

JULY 1998

**CENTRE FOR RESEARCH ON INTRODUCED MARINE PESTS**

**TECHNICAL REPORT NUMBER 18**

**A REVIEW AND EVALUATION OF BALLAST WATER  
SAMPLING PROTOCOLS**

**CAROLINE A. SUTTON<sup>1</sup>, KATE MURPHY<sup>2</sup>, RICHARD B. MARTIN<sup>1</sup>  
AND CHAD L. HEWITT<sup>1</sup>**

<sup>1</sup> CENTRE FOR RESEARCH ON INTRODUCED MARINE PESTS  
CSIRO DIVISION OF MARINE RESEARCH  
HOBART, TASMANIA

<sup>2</sup> DEPARTMENT OF ZOOLOGY  
UNIVERSITY OF TASMANIA  
HOBART, TASMANIA



**CSIRO**  
**MARINE RESEARCH**

A review and evaluation of ballast water sampling protocols.

Bibliography.

Includes index.

ISBN 0 643 06182 7.

1. Ballast water – Analysis. 2. Ballast water – Research – Australia. 3. Ballast water – Environmental aspects – Australia. 4. Marine pollution – Australia. I. Sutton, C. A. (Caroline Anne). II. CSIRO. Division of Marine Research. III. Centre for Research on Introduced Marine Pests (Australia). IV. University of Tasmania. Dept of Zoology. (Series: Technical report (Centre for Research on Introduced Marine Pests (Australia)); no. 18)

363.729464

## SUMMARY

This study was initiated to review and evaluate ballast water sampling techniques with a view to providing a basis for the development of a robust and effective sampling program. The proposed sampling program will support the risk assessment-based decision support system (DSS) currently under development for the Australian Quarantine and Inspection Service (AQIS) and provide verification of management activities. This study involved (i) a review of sampling protocols currently in use by national and international ballast water research and management groups and (ii) field trials and evaluations of a range of sampling methods.

Contact was made with 32 researchers who are or have recently participated in ballast water research. Information on sampling protocols was obtained from 14 research groups. The review indicated that the majority of programs were established to survey and document the range of organisms found in ballast tanks of incoming vessels to provide some measure of risk associated with ballast water discharge. Most were designed as monitoring programs and were carried out on an opportunistic basis. As a consequence, the sampling methods used were largely determined by available access to ballast tanks.

A total of nine ballast water sampling methods were identified from the review and seven were evaluated within this project. This involved opportunistic testing of different combinations of methods on a total of 9 vessels in the ports of Hobart, Devonport and Newcastle and a more detailed and controlled study on the MV *Iron Whyalla* during ballast water heat treatment trials between Pt Kembla and Pt Hedland. Sampling methods were evaluated for their practical (operational) application and their effectiveness in sampling the both the total zooplankton assemblage and a suite of target (or surrogate) taxa.

Access to ballast tanks and the stage of the ballasting cycle at which sampling occurs impose major restraints on the types of methods that can be employed. Operationally, net sampling through manholes was preferred for ease and speed of sampling but this method is only appropriate for cargo holds and wing tanks (when full). Sampling with pumps via sounding pipes or air vents provides access to a greater range of tanks but requires more cumbersome equipment and longer sampling times. In-line (ballast pump) sampling techniques also require relatively long sampling times and can only be used when ballast pumps are in operation, either during ballasting or deballasting.

The methods tested differed in the effectiveness with which they sampled the zooplankton community in ballast tanks and no single method effectively sampled all taxa. Overall, nets were more effective at sampling the total zooplankton assemblage and the suite of surrogate target taxa but some level of sampling bias was associated with all methods. Mobile zooplankters (such as crab zoea) were under-sampled by methods that relied on low flow rate pumps, while polychaete trochophores were adequately sampled by a range of methods.

These operational and biological uncertainties, make it inadvisable for sampling programs to rely on a single sampling method. The final selection of methods to be used in any instance will be influenced by the aims of the particular sampling program. For targeted sampling programs, the use of molecular techniques and a reduced reliance on traditional identification methods is likely to lead to the development of more efficient ballast sampling methods.

## ACKNOWLEDGEMENTS

Many people assisted over the course of this project. Special thanks go to all the national and international scientists who responded to the questionnaire and to those who provided valuable input into the development of the project.

We are also grateful to the crews of the various ships sampled during the project, in particular those on the *MV Iron Whyalla* and *MV Iron Sturt*, all of whom were very hospitable and helpful during our visits to their vessels. Thanks also to AQIS staff in Newcastle who provided support and assistance during the Newcastle component of the shipping survey. We are particularly grateful to those researchers who assisted in the collection and sorting of samples including, Dr Geoff Rigby, Dr Gustaaf Hallegraeff, Dr Julie Hall, Keith Hayes, and to Rod Turton and Nicole Mays who sorting the numerous plankton samples.

Funding for this project (AQIS 009/96) was provided by AQIS as part of the Strategic Ballast Water Research Program administered by the Ballast Water Research Advisory Group and the Australian Ballast Water Advisory Council.

## CONTENTS

<b>SUMMARY</b> .....	i
<b>ACKNOWLEDGMENTS</b> .....	ii
<b>CONTENTS</b> .....	v
<b>1 INTRODUCTION</b>	
1.1 Background.....	1
1.2 Approach to the project.....	3
<b>2 VESSEL CONFIGURATIONS AND SAMPLING OPTIONS</b>	
2.1 Vessel types and ballast tank configurations.....	5
2.2 Ballasting and deballasting procedures.....	5
2.3 Description and evaluation of potential sampling locations.....	5
2.3.1 Sounding pipes.....	7
2.3.2 Manhole access.....	8
2.3.3 Cargo hatch.....	8
2.3.4 Deck taps.....	9
2.1.5 Air vents and breather pipes.....	9
2.1.6 Ballast discharge.....	10
2.1.7 In-line (ballast pump).....	10
<b>3 REVIEW OF INTERNATIONAL BALLAST WATER SAMPLING PROTOCOLS</b>	
3.1 Development of the sampling protocols database.....	13
3.2 Summary of international sampling protocols.....	13
<b>4 DESCRIPTION AND EVALUATION OF SAMPLING PROTOCOLS</b>	
4.1 Background considerations.....	41
4.2 Sampling methods	
4.2.1 Sampling equipment.....	42
4.2.2 Description of sampling methods.....	43
4.3 Evaluation of sampling protocols.....	46
4.3.1 Operational assessment.....	46
4.3.2 Biological assessment.....	47
4.3.3 Suitability of different sampling methods for selected target taxa.....	50
<b>5 DISCUSSION AND CONCLUSIONS</b>	
5.1 Sampling methods.....	51
5.2 Identification of target taxa.....	53

---

5.3 Ballast water sampling and risk assessment. . . . .	53
5.4 Conclusions. . . . .	54
<b>REFERENCES. . . . .</b>	<b>55</b>
<b>APPENDICES</b>	
APPENDIX A Ballast water research groups. . . . .	59
APPENDIX B Ballast water sampling questionnaire. . . . .	69
APPENDIX C Vessel sampling – description and analysis. . . . .	81

## 1 INTRODUCTION

### 1.1 Background

In recent years world-wide attention has been focused on the problem of the introduction of marine species via ships' ballast water. This focus can be attributed to an increased awareness of the potential ecological and economic impacts associated with invasions of new environments by exotic species. Well documented examples of such invasions include the establishment of the European zebra mussel, *Dreissena polymorpha* in North America, resulting in the expenditure of millions of dollars per year on fouling removal and control (Gauthier and Steel 1996), and the introduction of American comb jelly, *Mnemiopsis leidyi*, to the Black and Azov Seas which is believed to have contributed to the failure of the anchovy and sprat fisheries in the area (GESAMP 1997). In Australia, the introduction of the Japanese seastar, *Asterias amurensis*, and toxic dinoflagellates in the genera *Gymnodinium* and *Alexandrium* have implications for both the wild fisheries and aquaculture industry (Davenport and McLoughlin 1993; Hallegraeff and Bolch 1992).

It has been estimated that as many as 3000 species are transported around the world each day by ballast water and that on any day the mix of species transported will be unique (Carlton *et al.* 1995). While the introduction of exotic organisms via ship's ballast water has been a known yet unquantified risk since the early 1900s (Carlton 1985, Gauthier and Steel 1996), it was not until the 1970's that the first documented ballast water sampling study was undertaken (Medcof 1975). Since then, and particularly following Carlton's (1985) influential review, research and management groups in numerous countries, including Australia, Canada, Germany, Israel, New Zealand, Norway, United Kingdom, and the United States have become involved in the sampling and monitoring of ballast water.

Until recently, many of these groups have been working independently and their sampling programs and the methods used have been developed to meet differing objectives. Some groups have been primarily concerned with obtaining baseline information to identify and assess the risks associated with ballast discharge (Medcof 1975; Carlton 1985; 1987; Williams *et al.* 1988; Hallegraeff and Bolch 1991; Subba Roa *et al.* 1994; Gosselin *et al.* 1995; Gollasch *et al.* 1995; Macdonald 1997), while others have focused on assessing compliance with existing guidelines (Locke *et al.* 1991; Michel Gilbert pers. comm.), managing high risk situations or assessing the effectiveness of ballast water treatments such as open ocean exchange (Williams *et al.* 1988; Rigby and Hallegraeff 1994; Carlton *et al.* 1995; Wonham *et al.* 1996), heat treatment (Rigby *et al.* 1997) and filtering (Cangelosi 1997).

At the 83rd ICES meeting in Aalborg (September 1995), participants in the Ballast Water theme session reported on ballast water projects undertaken by ICES member countries including Canada, Germany, UK, and USA and also in Australia and New Zealand. Concern was expressed by delegates at the lack of standard protocols and the potential for errors of interpretation that could result from the use of data obtained using different sampling methods (Thresher and Martin 1995; Carlton 1996). The need for a consistent approach to sampling ballast tanks was accepted by all delegates as was the need for participation by the shipping industry in the design of an effective sampling program. While the actual sampling procedures are only one part of an overall sampling and testing

program, developing methods that can provide representative samples from ballast tanks was seen as a key element in such a program.

In the absence of a comprehensive cost-effective means of treating ballast water, every ship entering Australian ports must be considered to be potentially capable of carrying exotic species that may pose a risk to coastal environments and their associated living resource-based industries. The Australian Quarantine and Inspection Service (AQIS) vessel sampling program for ships visiting Australian ports was aimed at identifying those vessels that posed a high risk of carrying toxic dinoflagellates in their ballast and assessing compliance with ballast water management guidelines. Sampling and testing of ballast water from identified high risk vessels was used to confirm the presence of target organisms and to establish the need for management responses to deal with the risk of their introduction. These management responses included requiring vessels to reballast at sea or denying vessels permission to discharge contaminated ballast in Australian ports.

This approach has to date been largely reactive and the sampling program, for logistic reasons, somewhat limited in its scope and effectiveness. In order to enhance the effectiveness of its barrier program and to become more proactive in the management of ballast water introductions, AQIS is moving to adopt a comprehensive quantitative risk assessment framework applicable to all domestic and international vessels and voyages. This framework will provide a qualitative hazard ranking of vessels in relation to a target group of pest species and will facilitate the operation of a cost effective sampling program by focusing sampling on the evaluation of identified high risk situations (Hayes and Hewitt 1998). The development of a risk analysis-based decision support system (DSS) is a key component of Australia's current ballast water management strategy and a major focus for the Strategic Ballast Water Research Program.

An effective sampling program is itself an important component of the DSS-based approach in that it provides:

- feedback on the general accuracy of biological risk assessments and management decisions made by the DSS;
- relevant information that will, over time, enhance the system;
- confirmation of the status of vessels identified as high risk on the basis of direct evidence as to the presence or absence of target organisms and;
- verification of the efficacy of ballast water treatments such as mid-ocean exchange.

To be effective in these roles, a ballast water sampling and testing program must be capable of providing an accurate estimate of target organisms in ballast tanks; this will likely involve the use of taxa-specific sampling methods. From an operational standpoint, sampling should result in only minor disruption to normal vessel operation and involve on-board sampling procedures that are safe and can be implemented by a few crew or shore-based staff.

From a management perspective, the capacity to rapidly screen ballast water samples and identify target species is a crucial element of a successful ballast water sampling and testing program. Delaying the unloading of a vessel while testing is undertaken is likely to be costly to the shipper and may cause major problems in relation to scheduling for port authorities. Ideally therefore, a testing or screening procedures should be quantitative, suitable for use

by appropriately trained staff (most likely non-biologists) outside a laboratory setting, and provide a rapid a turn around time of hours not days. Currently there are no generally available screening or testing procedures that meet these requirements.

### 1.1 Approach to the study

Ballast water is carried in tanks or holds that may differ in both their size and complexity depending on the type of vessel and the cargo it carries. In addition, each type of vessel will have specific requirements associated with the uptake and discharge of ballast water that will be influenced by cargo handling procedures and engineering constraints. Recognition of the complexity associated with monitoring the diversity of vessels entering Australian ports has led the Australian Ballast Water Management Advisory Council's Research Advisory Group to acknowledge that the current AQIS sampling program "...has some limitations in relation to uncertainties of sample representativeness and the quantitative nature of the analysis." In general terms, the objective of this study was to resolve some of these uncertainties and develop more robust sampling protocols. In acknowledgement of their management responsibilities in this area, it was AQIS's intention that the results of the study would provide the basis for the implementation of a routine monitoring program to support the DSS.

The development of an effective targeted sampling and testing program involves a number of logical stages:

1. Establishment of the criteria for the inclusion of a species on the target list.
2. Development of sampling methods for these species that;
  - are safe and comply with ship based safety regulation;
  - can be applied to a range of ballast tanks and tank configurations and;
  - reliably sample target species with quantified sampling biases and constraints.
3. Development of effective and timely screening tests for those target species that are known to survive transport in ballast water.
4. An assessment of the distribution of target species in different types of ballast tanks.

In practice there are a number of potential impediments to such an approach including:

- operational limitations on the use of standard sampling methods across a range of vessel types with different ballast tank configurations;
- the capacity of existing sampling methods to adequately describe the distribution and abundance of planktonic organisms in ballast tanks;
- the identification of the life history stages of target species that are likely to be present in ballast water; and
- a lack of information about the spatial and seasonal occurrence of target species in port environments, their availability for uptake in ballast water by vessels in infected ports and their subsequent survival in the ballast tank environment over the duration of the voyage.

This study dealt with only two of these issues and focused on an evaluation of a selected group of sampling methods in terms of their practicality for use with different ballast tank configurations, and the effectiveness with which they sampled the total zooplankton assemblage in ballast tanks and a range of surrogate taxa. Specifically the study involved:

1. A desk-top review to identify methods used by different international groups to sample ballast water, ascertain operational requirements for these methods and provide a preliminary assessment of the range of taxa sampled.
2. In conjunction with AQIS Project 006/96 (Heat Treatment), an assessment of the spatial distribution patterns of target or surrogate taxa in ballast tanks, and an evaluation of the effectiveness of various sampling methods in obtaining representative samples of target (or surrogate) taxa under semi-controlled conditions.
3. The development and opportunistic testing of a selection of sampling methods on different vessel types with different ballast tank configurations to evaluate operational requirements and sampling efficiencies for target (or surrogate) taxa under a variety of conditions.

## **2 VESSEL CONFIGURATIONS AND SAMPLING OPTIONS**

### **2.1 Vessel types and ballast tank configurations**

Ballast is taken on board vessels in order to meet operational and engineering safety standards for each particular vessel and voyage. A ship is said to be "in ballast" when it is empty of cargo and therefore fully or appropriately ballasted for the current voyage. Most vessels "in cargo" carry some ballast water for stability and trim.

Ballast water is carried in dedicated ballast tanks and/or cargo holds that vary in number, size and complexity. In general tank configurations are dependent on the type of ship and the cargo it carries (see Figure 2.1). Ballast tank arrangements for various types of vessels are documented in AQIS (1993b) and are summarised in Table 2.1. It should be noted that there are a great variety of vessel and tank configurations and the information presented in Table 2.1 is only indicative of this range.

### **2.2 Ballasting and deballasting procedures**

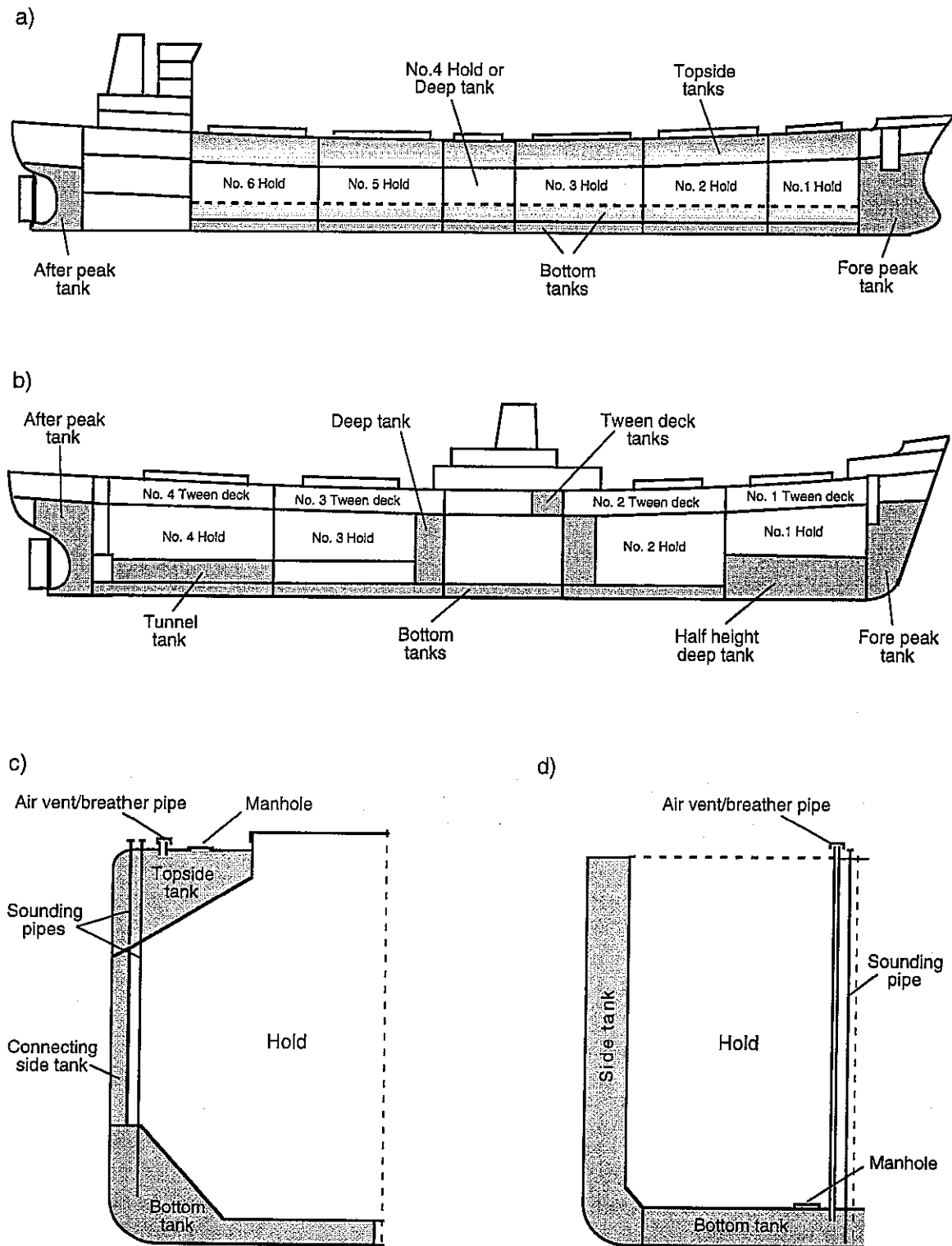
Ballast water is taken on board through inlets located on the bottom of the vessel or low down on the vessel's side plates on both sides of the hull. These intakes open into chambers ("sea chests") on the inside of the hull which in turn receive the intake pipes for the ballast water pumps. The intakes to the sea chests are typically covered by a coarse grid which excludes large objects; a second screen with openings of 5–10 mm is usually located between the sea chests and the ballast pumps.

Ballasting normally commences by gravity filling tanks to near draft and then by pumping to fill the tanks. A vessel's ballasting pipework system usually allows for the transfer of ballast between tanks and the sequential filling of double bottom, side and topside wing tanks, where these occur. Ballasting may take up to 3–4 days depending on the rate at which cargo is unloaded from the vessel and the ballast may be redistributed during this period to maintain the trim and stresses on the vessel within desired limits.

Deballasting is usually by gravity for topside wing and side tanks and by pumping out for double bottom tanks. Pumped discharge is either through the ballast water intakes or dedicated openings located low on the vessels sides. Deballasting may begin either while a vessel is approaching a berth or as soon as loading commences at the berth. Whether discharge of ballast occurs prior to berthing is determined by navigation and safety requirements for the vessels (and individual ports) and draft requirements at loading facilities. The majority of ballast is discharged at the berth and the rate of discharge is geared to the rate at which cargo is loaded. Detailed descriptions of ballasting operations are provided in AQIS Ballast Water Research Series Reports 1 and 4 (AQIS 1993a; 1993b).

### **2.3 Description and evaluation of potential sampling locations**

In general there are four techniques that can be used to obtain samples from ballast tanks: directly using nets, pumps or with discrete depth whole water samplers such as a Van Dorn sampler, and via the main ballast pump or sea chest (which may or may not require a pump). The methods that can be used on a particular vessel are largely determined by its ballast tank configuration and the available access to these tanks. These potential access points are summarised in Table 2.1 for different vessel types.



**Figure 2.1.** Ballast tank configurations for (a) a bulk carrier and (b) a general cargo vessel, and midships cross sections for (c) a typical bulk carrier or vessel with wing and double bottom tanks (these may or not be connected by side tanks), and (d) a general cargo/container vessel with side and bottom tanks. Typical positions of sounding pipes, manholes and air vents are indicated.

**Table 2.1.** Ballast tank configurations and sampling locations for different vessel types.

Vessel type	Ballast tanks	Possible access for sampling
Bulk carriers	topside, double bottom (which may be connected to topside), forepeak, afterpeak	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Ore carriers	wing, double bottom, forepeak, afterpeak, cargo hold(s)	hatch (if cargo hold is carrying ballast) manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Woodchip carriers	double bottom, forepeak, afterpeak, cargo hold(s)	hatch (if cargo hold is carrying ballast) manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Ore/bulk/oil carriers	side, double bottom, afterpeak, forepeak	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
LNG/LPG carriers	bottom, wing and double hulls	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Oil tankers	side (double hull tankers have a series of interconnected port and starboard tanks surrounding oil tanks some older vessel may carry ballast in oil tanks)	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Chemical tankers	double bottom and side	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Container ships	double bottom and side	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
General cargo	double bottom, forepeak, afterpeak	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty
Car carriers	double bottom, forepeak, afterpeak	manholes, sounding and breather pipes, main ballast pump, bottom of tank when empty

(Source: AQIS 1993b)

The following sections provide a brief descriptions of each access point and an evaluation of the limitations and advantages of each from a sampling perspective. This evaluation is based on the field surveys and ship-board experiments undertaken by CRIMP during this project and other related ballast water projects, and on discussions with other international groups carrying out ballast water sampling. More detailed evaluations are provided in Section 4.0.

### 2.3.1 Sounding pipes

A sounding pipe or tube is a 35–50 mm outside diameter (OD) perforated pipe that runs vertically from deck level to the bottom of a ballast tank. It enables the level of the water in the tank to be determined without opening a hatch or manhole. Sounding pipes are found on most vessels, each ballast tank has at least one, and they are always accessible provided there is deck access. Sampling via sounding pipes is carried out using pumps.

#### *Considerations when sampling via sounding pipes:*

- The distance between the level of ballast and the deck of the ship (i.e. the 'head') must be considered as the pumping capacity of most suction pumps is limited by the head. When ballast tanks are partially empty or when sampling bottom tanks it may be necessary for pumps to lift water 10–25 m.

- The sample may include sediments in addition to ballast water.
- If perforations in the walls of the pipe are absent or blocked (see below), samples may not come directly from the tank but from within the immediate confines of the sounding pipe. This factor may contribute to a sampling bias and the sample may not be representative of the tank as a whole.
- Perforations in the walls of the pipe may be blocked the sample may not necessarily come from the depth at which the intake hose for the pump is positioned.
- To filter a volume of water equivalent to that sampled by a net requires long pumping times, particularly when tanks are partially empty.
- For safety reasons, pumps driven by electric or petrol motors may not be used on some vessels, particularly during the loading of inflammable cargos.
- When using electric pumps, consideration must be given to the location and voltage of power on the vessel.

### 2.3.2 Manholes

Manholes refer to the openings that allow direct access to double bottom, top (wing) or side tanks. They provide access to the tanks for inspections or maintenance work. Manholes are usually oval in shape, up to 70 cm x 50 cm in size, and are almost always covered with a plate secured by bolts (12–20).

Manholes are one of two locations on a vessel where there is direct access to tanks and net and whole water sampling is feasible. Sampling through manholes is relatively fast and simple; it does however require opening of the manholes and is therefore not as convenient for the ship's crew as sampling via sounding pipes. In some situations it may be dangerous to open manholes as they are an integral part of the deck and opening them may compromise the structural integrity of the vessel.

#### *Considerations when sampling via manholes:*

- Pump, net or whole water sampling can be used but in many cases it will be only possible to sample the top 2–10 m of a ballast tank.
- The net diameter generally cannot not exceed 50 cm.
- There may be numerous obstacles and barriers within the tanks (such as ladders) that may catch sampling gear or tear nets.
- Nets generally catch larger organisms that may be able to avoid the relatively low suction rates of pumps that are used to sample via sounding pipes (see section 4).
- Light entering the tank via the open manhole may contribute to sampling bias. Most organisms are attracted to light (some may avoid it) which may lead to either an over or under (depending on the species response) estimate of a species abundance in a ballast tank (see section 4). To eliminate potential sampling bias associated with the entry of light, fittings can be attached to the manhole covers enabling the collection of pump samples without the need to remove the covers (see section 4). This, however, would not be appropriate for the majority of routine or opportunistic sampling programs where there is little prior contact made with the vessels to be sampled.

### 2.3.3 Cargo hatch

A hatch is the opening in the deck through which the cargo is loaded and unloaded from the cargo hold. Although holds are designed to carry cargo they may also be used to carry ballast. Woodchip carriers, for example, commonly use at least one cargo hold (usually located amidships) to carry ballast water in addition to that carried in dedicated bottom tanks. Some ore carriers will also use one of the amidships holds as a ballast tank in situations where it is necessary to increase vessel draft.

The hatch is the second location on a vessel where direct access to the ballast may be possible. Safety considerations may prevent the opening of hatches and the co-operation of the ship's crew is essential. Because of the depth of holds, appropriate safety precautions need to be considered when sampling through cargo hatches.

*Considerations when sampling via hatches:*

- Pump, net and whole water sampling can be used and the entire water column within the tank can be sampled; access for sampling sediments and macro zooplankton (fish, crabs) may also be possible.
- There are no restrictions on net dimensions however consideration should be given to tow rate and depth of tow.
- If the hold is near empty a sample can still be collected with a horizontal plankton tow.
- Light (or shadows) may contribute to sampling bias if the hold is only partially exposed.
- Hold water may have different characteristics (physical and biological) to that in dedicated ballast tanks.

### 2.3.4 Deck taps

Deck taps are connected to the vessels fire hose pump. These pumps usually supply ocean water, but can also be used to pump water from the ballast tanks.

*Considerations when sampling via deck taps:*

- Deck taps potentially provide a simple and rapid way to obtain a ballast water sample, however, planktonic organisms tend to be damaged by the high pressure pumps (Locke pers. comm.; Sutton unpublished observations; see section 4).

### 2.3.5 Air vents and breather pipes

#### *Air vents*

An air vent is an open ended pipe that extends into the ballast tank to allow ventilation and prevent the build up of pressure in the tanks during ballasting. Designs vary widely between vessels but in general air vents do not have perforations or extend down to the bottom of the tanks. From a sampling perspective, air vents are similar to sounding pipe in that water for the sample may be drawn from within the confines of a pipe. Water overflowing from air vents when tanks are being "pressed up" can be used to provide a qualitative sample (see "breather pipes" below).

*Considerations when sampling via air vents:*

- Air vents tend to be wider than sounding pipes. This can be a problem if an inertia pump is required for sampling (i.e. when there is a large head) as the hose tends to work within the pipe work and chafe the foot valve (see section 4).
- Air vent often have caps which might need to be removed to provide access to the pipe.

**Breather Pipes**

Breather pipes have the same purpose as air vents but are generally larger in diameter and covered. Some ships routinely "press up" the ballast tanks by allowing ballast water to overflow through the breather pipes to ensure the tanks are filled to capacity. This is done just prior to de-ballasting and loading cargo in order to make the tonnage loaded calculation easier. Sampling via the breather pipes was used during ballast water exchange (Rigby and Hallegraeff 1993), ballast water heat treatment trials (Rigby *et al.* 1997), and is presently in use by Scottish and Welsh research groups (see section 3).

*Considerations when sampling via breather pipes:*

- Sampling can only be carried out when tanks are overflowing.
- The sample is qualitative only.

**2.3.6 Ballast discharge sampling**

From a scientific view point, sampling ballast water as it is discharged from a vessel potentially provides the most reliable estimate of the diversity, number and viability of organisms entering a port environment via ballast water. From a management prospect, however, the detection of target pest species as they enter the port environment provides little scope for any meaningful barrier response.

For vessels where ballast is discharged from outlets above the unloaded or loaded waterline, sampling of water as it is discharged may be possible at certain stages of the deballasting process. In the case for bulk carriers, the discharge level descends below the waterline as cargo is loaded (Geoff Rigby pers. comm.). Ballast water may be discharged at a rate of 3000–5000 l min<sup>-1</sup> from 5–10 m above the waterline. While sampling this outflow is potentially possible, the volume and pressure of the outflow is likely to damage nets or other sampling equipment (Greg Ruiz pers. comm.). Because of the difficulties associated with obtaining a sample "bucket sampling" of the ballast discharge is usually undertaken as a last resort. Greg Ruiz (pers. comm.) however, is investigating the possibility of sampling the ballast overflow plume with plankton tows from small boat. This will provide interesting data from a scientific perspective but is too labour and equipment intensive to consider as routing monitoring or sampling option.

*Considerations when sampling ballast water discharge from hull outlets:*

- Sampling can only be attempted during ballast water discharge when outlets are above the waterline.
- Sampling ballast water at discharge is usually only contemplated as a last resort when tanks are almost empty.
- Sampling may be possible during gravity discharge from topside wing tanks.

### 2.3.7 In-line (or ballast pump) sampling

In-line sampling refers to a sample that is collected from the pipe work connected to the main ballast pump or from the ballast pump itself via the pressure gauge. Sampling at this location usually requires that the ballast pump be operating and hence samples must be taken while the ship is ballasting or deballasting. In some situations there may be sufficient head available to sample using this method when tanks are being ballasted by gravity. In order to generate a sufficient flow of water, samples are taken from the discharge side of the ballast pump.

Sampling at this location has not routinely been practised. Researchers that have employed this method have usually done so when no other sampling methods were available. However, the method was successfully used to collect ballast water and sediment samples on the BHP bulk carrier, *MV Iron Whyalla* (Rigby and Hallegraeff 1994; Rigby *et al.* 1993). With this method it is technically possible to sample all water entering and leaving the ballast tanks, however, to do so requires that sampling is undertaken over the entire ballasting period which, in the case of a bulk carrier, may be over a period of 1 to 5 days

#### *Considerations when in-line sampling:*

- In-line sampling has the potential to provide a truly representative sample of organisms entering or being discharged from ballast tanks.
- The main difficulty with this method is associated with the need to collect the sample over extended time frames (see section 4).
- Heterogeneity (both spatial and temporal) in the distribution of planktonic organisms in port water associated with different phase of the tidal cycle is likely to mean that spot samples taken by this method will not be representative of all water taken up by the vessel during ballasting.
- This method may not provide representative samples of planktonic organisms in ballast water because of lamina flow characteristics in the pipework accessed via the pressure gauge

### **3 REVIEW OF INTERNATIONAL BALLAST WATER SAMPLING PROTOCOLS**

#### **3.1 Development of the sampling protocols database**

In early 1997, CRIMP set up a "Ballast Water Contact List" to encourage discussion and transfer of information between the various international bodies involved in ballast water research. The list was developed with input from Dr Greg Ruiz (Smithsonian Environmental Research Centre), Dr Jim Carlton (Mystic Seaport), by requesting information via the CRIMP "Introduced Marine Pests" mailing list and the IUCN Invasive Species Specialist Group "Aliens" mailing list and through attendance at international conferences such as the recent ICES/IMO/IOC symposium. Following initial correspondence via the "Ballast Water Contact List" a questionnaire (see Appendix B) was sent to list members to obtain detailed information on ballast water sampling methods and analysis currently in use. An MS ACCESS database was developed to collate information on the sampling protocols of the various countries, institutions and scientists involved in ballast water sampling programs. The database is available upon request and is intended to be continued and developed with international input and eventually be available via the CRIMP web site.

The survey provided information on the various international sampling programs including, current sampling protocols and any associated operational problems, the effectiveness of these protocols in providing data to meet the program objectives, and an indication of the taxa most frequently sampled. This information was used to identify and select a subset of sampling methods for subsequent testing and evaluation during the project (see section 4).

#### **3.2 Summary of international sampling protocols**

The following tables summarise information obtained from completed questionnaires and through correspondence with members of the "Ballast Water Contact List". The tables were developed directly from the database and can be updated if or when further information becomes available.

The tables are designed to provide a quick reference guide to accessing information on current international sampling protocols. They are not comprehensive but present information provided to us through correspondence. General information on the various ballast water programs is provided in Appendix A. The contents of the tables are as follows:

Table 3.1: Lists all the individuals and institutions that are, or have recently been, involved in ballast water research.

Table 3.2: Lists the sampling locations and methods that have been used to sample various types of ships.

Table 3.3: Lists the details of each sampling method by institution.

Tables 3.4–6: List various methods of sample processing and analysis used by the various institutions.

**Table 3.1 Ballast Water Contact List**

<b>Name</b>	<b>Address</b>	<b>Telephone / Fax /Email</b>
<b>*Ben</b>	<b>Baghurst</b> Flinders University  G.P.O. Box 2100 Adelaide Australia	   South Australia 5001  bibcb@gamgee.cc.flinders.edu.au
<b>Christopher J.S. Bolch</b>	University of Tasmania Department of Plant Science PO Box 252-55 Hobart Australia	Tasmania 7001  61 (0)3 6226 1572 61 (0)3 6226 2698 Chris.Bolch@plant.utas.edu.au
<b>Helge</b>	<b>Botnen</b> University of Bergen (UNIFOB) Applied Environmental Research, UIFOB foundation Bergen High Technology Centre Bergen Norway	N-5020  + 47 55 58 44 65 + 47 55 58 45 25 helge.botnen@ifm.uib.no
<b>Malcolm I.</b>	<b>Campbell</b> Fisheries and Oceans  PO Box 5030 Moncton Canada	New Brunswick E1C 9B6  506 851 6247 506 851 2079 campbellM@gfc.dfo.ca
<b>Allegra</b>	<b>Cangelosi</b> Northeast-Midwest Institute  218 D Street SE. Washington USA	DC 20003  202 544 5200 202 544 0043 acangelo@nemw.org
<b>Jim</b>	<b>Carlton</b> Williams College, Mystic Seaport  PO Box 6000, 75 Greenmanville Ave Mystic USA	Connecticut 06355  860 572 0711 x519 860 572 5329 James.T.Carlton@williams.edu
<b>K.H</b>	<b>Chu</b> Chinese University of Hong Kong Department of Biology  Hong Kong	   b133707@mailserv.cuhk.hk
<b>Tim</b>	<b>Dodgshun</b> Cawthron Institute  98 Halifax St. E Nelson New Zealand	4811  64 3 548 2319 64 3 546 9464 timd@environment.cawthron.org.nz
<b>Clare</b>	<b>Eno</b> Joint Nature Conservation Committee  Monkstone House, City Road Peterborough UK England	Cambs PE1 1JY  44 17 33 866 833 44 17 33 555 948 eno_c@jncc.gov.uk

Name		Address	Telephone / Fax /Email
<b>Bella</b>	<b>Gallil</b>	National Oceanographic Institute  P.O.B. 8030 Tel Shikmoma Haifa Israel	04 851 5202 04 851 1911 gallil@math.tau.ac.il
<b>Michel</b>	<b>Gilbert</b>	Fisheries and Oceans Habitat Management and Environmental Science Division PO Box 1000, 850 Rue de la Mer Mont-Joli Quebec Canada G5H 3Z4	1 418 775 0604 1 418 775 0542 gilbertm@dfo-mpo.gc.ca
<b>Stephan</b>	<b>Gollasch</b>	University of Hamburg  Martin-Luther King- Platz 3 Hamburg Germany 20146	49 40 4123 4226 49 40 4123 3937 FB5A007@rrz-cip-1.rrz.uni-hamburg.de
<b>Julie</b>	<b>Hall</b>	National Institute for Water & Atmospheric Research  PO Box 11 115 Hamilton New Zealand	64 7 856 1709 64 7 856 0151 j.hall@niwa.cri.nz
<b>Gustaaf</b>	<b>Hallegraeff</b>	University of Tasmania Plant Science GPO Box 252-55 Hobart Tasmania Australia 7001	61 03 6886 8683 61 03 6226 2698 Hallegraeff@plant.utas.edu.au
<b>John</b>	<b>Hammer</b>	University of Wales School of Ocean Sciences  Bangor Anglesey UK, North Wales LL59 5EY	44 1248 382871 44 1248 382871 oss118@bangor.ac.uk
<b>Cameron</b>	<b>Hay</b>	Cawthron Institute  98 Halifax St. East Nelson New Zealand 4811	64 3 548 2319 64 3 546 9464 info@environment.Cawthron.org.nz
<b>Norbert</b>	<b>Huelsmann</b>	Free University Berlin Zoology Koengin-Luise-Strasse 1-3 Berlin Germany 14195	49 30 838 3921 49 30 838 3916 hulsmann@zwdat.fu-berlin.de
<b>*Andrea</b>	<b>Locke</b>	University of Toronto   Canada	   lockea@gfc.dfo.ca
<b>Josip</b>	<b>Lovric</b>	Polytechnic of Dubrovnik  20000 Dubrovnik Cira Carica 4 Croatia	+ 385 20 412811 (local 229) + 385 20 432563 kcetinic@galija.vdu.hr

Name	Address	Telephone / Fax /Email
<b>Elsbeth</b>	<b>MacDonald</b> Scottish Office of Agriculture, Environment & Fisheries Dept SOAEFD Marine Laboratories PO Box 101, Victoria Rd Aberdeen Scotland UK AB11 9DB	44 1224 876544 44 1224 295511 macdonaldem@marlab.ac.uk
<b>Tracy</b>	<b>McCollin</b> University of Wales School of Ocean Sciences Menai Bridge Anglesey North Wales Wales (and England) LL59 5E7	44 1248 382871 44 1248 382871 oss138@bangor.ac.uk
<b>Dan</b>	<b>Minchin</b> Fisheries Research Centre  Abbotstown, Dublin 15 Ireland	353 1 8210 111 353 1 8205 078 MINCHIN@frc.ie
<b>Kate</b>	<b>Murphy</b> University of Western Australia Marine Science 60 Bruce St Nedlands Western Australia Australia	61 (0)89 386 7872  murphyk@cw.uwa.edu.au
<b>Darren</b>	<b>Oemcke</b> James Cook University & CRC Reef Research Civil and Systems Engineering  Townsville Queensland Australia 4811	077 81 5081 077 75 1184 Darren.Oemcke@jcu.edu.au
<b>*Annette M.</b>	<b>Olson</b> University of Washington School of Marine Affairs Box 955685 Seattle Washington USA 98018 6715	1 206 685 2499 1 206 453 1417 olsonam@u.washington.edu
<b>Robert</b>	<b>Randall</b> Fisheries and Oceans  PO Box 5050 Burlington Ontario Canada L7S9 4A6	905 336 4496 905 336 6437 randallr@dfo-mpo.gc.ca
<b>Don</b>	<b>Reid</b> Consultant- for Ballast Water Filtration Project  Ottawa Ontario Canada	613 829 9642
<b>Geoff</b>	<b>Rigby</b> BHP Research  Newcastle Labs, off Vale St. Shortland NSW Australia 2307	61 (0)49 510 512 61 (0)49 501 336 rigby.geoffrey.gr@bhp.com.au
<b>Greg</b>	<b>Ruiz</b> Smithsonian Environmental Research Centre  PO Box 28 Edgewater Maryland USA 21037	1 301 261 4190 1 301 261 7954 ruiz@serc.si.edu

Name	Address	Telephone / Fax /Email
*Gary W. Sprules	University of Totonto  Canada	49510512 49501336 gsprules@cyclops.erin.utoronto.ca
Deborah Tanis	Battelle  397 Washington Street Duxbury MA USA 02332	tanisd@BATTELLE.ORG
Inger Wallentinus	Goteborg University Avd.Marin Botanik Carl Skottsbergs gata 22b Goteborg Sweden S41319	46 31 773 2702 46 31 773 2727 inger.wallentinus@marbot.gu.se

\* indicates that the person is not currently involed in ballast water research

**Table 3.2 Sampling protocols used on various ships**

Type of ship	Location sampled	Method employed	Institution
<b>Bitumen Carrier</b>	outflow	bucket	Scottish Office of Agriculture, Environment & Fisheries Dept.
<b>Bulk Carriers</b>	tank bottom	grab	University of Tasmania
	sounding pipe	Wattera interia pump	Cawthron Institute
		stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		portable pump	Northeast-Midwest Institute
		peristaltic pump	University of Tasmania
		peristaltic pump	James Cook University & CRC Reef Research
		hand pump	University of Wales
			University of Hamburg
		electric centrifugal	Scottish Office of Agriculture, Environment & Fisheries Dept.
		centrifugal- footvalve	Cawthron Institute
	outflow	net	Smithsonian Environmental Research Centre
		bucket	University of Wales
			James Cook University & CRC Reef Research
	manhole**	horizontal net haul	Northeast-Midwest Institute
	manhole	vertical net haul	University of Toronto
			University of Tasmania
			University of Hamburg
			Smithsonian Environmental Research Centre
			Scottish Office of Agriculture, Environment & Fisheries Dept.
			Fisheries and Oceans
		submersible net-pump	Flinders University
		stiff hose method	University of Wales
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		niskin bottle	Smithsonian Environmental Research Centre
		grab	University of Hamburg
			Smithsonian Environmental Research Centre
		during tank inspection	University of Hamburg
		diaphragm pump	University of Western Australia
		bucket	University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept.
			Northeast-Midwest Institute

Type of ship	Location sampled	Method employed	Institution
	manhole	bucket	James Cook University & CRC Reef Research
	in-line	pressure gauge	James Cook University & CRC Reef Research
	in line	pressure meter	University of Hamburg
		pressure gauge	University of Wales
	filter unit**	in-line	Northeast-Midwest Institute
	empty tank	grab	Northeast-Midwest Institute
	deck tap	ship's fire hose pump	University of Tasmania
		fire hose pump	University of Toronto
	ballast hold	net hauls	Cawthron Institute
		grab	James Cook University & CRC Reef Research
		centrifugal- foot valve	Cawthron Institute
		bucket	Cawthron Institute
<b>Car Carriers</b>	sounding pipe	hand pump	University of Hamburg
	outflow	bucket	University of Hamburg
	manhole	vertical net haul	University of Hamburg
		grab	University of Hamburg
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
<b>Chemical Tankers</b>	tank bottom	grab	University of Tasmania
	sounding pipe	stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		hand pump	University of Wales
		electric centrifugal	Scottish Office of Agriculture, Environment & Fisheries Dept.
	outflow	bucket	University of Wales
	manhole	vertical net haul	University of Wales
			University of Tasmania
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		stiff hose method	University of Wales
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		hand pump	University of Wales
		bucket	Scottish Office of Agriculture, Environment & Fisheries Dept.
	in line	pressure gauge	University of Wales
<b>Container Ship</b>	deck tap	deck tap	Smithsonian Environmental Research Centre
<b>Container Ships</b>	tank bottom	grab	University of Tasmania
	sounding pipe	Wattera interia pump	Cawthron Institute

Type of ship	Location sampled	Method employed	Institution
	sounding pipe	peristaltic pump	James Cook University & CRC Reef Research
		hand pump	University of Wales
			University of Hamburg
			Smithsonian Environmental Research Centre
	outflow	centrifugal- footvalve	Cawthron Institute
		net	Smithsonian Environmental Research Centre
		bucket	University of Wales
			University of Hamburg
	manhole	vertical net haul	University of Tasmania
			University of Hamburg
			Chinese University of Hong Kong
		submersible net-pump	Flinders University
		stiff hose method	University of Wales
		grab	University of Hamburg
			Smithsonian Environmental Research Centre
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
	in line	pressure meter	University of Hamburg
		pressure gauge	University of Wales
<b>Ferries</b>	outflow	bucket	University of Wales
	manhole	stiff hose method	University of Wales
	in line	pressure gauge	University of Wales
<b>General Cargo</b>	tank bottom	grab	University of Tasmania
	sounding pipe	stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		hand pump	University of Wales
			University of Hamburg
		foot valve pump	National Institute for Water & Atmospheric Research
		electric centrifugal	Scottish Office of Agriculture, Environment & Fisheries Dept.
		centrifugal- footvalve	Cawthron Institute
	outflow	bucket	University of Wales
			University of Hamburg
	manhole	vertical net haul	University of Wales
			University of Tasmania
			University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept.
			National Institute for Water & Atmospheric Research

Type of ship	Location sampled	Method employed	Institution
	manhole	vertical net haul	Fisheries and Oceans
		submersible net-pump	Flinders University
		stiff hose method	University of Wales
		hand pump	Scottish Office of Agriculture, Environment & Fisheries Dept. University of Wales
		grab	University of Hamburg
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept. National Institute for Water & Atmospheric Research
	in line	pressure meter	University of Hamburg
		pressure gauge	University of Wales
	Air vents	foot valve pump	National Institute for Water & Atmospheric Research
<b>LNG/LPG Carriers</b>			
	tank bottom	grab	University of Tasmania
	sounding pipe	stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		hand pump	University of Wales University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept.
	outflow	bucket	University of Wales University of Hamburg
	manhole	vertical net haul	University of Hamburg
		stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept. University of Wales
		hand pump	Scottish Office of Agriculture, Environment & Fisheries Dept.
		grab	University of Hamburg
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept.
	in line	pressure meter	University of Hamburg
		pressure gauge	Scottish Office of Agriculture, Environment & Fisheries Dept.
		pressure gauge	University of Wales
<b>Ocean Liner</b>			
	manhole	stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
<b>Oil Tankers</b>			

Type of ship	Location sampled	Method employed	Institution
	tank bottom	grab	University of Tasmania
	sounding pipe	stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		hand pump	University of Wales
			University of Hamburg
			Scottish Office of Agriculture, Environment & Fisheries Dept.
	outflow	net	Smithsonian Environmental Research Centre
		bucket	University of Wales
			University of Hamburg
	manhole	vertical net haul	University of Wales
			University of Tasmania
			University of Hamburg
			University of Bergen (UNIFOB)
			Smithsonian Environmental Research Centre
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		stiff hose method	University of Wales
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		Ruthner water sampler	University of Bergen (UNIFOB)
		niskin bottle	Smithsonian Environmental Research Centre
		hand pump	University of Wales
			Scottish Office of Agriculture, Environment & Fisheries Dept.
		grab	University of Hamburg
			Smithsonian Environmental Research Centre
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
			University of Bergen (UNIFOB)
			Scottish Office of Agriculture, Environment & Fisheries Dept.
	in line	pressure meter	University of Hamburg
		pressure gauge	University of Wales
<b>Ore Carriers</b>			
	tank bottom	grab	University of Tasmania
	manhole	vertical net haul	University of Tasmania
<b>Ore/Bulk/Oil Carriers</b>			
	tank bottom	grab	University of Tasmania
	sounding pipe	peristaltic pump	James Cook University & CRC Reef Research
		electric centrifugal	Scottish Office of Agriculture, Environment & Fisheries Dept.
	manhole	vertical net haul	University of Tasmania

Type of ship	Location sampled	Method employed	Institution
	manhole	vertical net haul	Scottish Office of Agriculture, Environment & Fisheries Dept.
		stiff hose method	Scottish Office of Agriculture, Environment & Fisheries Dept.
		bucket	Scottish Office of Agriculture, Environment & Fisheries Dept.
<b>Passenger</b>			
	sounding pipe	centrifugal- footvalve	Cawthron Institute
<b>Research Vessels</b>			
	sounding pipe	hand pump	University of Hamburg
	outflow	bucket	University of Hamburg
	manhole	vertical net haul	University of Hamburg
		grab	University of Hamburg
		during tank inspection	University of Hamburg
		bucket	University of Hamburg
	in line	pressure meter	University of Hamburg
<b>Tankers</b>			
	manhole	vertical net haul	Fisheries and Oceans
<b>Vessels without SPs</b>			
	in line	ballast pump	Cawthron Institute
<b>Woodchip Carriers</b>			
	tank bottom	grab	University of Tasmania
	sounding pipe	Wattera interia pump	Cawthron Institute
		centrifugal- footvalve	Cawthron Institute
	manhole	vertical net haul	University of Tasmania
	ballast hold	centrifugal- foot valve	Cawthron Institute

\* BWF: specific for the Ballast Water Filtration Demonstration Project, see Table 3.3

Table 3.3 International Ballast Water Sampling Protocols

institute	location	method	description	comments	sample details (type, method, mesh $\mu$ m)		
Cawthron Institute	sounding pipe	centrifugal-footvalve	A petrol or electric pump (rate = 100-200 LPM) depending on the circumstances. A flexible hose (20 mm OD) terminating with a foot valve is pushed to bottom of the sounding pipe. The hose is then jugged manually to fill the hose and prime the pump. The first part of the sample usually contains sediment. ** filtering details for Cawthron Institute: the discharge from both pumps passes through a flow meter and a filter bank consisting of 250, 100 and 40 $\mu$ m mesh inside a 100-120 mm plastic pipe	Preferred because most ships have sounding pipes. Used in situations where the head < 8 m.	sediment	filtered-preserved	100
					temperature	on site	-
					salinity	on site-probe	-
					phytoplankton	whole water	-
					phytoplankton	filtered-live	20
					phytoplankton	filtered-preserved	20
					zooplankton	filtered-preserved	100
					zooplankton	filtered-preserved	250
					sediment	filtered-preserved	100
					temperature	on site	-
					salinity	on site-probe	-
					phytoplankton	whole water	-
					phytoplankton	filtered-live	20
					phytoplankton	filtered-preserved	20
					zooplankton	filtered-preserved	100
					zooplankton	filtered-preserved	250
	manhole	Van Dorn sampler	An electric pump (rate = ~5 LPM). If ship's power supply is unavailable a wharf side generator is used. The same procedure as the petrol pump except for the use of flexible alkathene irrigation hose (20 or 12 mm OD) with fitted Wattera foot valves.	This pump is used sample tanks with no deck access or when the head is > 6-8m (can handle up to 25 m head).	sediment	filtered-preserved	100
					temperature	on site	-
					salinity	on site-probe	-
					phytoplankton	whole water	-
					phytoplankton	filtered-live	20
					phytoplankton	filtered-preserved	20
					zooplankton	filtered-preserved	100
					zooplankton	filtered-preserved	250
					phytoplankton	filtered-preserved	20
					phytoplankton	whole water	-
	in line	ballast pump	Sample collected from the bleeder valve (pressure gauge) of the main ballast pump during deballasting	To compare the efficiency of sounding pipe method and to assess the vertical stratification.	phytoplankton	filtered-preserved	20
					phytoplankton	whole water	-
					phytoplankton	filtered-preserved	20
					temperature	on site	-
					salinity	on site-probe	-
					plankton	filtered-preserved	-

institution	location	method	description	comments	sample details (type, method mesh um)
	ballast hold	bucket	Approximately 100L of water is collected from the hold with a bucket attached to a rope.	When tank is nearly empty and there is no other alternative.	salinity temperature zooplankton on site-probe on site filtered-preserved
		centrifugal-foot valve	Same procedure as sounding pipe, sample collected at 2 m depth. * filtering of sample	Same pump as used for sounding pipes. Net samples also collected.	salinity temperature phytoplankton phytoplankton zooplankton zooplankton on site-probe on site whole water filtered-live filtered-preserved filtered-preserved filtered-preserved
		net hauls	100 um mesh, x diameter plankton net. 1. When the hold is full (0-15m). 2. When the hold is empty (0.5 m) the net is towed around the perimeter and twice up through the middle along diagonals.	If possible two net samples are collected in addition to pumped samples.	zooplankton zooplankton filtered-preserved 100
<b>Chinese University of Hong Kong</b>					
	manhole	vertical net haul			zoo/phyto zoo/phyto filtered-live filtered-preserved
<b>Fisheries and Oceans</b>					
	manhole	vertical net haul	An 80um mesh, 30cm diameter net is lowered into the manhole then drawn back up at a steady rate	Ease of sampling, convenient, no power required.	phytoplankton zooplankton 80 (100, 80 (100,
	empty tank	scoop		Provided access is permitted, a sediment sample is scooped from the bottom of the empty tank/hold.	sediment whole water

institution	location	method	description	comments	sample details (type, method, mesh, um)
Flinders University	manhole	submersible net-pump	The unit (powered by a 12 volt battery, rate = 32.71 LPM) pumps and filters simultaneously. Water is pumped through a 150mL sample vial with 53 um mesh ports. The pump is lowered to desired depth in a horizontal orientation and turned on. Two 10 minute samples are collected (100mm below surface and 100mm from bottom).	Filters at a constant high rate irrespective of sampling depth. Very inexpensive equipment	filtered-preserved 53
					zooplankton
<b>James Cook University &amp; CRC Reef Research</b>					
	sounding pipe	peristaltic pump	Sounding pipes are pumped for 10 minutes prior to sample collection to reduce contamination from pipe corrosion. A teflon lined tubing was used to prevent contamination of iron samples. * in future would push tube to bottom of the sounding pipes to negate the need to pump large volumes of water to reduce the sounding pipe corrosion effect as it was time consuming.	Numerous tanks can be sampled randomly without interfering with the ship's operation. Will not operate with a head of >8m. Submerged pump would be ideal but not practical considering the narrow diameter of sounding pipes.	water chemistry
					whole water
outflow	bucket	Place appropriately cleaned glassware under the flow when it is being discharged onto the wharf.		Easy method if ballast is being pumped onto wharf.	phaeophytin
					whole water
manhole	bucket	Sample surface water with bucket. In future cases will use peristaltic pump as with the sounding pipes.		Good for surface samples, but does not sample tank bottom water and it takes up crew time to open the manholes.	chlorophyll a
					whole water
					water chemistry
					whole water

institution	location	method	description	comments	sample details (type, method mesh um)
	in-line	pressure gauge	Sample was collected by disconnecting the pressure gauge from the ballast pump. It requires the cooperation of the crew and was therefore considered inconvenient.	This method is used as a last resort to obtain a sample.	water chemistry phaeophytin chlorophyll a whole water whole water whole water
	ballast hold	grab	Grab sampler lowered from ship's deck into cargo hold ballast tank.	Very easy to obtain sediment sample.	sediment whole water
<b>National Institute for Water &amp; Atmospheric Research</b>					
	sounding pipe	foot valve pump			
	manhole	bucket	A bucket is attached to a rope and lowered into the tank via the manhole.	Sample can always be collected from this location.	plankton microbial phytoplankton zooplankton whole water filtered-preserved filtered-preserved filtered-preserved 2 20 100
		vertical net haul	50 cm diameter, 100 um vertical haul net. The net is lowered into the tank as deep as possible. It is then pulled up and lowered three times to maximize the volume filtered.	To collect whole water samples.	microbial phytoplankton composite filtered-preserved filtered-preserved whole water 2 20 -
	Air vents	foot valve pump	The hose from the pump 'flapped' against the relatively wide air vents causing substantial wear on the foot valve. This was prevented by putting buffers on the hose and housing the valve in metal casing.	Direct access to tank. When the cargo hold is full samples cannot be collected from the manholes.	zooplankton filtered-preserved 100
		foot valve pump		Direct access to tank as the air vents are open ended.	microbial phytoplankton zooplankton filtered-preserved filtered-preserved filtered-preserved 2 20 100

institution	location	method	description	comments	sample details (type, method mesh um)	
Northeast-Midwest Institute	sounding pipe	portable pump	Have not yet tested this method but this location will be sampled to obtain whole water sample for microbial analysis only.	Not yet tested. Is preferred for ready access to "empty" tank residuals. Allows relatively quick collection of samples with minimal disruption to crew.	microbial whole water	
	manhole (filtration project)	horizontal net haul	A specially fitted hinged manholes were installed to provide easy access to the tanks. Net tows were taken along a 10 m diagonal and pulled along by a trolley system. This method was used to monitor the treatment and control tank over the duration of the ballast water filtration experiment.	This is the preferred method as it provides direct access to the tank and replicate samples can be collected quickly.	phytoplankton suction/filtration-liv 20,30 zoo/phytoplankt filtered-live 20 zoo/phytoplankt filtered-preserved 20 zooplankton filtered-live 80 zooplankton filtered-preserved 80	
	manhole	bucket	A 3-10L whole water sample is collected via the manhole and filtered through 20 and 80um nytex filters for later chlorophyll extraction	This method is used to collect phytoplankton for chlorophyll extraction. Have both a treatment and control tank to assess the effectiveness of filtration.	phytoplankton whole water	20
	filter unit (filtration project)	in-line	Samples collected from the "filtration unit" designed to remove plankton from ballast and a primary treatment option. Samples (3 from inlet and outlet respectively) are taken periodically throughout the filtration operation. Samples are analysed using an optical particle sizing system. Used in conjunction with other methods to assess "biological efficiency" as it may not register translucent zooplanktoners, small sample volumes preclude adequate counts of larger (rare) particles/organisms.	Used to measure the "mechanical efficiency" of the filtering system and pump when the unit is in operation (see project summary)	phytoplankton partical size (PPS) whole water filtered-preserved	20

institution	location	method	description	comments	sample details (type, method, mesh um)
	empty tank	grab	Access via the manhole. 30-50 ml sample of sediment scooped off the tank bottom. Samples assessed for the total microbial content using cultures and molecular detection methodology.	To compare with sounding pipes samples and assess the extent to which they are effective for the sampling of "empty" tanks.	sediment whole water
<b>Scottish Office of Agriculture, Environment &amp; Fisheries Dept.</b>					
	sounding pipe	electric centrifugal	Battery operated- same as hand pump.	Used on ships other than oil or gas tanker (safety reasons) where the manholes are not available. Much preferable to hand pump.	sediment whole water
		hand pump	Same as manhole.	When manhole access is not available and safety requirements prevent the use of electric pumps. Vertically integrated sample also collected.	sediment whole water
		stiff hose method		When manhole access is not available.	nutrients salinity phytoplankton whole water whole water whole water
	outflow	bucket	Held a bucket on the end of a rope underneath the outflow and collected a known volume of water.	Did this once when no other access was available.	zooplankton filtered-preserved
	manhole	bucket	Bucket on a rope lowered into the tank.	This method is used when the tanks are too low to obtain a vertically integrated sample with the stiff hose method.	salinity nutrients phytoplankton whole water whole water whole water
		hand pump	A weighted hose is pushed to the bottom of the tank and used to pump sediment and water. Flow rate unknown.	Hand pump is used if safety requirements prevent the use of electricity. Collected in addition to vertically integrated sample.	sediment whole water

institution	location	method	description	comments	sample details (type, method mesh um)
		stiff hose method	A weighted hose is put into the ballast tank. When the hose reaches the bottom of the tank a valve at the top is closed and the hose is recovered. The hose is then emptied into a bucket. Mixed and subsamples are then collected.	Direct access to tank. To collect a vertically integrated sample	nutrients salinity phytoplankton whole water whole water whole water
		vertical net haul	50 cm diameter, 68 um mesh vertical haul net.	Direct access to tank.	zooplankton filtered-preserved 68
	in line	pressure gauge		Used when no other access available.	zooplankton whole water
<b>Smithsonian Environmental Research Centre</b>					
	sounding pipe	hand pump	Only useful for tanks where the head is <6m. Interested in testing an inertia pump to overcome this problem.	Only used as last resort when there is no access into the ballast tank for nets.	microbiological zooplankton whole water filtered-preserved 80
	outflow	net	Collect water in an 80um mesh net as it falls from the ship. Variable success and very unpredictable as to what ships will provide this opportunity. We have discontinued this method due to concerns about damage to organisms and net.	Very difficult and high pressure can damage the nets. Sampling the ballast plume is now being considering	zooplankton filtered-preserved 80
	manhole	grab		In order to collect a sediment sample	sediment whole water
		niskin bottle	A 2L niskin bottle is used to sample the surface water and the deepest water, usually 10m and is therefore only applicable on some ships, those with deep ballast tanks or cargo holds.	To obtain depth distributional data.	whole water

institution	location	method	description	comments	sample details (type, method, mesh, um)
University of Bergen (UNIFOB)		vertical net haul	80 um, 30 cm diameter net used to collect 2m tows and full-length tows from each manhole.	This is the preferred method as it is accessible, does not impose on the ships' crew, requires no power, not prone to mechanical failure.	zooplankton filtered-preserved 80
	deck tap	deck tap		Have only tried this method a few times, animals were badly damaged and therefore was not considered appropriate.	zooplankton filtered-preserved 80
	manhole	bucket	Only used on twice, samples were collected using bucket under the outflow. Zooplankton samples are course sieved after collection with 180 um mesh.	If ballast water has been dumped prior to sampling and there is little ballast left in the tanks	nutrients oxygen salinity phytoplankton zooplankton whole water whole water whole water whole water filtered-preserved 20-30
University of Hamburg		Ruthner water sampler			phytoplankton nutrients salinity oxygen whole water whole water whole water whole water
		vertical net haul	20 cm diameter, 20-30 um mesh vertical haul net. The depth (ie. volume) sampled is dependant on obstacles in the tank.	Very easy position to collect zooplankton samples.	zooplankton filtered-preserved 20-30
	sounding pipe	hand pump	Outflow is filtered through 10 and 55 um mesh nets. Flow rate is not controlled or measured.	Easy and fast for crew and staff, no power required.	zooplankton temperature salinity phytoplankton filtered-preserved 10 55

institution	location	method	description	comments	sample details (type, method, mesh um)
	outflow	bucket	Outflowing ballast is collected from outside the vessel with a bucket and then filtered through 10 and 55 um nets.	Easy to collect ballast sample and no pump required.	zooplankton phytoplankton filtered-preserved filtered-preserved 55 10
	manhole	bucket	Water at bottom of tank is collected with a bucket, then filtered through 10 and 55 um mesh nets.	Used when the tank is close to empty.	zooplankton temperature salinity phytoplankton filtered-preserved 55 - - 10
			during tank inspection	To obtain sediment samples.	sediment whole sediment -
		grab	Access to tank bottom is obtained via manholes.	To collect sediment samples when the tank is not empty	sediment whole sediment -
		vertical net haul	10 and x cm diameter, 10 and 55 um mesh nets respectively.	Direct access tank. Larger organisms caught and greater volumes sampled.	zooplankton salinity temperature phytoplankton filtered-preserved 55 - - 10
	in line	pressure meter	Collected from the pressure meter (gauge) of the ship's main ballast pump when it is operating. Requires ships crew to be available.	Last resort to obtain a sample.	zooplankton phytoplankton filtered-preserved filtered-preserved 55 10
<b>University of Tasmania</b>					
	tank bottom	grab	Access to tank bottom via manhole.	To obtain sediment samples.	sediment whole sample -

institution	location	method	description	comments	sample details (type, method mesh um)
	sounding pipe	peristaltic pump	Sample filtered through 20 um mesh plankton net	Not ideal to sample from the sounding pipes as the samples contained alot of rust.	temperature - salinity - phytoplankton filtered-preserved 20
	manhole	vertical net haul	20 cm diameter, 20um mesh net.		temperature - salinity - phytoplankton filtered-preserved 20
	deck tap	ship's fire hose pump	Sample filtered through 20 um mesh net.	Easy to obtain a sample from any ballast tank however the organisms are not in the best condition as the it purups at high pressure.	salinity - temperature - phytoplankton filtered-preserved 20
<b>University of Toronto</b>					
	manhole	vertical net haul	2 vertical haul nets: 41 micron, 13 cm diameter 110 micron, 30 cm diameter		temperature - salinity - zooplankton filtered-live 110 zooplankton filtered-live 41
	deck tap	fire hose pump	Use ship's fire hose pump to pump ballast out of any of the tanks onto the deck.	Easy and convenient, however the organisms are not in as good condition as net samples.	salinity - temperature - zooplankton filtered-live 110 zooplankton filtered-live 41

institution	location	method	description	comments	sample details (type, method, mesh um)	
University of Wales	sounding pipe	hand pump		When manhole access not available. Limited to head < ~8 m. Hand pump hose does not always fit down the sounding pipes.	zooplankton	filtered-preserved
					zooplankton	filtered-live
					sediment	whole water
					salinity	whole water
					heavy metals	whole water
	outflow	bucket			toxicity	whole water
					phytoplankton	whole water
	manhole	hand pump		When tanks too empty to operate hand pump.	sediment	whole water
					salinity	whole water
					heavy metals	whole water
toxicity					whole water	
phytoplankton					whole water	
hand pump			As per SOAEFD. Flow rate unknown and not measured.	Direct access to tank. Hand pump as opposed to electric or petrol pump used for safety reasons.	zooplankton	filtered-live
					zooplankton	filtered-preserved
					sediment	whole water
					salinity	whole water
					heavy metals	whole water
stiff hose method		As per SOAEFD	To collect an integrated sample (usually use a hand pump).	toxicity	whole water	
				phytoplankton	whole water	
				phytoplankton	whole water	
				phytoplankton	whole water	
				vertical net haul	Two tows- one fixed one live.	zooplankton
			Direct access to ballast tank.	zooplankton	filtered-live	

institution	location	method	description	comments	sample details (type, method)	mesh (um)
	in line	pressure gauge				
			Sample collected from tap of main ballast pump in the engine room. Difficult to sample large volumes.	When sounding pipe and manhole access unavailable.	zooplankton	filtered-live
					zooplankton	filtered-preserved
					sediment	whole water
					salinity	whole water
					heavy metals	whole water
					toxicity	whole water
					phytoplankton	whole water
<b>University of Western Australia</b>						
	manhole	diaphragm pump				
			see CRIMP protocol		zooplankton	filtered-preserved
		vertical net haul		Used for comparison and convenience.		100
			as above		zooplankton	filtered-preserved
				as above		100

Table 3.4 Phytoplankton Processing Details

Institution	Fixative Used	Storage of samples	processing details	abundance of taxa	Assessing Viability	details
<b>Cawthron Institute</b>	Lugol's iodine	-	The inertia pump is better than centrifugal. Nets and Van Dorn samples are good for fragile species.	no info	live inspection	Allowed to settle in Utermohl counting chambers. Then identified and scored alive/dead (IOC standard).
<b>Fisheries and Oceans</b>	10 % buffered formalin	70 % Alcohol		0 - 46 125 225 / cells m <sup>3</sup>	do not assess viability	
<b>Northeast-Midwest Institute</b>	frozen		Live whole water samples are vacuum filtered through 20 and 80µm nytex sorted with an optical particle sizing system after each filtration, then processed for chlorophyll extraction. Counts have been low therefore a pump 10µm mesh filter may be tested.		chlorophyll	Chlorophyll extraction pertains to organisms alive or recently alive at time of sampling.
<b>Scottish Office of Agriculture, Environment &amp; Fisheries Dept.</b>	Lugol's iodine or 2% formalin		no info	yes	culturing	Viability of dinoflagellates are assessed by establishing cultures.
<b>Smithsonian Environmental Research Centre</b>	bovins, lugols, formalin		Some collected in the 80µm net used for zooplankton sampling. Whole water samples collected with the Niskin bottle is the primary method for phytoplankton sampling.	yes	culturing	

Institution	Fixative Used	Storage of samples	processing details	abundance of taxa	Assessing Viability	details
<b>University of Bergen (UNIFOB)</b>	5% Pseudo-Lugol	chilled in dark	Particles are allowed to settle, water siphoned out for microscopic examination	no info	live inspection	Cells allowed to settle and examined with an inverted microscope (not part of the regular processing procedure, and has been done 3 times only).
<b>University of Hamburg</b>	4% SW/formalin	-	no info	110 specimens/L	culturing	no info
<b>University of Tasmania</b>	2% glutaraldehyde	4 degrees Celsius	-	variable	live inspection	Live inspection with a compound microscope onboard the ship
<b>University of Wales</b>	5% Lugols	-	-	-	live inspection	Live inspection under a microscope.

Table 3.5 Zooplankton Processing Details

institution	fixative used	storage	abundance of taxa	assessing viability	details
<b>Cawthron Institute</b>					
	5 % buffered formalin with rose bengal	isopropyl alcohol		culturing	sometimes try to culture obviously live larvae.
<b>Fisheries and Oceans</b>					
	10 % formal	70 % Alcohol	0-107447 / m <sup>3</sup>	not assessed	
<b>Flinders University</b>					
	10% formalin			no info	no info
<b>National Institute for Water &amp; Atmospheric Research</b>					
	10% formalin	70 % alcohol		do not	Samples are rinsed in water then examined under a dissecting microscope and identified to lowest possible taxa.
<b>Northeast-Midwest Institute</b>					
	10% formalin		1-4/L	live inspection	A live analysis is undertaken to ascertain any major trends in viability. Qualification in terms of zooplankton health (given mechanical impacts of filter screen) will also be undertaken
<b>Smithsonian Environmental Research Centre</b>					
	5-10 % formalin	70% alcohol	yes-no details	live inspection	Collect samples with vertical net haul. Also sample benthos and residual ballast after discharge. This is interesting as many species are very good at net avoidance.
<b>University of Bergen (UNIFOB)</b>					
	4% Formaldehyde			not assessed	

institution	fixative used	storage	abundance details of taxa	assessing viability	details
<b>University of Hamburg</b>	formaldehyde	70 % ethanol	1/L	live inspection	Try to cultivate live plankton for identification purposes.
<b>University of Toronto</b>	4% sucrose-formalin, buffered 10% Mg	4% sucrose-formalin, buffered 10% Mg	-	live inspection	Samples were examined within 2 hours of collection in order to determine if they were alive. The samples were then stained with a vital stain 'bacto-neutral red' and preserved
<b>University of Wales</b>	4 % neutralized formalin	70% alcohol and 2ml glycerol	-	live inspection	One sample (20L) placed in an insulated container and transported to laboratory for microscopic examination. Some larvae are cultured for identification.
<b>University of Western Australia</b>	4 % formalin, no buffer	-	< 5/m <sup>3</sup>	vital stain	stained live and dead material

Table 3.6 Sediment Processing Details

institution	method of analysis	method	details
Cawthron Institute			
University of Hamburg	microscope examination	as for phytoplankton	as for phytoplankton
University of Tasmania	no info	culturing	no info
Scottish Office of Agriculture, Environment & Fisheries Dept.	The sample is sonicated and sieved. It is then examined microscopically using fluorescent staining and density separated if required.	culturing	no info
Smithsonian Environmental Research Centre	Sample left in carboys to settle. The water is then siphoned off and sediment concentrated. Aliquot removed for heavy metal analyses, remained used for microscopic examination of cysts and culturing experiments	culturing	Cysts isolated and cultered- 50% hatching success
Northeast-Midwest Institute	Collect a sample with a grab via the manhole or at the bottom of an empty tank	culturing	
University of Wales	Total microbial content using culture and molecular detection methodology. Sample is allowed to settle for one week. The water is then siphoned off and the residue is centrifuged.	culturing not assessed	no assessed

## 4 DESCRIPTION AND EVALUATION OF SAMPLING PROTOCOLS

### 4.1 Background considerations

Developing an effective sampling program for ballast tanks involves balancing two conflicting requirements: the need for sampling methods that provide data to adequately answer the questions being posed, and the vessel's operational and structural restrictions that limit access to ballast tanks. The importance of clearly specifying the aims of the sampling program cannot be overemphasised as these must be clear before an effective sampling design or regime can be developed.

For most vessels ballast tank access will be the main factor influencing the selection of sampling methods. Obtaining representative samples of ballast water carried in cargo holds (as in the case of woodchip carriers) may present few technical difficulties but this form of ballasting is least common. The majority of vessels carry ballast in dedicated wing or bottom tanks that are complex in layout and often compartmentalised. Gaining access to these tanks to obtain samples at different stages of the ballasting or deballasting cycle may present significant operational difficulties.

All plankton sampling methods used in open water situations have some inherent biases and no one method will sample all planktonic taxa with equal efficiency. In open water situations methods can be developed that minimise these biases but there is usually little opportunity to do this in a ballast tank environment. Net diameters, are restricted by manhole dimensions and pump flow rates by the requirement to lift water over significant heads. For this reason sampling biases associated with standard sampling methods are likely to be greater in ballast tank situations. In addition, specific sampling methods will be limited in access to certain portion of the ballast tank. For example, net samples access is via manholes and therefore if the tanks are large (wing tanks) only the top few metres of the water column can be sampled.

As ballast pump intakes draw water from only a limited depth range, the planktonic species composition in ballast tanks will not be representative of the whole planktonic community of the port. In addition, sequential filling of tanks during prolonged ballasting may result in tanks within the same vessel having different planktonic assemblages because of diurnal and tidal changes in the planktonic communities in the port water adjacent to the vessel.

In assessing the efficiency of sampling techniques for ballast water sampling, comparisons both within and between vessels are likely to be confounded by a variety of factors including the type of vessel, the configuration of the ballast tanks, the age of the ballast water and the species specific survival characteristics of the entrained organisms. For example, temperature differences between the top and bottom of wing/bottom tanks and spatial differences in the distribution of planktonic organisms detected on the BHP vessel *Iron Whyalla* (Rigby and Hallegraeff 1994) suggest that structurally complex tanks may be neither physically or biologically homogeneous. Further support for this contention is provided by Murphy's (1997) sampling on the BHP vessel *MV Iron Sturt* which detected significant vertical stratification of the ballast tank community. This suggests that samples taken from any one point in a tank may not be representative of the tank as whole. It is not known if species distributions within tanks become more or less homogeneous with ballast water age.

Sampling programs that are aimed at the detection of target species in ballast water have two key requirements; (i) the availability of methods that can effectively sample those life history stages of the target species that are likely to be found in ballast tanks, and (ii) a capacity to unequivocally identify the relevant life history stages for each of the target species. These two requirements are interdependent because, in the absence of effective identification methods, it is not possible to directly assess the effectiveness of sampling methods for any target species. One approach to developing ballast water sampling methods for target species without taxonomic or other expertise to validate identifications is through the use of surrogate taxa. Surrogate species are usually a higher taxonomic grouping (e.g. families) of species which contain the target species. The assumptions that these taxa collectively are distributed and behave in ballast tanks in a similar manner to the target species, and that they will be sampled with similar efficiencies, in most cases, remain untested. However, using surrogates provides some indication of the suitability of available sampling methods for different groups of organisms by identifying those methods that might show significant sampling biases for different taxonomic groups.

## 4.2 Sampling methods

### 4.2.1 Sampling equipment

#### *Plankton nets*

A weighted free fall plankton net (70 cm mouth diameter, 100  $\mu\text{m}$  mesh) was used to sample port water adjacent to vessels. The net was fitted with Tekna maximum depth indicating gauge and the net had a fall rate of approximately 0.75  $\text{m s}^{-1}$ . A collar choke on the net prevented sampling during retrieval. Volume filtered was estimated from the formula  $\pi r^2 \text{depth}$ .

A 40 cm diameter, 53  $\mu\text{m}$  mesh vertical haul net with a heavy cod end was used for onboard sampling of ballast via manholes or in holds. Volume filtered was estimated by same formula as above and was generally 200–500 l, depending on the depth sampled. The same nets were used to filter pumped ballast water samples.

#### *Pumps*

A variety of pumps were used to sample different locations on vessels. The head of water to be pumped was the main influencing factor in pump selection. Sounding pipes and breather pipes were sampled using either a Mono cavity advancing pump or a Waterra foot valve (inertia) pump. These pumps were electrically driven and had pump rates of around 20  $\text{l min}^{-1}$  and 10  $\text{l min}^{-1}$  respectively, depending on the head. For manholes and fixed site sampling (see below), ASA diaphragm pumps (either electric or petrol driven) with a flow rate of around 100–130  $\text{l min}^{-1}$  were used. In some instances where it was unsafe to use electric or petrol powered pumps, a pneumatic pump capable of pumping at 40  $\text{l min}^{-1}$  was substituted and pump times were correspondingly increased. When using electrically powered pumps, an earth leakage circuit breaker was connected between the pumps and the vessel's power supply. In all cases the vessel's power was used; for international ships where the power supply was 110V, a step down transformer, international shipping plugs and adapters were required.

In most cases pump samples were standardised by pumping for 10 minutes; longer pump times were considered to be impractical in situations where multiple samples were collected

from each ship. Volume pumped was particularly important for sounding pipe samples as these samples compared more favourably with other methods when pump times were increased to 20–30 minutes.

## 4.2.2 Description of sampling methods

### *Sampling the port community*

The port community was sampled with drop nets which were taken from the wharf where the ships were berthed. The samples were collected opportunistically adjacent to vessels at various stages during the ballasting period. Net fall times were calculated to allow the net to sample from the surface to as close to the bottom as possible.

### *Sounding pipes*

Sounding pipe samples were collected by one of two methods depending on the distance between the deck and the level of the ballast (the head) in the tanks. If the head was greater than 6 m the Waterra inertia pump was used; for heads less than 6 m the Mono pump was used.

When sampling sounding pipes with the Mono pump a flexible 20 mm internal diameter (ID) hose was pushed down the sounding pipe to the desired depth. The outlet of the pump was directed through a 53  $\mu\text{m}$  mesh net sieve into a 20 l bucket which was allowed to overflow. Pumping rate were generally around 20 l  $\text{min}^{-1}$ , but was calibrated individually for each vessel to compensate for variations in head. The sample volumes were on average 200 l corresponding to 10 minutes of pumping. During sampling on the MV *Iron Whyalla* however, sample volumes were increased to 500 l to correspond with volumes sampled by the diaphragm pump (fixed site sampling) and net tows. Large sample volumes were not practical on other ships where sampling time was limited.

When sampling with the Waterra pump, a flexible 20 or 12 mm (depending on the width of the sounding pipe) OD hose with a Waterra foot valve (Waterra D25 and D32) fitted to the lower end was pushed down the sounding pipe to the desired depth. Care needed to be taken to ensure that the arm of the pump was located so the hose and the valve were vertical. The pump was secured with cables and ties to prevent it moving from the correct position. Sample filtering and calibration were the same as for the Mono pump. Pumping rate was dependant on the head and hose diameter and was calibrated individually for each tank.

### *Manholes*

The manholes were opened with shifters or a pneumatic drill with an appropriate fitting. In most cases this was done by the ship's crew. Samples were collected by vertical net hauls or pumps. When sampling using nets, the net was lowered through the manhole and allowed to sink slowly, cod end down, to the desired depth. The net was then retrieved by hand at a steady rate and sampled on the upward haul. Care had to be taken to avoid obstacles such as stairs and gratings that could easily catch the net. Sampled volume was usually around 200–500 l depending on the depth of the drop. Replicate hauls were taken as required.

Electrically powered diaphragm or pneumatic pumps were used to sample from open manholes. The inlet hose was placed in the open manhole to a depth of 0.25 m. No attempt was made to shade the manhole from light. The sample collection and processing was as

described for pump sampling via sounding pipes. Filtered volume per sample was around 400 l.

### ***Air vent and breather pipes***

Air vent sampling was carried out in the same manner as that for sounding pipes using the Waterra pump. For situations where the head was less than 6 m the Mono pump could be used in the same manner as for sounding pipes although this was not tested. When using the Waterra pump rubber buffers were slipped on the hose and placed at intervals along its length to keep the hose straight inside the air vent. This was essential as the foot valve only operates effectively when vertical. Care needed to be taken to ensure that the foot valve was not deployed beyond the lower end of the vent pipe as this allowed the hose curl up, making the valve sit on an angle and not operate effectively, and caused difficulties with retrieval.

Both air vents and breather pipes can be "pressed up" by pumping fresh ballast into the bottom of the tank and allowing the tanks to overflow. Zooplankton samples can be collected by holding the net in the stream of the overflow or by filling a bucket for the collection of whole water samples.

### ***In-line sampling***

For sampling from the ballast pump, a Mono-pump capable of pumping 20 l min<sup>-1</sup> was attached via a 20 mm ID reinforced plastic hose and an isolating valve to the discharge side of the ballast pump. The isolating valve was specially fitted for the sampling and was attached via an existing pipe fitting. The size of the opening into the pump housing was 20 mm. This opening was positioned in an area of high turbulence to increase the likelihood of obtaining representative samples from the flow in the pipe.

The outlet from the Mono pump was directed through a 53 µm net sieve into a 20 l drum that in turn drained via a drain at the top of the drum into the vessel's bilge. The flow through bucket kept the sample hydrated, cool and oxygenated throughout the lengthy sampling period. When sample collection was complete the net was rinsed and the sample concentrated into the codend and processed. When ballasting was prolonged, samples were preserved every 3–5 h over the duration of the sampling period.

Sea chest sampling was carried out in a similar manner. A 20 mm ID plastic hose was attached to a section of the sea chest's discharge pipe in the vicinity of the inlet to the ballast pump. Water was then drained from the sea chest using a Mono pump as described for ballast pump sampling.

Sampling from the pressure gauge was carried out by disconnecting the pipework leading to the pressure gauge and diverting the water flow to the bucket/sieve system described above. The flow rate from the pressure gauge was slow and variable (0.5–2 l min<sup>-1</sup>) and needed to be calibrated for each vessel where the method was used.

### ***Deck/fire taps***

This method was tested on the MV *Iron Whyalla*. The fire pump was used to pump ballast water from selected tanks for use in small scale heating trials (Rigby *et al.* 1997). Samples can be collected in this manner from any of the ballast water tanks.

### ***Fixed position sampling***

Fixed position sampling was used to examine the spatial distribution of plankton in ballast tanks. It is not a practical method of routine sampling or monitoring but rather a tool to assess the vertical and horizontal homogeneity of ballast tank zooplankton communities. Fixed position sampling was carried out on the BHP bulk carrier, MV *Iron Whyalla* (Rigby *et al.* 1997) and MV *Iron Sturt*.

On the MV *Iron Whyalla*, seven fixed positions were sampled in both of the port and starboard #2 double bottom and top wing tanks. Before the commencement of the voyage the tanks were fitted with fixed 50 mm Hardiflex® suction hose at depths of approximately 3, 10 and 22 m. At each of these depths there were 3, 2 and 2 fixed replicates, respectively. Each tank had 2 manholes with 4 camlock nipples permanently fitted to both the underside and deck side (even though there were only 7 sites). The hoses from each of the 7 replicates were fixed to the manhole covers with fire hose style camlock clamps. The deck side of each fitting was clearly marked with both depth and replicate number and when not in use was protected with a dust cover.

Two ASM diaphragm pumps were fitted with lockable wheels and rolled between the two manholes when required. Each pump had one inlet hose fitted with a camlock clamp which was clamped to the desired replicate camlock nipple. The pump outlet was allowed to flow into a 53 µm mesh sieve suspended in the flow-through drum described above.

Monitoring of the plankton community was undertaken at the beginning and the end of the voyage and whenever possible during the voyage. The pumping rates were calibrated at intervals throughout the voyage and varied depending on the depth of the fixed site. Initially, sample volumes were approximately 1 m<sup>3</sup> of water. Towards the end of the voyage, after high mortalities reduced the planktonic concentration, each sample volume was increased to 2–3 m<sup>3</sup>.

Similar trials were conducted on the MV *Iron Sturt*, although the depths (0.25, 3 and 7 m) were not spatially replicated. The manhole covers were not modified as above and had to be opened for sampling. The same ASM diaphragm pump as above was used. Initially this was powered by a petrol motor (Murphy 1997) but was changed to electric when the vessel began regularly transporting acid and the petrol motor was considered to pose a safety hazard. In situations where sampling was taking place as the vessel was loading acid, even electrical pumps were considered to pose a potential hazard and a pneumatic pump was trialed. The inlet for this pump was inserted into the fixed position pipes.

### ***Sample processing***

All nets and sieves had the same cod end design. After sample collection the net or sieve was washed down and the sample concentrated in the cod end with repetitive straining and rinsing. The tap on the cod end was then opened and the sample allowed to drain into a 300 ml sample jar and preserved with 10% (30 g of borax per litre) buffered formalin (Omori and Ikeda 1976). After fixing for at least one week in formaldehyde the samples were then transferred to 70% ethanol for storage and sorting.

### 4.3 Evaluation of sampling protocols

The sampling methods outlined above were evaluated for their practicality and application for routine monitoring (operational assessment) and their relative effectiveness in providing a representative sample of the ballast water zooplankton community (biological assessment). Effectiveness in this context was measured by comparing species (taxa) density and richness for each set of method comparisons. Various combinations of the different sampling methods were trialed on nine commercial vessels. The results of these assessments are summarised below and detailed results and analysis are provided in Appendix C.

#### 4.3.1 Operational assessment

##### *Sounding pipes*

If large volumes of water are required, as is the case for zooplankton samples, the most practical method of sampling via the sounding pipe is with a pump. The two pumps trialed were both electrically powered and required additional equipment such as cables, transformers, circuit breakers and adapters all of which were cumbersome, making this method extremely labour intensive. Other methods such as the "stiff hose method" (see Table 3.3) were not suitable for collecting zooplankton samples.

Of the two pumps, the Mono pump was the easiest to use, mainly because the Waterra pump, being an inertia pump, required restraining to prevent it moving out of alignment. The Mono pump was lighter and had a higher pumping rate (20 l min<sup>-1</sup>) than the Waterra pump (10 l min<sup>-1</sup>) making sampling times shorter for comparable volumes. The Waterra pump is, however, the only method that can be used to collect sounding pipes samples from double bottom tanks, or tanks with heads > 6 m. An alternative would be to collect an in-line sample.

##### *Manholes*

Sampling via the manholes with nets was the least labour and equipment intensive method trialed. However, access via manholes is not guaranteed; two of the nine ships surveyed had manholes that were cemented over. Problems with access should not be a deterrent for the use of this method for routine monitoring, as with the proper authority and prior notification, a ship can be required to open manholes (AQIS pers. comm.).

Sampling with pumps via the manholes was more labour intensive. The electrically operated pump required all the equipment needed for sounding pipe sampling and the same practical disadvantages apply. In addition, the diaphragm pump was extremely heavy, difficult to manoeuvre, and required the operation of the ship's crane to get it onboard. Because of its size and weight alone, the use of this pump for routine sampling cannot be recommended. In contrast, the pneumatic pump required less equipment, the pump and cables were light and could be carried onboard manually, and most vessels have compressed air available on deck (Geoff Rigby pers. comm.).

##### *Air vents and breather pipes*

Sampling via the air vents was difficult mainly because of the problem of keeping the Waterra foot valve vertical in the air vent. Sampling from the air vents would have been far easier with the Mono pump, however, this was not possible on the ship on which this method

was tested because the head was greater than 12 m. If access determines that a Waterra pump or equivalent must be used, it is recommended that samples be collected from sounding pipe and not air vents.

Sampling the overflow from air vents or breather pipes while tanks are being "pressed up" is a very easy and practical method of obtaining qualitative samples and excellent for the collection of whole water samples. However, pumping rates during "pressing up" are high and small diameter/small mesh nets may not adequately filter the large volume of water that overflows. The method is also not suitable in situations where vessels at berth are prohibited from allowing deck washings to run off from the deck into the port.

### ***In-line sampling***

Collection of in-line samples was the simplest and least equipment intensive of all the methods tested. However, this method can only be used when the vessel's ballast pump is in operation and requires a person to remain on hand during the whole period, which could take many hours depending on the number of tanks to be sampled. As it is unlikely that a single 10–20 minute sample would be representative of the plankton entrained in a ballast tank, the sampling should be continued over the entire de/ballasting period for the particular tank(s) of interest.

Sampling via the pressure gauge requires little assistance from the vessel's crew. However, it is important to discuss the procedure with the engineers so that they are aware that the filtered water is overflowing into the bilge so they can take appropriate measures of dealing with the build up of bilge water.

In-line sampling via the pipe work (ballast pump and sea chest) requires certain modifications to the vessel and prior cooperation and/or communication with the ship's engineers. However, as the necessary modifications are relatively simple it may be worth pursuing this type of sampling on a routine basis.

### ***Deck/fire taps***

This method provides an extremely rapid and simple method of obtaining ballast water on all vessels where the fire pump can be used to pump ballast water from any of the ballast tanks. The high pressures and flow rates generated by fire pumps are capable of damaging nets so some alternative filtering arrangements may need to be developed.

## **4.3.2 Biological assessment**

A summary of the biological assessments is provided in Table 4.1

### ***Sounding pipes***

The type of pump used greatly influences the sample obtained from the sounding pipe. For example the Waterra pump performed very poorly in comparison to the Mono pump and was, in fact, inferior to every method it was compared to in terms of zooplankton density and taxa richness. This appears to be a function of the low pumping rate of the Waterra pump (5–10 l min<sup>-1</sup>) which would allow for considerable avoidance for larger zooplankton such as decapod larvae, chaetognaths and large copepods which were under-represented by this method. In trials on the MV *Iron Sturt* where the head was negligible, and therefore the

pumping rate of the two pumps similar, species richness and taxa density were similar for both pumping systems.

### ***Manholes***

Manhole samples were, on average, the most efficient at providing representative samples of the ballast tank community. In particular, the manholes were the best location for sampling larger, highly mobile plankton such as decapod larvae, chaetognaths and ostracods. Open manholes, by exposing the plankton community to light may bias samples by over estimating the abundance of positively phototactic taxa. An underestimation of negatively phototactic taxa may also occur, however, there did not appear to be any taxa that were underestimated due to a negative phototactic response when sampled via this method within the limits of taxonomic resolution.

Samples collected from open manholes using pumps were generally more diverse than those collected using nets. This was particularly evident for crustacean larvae, which are positively phototactic and are preferentially distributed in the surface water (Murphy 1997). Nets were as efficient as pumps at sampling the suite of surrogate target taxa.

Notwithstanding any possible light induced bias, samples collected from open manholes had greater over all densities for all target taxa, including polychaetes, echinoderm larvae and crab zoea (decapods and crustaceans in general). Of the two methods pump samples were more diverse but not significantly so to outweigh the practical advantage of using nets.

### ***Air vent and breather pipes***

Sounding pipe and air vent samples were virtually identical in terms of total zooplankton density and taxa richness. It might have been expected that samples taken from air vents (which are open ended and larger in diameter) would be more representative than those from sounding pipes. This however, was not reflected in the data and suggests that sampling may have been biased by the poor performance of the Waterra pump when sampling air vents. Further trials with high capacity pumps are required to test this assumption. Based on the results from this survey, pump sampling via air vents is not recommended as it provides no better assessment of the zooplankton community than sampling via sounding pipes.

Sampling from air vents or breather pipes when tanks were being "pressing up" was suitable for whole water samples or the collection of samples that required smaller volumes, such as phytoplankton samples. However, it is not recommended for the collection of zooplankton samples because it cannot be used to sample large volumes of water while in port.

### ***In-line sampling***

In terms of zooplankton density and taxa richness pressure gauge samples rated very poorly in comparisons with manhole samples. It was comparable to the Waterra and Mono pump samples in taxa richness but generally had lower estimates of density. Pressure gauge sampling was trialed on the Newcastle field sampling exercise where overall densities and richness were relatively low. In these ships larger plankton such as decapods and chaetognaths were not present even in the net hauls. However, despite this, and given the low flow rates and the lamina flow characteristics typical of pipe flow dynamics, it is unlikely that this method would effectively sample larger zooplankton.

**Table 4.1.** Method comparisons for (a) taxa richness and (b) total zooplankton density for the various sampling methods. The sum of the numbers in each cell of the table represents the number of comparisons between gear types conducted on the same ship and tank at the same time. Number designations in each cell are as follows: top right – number of comparisons where column variable was higher than the row variable; bottom left – number of comparisons where row variable was higher than the column variable; and top left – number of comparisons where row and column variables were tied. For each cell, an arrow points to the method which gave the highest estimate of taxa richness or zooplankton density; ties are indicated by horizontal lines. Shaded arrows or lines indicate that there were too few comparisons to test the significance of an indicated trend.

a)

RICHNESS	Net haul	Mono pump	Waterra pump	Pressure gauge	Pneumatic pump	Ballast pump	Sea chest	Diaphragm pump	Drop net	Fixed site
Net haul										
Mono pump	2 —									
Waterra pump	↑ 3	↑ 1								
Pressure gauge	↑ 3	↑ 2	↑ 1	← 1						
Pneumatic pump	↑ 1	← 1	← 1							
Ballast pump	← 1	↑ 1								
Sea chest	↑ 1	↑ 1				↑ 1				
Diaphragm pump	2 ← 1	← 1				← 1	← 1			
Drop net	← 1	← 2		← 1						
Fixed site	↑ 1	↑ 1						← 1		

b)

DENSITY	Net haul	Mono pump	Waterra pump	Pressure gauge	Pneumatic pump	Ballast pump	Sea chest	Diaphragm pump	Drop net	Fixed site
Net haul										
Mono pump	1 ↑ 2									
Waterra pump	↑ 2	1								
Pressure gauge	↑ 3	3 —	↑ 1	← 1						
Pneumatic pump	← 1	1	← 1							
Ballast pump	↑ 1	↑ 1								
Sea chest	↑ 1	↑ 1				1				
Diaphragm pump	5 — 1	↑ 1				1 —	1 —			
Drop net	↑ 1	↑ 2		1						
Fixed site	↑ 1	↑ 1						1		

Samples collected from the ballast pump were more diverse than those from the sea chest, although over all sample densities were comparable. Sampling in the turbulent zone of the pipe work may have contributed to the collection of a more representative sample as those from the sea chest came from an area of the pipe work subject to more lamina flow.

In general, despite the large volumes of water sampled, in-line sampling from the pipework (ballast pump and sea chest) did not provide a more representative sample than the Mono pump and net hauls except for the collection of some very rare taxa (fish larvae and rotifers). The method unquestionably under sampled larger more common taxa such as decapod larvae, but was only trialed on the MV *Iron Whyalla* and the results are sufficiently promising to warrant further exploration and development.

### **Deck/fire taps**

Zooplankton collected from the fire hose outlets on the MV *Iron Whyalla* were generally in poor condition, probably as a result of the high sheer forces experienced in the high pressure fire pump. It is likely that many delicate organisms such as invertebrate larvae would be indistinguishable after passage through this pump, if in fact they were present in the samples. However, the development of molecular identification techniques capable of detecting target taxa in mixed plankton samples suggests that this sampling option should be examined further.

### **Port water sampling**

Certain obvious biases are inherent in sampling adjacent port waters with drop nets. A single net drop represents an integrated water column sample taken at one point during the ballasting period. Various physical factors such as time of day and the tidal cycle that effect the horizontal and vertical distribution of plankton will in turn effect the suite of taxa entrained in ballast water. Overall however, port samples contained all of the taxa sampled from ballast tanks. However, drop net samples were generally more diverse and were significantly more efficient at sampling cnidarians, hydroids and ctenophores than the other onboard methods. It is unclear if these taxa were relatively under sampled or were not surviving the ballasting process. Decapod larvae and shrimp densities were comparable to net hauls and higher than the pumps and in-line sampling. Larger taxa such as fish larvae were under sampled by drop nets.

### **4.3.3 Suitability of different sampling methods for selected target taxa**

The assessment of the suitability of different sampling methods for surrogate taxa was restricted to those taxa taken during the vessel sampling survey and the MV *Iron Whyalla* heat treatment trial. Descriptions of sampling methods and detailed analysis of the results of these studies are given in Appendix C and Rigby *et al.* (1997). A summary of the taxa/sampling methods assessments are given in Table 5.1.

Phytoplankton can be sampled by all methods although deck tap samples were usually found to be in poor condition (Rigby *et al.* 1997). Sampling for phytoplankton requires mesh size of nets or sieves to be reduced to 20  $\mu\text{m}$ . Sampling for macrophyte life history stages was not attempted. Motile dinoflagellates are likely to be appropriate surrogates for zoospores in terms of their distribution in ballast water and potential susceptibility to different sampling methods.

Crab zoea were poorly represented by sounding pipe and air vent sampling (using both the Waterra and Mono pumps) and in-line sampling but were well represented in manhole samples taken with both nets and pumps. Positively phototactic crab zoea may be overestimated by manhole sampling if the manholes are left uncovered. Polychaete trochophores and bivalve larvae were sampled by most methods but were most abundant in manhole samples taken by net hauls and pumps. Sampling for polychaetes via sounding pipes and air vents is appropriate when ballast tanks are full and pump flow rates are relatively high. Echinoderm larvae were detected in both net haul and fixed site samples taken on the MV *Iron Sturt*.

**Table 5.1.** Summary of the suitability of sampling methodologies for different target taxa (see Appendix C for detailed information).

Method	Target taxa sampled	Target taxa not sampled
Sounding pipe (Waterra pump)	phytoplankton, polychaetes	crab zoea, crab megalopae
Sounding pipe (Mono pump)	phytoplankton, polychaetes, bivalves	crab zoea, crab megalopae
Manhole (diaphragm pump)	phytoplankton, polychaetes, bivalves, crab zoea, echinoderm larvae	
Manhole (net haul)	phytoplankton, polychaetes, bivalves, crab zoea, crab megalopae, echinoderm larvae	
Air vents (Waterra pump)	phytoplankton	
In-line (pressure gauge)	phytoplankton	(1)
In-line (ballast pump)	phytoplankton, polychaetes, bivalves	crab zoea
In-line (sea chest)	phytoplankton, polychaetes, bivalves	crab zoea
Deck/fire tap	potentially all target taxa if morphological identification are not required	unsuitable for all taxa if morphological identifications are required

(1) not tested at low zooplankton densities.

## 5 DISCUSSION AND CONCLUSIONS

### 5.1 Sampling methods

The review of national and international ballast water research programs indicated that most of these programs were opportunistic and had been established to survey incoming vessels for the purpose of quantifying and identifying the range of organisms transported via ballast water. In most cases the sampling methods were determined by available access to ballast tanks and the stage in the ballasting cycle when sampling could take place. Some of these monitoring programs targeted specific taxonomic groups (mainly toxic dinoflagellates) but efficacy of sampling was not critically evaluated. Of the programs surveyed only the Cawthron Institute's contained a method evaluation component but this focussed mainly on operational aspects of sampling (Hay *et al.* 1997).

The operational assessment of methods evaluated in this project confirmed that access to tanks and the stage of the ballasting cycle at which sampling occurs impose major restraints on the types of methods that can be employed. For example net hauls are simple, quick and effective, but can only be used for holds and tanks, and, in the latter case, only when manholes are accessible. In addition, top wing and side tanks can only be sampled with nets when these tanks are full of ballast water. At the other extreme, sampling via sounding pipes with an inertia pump can be used to obtain a ballast water sample at any stage of the ballasting cycle on most vessels. However, the equipment is relatively cumbersome and requires prolonged pumping times to achieve adequate sample volumes. In-line sampling methods offer simplicity and the opportunity to obtain samples whenever the ballast pumps are running, but requires sampling to continue over the entire de/ballasting period to achieve adequate sample volumes. In situations where available "on board" time is short, methods that require prolonged sampling times will limit the number of replicates that can be taken and the number of tanks that can be sampled. From an operational perspective therefore, sampling methods that are simple, easy and rapid to employ, and require the minimum of infrastructure are to be preferred.

Given that access to ballast tanks will largely determine the method(s) that can be used in any situation, the sampling characteristics of these methods (i.e. the efficiency with which they sample the suite of target species) are crucial in determining their suitability for use in a targeted sampling program. For phytoplankton and microbial studies any one of the methods tested is likely to yield an adequate sample for most monitoring or testing purposes. This includes the detection of *Vibrio cholerae* as copepods were well represented in almost all samples. With the possible exception of the sounding pipe method, none of the methods are suitable for obtaining sediment samples for detecting dinoflagellate cysts unless these have been resuspended within the tanks.

The biological assessment of sampling methods confirmed that there were significant differences between methods in their capacity to sample the zooplankton assemblage as a whole and selected taxa. This indicates that all methods showed some level of sampling bias. For example, net sampling through manholes was overall the most effective method for both the total zooplankton assemblage and the suite of surrogate target taxa. However, some taxa, polychaetes for example, were effectively sampled by all the methods tested, including pumps via sounding pipes, while others, such as crab zoea, were under-sampled by low flow

rate pumps. Possible explanations for this include net or pump avoidance and/or heterogeneous distributions for some species.

Avoidance of sampling gear, both nets and pumps, by actively swimming species is a well documented source of bias associated with sampling planktonic communities. Ballast tank zooplankton assemblages are no exception and no one sampling method will be capable of providing representative samples of the whole ballast tank community or the entire suite of target species. There are a number of anecdotal reports that support this contention including, a school of mullet that evaded net tows but were observed in the residual 40 cm of ballast water in an emptied tank (Greg Ruiz pers. comm.), negatively phototactic copepods that dive to the bottom of ballast holds when exposed to the light of an open manhole (Jim Carlton pers. comm.), and juvenile fish that evade net tows but are observed in the light of a manhole (CSIRO unpublished observations). In general terms, gear avoidance by any species will lead to an under-estimation of their density and may contribute to that species not being detected by some sampling methods, particularly when densities are low. This has implications when sampling vessels to validate biological risk assessments made by the DSS (see below).

Heterogeneity in the spatial distribution the intrained planktonic community is potentially a major contributor to bias when sampling ballast tanks. If the planktonic community in a ballast tank is evenly distributed, any sampling bias shown by different methods will be attributable to species-specific avoidance. In situations where the distribution or density of target species is patchy (for whatever reason), methods that do not sample the same body of water within a tank (e.g. net and sounding pipe pump sampling) will have additional biases resulting from the species-specific distributions of the target species. This may result in either an under or an over estimate of the abundance of target species in a ballast tank depending on their spatial distribution and the method used to sample them.

Unfortunately data relating to spatial differences in zooplankton distribution in ballast tanks are both limited and equivocal. In this study, no significant differences in total zooplankton density or taxa richness were detected at three fixed sites located at three discrete depths within the ballast tanks on the MV *Iron Whyalla*. However, Murphy (1997) found that in the top 6 m of ballast water in tanks on the MV *Iron Sturt*, crustaceans were most abundant in surface layers while chaetognaths, polychaete larvae and bivalves veligers were sampled equally at all depths. At least two factors are likely to be influencing these results. First, light, which was totally excluded during sampling on the MV *Iron Whyalla* but not on the MV *Iron Sturt*, has a considerable influence on the distribution of planktonic organisms and in the absence of light, taxa that show either avoidance or attraction to light are likely to be distributed more homogeneously. Second, Murphy's results suggest that some species were significantly more abundant in the very surface layers and may have been responding to the air/water interface. Such small scale variations in depth distribution would not have been detected on the MV *Iron Whyalla* as the upper most sampling site was 3 m below the surface.

Clearly without some independent method of assessing the sampling efficiencies of different methods the interpretation of the results of ballast water sampling will always be difficult. In this study, comparisons of sampling methods between vessels (and in some case between tanks on the same vessel) were confounded by a range of factors including, differences in season, the age of the ballast, whether or not the vessel had exchanged ballast, and the period

of day over which ballasting occurred. Ballast age in particular contributes to low zooplankton densities, and high variances associated with low and patchy zooplankton densities will often preclude meaningful statistical analysis. On several occasions during this study, tanks on the same vessel, which had apparently been ballasted at the same time, showed sufficient variability to invalidate method comparisons between tanks. In the absence of species-level identifications, the use of "morpho species" will continue to obscure possible species-specific patterns of behaviour. This in turn limits our capacity to differentiate between the confounding effects of individual method biases and the distribution of target species within ballast tanks.

## **5.2 Identification of target taxa**

The capacity to identify target species in ballast water is implicit in any approach to ballast water management based on target species. The development of genetic (molecular) or similar techniques that are capable of providing unequivocal species-level identifications of larval forms is a key requirement for the implementation of a targeted sampling program. The development of a genetic identification capability also opens up the possibility of utilising alternative sampling methods by eliminating the need to retain specimens in good condition to facilitate species identifications based on morphological characteristics. Deck taps supplied by the vessels fire pump potentially offer a rapid and efficient method of delivering ballast water from any ballast tank on the vessel. Sampling via deck taps has generally been disregarded because the high pressures generated by the pumps usually result in plankton samples in poor condition.

## **5.3 Ballast water sampling and risk assessment**

An effective sampling program is an important component of the DSS-based approach to the management of ballast water in that it provides two key elements:

- feedback on the general accuracy of biological risk assessments decisions made by the DSS including confirmation of the status of vessels identified as high risk on the basis of direct evidence as to the presence or absence of target organisms; and
- relevant information that will, over time, enhance the risk assessment framework.

To be effective in the first of these roles, a ballast water sampling and testing program must be capable of providing accurate estimates of target organisms in ballast tanks; this will likely involve the use of taxa-specific sampling methods. At this stage of our understanding, a knowledge of the infectious state of any port or ship will be broadened by a positive result (i.e. a positive identification of a target taxa). However, given the limitations of sampling discussed above, it is important to acknowledge that a negative result does not mean that a target taxa was not present, only that it was not detected. It may mean that the species in question was present, but in densities too low to detect using the available sampling methods. In this context it is also important to acknowledge that where operational factors limit sampling options, methods may be used that are inappropriate for sampling some target species. If the probabilities of detection are sufficiently low, no inference can be drawn from the failure of the method to detect the species. In short, it is important not to expect a sampling protocol to provide information to answer inappropriate questions.

An increased knowledge of the ecology of ballast water communities and the survival patterns of the target species, will, in future, allow the interpretation of the "negative" result.

For example, with an ability to identify the larvae of individual target species and an enhanced understanding of their ecology, research can then focus on the risk posed by the transport of the larvae. Specifically, this involves an examination of the uptake of larvae in ballast, larval density in ballast as compared to port communities, and most importantly, the survival of larvae in ballast over the period of a voyage. This information will feed directly into the risk assessment framework and provide the empirical data necessary to predict densities of larvae in tanks when real densities are too low to detect and estimate by sampling alone. This will allow us to move beyond simple issues of "presence" or "absence" to questions such as is the species likely to be present in sufficient densities to pose a significant risk?

## **5.4 Conclusions**

This study has identified a variety of methods appropriate and practical for ballast water sampling in general, and for specific taxa. No single method is appropriate for all vessel situations or will adequately sample all taxa. Uncertainties in relation to sampling biases associated with different techniques and the distribution of target taxa in ballast tanks make it advisable that sampling programs not rely on a single method. The application of at least two methods will increase our understanding of the biases of individual methods and enable more accurate interpretation of the results obtained. This is particularly important on those vessels where it is impractical to use preferred sampling methods. A better understanding of the biases of each particular method along with a more detailed knowledge of the ecology and survival of specific taxa will enhance the effectiveness of sampling as a tool to validate the risk assessment framework.

## 7 REFERENCES

- Australian Quarantine and Inspection Service (AQIS). 1993a. *Ballast Water Research Series*, Report No. 1: Ballast water treatment for the removal of marine organisms. Australian Government Publishing Service, Canberra, 99 p.
- Australian Quarantine and Inspection Service (AQIS). 1993b. *Ballast Water Research Series*, Report No. 4: Ballast water management. Australian Government Publishing Service, Canberra, 254 p.
- Bolch, C.J. and Hallegraeff, G.M. 1992. Transport of dinoflagellate cysts in ships' ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res.* 14: 1067-1084.
- Bolch, C.J. and Hallegraeff, G.M. 1993. Chemical and physical options to kill toxic dinoflagellate cysts in ships' ballast water. *J. Mar. Environm. Engineering.* 1, 23-29.
- Bruce B.D., Sutton C.A., and Lyne, V. 1995. Laboratory and field studies of the larval distribution and duration of the introduced sea star *Asterias amurensis* with updated and improved prediction of the species spread based on a larval dispersal model. Final report to Fisheries Research and Development Corporation #93/235, CSIRO Division of Fisheries, Hobart, 66 p.
- Cangelosi, A. 1997. The *Algonorth* experiment. *Seaway Review*, 25(3), 4 p.
- Carlton, J.T. 1985. Transoceanic and inter-oceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanogr. Mar. Biol. Rev.* 23, 313-317.
- Carlton, J.T. 1996. The nature of ballast water. *ICES CIEM Information Newsletter*, Issue No. 27, March 1996.
- Carlton, J.T. 1997. Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bull. Mar. Sci.* 41, 452-465.
- Carlton, J.T. and Geller, J.B. 1993. Ecological roulette: the global transport of non-indigenous marine organisms. *Science* 261, 78-82.
- Carlton, J.T., Reid, D.M. and van Leeuwen, H. 1995. The role of shipping in the introduction of non indigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. The National Biological Invasions Shipping Study. Prepared for the United States Coast Guard and the US Department of Transport; National Sea Grant Program/Connecticut Sea Grant Project (R/ES-6), Report No. CG-D-11-95, 345 p.
- Davenport, S. and McLoughlin, R.J. (1993). Preliminary assessment of the distribution and potential impact of the introduced starfish *Asterias amurensis* in Tasmanian waters. Fisheries Research and Development Corporation Status Report. CSIRO Division of Fisheries, Hobart, 38 p.
- Gauthier, D. and Steel, D.A. 1996. A synopsis of the situation regarding the introduction of non indigenous species by ship-transported ballast water in Canada and selected countries. *Can. Man. Rep. Fish. Aquatic. Sci.* 2380.

- GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) 1997. Opportunistic settlers and the problem of the ctenophore *Mnemiopsis leidyi* invasion in the Black sea. *Rep. Stud. GESAMP* 53, 84 p.
- Gollasch, S., Dammer, M., Lenz, J. and Anders, H.G. 1995 Non-indigenous organisms introduced via ships into German waters. Theme session: *Ballast water: ecological and fisheries implications*, ICES Annual Science Conference 1995. Aalborg, Denmark (ICES CM 1995/O:13), 21 p.
- Gosselin, S., Levasseur, M. and Gauthier, D. 1995. Transport and deballasting of toxic dinoflagellates via ships in the Grande Entrée Lagoon of the Iles-de-la-Madeleine (Gulf of St Lawrence, Canada) In: Lassus, P. *et al.* (eds), *Harmful Marine Algal Blooms*, Lavoisier Publ. Inc., 591–596.
- Hallegraef, G.M. and Bolch, C.J. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. *Mar. Poll. Bull.* 22: 27–30.
- Hay, C., Handley, S., Dodgshun, T., Taylor, M. and Gibbs, W. 1997. Cawthron's Ballast Water Research Programme: Final report 1996–1997 to MAF Policy. *Cawthron Report* No. 417, 139 p.
- Hayes, K.R. and Hewitt, C.L. 1998. Risk assessment framework for ballast water introductions. *Centre for Research of Introduced Marine Pests, Tech. Rep. No. 14*. CSIRO Marine Laboratories, Hobart, 75 p.
- Locke, A., Reid, D.M. and Sprules, W.G., Carlton, J.T. and van Leeuwen, H.C. 1991. Effectiveness of mid-ocean exchange in controlling freshwater and coastal zooplankton in ballast water. *Can. Tech. Rep. Fish Aquat. Sci.* 1822, 93 p.
- McMinn, A. Hallegraef, G.M. and Short S.A. 1997. Microfossil evidence for the recent introduction of the toxic dinoflagellate *Gymnodinium catenatum* into Tasmanian waters. *Mar. Ecol. Prog. Ser.* 161, 165–172.
- Macdonald E.M. 1995. Dinoflagellate resting cysts and ballast water discharges in Scottish ports. Theme session: *Ballast water: ecological and fisheries implications*, ICES Annual Science Conference 1995, Aalborg, Denmark (ICES CM 1995/O:10), 17 p.
- Medcof, J.C. 1975. Living marine animals in a ship's ballast water. *Proc. Natl. Shellfish Ass.* 65: 11–12.
- Murphy 1997. The survival and sampling of zooplankton in ballast water. BSc Hons dissertation, University of Tasmania, Australia, 31 p.
- Oemcke, D.J. & van Leeuwen, J. (1998) *Potential of ozone for ballast water treatment*. Ports Corporation of Queensland and CRC Reef Research Centre report, 40 p.
- Omori, M. and Ikeda, T. 1984. *Methods in marine zooplankton ecology*. Wiley, NY.
- Rigby, G. and Hallegraef, G.M. 1994. The transfer and control of harmful marine organisms in shipping ballast water: behaviour of marine plankton and ballast water exchange trials on the MV *Iron Whyalla*. *J. Mar. Environ. Engineering* 1: 91–110.

- Rigby, G.R., Steverson, I. and Hallegraeff, G.M. 1993. Shipping ballast water trials on the MV *Iron Whyalla*. In: *AQIS Ballast Water Research Series*, Report No. 4: Shipping ballast water trials on the bulk carrier MV *Iron Whyalla*. Australian Government Publishing Service, Canberra, p 1–41.
- Rigby, G.R., Hallegraeff, G.M. and Sutton, C.A. 1997. Ballast water heating and sampling trials on the BHP ship MV *Iron Whyalla* in Port Kembla and *en route* to Port Hedland. Report prepared for the Australian Quarantine and Inspection Service (AQIS) October 1997, 40 p.
- Subba Roa, D.V., Sprules, W.G., Locke, A. and Carlton, J.T. 1994. Exotic Phytoplankton from ships' ballast waters: Risk of potential spread to mariculture sites on Canada's east coast. *Can. Data Rep. Fish. Aquatic. Sci.* 937, 51 p.
- Thresher, R.E. and Martin, R.B. 1995. Reducing the impact of ship borne marine introductions: focal objectives and development of Australia's new centre for research on introduced marine pests. Theme session: *Ballast water: ecological and fisheries implications*, ICES September 1995, Aalborg, Denmark (CM 1995/O:4).
- Williams, R.J., Griffiths, F.B., Van der Wal, E.J. and Kelly, J. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine, Coastal and Shelf Science*. 26: 409–420.
- Wonham, M.J., Walton, W.C., Frese, A.M. and Ruiz, G.M. 1996. Transoceanic transport of ballast water: biological and physical dynamics of ballasted communities and the effectiveness of mid-ocean exchange. Final Report report submitted to U.S. Fish and Wildlife Service and the Compton Foundation, Smithsonian Environmental Research Centre, MD 21037.

## **APPENDIX A: INTERNATIONAL BALLAST WATER PROGRAMS**

There are a number of international groups currently undertaking ballast water research. Projects range from purely descriptive studies of the contents of ballast tanks to exploring specific questions in relation to ballast water ecology, biological and physical interactions in ballast tanks, and the assessment of treatment options. Gauthier and Steel (1996) have provided an excellent synopsis of the ballast water research activities and management practices in use internationally. This section summarises information on projects currently under way, or recent projects where information has yet to be published. Much of the information included in these summaries was obtained through correspondence with individual research groups and from unpublished ICES meeting reports (in particular those of the Working Group on the Introduction and Transfer of Marine Organisms (WGITMO) held at La Tremblade, France, April 1997 and The Hague, March 1998) and, in such cases, will not be referenced formally. Publications or reports referred to in this section are included in section 7 (References) above.

### **A1 United States**

#### **Smithsonian Environmental Research Centre (SERC)**

Contact: Dr Gregory Ruiz

The SERC Marine and Estuarine Invasions Biology program, has been working on ballast water issues since the early 1990s. The aims of program are to:

1. Measure the patterns of non indigenous species transfer, invasion and impact,
2. Test the mechanisms that underlie these patterns, and
3. Assess the efficacy of management options .

SERC's ballast water research has included quantifying the species composition of ballast water entering Chesapeake Bay, assessing ballast water management, delivery patterns and treatment options, and the condition, survival and viability organisms in ballast tanks. The protocols developed by SERC to facilitate this research are now standard for most groups conducting ballast water sampling in the US. The group has concentrated on the collection of plankton net samples from bulk holds and tanks. They have also opportunistically sampled the sediments and benthos of empty tanks to detect species that may not have been caught by nets.

During the course of this research (5–6 years) SERC has sampled over 250 vessels, including bulk carriers (175), ore carriers (10), oil tankers (25), container ships (20), general cargo (10) and military ships (25). Most of this sampling has focused on the description of the physical and biological (plankton and more recently microbial) properties of ballast water in ships entering Chesapeake Bay, and more recently, Alaskan ports (Ruiz pers. comm.). 90% of these ships were found to contain live organisms and over 280 distinct taxa have so far been reported. The abundance of organisms was found to be negatively correlated with ballast water age.

To assess the survival of organisms in ballast, (transit success) 15 vessels were sampled at the beginning and end of the voyage and for one vessel over the duration of the cruise

(Wonham *et al.* 1996). Voyage mortality was found to be very high for phytoplankton and zooplankton (average survival rates of 5%), but low for microbial communities (Ruiz pers. comm.).

Ballast exchange as a treatment option was trialed on 6 ships by measuring the % removal of the ballast organisms and by using tracers such as inert bio-balls, *Artemia* cysts and rhodamine dye. Of these methods rhodamine dye and directly measuring the change in the community after exchange have proven the most useful indicators of water exchange. A more extensive series of experiments to assess exchange effectiveness is currently underway.

SERC is also stepping up research on microbial communities in ballast water with a focus on toxicogenic *Vibrio cholera*. Funding has been secured for a two year project to characterise the transfer, dynamics and risk of invasion for microbial communities associated with ballast water. This work will include the examination of the interactions between bacteria and *Vibrio* spp with plankton communities and specific taxa.

### **Mystic Seaport**

Contact: Dr Jim Carlton

Jim Carlton's group at Mystic Seaport, Connecticut, and Dr Lu Eldredge (Bishop Museum, Hawaii) have commenced a project to characterise the biological and physical properties of ballast arriving in Hawaii. They are using the SERC sampling protocols for the collection of phytoplankton and zooplankton samples.

### **Northeast Midwest Institute**

Contact: Dr Allegra Cangelosi

Dr Allegra Cangelosi of the Northeast Midwest Institute is the principal investigator for the Ballast Water Filtration Demonstration project. This is a 2+ year project funded by the Great Lakes Protection Fund which has a primary aim of developing a widely applicable ballast water treatment method. The project is testing the biological and mechanical effectiveness of filtration as a primary ballast treatment process and will assess the need for additional treatments to target smaller organisms such as pathogens. To this end the project has, in collaboration with Dr Ivor Knight (James Madison University), recently secured additional funding for microbial analysis of ballast water. The second stage of the project (commencing May/June 1998) will involve a survey and sampling of incoming vessels to assess the effectiveness of filtration for reducing the prevalence of detected organisms. The group expects the treatment to be effective in not only removing plankton but also pathogens, as many of the pathogens attach to larger organisms such as copepods.

The ship-board trials were conducted in mid 1997 on board a specially equipped bulk carrier *Algonorth* (Cangelosi 1997). The project description and protocols have been published in a technical report manual and the results of the trials should be available in late 1998. Although filtration looks promising (Cangelosi pers. comm.) it was designed as a primary treatment to be used in combination with secondary treatments. There are plans to follow up the filtration project with a desk-top study to review secondary treatment options (such as UV light, ultrasound, heat) and to subsequently undertake initial trials of selected options aboard the *Algonorth*, perhaps by mid 1998.

## **Battelle**

Contact: Dr Deborah Tanis

Battelle has been contracted by the US Coast Guard to work on the Ballast Water Exchange Verification Program. This program was initiated in response to recent amendments to the Nonindigenous Species Act which addressed the issue of ballast water mediated introductions by specifying the need to develop provisions for controlling the discharge of ballast water in US waters. The US Coast Guard, as the body responsible for monitoring the voluntary regulations, identified the need to develop methods to verify that vessels were conducting mid-ocean exchange of ballast water. Battelle aims to develop techniques to verify that incoming vessels have conducted the required mid-ocean exchange prior to their entering US ports. The project commenced in late 1997 and a laboratory-based study to assess potential indicators of ballast exchange is in progress. At this stage Battelle is tending to favour an approach based on optical properties (excitation and emission spectra) and is currently testing water samples from an range of environments from different parts of the world with a view to identifying unique optical signatures. The program is running concurrently with other programs which are assessing shore-based water treatment options.

Battelle is also involved in a study of ballast water discharges in the Gulf of Mexico and is a collaborator with Cawthron Institute to review and develop ballast exchange verification methods for the NZ Ministry of Fisheries (see below). Battelle has also been contracted by the Northeast-midwest Institute to undertake a desktop study to review the feasibility of secondary treatments such as UV irradiation, heat and ultrasonics.

## **A2 Canada**

### **Fisheries and Oceans, Canada**

Contact: Dr Michel Gilbert

The Fisheries and Oceans project was initiated in 1994 to assess the risks associated with ballast water-mediated introductions of nonindigenous marine organisms to the estuary and Gulf of St Lawrence. Assessments of potential risks are based on the (1) foreign vessel traffic, (2) deballasting statistics, and (3) biodiversity and richness of protistan and metazoan taxa found in ballast water and ballast tank sediments. In 1995, manhole sampling with nets was carried out on 93 foreign vessels, most of which had conducted a mid-ocean exchange of ballast water. The main objective of this study was to assess the species composition and abundance of organisms in ballast water upon arrival. An extensive species lists of over 200 taxa have been compiled and nonendemic species are to be identified (Gilbert and Campbell unpublished data).

## **A3 Australia**

### **Flinders University, SA**

Contact: Ben Baghurst

The ballast water of 10 vessels arriving into South Australia and 5 vessels ballasting in the Port River, were sampled by Ben Baghurst for a BSc Hons project in 1995. All major trophic groups were represented in ballast water samples with the most common taxa being

copepods, tintinnids, diatoms, gastropod larvae, barnacle nauplii, dinoflagellates and bivalve larvae. Comparisons between recently ballasted water and the port community revealed that all taxa sampled in ballast tanks were present in port water. Densities of most taxa in the ballast tanks were much lower than in the port community. A variety of potential ballast water treatments were trialed in the laboratory, including chlorine and hydrogen peroxide, to determine the acute tolerances of species that had been laboratory reared.

### **University of Tasmania**

Contact: Dr Gustaaf Hallegraeff

Dr Gustaaf Hallegraeff was the first scientist to document the occurrence of toxic dinoflagellates in Australian waters and has been involved in various aspects of ballast water research since then. His work has included two extensive surveys of over 345 ships in numerous ports around Australia (Hallegraeff and Bolch 1991; Bolch and Hallegraeff 1992). These studies found that 65% of ships had sediments containing diatoms, 50% contained viable dinoflagellate cysts and 5% contained toxic dinoflagellates (*Alexandrium* spp and *Gymnodinium catenatum*). Much of the *en route* ballast water research undertaken by Gustaaf Hallegraeff has been carried out in collaboration with Dr Geoff Rigby of BHP research (see below). This has involved various laboratory and field trials to test the lethal levels of various chemicals (Bolch and Hallegraeff 1993) and the potential for heat treatment of ballast water (Hallegraeff *et al.* 1997).

Recent collaborative studies with micropaleontologist Andrew McMinn (McMinn *et al.* 1997) has focused on tracing the history and origins of introduced phytoplankton and have confirmed and dated the introduction of the toxic dinoflagellate *Gymnodinium catenatum* to have occurred in 1972. Following on from this work, Chris Bolch (PhD student) is now using molecular genetic PCR techniques to map the biogeography of this species and trace its movement and introduction on an international scale.

### **James Cook University and CRC for Reef Research**

Contact: Darren Oemcke

As part of a PhD project in the Department of Civil and Environmental Engineering at James Cook University, Darren Oemcke carried out a ballast water sampling project in 1995/96, sampling bulk carriers (8) and container ships (2). The project focused on measuring the physical and chemical properties of ballast water that could impact on different treatment options. Treatment options explored include ozonisation and UV radiation particularly for the purpose of killing dinoflagellate cysts and *Vibrio cholerae* (Oemcke and van Leeuwen 1998).

### **BHP Research**

Contact: Dr Geoff Rigby (resigned from BHP, February 1998)

BHP Research involvement with ballast water issues has focused primarily on ballast water treatment. Dr Geoff Rigby has been the main driving force in this research which has included ballast exchange and heat treatment trials on the BHP bulk carrier *MV Iron Whyalla* (Rigby and Hallegraeff 1993; Rigby *et al.* 1997).

In the exchange trial, methylene blue dye and change in the plankton community composition was used to measure exchange efficiency. In this study fixed points at different positions (depths) in the wing and bottom tanks of the MV *Iron Whyalla* were sampled using propositioned pipes connected to a deck pump. It was found that exchanging three tank volumes was required to remove 95% of the original ballast water and 75% of the sediment. The exchange trials were the precursor for the heat treatment trials which were carried out in 1997. In this latter trial, cooling water heated by the ship's engine was used to gradually heat a test ballast tank to around 38°C. This treatment killed all zooplankton and, based on laboratory trials, the temperatures were sufficient to kill dinoflagellate cysts if maintained for at least 8 hours (Rigby *et al.* 1997).

The sampling component of the heat treatment trials were carried out by Dr Gustaaf Hallegraeff (University of Tasmania) and Caroline Sutton (CRIMP) and is included in the relevant sections detailing these researchers protocols. Although BHP does not undertake any routine ballast water sampling, Dr Rigby has provided advice on methods of sampling ballast, particularly the in-line method.

### **CSIRO Centre for Research on Introduced Marine Pests (CRIMP)**

Contact: Richard Martin/Chad Hewitt

#### ***ABWMAC Strategic Ballast Water Research Program:***

CRIMP has or is involved in a number of projects funded under this program. These include:

- Ballast Water Sampling and Testing (Project 8/96) – this project is the subject of this report;
- Heat Treatment (Project 6/96) – ballast water heat treatment trials carried out on the MV *Iron Whyalla* in collaborative with Dr Geoff Rigby (BHP) and Dr Gustaaf Hallegraeff (University of Tasmania);
- Risk Assessment Methodology (Project 2/96) – the development of a risk assessment methodology to facilitate port to port risk assessments for the AQIS ballast water decision support system;
- Evaluation of Techniques for Detecting Toxicogenic *Vibrio cholerae* in Ballast Water (Project 9A/97) – an evaluation of current detection methods and development of new techniques to detect cholera in ballast water being carried out by the CSIRO Division of Food Science and Technology; CRIMP is providing advice and field assistance with the development of sampling protocols.

#### ***University of Tasmania/CRIMP collaborative projects:***

Kate Murphy completed an honour project which evaluated the survival and sampling of zooplankton in ballast water in conjunction with the University of Tasmania and CRIMP. Survival of organisms was monitored during four 4–6 day voyages on the BHP vessel MV *Iron Sturt*. Although mortality rates were high, it was found that many individuals were capable of surviving domestic transport in ballast tanks. The patterns of survival for individual taxa varied over the four voyages and various physical and biological processes

associated with sampling of the initial population contributed to the observed differences (Murphy 1997).

## **A4 New Zealand**

### **Cawthron Institute**

Contacts: Dr Cameron Hay/Tim Dodgshun

Cawthron have recently completed a 2 year ballast water sampling project (Hay *et al.* 1997). The main aim of the project was to develop a standard method of sampling ballast that could be applied to all ships and ballast tank configurations. 75 vessels were boarded and 161 ballast tanks on 50 of these vessels were sampled. Vessel types included in the survey included container ships (55% of tanks sampled), bulk carriers (32%) and break bulk carriers (5%); car carriers, general cargo, refrigerated carriers and one passenger vessel made up the remainder. The bias of the survey towards container ships may explain why sampling via sounding pipes was adopted as the favoured method; in most cases sounding pipes provide the only access to ballast tanks on such vessels.

The study provided a descriptive account of the physical and biological characteristics of ballast water arriving into the ports of Lyttleton and Nelson. 80% of the vessels sampled had live plankton and approximately half of these had reported to have exchanged ballast water. However, the presence of coastal and oceanic plankton species in many of the exchanged tanks suggested the exchanges were not wholly effective. Various physical properties of ballast water were measured in an attempt characterise exchanged and non exchanged tanks. Although there was some evidence of a relationship between the temperature and salinity of ballast water and ballast water exchange, this correlation was not strong enough to be used as a method to verify that exchange had occurred.

Cawthron is now focusing on ballast water treatment and management and has commenced a ballast water treatment project headed by Dr Doug Mountford. The group has conducted laboratory experiments to test the effectiveness of heat treatment, deoxygenation with glucose and nitrogen sparging on *Undaria pinnatifida* zoospores and echinoderm larvae (AMSA Conference paper, Auckland 1997).

Cawthron and Battelle were the successful co-tenderers for a contract let by the NZ Ministry of Fisheries to examine the efficiency of ballast water exchange and the degree of compliance with NZ ballast water mandatory controls and voluntary guidelines. The project, which began in early 1998 and will run for 17 months, will review the availability of existing methods to monitor ballast water exchange and develop and test a practical compliance verification methodology. Battelle and Cawthron staff involved in the project attended a workshop convened by AQIS in Melbourne (3 March 1998) to review possible approaches to ballast exchange verification.

### **National Institute for Water and Atmospheric Research (NIWA)**

Contact: Dr Julie Hall

Julie Hall undertook a pilot study to evaluate the methods for investigating the survival of organisms (bacteria, phytoplankton and zooplankton) in ballast water tanks during a trans-

Tasman voyage on board the general cargo vessel the MV *Tasmanian Enterprise*. The voyage was a joint project with CRIMP (zooplankton) and this was the first time changes in the bacterial community have been measured during a voyage. Rhodamine dye was used to assess the efficiency of ballast exchange.

The study found that the density of phytoplankton and zooplankton decreased over time while bacterial numbers and production increased over the voyage duration. The ballast exchange removed 95% of the original ballast. The results from this study suggest that timing of ballast exchange is likely to influence its effectiveness.

Dr Julie Hall is continuing to research the dynamics of ballast water microbial communities and is currently collaborating with Dr Greg Ruiz (SERC) on a microbial project to measuring the transfer, dynamics and risk of invasion for microbial communities associated with ballast water (see SERC entry above).

## **A5 Europe and other areas**

### **University of Bergen, Norway**

Contact: Helge Botnen

In 1996, the Applied Environmental Research group at the University of Bergen commenced a 4+ year ballast water sampling program to characterise the biological (phytoplankton and zooplankton) and physical component of ballast water discharged into the Sture Harbour. To date the sampling has focused on oil tankers (28) but will include other types of ships as opportunities arise. The program was initiated as an extension of the 1985 "Sture Study" which included comprehensive baseline environmental surveys of the flora and fauna, hydrography, benthos, littoral and oil hydrocarbon concentration of the area before it became a commercial port in 1988. Since 1989 the baseline surveys have been followed up by an annual monitoring program.

### **National Oceanographic Institute, Israel**

Contact: Dr Bella Galil

Dr Bella Galil commenced collaborative ballast water research with SERC in 1994, in particular the sampling of a coal vessel on transit from Israel to the United States (Wonham *et al.* 1986). In 1996, a survey of 3000 vessels was commenced with the aim of assessing and quantifying source and volume of ballast water arriving in Israeli ports. 60 vessels were sampled using SERC plankton (zooplankton and phytoplankton) sampling protocols. Collaboration with SERC ended in 1997 with the conclusion of the project. Dr. Galil is now collaborating with Dr Norbert Huelsmann of the Free University Berlin, and focusing research on the ballast water trophic dynamics.

### **University of Hamburg, Germany**

Contact: Dr Stephan Gollasch

From 1992–96, the Zoologisches Institut and Museum of the University of Hamburg and Institut für Meereskunde Kiel conducted a collaborative study of German shipping (*Introduction of non-indigenous organisms into the North Sea and Baltic Sea:*

*investigations on the potential ecological impact by international shipping*). As part of the study, two PhD students, Stephan Gollasch and Mark Dammer, were enlisted to describe and quantify the zooplankton and phytoplankton components of ballast water. Over the course of the project 186 of 211 vessels were successfully sampled, 132 for ballast water, 71 for sediments and 131 for hull fouling. 73 % of the ballast water samples were found to contain live organisms. Survival of organisms was studied on one voyage from Singapore to Bremerhaven, Germany. Temperature and oxygen were found to be variable and therefore were thought to be important factors in determining survival (Gollasch *et al.* 1995).

More recently, the European Union (EU) countries; Finland, Ireland, Lithuania, Sweden, United Kingdom and Germany, coordinated by Prof. H. Rosenthal (Institut für Meereskunde) and Dr Stephan Gollasch, are participating in a Concerted Action Plan (CAP) entitled: *Testing monitoring systems for risk assessment of harmful introductions by ships to European waters*. The CAP aims to develop a monitoring system appropriate for international use by in-transit evaluation and validation of existing methodologies and the compilation of case histories of various introductions.

The project will bring together experts from EU countries (and other invited guests) and will involve a series of workshops (including a number of sea going workshops) over a two year period commencing in early 1998. These workshops will provide a forum for exchange of ballast water research expertise ranging from qualitative and quantitative techniques used for on-board ship sampling and in-transit survival, sample processing, laboratory procedures, and statistical analysis. An initial meeting of the group in Kiel (Germany) looked at inter-calibration exercises prior to longer sea-going workshops on major European shipping routes. A second meeting was held in March 1998 in the Hague (Netherlands.)

### **Scottish Office of Agriculture, Environment and Fisheries Department (SOAEFD)**

Contact: Elspeth McDonald

In 1994, SOAEFD commenced a 3 year ballast water sampling project which targeted Scottish ports that had been identified as being important areas for ballast water discharge. Over the duration of the project 108 ships were sampled, mainly oil tankers (54), general cargo vessels (41), bulk carriers (21) and gas ships (7). The project described the biological and physical characteristics of ballast water in relation to the risk of introducing non-indigenous species, particularly those that could harm valuable fin and shellfish industries (Macdonald 1995).

Much of the biological component focused on phytoplankton and sediment sampling. Preliminary results from 32 ships sampled indicated that dinoflagellate cysts were present in 90% of samples, and four samples were found to contain *Alexandrium sp* cysts. It was concluded that the common occurrence of dinoflagellate cysts in ships visiting Scottish ports could pose a risk to Scottish waters and therefore warranted investigations into control measures.

SOAEFD have undertaken preliminary studies to assess the effectiveness of ballast water exchange by comparing the diversity and density of the ballast community before and after exchange. SOAEFD's Elspeth MacDonald is currently participating in the EUCAP and is a collaborator in the ballast water project underway at Bangor University (see below).

## **Fisheries Research Institute, Ireland**

Contact: Dan Minchen

Dan Minchen, is Ireland's main ballast water representative and is currently participating in the EUCAP. Other than this recent involvement in EUCAP, Ireland has no formal ballast water sampling projects but is conducting an ongoing survey of shipping movements and volumes of ballast discharged to identify high risk ports. This has for the most part been a desk top study using shipping and port office records, and only on a few occasions have ships been boarded. Cork Harbour and Shannon Estuary have been identified as important Irish ports but are low risk relative to other European ports in terms of the number of ship visits and volume of ballast discharged .

Ireland is particularly concerned about haul fouling and the high prevalence of fouling organisms despite the use of organotin antifoulants. Limited, opportunistic sampling of ship's hulls during dry docking has been carried out.

## **Bangor University, Wales**

Contact: Tracy McCollin

The School of Ocean Science of the University of Wales commenced a three year government funded project in 1996 entitled: *Marine organisms transported in ship's ballast*. The work is complimentary to research undertaken by SOAEFD and data from the current project will be added to the existing SOAEFD database. The project is largely descriptive with the main objectives to (1) describe the range of organisms present in ship's ballast water and sediment in relation to port of origin and season, (2) monitor the quality and toxicity of ballast water through oyster and algae bioassays and (3) assess the risk to the coastal waters of England and Wales associated with ballast water and sediment discharge. All major ports will be targeted and as of May 1997 the project had sampled 29 ships which included LNG/LNG carriers (9), bulk carries (5), general cargo (6), container ships (4) and ferries (2). The initial focus for the project will be on ballast water and sediments but could expand to include hull and sediment sampling during dry docking.

## **Polytechnic of Dubrovnik, Croatia**

Contact: Joseph Lovric

The Polytechnic of Dubrovnik has very recently commenced a ballast water project headed by Joseph Lovric but has not yet commenced field work. The project aims to investigate various methods of treating ballast water and destroying organisms during transit. They are particularly interested in the physico-chemical properties of ballast waters and how these factors impact on survival. The project will develop in three phases: (1) collecting baseline information on potential pest species whose introduction may result in significant problems once established, (2) research into and trialing of effective ship-board treatment (chemical or physical) for the "target" species and (3) implementation of accepted treatment on ships. As this project is only new no protocols or preliminary results are available.

**APPENDIX B: BALLAST WATER RESEARCH QUESTIONNAIRE****1 Personal details**

- 1.1 Name:
- 1.2 Institution:
- 1.3 Country:
- 1.4 Mailing address:
- 1.5 Telephone number:
- 1.6 Fax number:
- 1.7 E-mail address:
- 1.8 Project title:
- 1.9 Names of colleagues:
- 1.10 Project start date:
- 1.11 Project finish date (if appropriate):
- 1.12 International collaborations:

**2 Vessel details**

- 2.1 From what type(s) of ships have you collected ballast water samples? Please indicate with numbers approximately how many in each category you have sampled.

If yes please tick	Type of ship	# of ships sampled	# of tanks sampled	# of samples per tank	Port of origin	Origin of ballast
	Bulk carriers					
	Ore carriers					
	Woodchip carriers					
	Ore/Bulk/Oil carriers					
	LNG/LPG carriers					
	Oil tankers					
	Chemical tankers					
	Container ships					
	General cargo					
	Other: (give details)					
	Other:					
	Other:					
	Other:					

## 2.2 Where on these ships have you collected your samples?

If yes please tick	Location	Type of ship you sample at this location	Is this your preferred location?	Why do you sample in this location?
	sounding pipes			
	manholes			
	deck tap			
	ballast outflow			
	in line sampling			
	other: (give details)			
	other:			
	other:			
	other:			

## Sampling details:

Do you have any published material detailing your methods and results?  yes  no

(Please give references in the sections where it is most appropriate.)

Can you provide a copy of the report/paper/memo?  yes  no

Are you willing to provide details of your methodology and results?  yes  no

### 3 Sounding pipes

3.1 When sampling via a sounding pipe what method of collection do you use?

*If sounding pipes not sampled – go to section 4.*

If yes please tick	Method	Make/brand	# of samples	Volume of water per sample	Time per sample	Depth sample taken
	foot valve pump					
	peristaltic pump					
	stiff hose method					
	hand pump					
	Ruthner sampler					
	electric centrifugal					
	other: (give details)					
	other:					
	other:					

3.2 Why do you use this method? (e.g. convenience, lack of power etc.)

3.3 Is this your preferred method of sampling in this location?

## 3.4 Sample details:

If yes please tick	Type of sample	Mesh size/ volume filtered	Are sub samples taken? What volumes?
	filtered sample		
	whole water samples		
	other: (give details)		
	other		
	other		

## 4 Manhole sampling

4.1 When sampling via a manhole what sampling method do you use? Please state the name or brand of pump where applicable.

*If manhole not sampled – go to section 5.*

If yes please tick	Method	Make/brand or mesh size and ring diameter	# of samples	Volume of water per sample	Time per sample	Depth sample taken
	diaphragm pump					
	foot valve pump					
	peristaltic pump					
	stiff hose method					
	hand pump					
	Ruthner sampler					
	electric centrifugal					
	vertical net haul					
	other: (give details)					
	other:					
	other:					

4.2 Why do you use this method? (e.g. convenience, lack of power etc.)

4.3 Is this your preferred method of sampling in this location?

## 4.4 Sample details:

If yes please tick	Type of sample	Mesh size / volume filtered	Are sub samples taken? What volumes?
	filtered sample		
	whole water samples		
	other: (give details)		
	other		
	other		

## 5 Deck tap sampling (via fire pump)

5.1 When sampling via a deck taps what sampling method do you use? Please state the name or brand of pump where applicable.

*If deck tap not sampled – go to section 6.*

If yes please tick	Method	Make/brand	# of samples	Volume of water per sample	Time per sample	Depth sample taken
	foot valve pump					
	peristaltic pump					
	stiff hose method					
	hand pump					
	Ruthner sampler					
	electric centrifugal					
	other: (give details)					
	other:					
	other:					

5.2 Why do you use this method? (e.g. convenience, lack of power etc.)

5.3 Is this your preferred method of sampling in this location?

5.4 Sample details:

If yes please tick	Type of sample	Mesh size/ volume filtered	Are sub samples taken? What volumes?
	filtered sample		
	whole water samples		
	other: (give details)		
	other		
	other		

## 6 Outflow sampling

6.1 If you sample the ballast outflow please give details

*If outflow not sampled – go to section 7.*

Description of method used (please provide diagrams or photos if available):

Details	Answer	Comments
# of samples:		
Volume of water per sample:		
When is sample taken?		
Time per sample:		

6.2 Why do you use this method? (e.g. convenience, lack of power etc.)

6.3 Is this your preferred method of sampling in this location?

6.4 Sample details:

If yes please tick	Type of sample	Mesh size/ volume filtered	Are sub samples taken? What volumes?
	filtered sample		
	whole water samples		
	other: (give details)		
	other		
	other		

## 7 In-line sampling (main ballast pump)

7.1 If you "in-line" sample please give details and provide a diagram or photograph (if available) of your set up.

*If "in-line" sampling not carried out – go to section 8.*

Details	Answer	Comments
# of samples		
Volume per sample		
Time per sample		
When is sample taken? (ballasting or de-ballasting?)		
Other details		
Other details		

7.2 Why do you use this method? (e.g. convenience, lack of power etc.)

7.3 Is this your preferred method of sampling in this location?

7.4 Sample details:

if yes please tick	Type of sample	Mesh size / volume filtered	Are sub samples taken? What volumes?
	filtered sample		
	whole water samples		
	other: (give details)		
	other		
	other		

## 8 Biological and physical data

8.1 Do you collect zooplankton samples?  yes  no

*If zooplankton samples not collected – go to section 8.2.*

(a) Method used:

pump

net (if you have already given details what question should I refer to?)

other (give details)

If pump used:

Depth(s) sampled (m):

Sample type:

filter/sieve

Filter mesh size ( $\mu\text{m}$ ):

Filter/sieve design:

whole water sub sample

Volume (l):

other (give details):

(b) Fixative used for zooplankton (%):

(c) Do you assess viability / health of the plankton in samples?

Method used:

Vital stain? What stain?:

Did it stain only live material?  yes  no

Was it best for certain taxa?  yes  no

Which taxa?:

Live inspection

Video recording

Other (give details):

**8.4 Do you collect nutrient samples?**  yes  no

*If nutrient samples not collected samples – go to section 8.5.*

(a) Method of collection:

sub sample of method used for phytoplankton

pump (refer to question):

other (give details):

(b) Volume collected (l):

(c) Nutrients analysed:  NO<sub>3</sub>,  PO<sub>4</sub>,  total P,  total N,  other:

(d) Method of analysis:

(e) Can you provide details of analysis method?  yes  no

(f) Do you have any archived data?  yes  no

Maintained where?:

(j) Do you have any published material detailing your methods and results. (Please give full references):

**8.5 Do you take salinity measurements**  yes  no

*If salinity measurements not taken – go to section 8.6.*

(a) Method of collection/analysis

as above

Sub-sample volume (l):

Method of analysis:

Reference:

probe

Brand/make:

other (give details):

**8.6 Do you take oxygen measurements**  yes  no

*If oxygen measurements not taken – go to section 8.7.*

(a) Method of collection

as above

Sub sample volume (l):

Method of analysis:

Reference:

probe

Brand/make

other (give details):

**8.7 Do you record temperature**

yes  no

*If temperatures not recorded – go to section 8.8.*

(a) Method of collection

probe

other

Give details (e.g. depth measured / profile / single measurement):

**8.8 Do you collect microbial samples?**

yes  no

*If microbial samples not collected – go to section 8.9.*

(a) Method of collection:

whole water samples as with phytoplankton/nutrients

other (give details):

(b) Method of processing /fixation:

preserved in formalin

freezing (liquid nitrogen)

filtered, stained, frozen

other (give details):

Reference:

Please give details or references to your processing and analysis methods.

**8.9 Do you collect samples from the port community?** yes  no*If samples from the port community not collected – go to section 8.10.*

(a) What information?

 plankton samples zooplankton net; mesh size ( $\mu\text{m}$ ) and ring diameter (cm): other methods (give details): phytoplankton net; mesh size ( $\mu\text{m}$ ) and ring diameter (cm): other method : nutrients salinity temperature other information

Depth(s) sampled (m):

(b) When is sample generally taken?:

 opportunistically high tide  low tide time of ballasting other (give details):

Please give references for you methods if they differ to those details in the above sections.

**8.10 Ballast exchange****Have you tested the effectiveness of ballast water exchange** yes  no*If no, go to section 8.11.*

(a) What method did you use?

 dye

- bio beads (brand/make):
- other method of seeding (give details):
- water chemistry
- salinity
- isotope

Reference:

- (b) How effective was the method used?:

### 8.11 Vessel details

- (a) Do you collect vessel details?  yes  no

If yes please tick	Details
	Last port of call
	Last place ballasted
	Ballast log
	Ballast exchanged
	Length of voyage
	Taking on ballast
	Tides
	Rainfall
	Shipping traffic in the immediate area
	Other
	Other
	Other

- (b) Can you provide a photocopy/attachment of a questionnaire/form that you give to the vessel's master to fill out?  yes  no  not available

## 9 Future directions

Briefly state where your project is heading and further ballast water related research you plan to undertake:

Are you willing to be involved in collaborative research for this project and for future projects?  yes  no

Would you like to receive a copy of the review for your information?  yes  no

Who should we contact to check details and answer questions about your project (if necessary)?

## APPENDIX C: VESSEL SAMPLING – RESULTS AND ANALYSIS

### C1 Methods

#### C1.1 Vessels sampled

The various methods of sampling were tested by the opportunistic sampling of nine vessels; where possible as many methods as practicable were compared on each vessel. On all but two vessels, the MV *Tasmanian Enterprise* and MV *Iron Whyalla*, samples were collected while in port at either the beginning or end of a voyage. The MV *Tasmanian Enterprise* was sampled in Burnie, Tasmania and *en route* to New Zealand by Dr Julie Hall (NIWA). The MV *Iron Whyalla* sampled *en route* from Port Kembla to Port Hedland in conjunction with the ballast water heat treatment project (Rigby *et al.* 1998). The MV *Iron Sturt* was sampled in the port of Hobart and during two voyages *en route* between Hobart and Burnie, Geelong and Port Pirie. Six other ships were sampled in Newcastle as part of a risk assessment field survey. Table C.1 provides a summary of the vessels sampled during this project with information on the sampling methods tested on each vessel. The sampling methodologies were as described in section 4.2.2.

#### C1.2 Laboratory procedures

Preserved zooplankton samples were transferred to 70% alcohol one month after initial preservation in formalin diluted to 10% in seawater. Prior to laboratory examination, samples were rinsed in fresh water to remove alcohol and then sorted under a binocular dissecting microscope. Where possible entire samples were sorted, however, many samples required splitting using a Folsom plankton splitter (1:2 reduction), in particular those collected at the beginning of a voyage when counts were usually high (i.e. before significant mortality had occurred). The number of sample splits depended on the density of individual taxa present; in some cases different split fractions were required for copepods, chaetognaths and nauplii. In general, copepods were split 4–5x, nauplii 4–6x and chaetognaths 2x. The total number of individuals was estimated by the formula: count  $\times 2^n$ , where n = number of splits (Omori and Ikeda 1976).

Most organisms were identified to the lowest taxa possible. Decapods were identified to species "types". Copepods, when not identified to species, were grouped by size categories: small, medium and large calanoids (< 300, 300–750 and >750  $\mu\text{m}$  respectively); small, medium and large cyclopoids (< 400, 400–850 and >850  $\mu\text{m}$  respectively); medium and large harpacticoids (200–500 and >500  $\mu\text{m}$ ); and nauplii.

#### C1.3 Statistical analysis

Zooplankton densities were standardised to numbers per 100 l and, unless otherwise stated, logarithmically transformed ( $\ln(x+1)$ ). In most cases the logarithmic transformations provided the best fit of residuals versus fitted values. All analyses of total zooplankton densities were made with either fully factorial analyses of variance (ANOVA) or with a General Linear Model (GLM) depending on whether the sample sizes were even and orthogonal or uneven respectively. In cases where the data displayed uneven variances, paired t-tests for unequal variances were used. Total zooplankton was chosen for most comparisons as individual taxa density was usually too low to allow adequate comparisons. Densities of surrogate target taxa (polychaetes, crab zoea and megalopae, were compared

Table C.1. Summary of vessel information, ballasting and deballasting details, and tanks sampled.

Vessel	Origin of ballast	Ballasting date	Sampling date	Exchange location	Exchange date & time	Age of ballast	Ballast tanks and locations sampled
<i>Tasmanian Enterprise</i> containers	Devonport, Australia	January 11 1997	January 11 1997	not this tank	not applicable	1 day	# 3 forward and aft; manholes and sounding pipes
<i>Iron Whyalla</i> bulk carrier (coal/iron ore)	Port Kembla, Australia	April 5 1997	January 15-16 1997 April 6-8 1997	not before sampling not applicable	not applicable	4-5 days 0-2 days	#7 forward and aft; sounding pipes and air vents # 2 and # 3; port, in-line, manholes and sounding pipes
<i>Pacific Ocean</i> bulk carrier (grain)	New Zealand various ports	June 24-July 2 1997	July 15 1997	not applicable	not applicable	3-4 days	# 2 and # 3; fixed site, manholes and sounding pipes
<i>Eternal Wind</i> bulk carrier (coal/general)	Imary, Japan maiden voyage; (fresh waterballast)	June 19 1997	July 16 1997	45°22' N, 164°02' E 43°35' N, 159°20' E	0405, July 4 to 2100, July 4 1997	27 days; 18-19 days from exchange	# 3 and # 5; manhole, sounding pipes and in-line (deballasting tank #1 and 5) # 2 and # 4; manhole, sounding pipes and in-line (deballasting)
<i>Saryo Maru</i> bulkcarrier (coal)	Kudamatsu, Japan	June 13 1997	July 16 1997	09°28' N, 148°53' E 03°24' N, 151°04' E	0830-700 June 23 to 0830-1130 June 24 1997	33 days; 22-23 days from exchange	# 4 and # 2; manhole (no power available or access to engine room)
<i>Prince Field</i> bulk carrier (general cargo)	Osaka, Japan & Kunsan, Korea	June 25 1997, Osaka ; July 1 1997, Kunsan	July 17 1997	18°36' N, 136°17' E 08°12' S, 153°53' E	0800-2330, July 7 to 0700-2300, July 12 1997	22 days; 5-12 days from exchange	# 1 and # 4; manholes, sounding pipe and in-line (deballasting)
<i>New Harmony</i> bulk carrier (ore)	Newcastle	July 17 1997	July 17 1997	not applicable	not applicable		# 4 (TST and BT); manhole, sounding pipes and in-line (ballasting)
<i>Stella Benry</i> bulk carrier (grain)	Torna Komat, Japan	July 3 1997	July 18 1997	12°24' N, 150°09' E 04°34' S, 153°20' E	0800, July 9 to 0000, July 12 1997	15 days; 6-9 days from exchange	# 2 and # 4; sounding pipes and in-line (ballasting)
<i>Iron Sturt</i> bulk carrier	Hobart, Australia	September 4 1997	September 4 1997	not applicable	not applicable	0 days	# 3; manhole and sounding pipes
		January 16 1997	January 16, 19, 21 1997	not applicable	not applicable	1, 3, 5 days	# 3 forward; fixed site and manhole
		February 25 1997	February 25, 28, March 2 1997	not applicable	not applicable	1, 3, 5 days	# 3 forward; fixed site and manhole

when possible in the same way as total zooplankton density. Taxa richness was compared by the compilation of taxa lists for the various sampling methods.

## **C2 Results and analysis of the vessel survey**

Taxa diversity and density varied widely between vessels and was strongly influenced by the age of the ballast water; both taxa density and diversity declined with increased ballast age, a phenomenon reported in other ballast water studies (Rigby *et al.* 1997, Murphy 1997). This was particularly evident during the Newcastle sampling exercise where the ballast water age was greater than 20 days for most vessels sampled. Because of an industrial dispute, many of the vessels sampled had been at anchor for several weeks waiting for a berth to become available. It is not surprising then, given most ballast tank mortality occurs in the first week (Rigby *et al.* 1997), that both taxa density and richness was very low for these vessels.

Many of the ships were sampled in winter when both the density and diversity of wild plankton communities is lowest thereby reducing taxa richness and increasing plankton patchiness. An assessment of this seasonal influence was beyond the time frame of this study. All the ballast tank communities were dominated by copepods, however some tanks (except for the Newcastle vessels) contained sufficient surrogate target taxa, such as polychaetes and crab zoea (and decapod larvae in general), to permit evaluations of sampling efficacy for individual taxa.

### **C2.1 MV *Tasmanian Enterprise***

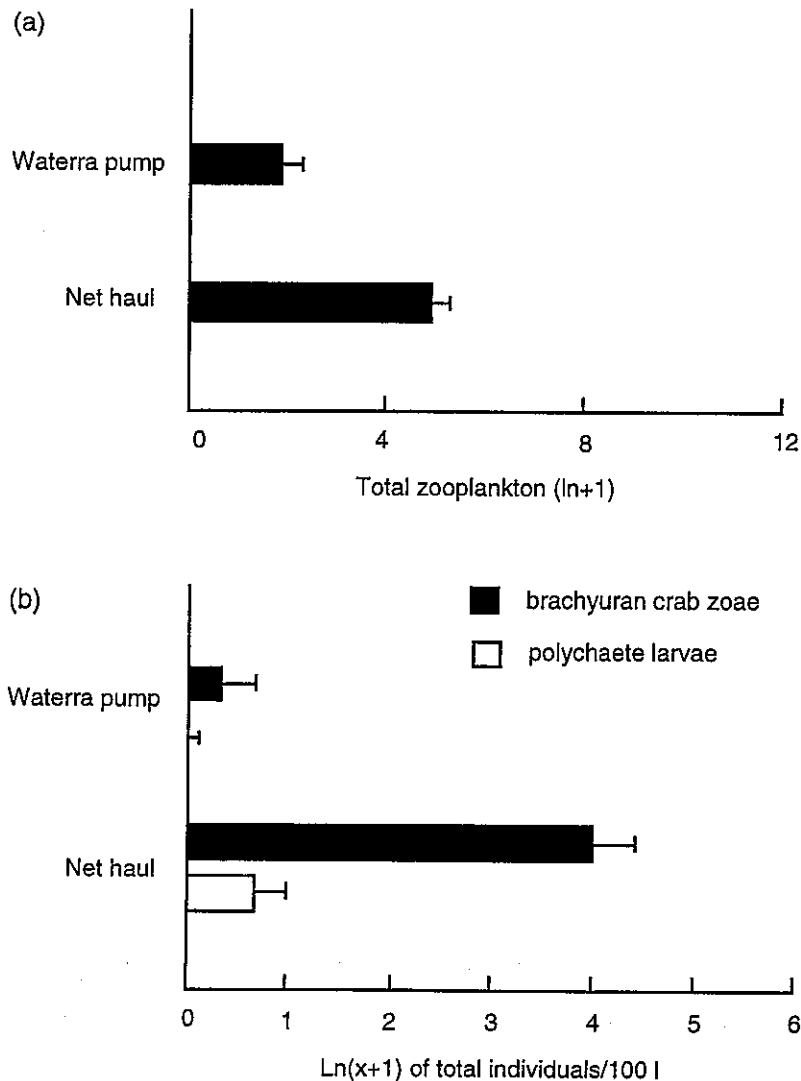
Sampling methodologies compared on the MV *Tasmanian Enterprise*:

Manholes:	vertical net hauls
Sounding pipes:	Waterra pump
Air vents:	Waterra pump

The three methods were compared in two separate analyses as collection took place on different days. Net haul samples were collected two hours after ballasting from #3 aft and forward, starboard and port tanks, respectively. The comparison between the sounding pipes and net hauls was conducted on #3 aft starboard tanks only as comparable samples from the other tanks for the sounding pipes were not available. The comparison of sounding pipes and air vents was conducted on samples collected from #7 aft and #7 forward tanks, five and six days after ballasting. The sounding pipe samples used for the comparison were part of series of samples taken for monitoring survival of zooplankton over the entire voyage as part of a joint NIWA/CRIMP study.

#### ***Manholes and sounding pipes comparison***

Total zooplankton density estimates were significantly lower ( $F_{[1,6]}=81.07$ ,  $p<0.001$ ) in sounding pipe samples than those taken with vertical net hauls via manholes (Figure C.1a). When broken up into individual taxa, the densities of crab zoea and shrimp larvae were significantly lower (two sample t-test for uneven variances,  $p<0.001$ , Figure C.1b) when sampled from sounding pipes than from manholes. Although not significant, all other taxa examined showed a similar trend.



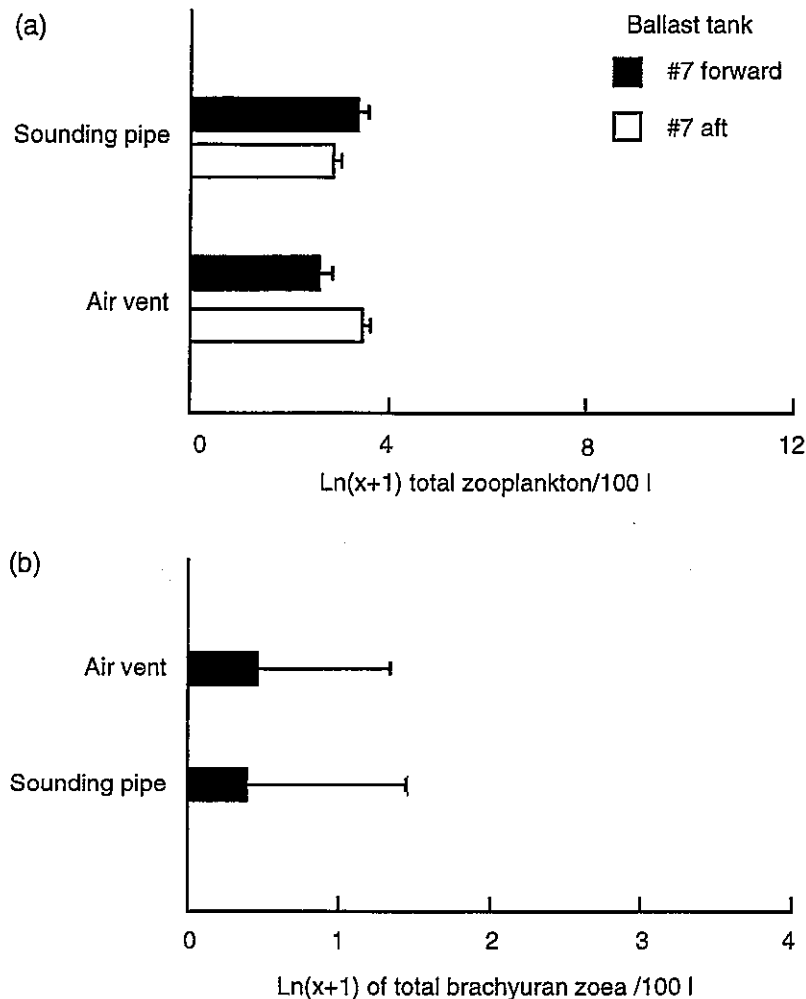
**Figure C.1.** Comparison of sounding pipe (Waterra pump) and manhole (net haul) samples from MV *Tasmanian Enterprise*, 11 January, 1997 with respect to (a) total density of zooplankton and (b) surrogate target taxa. Samples collected from #3 port and starboard ballast tanks. Standard errors are indicated.

Species richness was greater in samples collected by vertical haul net samples (8) than in those collected with the Waterra pump (5), however, this difference was not significant (two sample t-test using separate variances  $p=7.82$ ). Polychaetes and ostracods were present in very low numbers in net hauls and not present in the sounding pipe samples; the counts were too low to test if these taxa were preferentially selected by either method. However, the low densities of shrimp and crab zoea in sounding pipe samples (present in 100% of net tows but only 17–33% of sounding pipe tows; Table C.2) suggests that pump avoidance by larger taxa with relatively well developed swimming capabilities, such as the decapod larvae, resulted in the under sampling of such taxa by the Waterra pump.

#### ***Sounding pipe and air vents (Waterra inertia pump) comparison***

Densities had declined from pre-voyage levels, although total taxa richness for the samples increased from 6 to 7 taxa (Figure C.2a; Table C.3). There were no significant differences

between density or richness of total zooplankton collected from the sounding pipes and air vents ( $F_{[1,20]}=0.004$ ,  $p=0.68$  and two sample t-test  $p=0.56$ , respectively). Similarly crab zoea were not preferentially selected by either method (two sample t-test  $p=0.91$ ; Figure C.2b). This comparison was conducted over two days and "day" was found to be significant for total zooplankton density with densities declining over time. There was, however, no day x method interaction and samples were from the two days were pooled for comparisons. Densities of other taxa such as ostracods and polychaetes were too variable for statistical comparisons; differences in presence/absence of these taxa between the two methods were probably due to plankton patchiness rather than preferential selection.



**Figure C.2.** Comparison of sounding pipe and air vent samples taken with the Waterra pump from *MVTasmanian Enterprise*, 15–16 January 1997, with respect to (a) total zooplankton and (b) brachyuran crab zoea. Samples collected from #7 port and starboard, forward and aft ballast tanks. Standard errors are indicated.

**Table C.2.** Mean density and occurrence of taxa sampled via manholes (net haul) and sounding pipes (Waterra pump), MV *Tasmanian Enterprise*, 11 January 1997. The first set (n=11 and 6) includes all samples; the second set (n=3) includes only those samples taken from the #3 aft starboard tank. Nauplii are not included in taxa counts as they may be represented under class Copepoda.

Phylum	Class	Order	Lowest taxa	Net haul (n=11)		Waterra pump (n=6)		Net haul (n=3)		Waterra pump (n=3)		
				Mean	#	Mean	#	Mean	#	Mean	#	
Annelida	Polychaeta	Spionoida	polychaetes	1.35	6			2.71	2			
Arthropoda	Copepoda	Calanoida	calanoids - large	7.57	11			16.71	3			
			calanoids - medium	4.56	11	2.22	1	3.16	3	1.19	1	
			calanoids - small	4.59	11	2.80	3	4.18	3	1.19	1	
		Cyclopoida	cyclopoids - large	1.02	10	1.19	1	1.47	3	1.19	1	
			cyclopoids - medium	6.59	11	9.13	3	8.01	3	2.38	3	
			nauplii	29.77	11	3.87	5	35.44	3	1.19	3	
		Malacostraca	Decapoda	brachyuran zoea	42.45	11	8.10	2	45.38	3	1.19	1
				decapod shrimp zoea	2.46	11	1.19	1	3.84	3		
		Ostracoda		ostracods	0.34	2						
Cnidaria	Hydrozoa	Hydroida	cnidarians	0.34	1							
# of taxa (10)				10		6		8		5		
% of total taxa				100%		60%		80%		50%		

**Table C.3.** Mean density and occurrence of taxa sampled via sounding pipes and the air vents with the Waterra pump, MV *Tasmanian Enterprise*, 15–16 January 1997. Nauplii are not included in taxa counts as they may be represented under class Copepoda.

Phylum	Class	Order	Lowest taxa	Sounding pipes (n = 12)		Air vents (n = 8)		
				Mean	#	Mean	#	
Annelida	Polychaeta	Spionoida	polychaetes			1.67	1	
Arthropoda	Copepoda	Calanoida	calanoids - large	3.17	10	2.50	4	
			calanoids - medium	3.92	10	3.61	6	
			calanoids - small	1.46	4	1.33	5	
		Cyclopoida	cyclopoids - large	1.39	4	1.94	5	
			cyclopoids - small	6.60	12	5.83	7	
			nauplii	6.46	8	7.36	8	
		Malacostraca	Decapoda	brachyuran zoea	6.46	4	6.67	2
		Ostracoda		ostracods	0.83	1		
		# of taxa (9)				7		7
% of total taxa				87%		87%		

## C2.2 MV Iron Whyalla

Sampling methodologies compared on the MV *Iron Whyalla*:

Engine room	sea chest and ballast pump
Manholes:	net hauls and diaphragm pump
Sounding pipes:	Mono pump
Fixed position:	diaphragm pump
Port sample:	drop net

In addition to these comparisons sampling *en route* provided an opportunity to explore questions regarding tank homogeneity, sample volume and variations in sampling depth in sounding pipes.

### C2.2.1 Within-tank variability

#### *Ballast tank homogeneity*

There were no significant differences in zooplankton density or taxa richness between the three depths or between the port and starboard tanks (density:  $F_{[2,12]}=0.22$ ,  $p=0.81$ ,  $F_{[1,12]}=0.03$ ,  $p=0.87$ , richness:  $F_{[2,12]}=0.02$ ,  $p=0.89$ ,  $F_{[1,12]}=1.04$ ,  $p=0.41$  respectively) indicating that tank distributions were homogeneous. This result is at variance with the observations on the MV *Iron Sturt* by Murphy (1997) who found that some taxa (crustacean larvae) were more abundant in surface samples. Murphy, however, examined three depths, 0.25, 2 and 6 m, which were all included within the top fixed depth sampled on the MV *Iron Whyalla*. If stratification of crab larvae was occurring within the top 6 m of the ballast tank, the fixed site sampling on the MV *Iron Whyalla* would not have detected it. This explanation is supported by the fact that Murphy found the most striking differences in density was between the surface and 2 m depth. The higher occurrence of crustacean larvae in the surface waters on the MV *Iron Sturt* was most likely influenced by light which, by contrast, was completely excluded on the MV *Iron Whyalla*, and in the absence of which, larvae may not have had sufficient cues to orient with respect to depth.

#### *Sampling depth*

It has generally been assumed that pump sampling via sounding pipes will sample pipe and bottom water only and may not be representative of the ballast tank as a whole. However, given that sounding pipes are perforated it is not unreasonable to expect that samples may be representative of the depth at which the pump inlet pipe is positioned. To address this question, replicate samples were collected at three depths (5, 10, 20 m) from the #3 ballast water tank and compared using a one way ANOVA.

There were no significant differences in zooplankton density or richness between the three depths sampled ( $F_{[2,7]}=0.42$ ,  $p=0.68$  and  $F_{[2,7]}=0.39$ ,  $p=0.7$  respectively). Given that there was no difference with depth of fixed site samples (see ballast tank homogeneity above), the analysis was not able to determine if sounding pipe samples were indicative of bottom water only.

### **Sample volume**

Taxa richness increased with increased sample volumes and did not show any signs of levelling off even after sampling 4000 l of water. While increased volumes provided more representative samples, most surrogate target taxa were sampled in the first 500 l.

#### **C2.2.1 Method comparison**

Fixed site samples were collected from the #2 ballast water tank, the manholes of which were securely closed (see section 4.2.2). This precluded sampling with vertical net hauls so net samples were taken through the manholes on #3 tank. Both tanks were ballasted within five hours of each other on the same night, and were considered to be the most uniform of all the tanks on the ship. To establish any differences between the tanks that might preclude their use in method comparisons, sounding pipe samples taken from each of the tanks on two separate days were compared. There were no significant differences between the two tanks for both species density and richness ( $F_{[1,12]}=0.05$ ,  $p=0.83$  and  $F_{[1,12]}=2.1$ ,  $p=0.19$  respectively). Subsequent comparisons were conducted without including "tank" as a factor in the analyses.

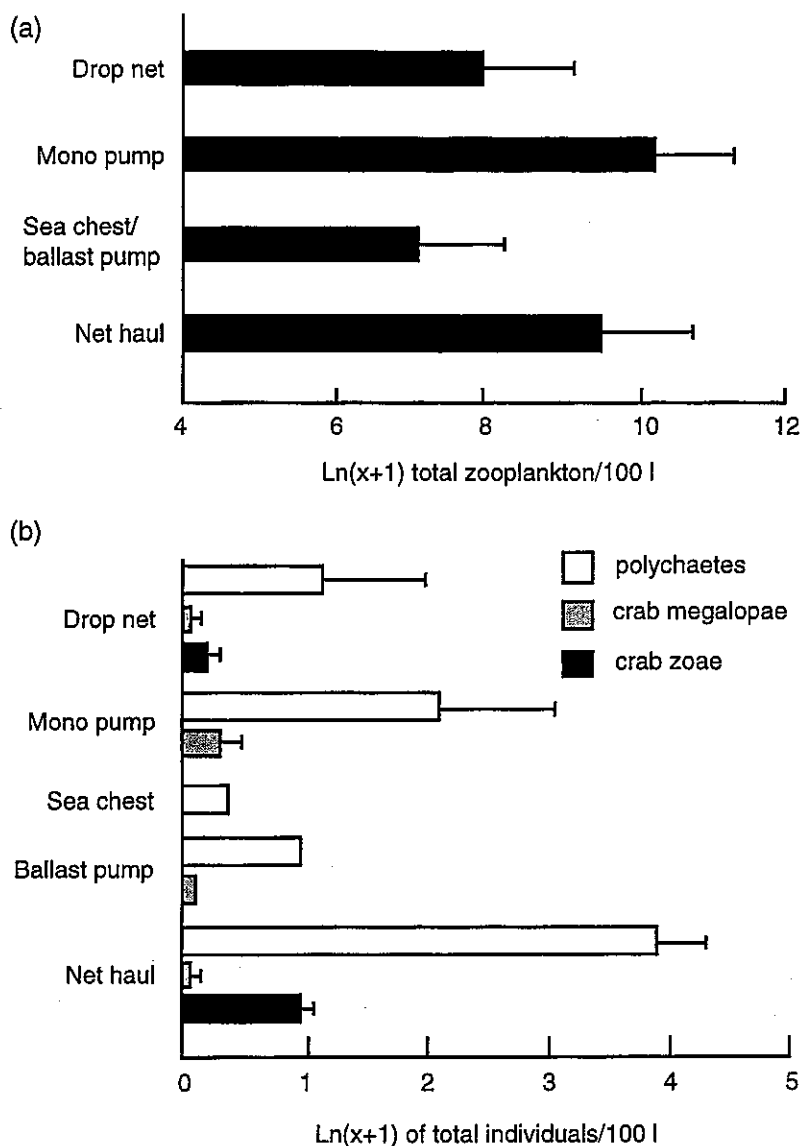
Sampling was carried out as described in section 4.2.2. Unfortunately, not all the trials could be conducted on the same day. Therefore, two analyses were conducted: the first compared engine room (sea chest and ballast pump), sounding pipe, port and manhole net samples collected 6–8 April; the second, compared fixed depth (top depth only), sounding pipe, vertical net haul, and diaphragm pump samples collected 14–15 April.

#### **Port, manhole, ballast pump/sea chest, sounding pipe comparison**

The sea chest and ballast pump samples were pooled as there was little difference in density between the two methods, Combining data for these two methods enabled statistical comparisons to be made with the other methods by providing replicates of samples taken in the engine room. There was a significant difference between the methods ( $F_{[3,14]}=14.71$ ,  $p<0.01$ ). *Post hoc* pair-wise comparisons indicated that estimates of overall taxa density were highest for samples collected from ballast tanks (sounding pipes and manholes) and that engine room and drop net samples were not significantly different (Figure C.3a).

For individual taxa, there was a tendency for polychaete larvae, crab zoea to occur in higher densities in net haul samples, while crab megalopae were better represented in Mono pump samples (Figure C.3b). Uneven sample variances precluded statistical comparison of these trends. Average densities of target taxa surrogate species are shown in Table C.4.

The percentage of taxa present in samples collected by the various methods was relatively low (39–63%) indicating considerable between sample variability (Table C.4). Species richness was greatest in the port samples (29) followed by the Mono pump samples (27), ballast pump samples (26), vertical net haul samples (24), with the lowest species richness (19) occurring in the sea chest samples. However this difference was only significant for the engine room/Mono pump comparison (pairwise comparison,  $F_{[3, 14]}=12.29$ ,  $p<0.01$ ). In general, net haul samples contained more larger taxa, such as crab and shrimp larvae, while polychaete larvae were adequately sampled by all methods (with higher densities for net samples).



**Figure C.3.** Comparison of Mono pump (sounding pipe), net haul (manhole), engine room (sea chest and ballast pump) and drop net (port) samples from the MV *Iron Whyalla*, 6–8 April 1997, with respect to (a) total zooplankton and (b) surrogate target taxa. Samples collected from #2 and #3 ballast water tanks. Standard errors are indicated.

#### ***Fixed site, manhole (net haul, diaphragm) and sounding pipe***

Densities estimates and taxa richness were significantly higher in both manhole samples (net and pump) compared to the Mono pump (sounding pipe) and fixed site (pooled top and bottom depth only) samples ( $F_{[3,23]}=15.88$ ,  $p<0.001$ ,  $F_{[3,23]}=7.79$ ,  $p = 0.001$  respectively; Figure C.4a). Intra-sample variability precluded rigorous statistical analysis of the individual taxa, however, the same trend as above was evident for individual taxa such as polychaetes, crab zoea, crab megalopae and chaetognaths with densities higher in the manhole samples (highest in manhole net hauls). Densities of surrogate target taxa are shown Figure C.4b).

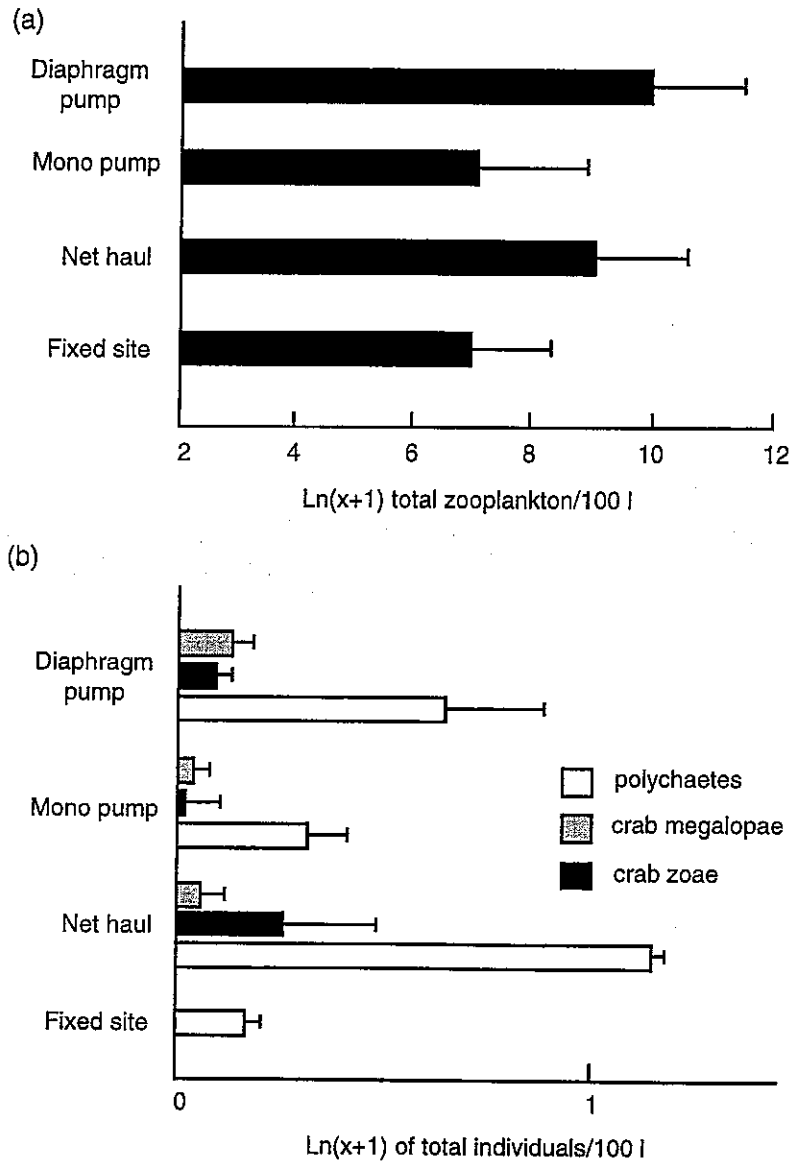
The Mono pump in this instance did not sample as efficiently in comparison to net hauls (with the exception of decapods, which were consistently under sampled). This suggests

Table C.4. Average density and occurrence of taxa sampled from the engine room (ballast pump and sea chest), port plankton community (drop net), sounding pipes (Mono pump), manholes (net haul), MV Iron Whyalla, 6-8 April 1997. Nauplii are not included in taxa counts as they may be represented in the class Copepoda.

Phylum	Class	Order	Lowest taxa	Drop net (n = 3)	Sea chest (n = 1)	Ballast pump (n = 1)	Mono pump (n = 5)	Net haul (n = 4)		
				Mean #	Mean #	Mean #	Mean #	Mean #		
Annelida	Polychaeta	Spionida	polychaetes	5.06	0.41	1.68	26.95	48.54		
			Cladocera	0.71	1.19	1	0.71	2		
Arthropoda	Cirripedia	Calanoida	barnacle cyprids	143.88	13.57	37.83	0.76	478.57		
			cirripedia nauplii			1	4.78	3		
	Copepoda			calanoids - large	880.15	85.78	20.94	2.39	23.04	
				calanoids - medium	156.83	4.51	148.54	2691.3	2026.19	
				calanoids - small			1	891.86	3	
				cyclopoids - large			1	20.94	3	
				cyclopoids - medium			1	7.11	1	
				cyclopoids - small			1	21.73	4	
				harpacticoid sp 1			1984.4	192.96	263.90	11452.02
				harpacticoid sp 2			55.00	0.03	7.11	1566.98
				nauplii			57.32	4.27	43.75	14253.49
				amphipods				14.50	0.10	6.53
Malacostraca			gammarid amphipods			0.40		9097.62		
			caprellid amphipods							
			cumaceans		0.03		0.76	1		
			brachyuran megalopae sp 1			0.10		0.94	2	
			brachyuran megalopae sp 4					0.17	2	
			brachyuran zoea sp 1			0.23			0.20	
			brachyuran zoea sp 2						1.32	
			brachyuran zoea sp 3						0.20	
			brachyuran zoea sp 4			0.19			0.40	
			penaid shrimp			1.78	0.03	0.49	0.71	1.19
						2	1	1	2	1



that sampling efficiency was reduced after tank densities had declined. This was shown by the relatively high intra-sample variability for both Mono pump and fixed site samples which resulted in an lower average taxa richness for these methods, despite the combined value for species richness being similar to net hauls (Table C.5). Although the diaphragm pump sampled the greatest number of taxa, many of these taxa (particularly certain decapod species) were rare and present in less than 20% of the samples. This difference could result from a situation where a higher number of taxa are distributed in the top 25 cm of the tank (perhaps attracted by light entering through the manhole); samples taken by the diaphragm pump would therefore contain more taxa than net hauls which sample over a greater depth range.



**Figure C.4.** Comparison of diaphragm pump (manhole), Mono pump (sounding pipe), net haul (manhole) and diaphragm pump (fixed site) samples from the MV *Iron Whyalla*, 14–15 April 1997, with respect to (a) total zooplankton and (b) surrogate target taxa. Samples collected from #2 and #3 ballast water tanks. Standard error are indicated.

**Table C.5. Average density and occurrence of taxa sampled via manholes (diaphragm pump and net haul), sounding pipes (Mono pump) and fixed sites (diaphragm pump), MV Iron Whyalla, 14-15 April 1997. Nauplii are not included in taxa counts as they may be represented in the class Copepoda.**

Phylum	Class	Order	Lowest taxa	Diaphragm (n = 5) Mean #	Mono pump (n = 4) Mean #	Net haul (n=4) Mean #	Fixed bottom (n = 4) Mean #	Fixed middle (n= 4) Mean #	Fixed top (n =4) Mean #		
Annelida	Polychaeta	Spionoida	polychaetes	8.25	1.51	14.04	1.72	0.87	1.34		
			calanoids - large	3.37	1.09	6.55	1.10	0.22	1.41		
Arthropoda	Copepoda	Calanoida	calanoids - medium	567.79	86.82	246.56	63.00	46.78	106.46		
			calanoids - small	359.62	58.71	333.33	27.97	15.65	51.82		
			cyclopoids - medium	188.37	772.26	197.88					
			cyclopoids - small	5454.77	1074.46	3240.21					
			nauplii	8060.01	840.27	4440.21	1046		762.62	2111.07	
		Malacostraca	Decapoda	brachyuran megalopae	0.77						
				brachyuran megalopae sp 1	0.18	0.67	0.40				
				brachyuran megalopae sp 2	0.26						
				brachyuran megalopae sp 3	0.26						
				brachyuran megalopae sp 5	0.26						
Chaetognatha	Ostracoda	Hydrozoa	brachyuran zoea sp 1	0.29		1.79					
			brachyuran zoea sp 3	0.51							
			decapod shrimp zoea sp 2	0.51	0.33	0.40				0.09	
			decapod shrimp zoea sp 3			0.20					
			ostracods	6.60	1.06	3.37	0.68	0.32		0.89	
			chaetognaths	99.32	15.29	202.80	28.1	19.77		53.56	
			cnidarians	2.11	0.28	0.30					
			bivalves	7.83	1.42	5.12	0.26	0.21		0.20	
			gastropods	0.26			0.25			0.09	
			Nemertea								
			nemertea	0.11							
# of taxa (22)				20	13	14	8	7	9		
% of total taxa				91%	58%	64%	36%	32%	41%		

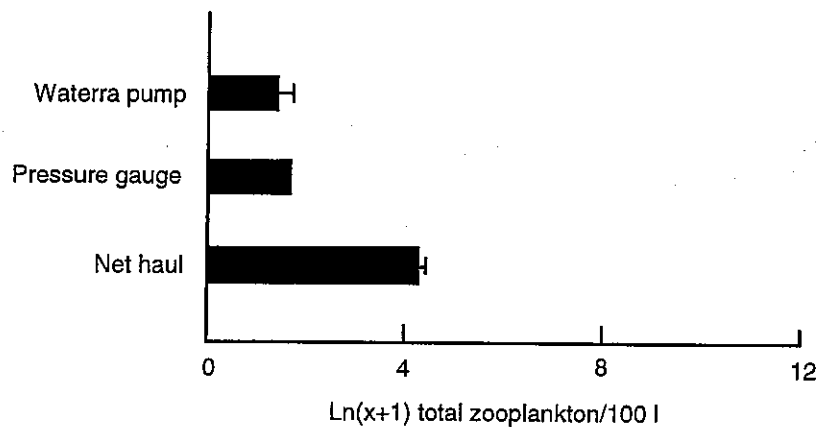
## 2.3 Pacific Ocean

Methods compared on MV *Pacific Ocean*:

Sounding pipes:	Waterra pump
Manholes:	net hauls
Engine room:	pressure gauge

All the methods tested on the MV *Pacific Ocean* were sampled on the same day as part of the Newcastle field sampling exercise. Samples for the comparison of the Waterra pump and net hauls were taken from the #3 and #5 port and starboard topside tanks, the pressure gauge sample was collected during the deballasting of the #1 and #5 double bottom tank. The comparisons were justified on the basis that the tanks were ballasted at the same time.

Total zooplankton densities in Waterra pump samples were significantly lower than those in net haul samples ( $F_{[1,10]}=154.88$ ,  $p<0.001$ ; Figure C.5). There was also a significant tank effect ( $F_{[1,10]}=11.43$ ,  $p<0.02$ ) with density estimates from both methods lower in #5 topside tank. There was no significant interaction between tank and method ( $F_{[1,10]}=0.39$ ,  $p=0.56$ ). Zooplankton density in the pressure gauge sample was comparable to that from the Waterra pump, but far lower than that in net haul samples (Figure C.5).



**Figure C.5.** Comparison of Waterra pump (sounding pipe), pressure gauge and net haul (manhole) samples from the MV *Pacific Ocean*, 15 July 1997 with respect to total zooplankton. Samples collected from #3 and #5 ballast water tanks. Standard errors are indicated.

Comparisons of species richness (Table C.6) indicated that the net hauls sampled a significantly greater number of taxa than the Waterra pump ( $F_{[1,10]}=18.52$ ,  $p=0.005$ ) and there was no tank or interaction effect ( $F_{[1,10]}=0.007$ ,  $p=0.94$  and  $F_{[1,10]}=2.38$ ,  $p=0.17$  respectively). The taxa richness observed on MV *Pacific Ocean* was low and was dominated by copepods. No target taxa were found in samples taken on this vessel. Net hauls sampled a greater diversity of taxa than either the pressure gauge or the Waterra pump. This result is probably a reflection on the low volume sampled with Waterra pump as compared to the net hauls, and avoidance of the pump by some taxa (chaetognaths) because of the low pumping rates.

**Table C.6.** Average density and occurrence of taxa sampled via manholes (net haul), pressure gauge and sounding pipes (Waterra pump), MV *Pacific Ocean*, 15 July 1997. Nauplii are not included in taxa counts as they may be represented in the class Copepoda.

Phylum	Class	Order	Lowest taxa	Net haul (n = 6)		Pressure gauge (n = 1)		Waterra pump (n = 4)		
				Mean	#	Mean	#	Mean	#	
Arthropoda	Copepoda	Calanoida	calanoids - large	1.23	6			3.33	1	
			calanoids - medium	3.31	5	1.23	1	2.78	3	
			calanoids - small	1.79	4					
		Cyclopoida	cyclopoids - medium	3.97	4					
			cyclopoids - small	9.60	5	0.62	1	0.67	1	
		Harpacticoida	harpacticoids	1.69	5	1.23	1	1.17	2	
			nauplii	57.47	6	1.23	1			
		Malacostraca	Isopoda	cryptoniscids			1.23	1		
			Tanaidacea	tanaids	0.26	1				
		Chaetognatha		chaetognathids	0.26	1				
# of taxa (10)				8		4		3		
% of total taxa				80%		40%		30%		

## 2.4 *Eternal Wind*

Methods compared on the MV *Eternal Wind*:

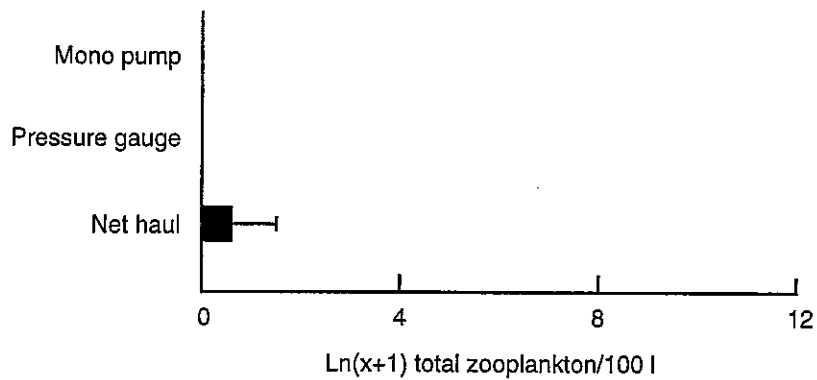
Sounding pipes: Mono pump

Manholes: net hauls

Engine room: pressure gauge

All methods compared on the MV *Eternal Wind* were collected from the #4 ballast water tank; nets hauls were also taken from the #2 tank. No statistical comparisons could be made between methodologies used on this ship because no plankton were found in the samples collected from the Mono pump or the pressure gauge. Samples collected via the nets from the manholes had extremely low densities (Figure C.6) and consisted of copepods, which were probably oceanic species (Table C.7).

This was the maiden voyage for this vessel and upon departure from Japan the ballast tanks were filled with fresh water, the ship had undergone a mid-ocean exchange and based on the salinity readings (33.2‰) the exchange was reasonably complete. The low densities were most likely the result of a mortality induced by low salinity levels during the early stages of ballast water exchange. Only in the latter stages of the exchange, when salinities had increased, would the oceanic plankton have been able to survive.



**Figure C.6.** Comparison of Mono pump (sounding pipe), pressure gauge and net haul (manhole) samples from the MV *Eternal Wind*, 16 July 1997 with respect to total zooplankton. Samples collected from #2 and #4 (manhole) and #3 (sounding pipe) ballast water tanks. Standard errors are indicated.

**Table C.7.** Average density and occurrence of taxa sampled via manholes (net haul), pressure gauge and sounding pipes (Mono pump), MV *Eternal Wind*, 16 July 1997.

Phylum	Class	Order	Lowest taxa	Net haul (n = 6)		Pressure gauge (n=1)		Mono pump (n=4)	
				Mean	%	Mean	%	Mean	%
Arthropoda	Copepoda	Calanoida	calanoids - medium	0.84	6				
		Cyclopoida	cyclopoids - medium	0.42	6				
			cyclopoids- small	0.42	6				
# of taxa (6)				3		-		-	
% of total taxa				100%		0%		0%	

## 2.5 Sanyo Maru

On the MV *Sanyo Maru* there was no power available for sounding pipe samples and no access to the engine room. Net hauls were taken via manholes from #4 and #2 port and starboard ballast water tanks. No zooplankton were found in net haul samples taken from either tank. This could be attributed to ballast age, which was 33 days from ballasting and 23 days from mid-ocean exchange, the oldest ballast of all the ships surveyed.

## 2.6 Prince Field

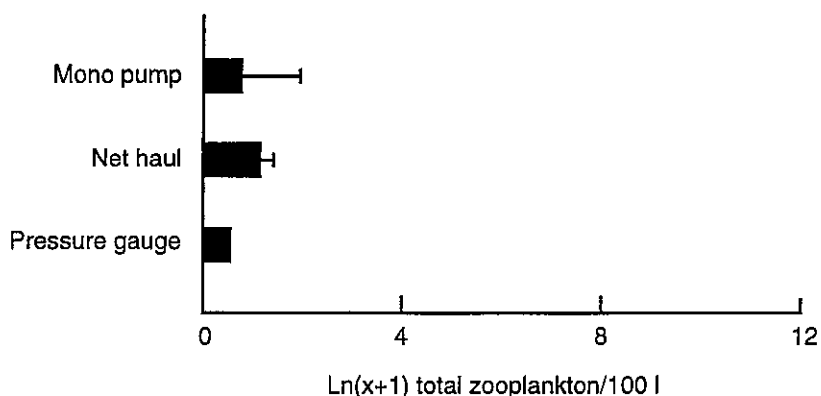
Methods compared on the MV *Prince Field*:

Sounding pipes: Mono pump  
 Manholes: net hauls  
 Engine room: pressure gauge

Samples from the MV *Prince Field* were collected on the same day from the #1 and #4 port and starboard ballast water tanks; both tanks were deballasted together and the pressure

gauge sample was a combination of water from both tanks. The statistical analysis included only the #4 ballast water tank as deballasting of #1 tank occurred before net hauls were taken.

Species richness was greatest for the net haul samples (Table C.8) but was very low and consisted entirely of copepods. The net hauls filtered more water than the pumped samples which most likely contributed to higher taxa richness in net haul samples. Most of the copepods sampled were very small and it is unlikely that they would have been able to avoid the Mono pump; smaller volumes rather than avoidance is the most likely explanation of the difference in taxa richness between methods.



**Figure C.7.** Comparison of Mono pump (sounding pipe), net haul (manhole) and pressure gauge samples from the MV *Prince Field*, 17 July 1997, with respect to total zooplankton. Samples collected from #1 and #4 ballast water tanks. Standard errors are indicated.

**Table C.8.** Average density and occurrence of taxa sampled via sounding pipes (Mono pump), manholes (net haul) and pressure gauge, MV *Prince Field*, 17 July 1997. Nauplii are not included in taxa counts as they may be represented in the class Copepoda.

Phylum	Class	Order	Lowest taxa	Mono pump (n = 4)		Net haul (n = 6)		Pressure gauge (n = 1)	
				Mean	%	Mean	%	Mean	%
Arthropoda	Copepoda	Calanoida	calanoids - large					0.14	1
			calanoids - medium			0.32	3	0.14	1
			calanoids - small			0.16	3	0.14	1
		Cyclopoida	cyclopoids - medium			0.16	3		
			cyclopoids - small	1.33	4				
		Harpacticoida	harpacticoids - medium			1.11	3		
			nauplii			0.16	6	0.14	1
	Ostracoda		ostracods			0.32	3		
# of taxa (7)				1		5		3	
% of total taxa				14%		72%		43%	

## 2.7 Stella Benny

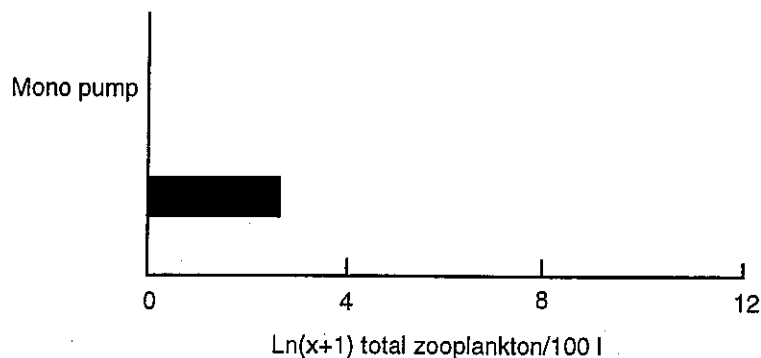
Methods compared on the *MV Stella Benny*:

Sounding pipes: Mono pump

Engine room: pressure gauge

On the *MV Stella Benny* samples for comparison of both methods were taken from the #2 and #4 port and starboard ballast water tanks. Net hauls were not collected as the manholes were cemented over.

No zooplankton were found in the samples collected via the sounding pipes. Densities of plankton collected via the pressure gauge were relatively low (Figure C.8). Species richness was also low (Table C.9), the pressure gauge sample containing only copepods. It is unclear why densities were so low for this vessel as the age of ballast was only 15 days from last port of call and 6–9 days after ballast exchange.



**Figure C.8.** Comparison of the Mono pump (sounding pipe) and pressure gauge samples from the *MV Stella Benny*, 18 July 1997, with respect to total zooplankton. Samples collected from #2 ballast water tank.

**Table C.9.** Average density and occurrence of taxa sampled via the sounding pipes (Mono pump) and pressure gauge, *MV Stella Benny*, 18 July 1997.

Phylum	Class	Order	Lowest Taxa	Mono pump (n = 4)		Pressure gauge (n = 1)	
				Mean	#	Mean	#
Arthropoda	Copepoda	Calanoida	calanoids - large			2.65	1
			calanoids - small			2.65	1
		Cyclopoida	cyclopoids - small			4.41	1
		Harpacticoida	harpacticoids - medium			3.53	1
			# of taxa (4)	–		4	
			% of total taxa	0%		100%	

## 2.8 New Harmony

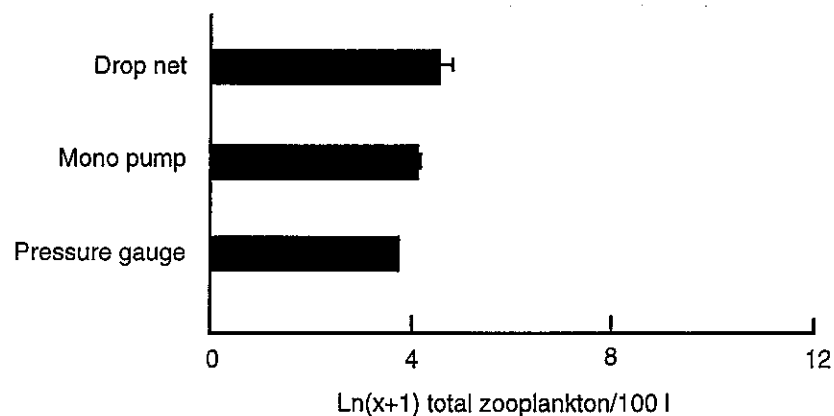
Methods compared on the MV *New Harmony*:

Sounding pipes: Mono pump  
 Engine room: pressure gauge  
 Port community: drop net

Pressure gauge and drop net samples were collected from the MV *New Harmony* while the ship was ballasting the #4 topside tank. Mono pump samples were collected from the same tanks one hour after ballasting.

Because of uneven sample variances a two sample t-test (using separate variances) was used to compare sampling methods. There was no significant difference between drop net and Mono pump samples ( $p=0.145$ ). The species density estimated by the pressure gauge sampling was lower but in the same range as the other two methods (Figure C.9).

In terms of taxa richness, the drop nets sampled significantly more taxa than either the Mono pump ( $F_{[1,7]}=16.59$ ,  $p=0.01$ ) or the pressure gauge; samples from the pressure gauge and Mono pump contained a similar number of taxa (Table C.10). The main difference in taxa composition between the methods was the absence of decapods from the pressure gauge and Mono pump samples. This result was probably a sampling artefact attributable to avoidance and low sample volumes rather than to the absence of these taxa in the ballast tanks.



**Figure C.9.** Comparison of drop net (port), Mono pump (sounding pipe) and pressure gauge samples from the MV *New Harmony*, 17 July 1997, with respect to total zooplankton. Samples collected from #4 ballast water tank. Standard errors are indicated.

**Table C.10.** Average density and occurrence of taxa sampled from the port water (drop net), sounding pipes (Mono pump) and pressure gauge, MV *New Harmony*, 17 July 1997. Nauplii not included in taxa counts as they may be represented in the class Copepoda.

Phylum	Class	Order	Lowest taxa	Drop net (n = 3)		Mono pump (n = 4)		Pressure gauge (n = 1)		
				Mean	#	Mean	#	Mean	#	
Annelida	Polychaeta	Spionoida	polychaetes	0.15	3					
Arthropoda	Copepoda	Calanoida	calanoids - large	23.74	3	14.33	4	2.67	1	
			calanoids - medium	34.33	3	19.33	4	16.67	1	
			calanoids - small	16.41	3	7.00	4	13.33	1	
		Cyclopoida	cyclopoids - large			2.00	2			
			cyclopoids - medium	2.37	3	2.00	2	0.67	1	
			cyclopoids - small	9.52	3	11.00	4	4.67	1	
		Harpacticoida	harpacticoids - medium	3.06	2	0.67	2	0.67	1	
			nauplii	13.93	2			3.33	1	
		Malacostraca	Decapoda	brachyuran zoea	0.14	2				
				decapod shrimps	0.42	2				
				decapod shrimp larva	0.11	1				
				decapod shrimp zoea	0.15	1				
				Tanaidacea	tanaids	0.10	1			
Chaetognatha			1.74	3	8.00	4				
Cnidaria	Hydrozoa	Hydroida	cnidarians	0.09	3					
Ctenophora				0.19	1					
Nemertea				0.04	2					
# of taxa (16)				16		8		6		
% of total taxa				100%		50%		38%		

## 2.9 Iron Sturt

Methods compared on the MV *Iron Sturt*

Sounding pipes: Waterra and Mono pump

Manholes: net hauls

Fixed sites: pneumatic pump

Method comparisons on the MV *Iron Sturt* were all carried out on the same day using the #3 forward port and starboard tanks. The fixed site sampling was conducted using the ship's pneumatic pump. The diaphragm pump, normally used for fixed site sampling (Murphy 1997) was not used as the ship was taking on acid and the use of an electrically powered pump was considered dangerous (see section 4.2.2). For the purpose of the method comparison the top two fixed depths were used in the analysis as the 7 m site was beyond the reach of the net.