsed with freshwater and preserved in a solution of 70 % ethanol or other preservative (Table 16).

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides

Quality assurance

Personnel work with taxonomists to verify sorting accuracy and target species identity.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Underwater visual / video surveys

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
With the exception of microscopic species, underwater visual surveys can potentially detect many species on the target list. However, it is most suited to the following species: *Asterias amurensis* – Northern Pacific seastar *Caulerpa racemosa* – Green macroalga *Carcinus maenas* - European green crab *Charybdis japonica* – Lady crab *Caulerpa taxifolia* – Mediterranean seaweed *Codium fragile* ssp. *fragile* – Green macroalga *Crassostrea gigas* – Feral Pacific oyster *Didemnum* spp. – Ascidian *Eriocheir* spp. – Mitten crab *Hemigrapsus sanguineus* – Japanese shore crab *Mytilopsis sallei* – Black striped mussel *Musculista senhousia* – Asian bag mussel *Mnemiopsis leidyi* – Comb jelly *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Sabella spallanzanii* – Meditteranean fan worm *Undaria pinnatifida* – Japanese kelp *Womersleyella setacea* – Red macroalga	Specimens labelled with site code/ location details and fixed according to taxon (see Table 16). Samples for DNA analysis must be frozen at -80 °C, fixed in 90 % ethanol or put into desiccant. Still and video images must be clear and of sufficient resolution to determine at least one of the specimen's unique identifying characteristics. The characteristics observed on the image must match those of the physical specimen before identification.	Dissecting microscope.

Sample processing (Level 2 expertise)

Specimens are left in the fixative for 7-10 days, before they are thoroughly rinsed with freshwater and preserved in a solution of 70 % ethanol or other preservative (see Table 16).

Photographs should be labelled and may be kept at room temperature. Note that video material has an effective life of approximately 5 years under archival conditions.

Photographs and video images are compared to physical specimens for identification.

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides

Quality assurance

Specimen labels must match diver records and samples must be intact for identification.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Visual surveys / beach walks

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Visual surveys may involve collection of living organisms, or those washed up in wrack. Potential target species include: *Asterias amurensis* – Northern Pacific seastar *Balanus eburneus* – Ivory barnacle *Balanus improvisus* – Bay barnacle *Bonnemaisonia hamifera* – Red macroalga *Carcinus maenas* – European green crab *Caulerpa racemosa* – Green macroalga *Caulerpa taxifolia* – Mediterranean seaweed *Charybdis japonica* – Lady crab *Codium fragile* ssp. *fragile* – Green macroalga *Corbula amurensis* – Brackish water bivalve *Crepidula fornicata* – American slipper limpet *Crassostrea gigas* – Feral Pacific oyster *Ensis directus* – Jack-knife clam *Eriocheir* spp. – Mitten crab *Grateloupia turuturu* – Red macroalga *Hemigrapsus sanguineus* – Japanese shore crab *Mytilopsis sallei* – Black striped mussel *Musculista senhousia* – Asian bag mussel *Mya arenaria* – Soft shell clam *Perna perna* – South African brown mussel *Perna viridis* – Asian green mussel *Rapana venosa* - Gastropod *Undaria pinnatifida* – Japanese kelp *Varicorbula gibba* – European clam *Womersleyella setacea* – Red macroalga	Specimens labelled with site code/location details, photographed and fixed according to taxon (see Table 16). Samples for DNA analysis must be frozen at -80 °C, fixed in 90 % ethanol, or put into desiccant. Still and video images must be clear and of sufficient resolution to determine at least one of the specimen's unique identifying characteristics. The characteristics observed on the image must match those of the physical specimen before identification.	Dissecting microscope.

Sample processing (Level 2 expertise)

Specimens are left in the fixative for 7-10 days, before they are thoroughly rinsed with freshwater and preserved in a solution of 70 % ethanol or other preservative (see Table 16).

Photographs and video images are compared to physical specimens for identification.

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides.

Quality assurance

Specimen labels must match log sheet records and samples must be intact for identification.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Sample processing method: Macroalgae

TARGET SPECIES	FIELD PRE-PROCESSING	EQUIPMENT SPECIFICATIONS
Bonnemaisonia hamifera – Red macroalga *Caulerpa taxifolia* – Mediterranean seaweed *Caulerpa racemosa* – Green macroalga *Codium fragile* spp. *fragile* – Green macroalga *Grateloupia turuturu* – Red macroalga *Sargassum muticum* – Asian seaweed *Undaria pinnatifida* – Japanese seaweed *Womersleyella setacea* – Red macroalga	After collection, samples need to be processed quickly to avoid DNA damage and deterioration of structures important for identification. If necessary, samples can be kept for up to 6 hours before being preserved, but only if they are kept cool in low light or darkness. If samples are to be examined fresh, then specimens should be kept submerged in water from the collection site (flow-through tanks are best). If epiphytic organisms are present, separate subsamples should also be retained for the purposes of identification.	Dissecting microscope.

Sample processing (Level 2 expertise)

On site or in the laboratory: Specimens for DNA analysis should be sub-sampled first. Relatively small amounts of material are required (a few cm^2), although more may be taken. The specimen needs to be as healthy as possible, free of other organisms (e.g., epiphytes, pieces of substrate) and reproductive structures should be evident (if at all possible). Place the selected portion to be preserved for DNA analysis in a screw capped container filled with 95-100 % ethanol. Alternatively, wrap the algae in a small square of Chux cloth (to keep material together) and place it in a zip-loc plastic bag with silica gel (Harvey et al., 2005). Samples should be labelled in accordance with the principles outlined in section 4.1.2. Samples for DNA analysis should be kept well away from formalin-fixed samples as the formalin vapour can cause deterioration of DNA.

Using a shallow tray, float the remainder of the specimen in water and slide a piece of archive-quality herbarium paper beneath, lifting it out carefully so that the specimen is spread out. Press the mounted specimen between absorbent layers of inkless newsprint or other paper, replacing the paper periodically to prevent mould.

For longer-term storage, specimens should be fixed in formalin and then preserved in a glycerol-ethanol solution (or air-dried; Harvey et al. 2005). For fixation, samples are placed in a double layer of heavy-duty plastic bags with labels (see section 4.1.2) and full strength formalin added. Bags are sealed immediately, without removing excess air (this helps protect specimens from damage). Sealed bags are then placed in screw-top black plastic barrels (or similar) for storage and transport.

After initial fixation, specimens must be washed thoroughly with freshwater in a fume hood. After all the formalin is gone, a 1:7:2 (10 parts total) glycerol:ethanol:water solution is prepared by adding 100 ml of glycerol to 900 ml of 80 % ethanol. As a general principle, a minimum of two 250 ml screw-cap jars worth of material should be preserved and any excess material air-dried. Specimens should not be closely-packed in the jar as this inhibits infusion of the glycerol-ethanol solution.

Sample identification (Level 3 expertise)

Identify the organisms using taxonomic keys or identification guides.

Quality assurance

Specimen labels must match log sheet records and samples must be intact for identification.

Parataxonomic / Identification guides

- NIMPIS, www.marinepests.gov.au/nimpis
- PaDIL, www.padil.gov.au
- BIPW, www.tdwg.org/biodiv-projects

Attachment C Taxonomic tools and sources for marine species identification

There are a number of tools available to provide information on the ecology and identification of marine pest species.

NIMPIS (www.marinepests.gov.au/nimpis)

The National Introduced Marine Pest Information System (NIMPIS) is a central repository of information on the biology, ecology and distribution (international and national) of invasive marine pest species. It includes known species that have been introduced to Australian waters and species that are considered to pose a potential for future introductions. The information within NIMPIS is also used to support rapid responses to new incursions, and assist in the management of existing introduced species in Australian waters. The NIMPIS database provides managers with information to assist in developing policies and responses to manage marine pests, including reducing the likelihood of new introductions of invasive marine species.

PaDIL (www.padil.gov.au)

The Pests and Diseases Image Library (PaDIL) is a Commonwealth Government initiative, developed and built by Museum Victoria's Online Publishing Team, with support provided by DAFF and PHA (Plant Health Australia). PaDIL's content is dominated by terrestrial insect pests, however many marine introduced species (and similar native species for comparative purposes) are now being added. The high quality images produced via PaDIL have the potential to be a valuable resource for introduced marine pest surveys.

BIPW (www.tdwg.org/biodiv-projects)

Biodiversity Information Projects of the World (BIPW) was formed to help establish international collaboration among biological database projects. This website promotes the wider and more effective dissemination of information and provides searching and links to many other marine pest websites and information systems.

PARATAXONOMIC TOOLS

These tools may, for example, consist of field cards with photos and key features to look for to identify species. A number of these tools have been produced by state/territory government agencies and are available on their respective websites.

Table C1 provides a starting point for taxonomic expertise and tools for marine species identification. This information is also available and updated via the Taxonomy Research and Information Network (TRIN) at www.taxonomy.org.au and TRIN Wiki at http://wiki.trin.org.au.

Table C1. Taxonomic expertise and tools for marine species identification

TAXON	TAXONOMIC EXPERTISE1,2	MAJOR IDENTIFICATION TOOLS AND OTHER SOURCES4
Algae	Macroalgae: C.F. Gurgal (SHSA); J.Huisman (MRD); G.Kraft[3] (UME); J.A.Lewis (ESL); A.Millar (RBGS); W.Nelson (NIWA); J.Phillips (UQ); B.Womersley (SHSA); Microalgae: S.Brett (MSV); G.Hallegraeff (UTAS); K.Heimann (JCU)	www.algaebase.org http://seaweed.ucg.ie/defaultwednesday.html No single comprehensive guide. See Womersley (1984-2003), Huisman (2000, 2006), Kraft (2007), Adams (1994), McCarthy & Orchard (2007).
Annelida: Hirudinea (Leeches)	None	See Australian Biological Resources Study (1994-2005) for checklist last updated by Govedich in 2002.
Annelida: Oligochaeta	A.M.Pinder (DEC) [to be confirmed]	None for marine taxa. See Australian Biological Resources Study (1994-2005) for checklist for Order Tubificida only last updated by Pinder in 2003.
Annelida: Polychaeta	C.J.Glasby (MAGNT); P.A.Hutchings (AM); H.Paxton[3](AM); G.Read (NIWA); R.S.Wilson (MV)	Wilson et.al. (2003) and Rouse & Pleijel (2001) should allow identification of most Australian species. See Australian Biological Resources Study (1994-2005) for checklist last updated by Hutchings & Johnson in 2003
Brachiopoda	J.Richardson[3](MV)	See Australian Biological Resources Study (1994-2005) for checklist last updated by Middelfart & Reid in 2001. Richardson (1997) contains a key to about half of all species known from southern Australia.

Table C1. continued

TAXON	TAXONOMIC EXPERTISE1,2	MAJOR IDENTIFICATION TOOLS AND OTHER SOURCES4
Bryozoa (=Ectoprocta)	P.Bock[3] (MV); P.Cook[3] (MV); D.Gordon (NIWA)	Very diverse, and very poorly known in Australia. There is no comprehensive reference. Bock (1982) includes only a few of the most common species.
Crustacea: Amphipoda	P.Berents (AM); J.K.Lowry (AM); G.C.B.Poore (MV); W.Zeidler[3] (SAM)	Barnard and Karaman (1991) allow identification of families and genera. Keys to families, and to Australian species for some families, can be found in Lowry & Springthorpe (2001).
Crustacea: Isopoda	N.L.Bruce (MTQ); C.Hass[3] (WAM); S.Keable (AM); G.C.B.Poore (MV); G.D.F.Wilson (AM)	Keys to families can be found in Keable et al. (2002). Also see Hass & Knott (1998).
Crustacea (other Peracarida)	G.C.B.Poore (MV)	Keys to Tanaidacea families can be found in Larsen (2002). Keys to Mysidacea families can be found in Meland (2002).
Crustacea: Cirripedia	D.Jones (WAM); J.A.Lewis (ESL)	See Jones (1990), Poore (2008), Henry & McLaughlin (1975, 1986), Foster (1978), Hosie & Ayhong (2008), Underwood (1977).
Crustacea: Copepoda	G.Walker-Smith (MV)	Keys to families of Calanoida can be found in Bradford-Grieve (2002).
Crustacea: Decapoda	S.Ahyong (NIWA); P.J.F.Davie (QM); G.C.B.Poore (MV); J.Yaldwyn (TP)	Poore (2004) provides a comprehensive treatment of the fauna of southern Australia. Keys to families of Stomatopoda can be found in Ahyong & Lowry (2001) and for Anomura families in McLaughlin et al. (2002).
Crustacea: Leptostraca	G.Walker-Smith (MV)	See publications of Walker-Smith (1998; 2000; 2001).
Cephalochordata	B.J.Richardson (UWS)	See Australian Biological Resources Study (1994-2005) for checklist last updated by Richardson in 1997.
Cnidaria: Anthozoa	C.Wallace (MTQ)	Some of the temperate fauna is covered by in part in The Marine Invertebrates of South Australia Part 1 (Thomas & Shepherd 1982).
Cnidaria: Hydrozoa	J.Watson[3] (MV)	Parts of the fauna are covered by Watson and colleagues (Vervoort & Watson 2003; Watson 1982; 2000).

Table C1. continued

TAXON	TAXONOMIC EXPERTISE1,2	MAJOR IDENTIFICATION TOOLS AND OTHER SOURCES4
Cnidaria: Octocorallia	P.Alderslade[3] (contactable via CSIRO)	Fabricius & Alderslade (2001) is a guide tropical genera only; also see Alderslade (1998). Some of the temperate fauna is covered by in part in The Marine Invertebrates of South Australia Part 1 (Grasshoff 1982a, b; Utinomi & Shepherd 1982; Verseveldt 1982).
Cnidaria: Scleractinia	J.E.N Veron[3](AIMS); C.Wallace (MTQ);	For the tropical fauna see Wallace (1999), Veron (1996; 2000; 2003) and Veron & Stafford-Smith (Veron & Stafford-Smith 2002). Temperate fauna treated by Shepherd & Veron (1982).
Cnidaria Scyphozoa	L.Gershwin (QVMAG)	Some of the temperate fauna is covered in Southcott (1982).
Ctenophora	L.Gershwin (QVMAG)	None
Echinodermata: Asteroidea	L.Marsh[3](WAM); T.O'Hara (MV); M.O'Loughlin[3] (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Zeidler & Shepherd (1982) is useful for the temperate fauna.
Echinodermata: Crinoidea	T.O'Hara (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Shepherd et al. (1982) is useful for the temperate fauna.
Echinodermata: Echinoidea	K.McNamara (WAM); T.O'Hara (MV); A.Miskelly (AM)	Much of the tropical fauna is covered by Clark & Rowe (1971). Baker (1982b) is useful for the temperate fauna. Also see Miskelly (2003).
Echinodermata: Holothuroidea	T.O'Hara (MV); M.O'Loughlin[3](MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Rowe (1982) is useful for the temperate fauna.
Echinodermata: Ophiuroidea	T.O'Hara (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Baker (1982a) is useful for the temperate fauna.
Echiura	None	See publications of Edmonds and colleagues (Edmonds 1987; Stephen & Edmonds 1972).
Kamptozoa	None	Introductions to the fauna and the literature have been published by Wasson (Wasson 2002; Wasson & Shepherd 1997).
Hemichordata	None	See Burdon-Jones (1998) and Australian Biological Resources Study (1994-2005) for checklist last updated by Burdon-Jones in 1997.

Table C1. continued

TAXON	TAXONOMIC EXPERTISE1,2	MAJOR IDENTIFICATION TOOLS AND OTHER SOURCES4
Mollusca: Aplacophora	None	See Scheltema (1989; 1999).
Mollusca: Polyplacophora	K.Gowlett-Holmes (CSIRO)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Macpherson & Gabriel 1962; Ponder, et al. 2000).
Mollusca: Bivalvia	S.Boyd[3] (MV); T.Darragh[3] (MV); J.M.Healy (QM); S. Slack-Smith (WAM); R.C.Willan (MAGNT)	No single comprehensive guide; a variety of publications are relevant in part (Lamprell & Healy 1992, 1998a; Macpherson & Gabriel 1962; Ponder, et al. 2000) .
Mollusca: Cephalopoda	M.Norman (MV)	See Norman & Reid (2000).
Mollusca: Gastropoda: Prosobranchia	T.Darragh[3] (MV); W.Ponder[3] (AM); S. Slack-Smith (WAM); F.Wells (ENZ); R.C. Willan (MAGNT)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Ponder, et al. 2000; B.R. Wilson & Gillett 1971).
Mollusca : Gastropoda: Opisthobranchia	C.Bryce (WAM); R.Burn[3] (MV); W.Rudman[3] (AM); R.C.Willan (MAGNT)	No single comprehensive guide. About one-quarter of all temperate species are treated by Burn (1989), and many tropical species are illustrated by Coleman (2001).
Mollusca : Gastropoda: Pulmonata	S.Slack-Smith (WAM)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Macpherson & Gabriel 1962; Ponder, et al. 2000).
Mollusca: Scaphopoda	J.M.Healy (QM)	See Lamprell & Healy (1998b; 2001).
Nemertea	None	A difficult and diverse group, poorly known in Australia; for an introduction see Gibson (1997) and references cited therein.
Phoronida	None	The small fauna is adequately covered by Emig and colleagues (Emig, et al. 1977; Emig & Roldan 1992; Shepherd 1997).
Platyhelminthes	L.Winsor (JCU)	A difficult and diverse group. The temperate fauna, especially, is poorly known in Australia (Cannon 1986; Newman & Cannon 2003, 2005).

Table C1. continued

TAXON	TAXONOMIC EXPERTISE1,2	MAJOR IDENTIFICATION TOOLS AND OTHER SOURCES4
Porifera	J.Fromont (WAM); J.N.A.Hooper (QM); B.Alvarez de Glasby (MAGNT); L.J.Goudie, (via MV); S.Sorokin (SARDI)	A difficult and diverse group, poorly known in Australia. Higher level classification covered in Hooper & Soest (2002). A species level classification web tool is in progress.
Pycnogonida	C.Arango (QM); D.Staples (MV)	See Staples (1997).
Sipuncula	None	See publications of Edmonds and colleagues (Edmonds 1980; Stephen & Edmonds 1972).
Tunicata	P.Mather[3] (QM) [publishes as P.Kott]	See Kott (1985; 1990a; 1990b; 1992a; 1992b).
Chordata: Osteichthyes	M.Gomon (MV); J.Johnson (QM); R.Kuiter	See Australian fish collections website at http://archive.amol.org.au/collection/hostedwebs/fish/fish_intro.html. See Kuiter (1996), Gomon et al. (1994), Kuiter & Debelius (1994)

Key

1 - Institution abbreviations:

Abbreviation	Institution
AU	Auckland University, Auckland
AIMS	Australian Institute of Marine Science, Townsville
AKM	Auckland Museum, Auckland
AM	Australian Museum, Sydney
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEC	Department of Conservation and Land Management, Perth
ENZ	Enzer Marine Environmental Consulting
ESL	ES Link Services
JCU	James Cook University, Townsville
MAGNT	Museum and Art Gallery of the Northern Territory, Darwin
MRD	Murdoch University, Perth
MSV	Microalgal Services Victoria
MTQ	Museum of Tropical Queensland, Townsville
MV	Museum Victoria, Melbourne
NIWA	National Institute of Water and Atmospheric Research, Wellington
QVMAG	Queen Victoria Museum and Art Gallery, Launceston
QM	Queensland Museum, Brisbane
RBGS	Royal Botanic Gardens, Sydney
SAM	South Australian Museum, Adelaide
SHSA	State Herbarium of South Australia, Adelaide
TMAG	Tasmanian Museum and Art Gallery, Hobart
TP	Museum of New Zealand Te Papa Tongarewa, Wellington

UTAS	University of Tasmania
UQ	University of Queensland
UME	University of Melbourne
UWS	University of Western Sydney, Hawkesbury
WAM	Western Australian Museum, Perth

2 - A few taxonomists based in New Zealand have also been included, but their expertise is not yet fully surveyed here.

3 - Retired or honorary.

4 - Full citations in the comprehensive reference list in the main section of this manual.

Attachment D Contacts for marine invertebrate and herbarium curators/collection managers

MUSEUM	CONTACT DETAILS
Australian Museum, Sydney (AM)	Web: http://australianmuseum.net.au Phone: (02) 9320 6000
Museum and Art Gallery of the Northern Territory, Darwin (MAGNT)	Web: http://www.nt.gov.au/nreta/museums/index.html Phone: (08) 8999 8264
Museum Victoria, Melbourne (MV)	Web: http://museumvictoria.com.au Phone: 13 11 02
Queensland Museum, Brisbane (QM)	Web: http://www.qm.qld.gov.au Phone: (07) 3840 7555
South Australian Museum, Adelaide (SAM)	Web: http://www.samuseum.sa.gov.au Phone: (08) 8207 7500
Tasmanian Museum and Art Gallery, Hobart (TMAG)	Web: http://www.tmag.tas.gov.au Phone: (03) 6211 4177
Western Australian Museum, Perth (WAM)	Web: http://www.museum.wa.gov.au/ Phone: (08) 9212 3700
HERBARIUM	**CONTACT DETAILS**
National Herbarium of New South Wales	Web: http://www.rbgsyd.nsw.gov.au/science/nsw_herbarium Phone: (02) 9231 8111
Northern Territory Herbarium	Web: http://www.nt.gov.au/nreta/wildlife/plants/index.html Phone : (08) 8999 5511
Queensland Herbarium	Web : http://www.epa.qld.gov.au/nature_conservation/plants/queensland_herbarium/index.html Phone : (07) 3896 9326
State Herbarium of South Australia	Web: http://www.environment.sa.gov.au/science/bio-discovery/overview.html Phone: (08) 8222 9308
Tasmanian Herbarium	Web: Web: http://www.tmag.tas.gov.au Phone: (03) 6226 2635
National Herbarium of Victoria	Web: http://www.rbg.vic.gov.au/ Phone: (03) 9252 2429
Western Australian Herbarium	Web: http://www.dec.wa.gov.au/science-and-research/wa-herbarium/wa-herbarium.html Phone: (08) 9334 0500

This information is also available and updated via the Taxonomy Research and Information Network (TRIN) at www.taxonomy.org.au and TRIN Wiki at http://wiki.trin.org.au

Attachment E Professional links and societies

SOCIETY/LIST SERVER	URL	COMMENTS
All organisms	http://tolweb.org/tree	Tree of life project
Australasian Society for Phycology and Aquatic Botany	www.aspab.cjb.net	A forum for anyone interested in phycology and/or aquatic botany.
Cnidaria Newsgroup	www.ucihs.uci.edu/biochem/steele/newsgroup.htm	A forum for cnidarian (coelenterate) biologists including taxonomists.
Crustacean Society	www.vims.edu/tcs	A forum for crustacean biologists including taxonomists.
Echinoderm-l	http://nic.museum/archives/echinoderm-l.html	A forum for echinoderm biologists including taxonomists.
International Bryozoology Association	www.nhm.ac.uk/hosted_sites/iba	A forum for bryozoan (Ectoprocta) biologists including taxonomists.
International Polychaetology Association	http://biocollections.org/pub/worms/annelid.html	A forum for polychaete biologists including taxonomists.
Leech-l	http://archiver.rootsweb.com/th/index/LEECH	A forum for leech (Hirudinea) biologists including taxonomists.
Malacalogical Society of Australia	www.amonline.net.au/invertebrates/mal/malsoc	A forum for Australian mollusc biologists including taxonomists. Includes links to international mollusc societies.
Nemertes forum	http://nemertes.si.edu/mod/forum	A forum for nemertean biologists including taxonomists.
Porifera list	www.jiscmail.ac.uk/lists/porifera.html	A forum for sponge (Porifera) biologists including taxonomists.
Society for the Preservation of Natural History Specimens (SPNHC)	www.spnhc.org	A multidisciplinary organisation composed of individuals (including many museum professionals) who are interested in development and preservation of natural history collections.
Southern California Association of Marine Invertebrate Taxonomists (SCAMIT)	www.scamit.org	Promotes the study of marine invertebrate taxonomy in southern California and developing a regionally standardised taxonomy. Connects taxonomists with ecologists and consultants in southern California through newsletters and meetings.

Attachment E. continued

SOCIETY/LIST SERVER	URL	COMMENTS
TAXACOM	http://biodiversity.bio.uno.edu/mail_archives/taxacom	List server for taxonomists.
Taxonomic Databases Working Group (TDWG)	www.tdwg.org	A forum for biological data projects, develops and promotes the use of data standards and facilitates exchange of taxonomic data.
Taxonomy Research and Information Network & TRIN Wiki	www.taxonomy.org.au http://wiki.trin.org.au	A group of leading Australian scientists in the field of taxonomy. TRIN Wiki includes a collaborative national electronic framework for taxonomic knowledge exchange.
Tunicata list	www.jiscmail.ac.uk/lists/tunicata.html	A forum for tunicate (ascidian) biologists including taxonomists.

This information is also available and updated via the Taxonomy Research and Information Network (TRIN) at www.taxonomy.org.au and TRIN Wiki at http://wiki.trin.org.au.