

## SHORT COMMUNICATION

Heterogeneous zooplankton distribution  
in a ship's ballast tanksKATE R. MURPHY<sup>1\*</sup>, DAVID RITZ<sup>1</sup> AND CHAD L. HEWITT<sup>2</sup><sup>1</sup>DEPARTMENT OF ZOOLOGY, UNIVERSITY OF TASMANIA, HOBART, TASMANIA, AUSTRALIA, AND <sup>2</sup>CENTRE FOR RESEARCH ON INTRODUCED MARINE PESTS, C.S.I.R.O. MARINE RESEARCH, HOBART, TASMANIA AUSTRALIA 7001

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*We examined the vertical distribution of zooplankton in a ship's ballast water. Some crustacean taxa were more abundant at 0.5 m and 2 m than at 6 m depths. Non-random distributions affect population size estimations and are an important consideration for invasion risk assessment.*

The unintentional transport of aquatic organisms with ships' ballast water has apparently enabled numerous species to expand their ranges to environments in which they did not evolve (Carlton 1998; Cohen and Carlton, 1998; Ruiz *et al.*, 2000). In normal trading operations, ships load seawater ballast in one port, then discard it at another in exchange for cargo. Modern international voyages span just a few days or weeks, affording entrained organisms a unique opportunity for transport to biogeographically remote locations which would otherwise be rarely accessible.

Severe, and potentially irreversible, impacts of introduced aquatic organisms have been demonstrated in many parts of the world, for example, the zebra mussel *Dreissena polymorpha* in the Great Lakes of North America (Roberts, 1990; Strayer *et al.*, 1998), and the toxic dinoflagellate *Gymnodinium catenatum* in southern Australia (Bolch and Hallegraeff, 1990). The identification of ballast water as a probable dispersal mechanism for these and numerous other exotic organisms has prompted numerous international agencies to set-up ballast water sampling programmes. However, recent surveys reveal the use of a wide range of zooplankton sampling equipment and methodologies (Sutton *et al.*, 1998; Gollasch, in preparation); including pump sampling via sounding pipes (Hay *et al.*, 1996), bucket hauls (Gollasch *et al.*, 2000) and full-depth (Wonham *et al.*, 2001) and near-surface (Ruiz *et al.*, 2000; Smith *et al.*, 2000) plankton net tows.

The assessment of risk associated with ballast water

discharge depends on reliable knowledge of the identity, viability and quantity of its inhabitants (Hayes and Hewitt, 1998). Implicit in any estimation of the quantity of organisms in a ballast tank is the assumption that the samples provide an unbiased estimate of the zooplankton densities throughout the tank. While the difficulties inherent in obtaining quantitative and representative samples of ballast organisms have long been recognized (Carlton, 1982, 1985; Gollasch *et al.*, 2000), even basic assumptions relating to zooplankton distribution have remained untested.

Vertical concentration gradients of zooplankton are well studied in open water [e.g. (Pearre, 1973; Lampert and Taylor, 1985)]. However, several factors peculiar to ballast tank environments might be expected to distinguish the distribution of their zooplankton populations—first, the complete absence of light; second, a potentially diminishing food supply imposed by the isolation of the tank from external inputs and third; relatively rapid changes in water temperature associated with voyages between different latitudes [e.g. (Carlton, 1982; Gollasch *et al.*, 2000)].

We assessed vertical trends in zooplankton abundance within the ballast tanks of the MV 'Iron Sturt' against the null hypothesis that zooplankton are randomly distributed in order to identify potential problem areas for quantifying zooplankton in ballast water.

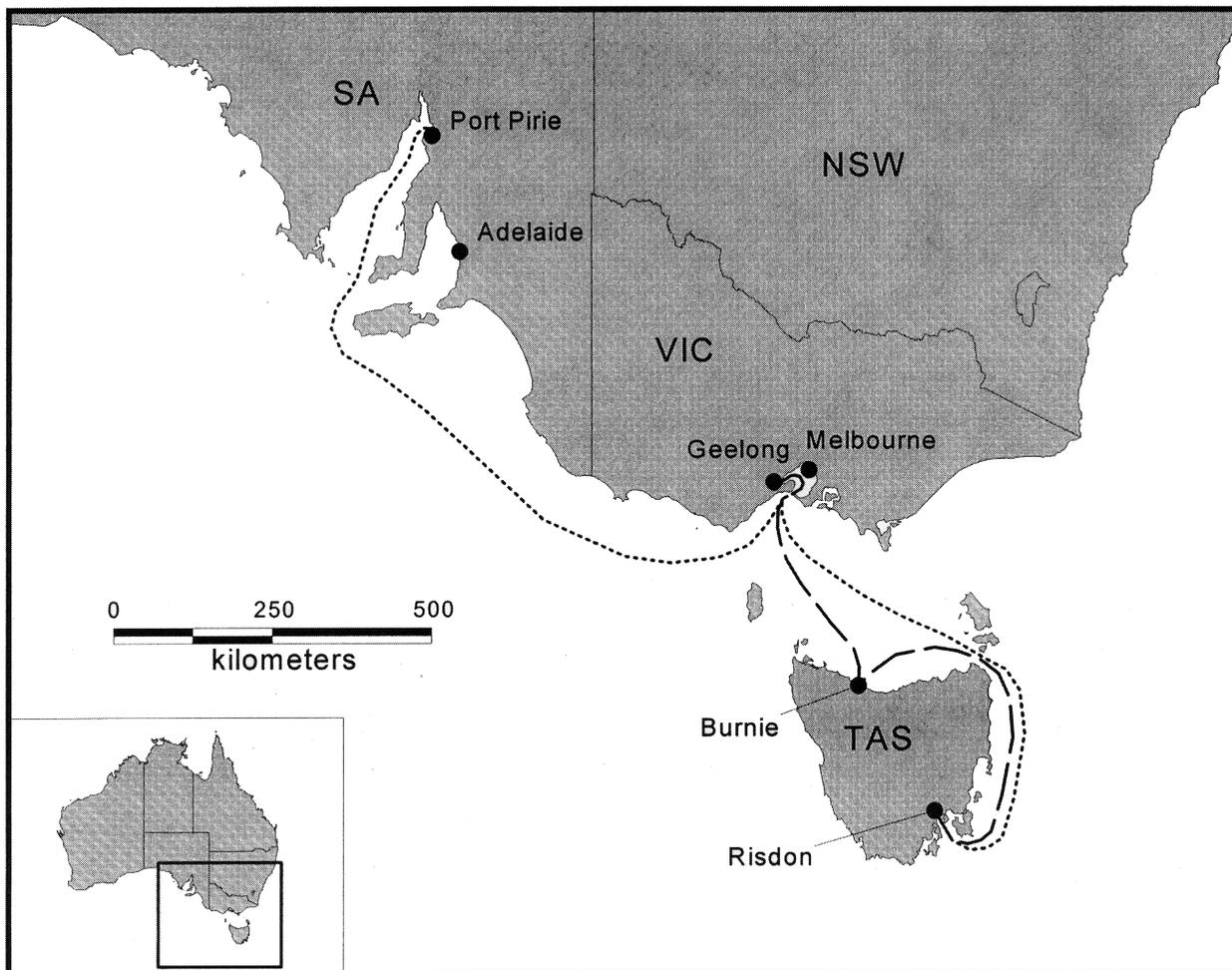
Ballast water sampling was conducted on the bulk carrier MV 'Iron Sturt' during two commercial voyages

on January 15–19 and February 24–28, 1997 (hereafter referred to as voyages 1 and 2, see Figure 1). In both cases, ballast water carried by the vessel originated from the Derwent Estuary at Risdon, in southern Tasmania (intake depth 3–4 m). Ballast water was drawn from the Risdon dock during the rising tide (voyage 1) or the high tide (voyage 2). After leaving Risdon, the vessel travelled to Geelong before discarding ballast in the Spencer Gulf near Port Pirie (voyage 1) or to Burnie before discarding ballast in the port of Geelong (voyage 2). Weather conditions on both voyages were calm except during the crossing of Bass Strait en route to Geelong. Water temperatures in the ballast tanks increased by less than 3°C from the beginning to the end of each voyage.

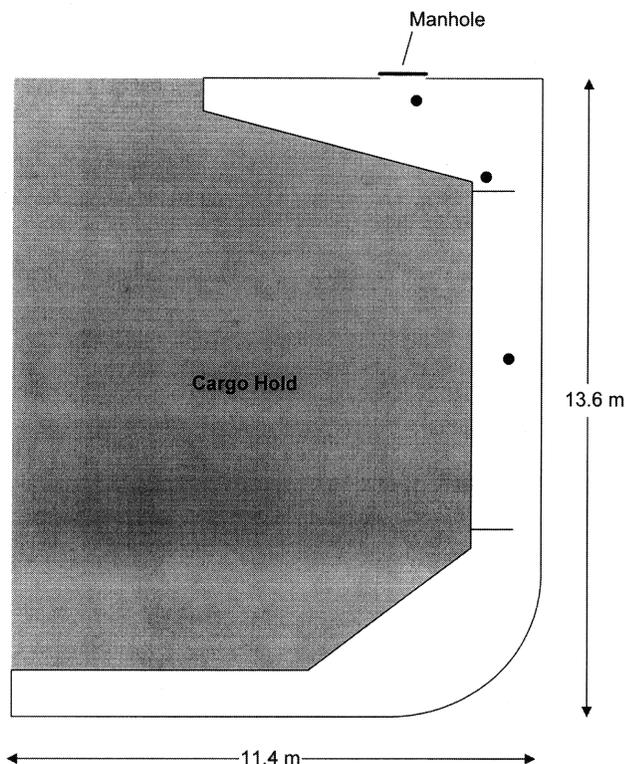
Sampling on each voyage was restricted to a single pair of wing tanks (port and starboard 4-aft tanks) containing water of the same physical and temporal origin. The port and starboard 4-aft tanks are approximately 13 m deep and irregularly shaped with a narrow connection between

top and bottom wing tanks to accommodate the cargo hold in the centre of the vessel (Figure 2). A manhole with a removable cover on the foredeck provides access to each tank. Approximately 3 m below the manhole, a horizontal partition forms an incomplete barrier between the top and mid-sections of the tank, with further partitions located at intervals deeper in the tank. When fully ballasted, the 4-aft tanks each hold 600.5 m<sup>3</sup> of sea water. Zooplankton samples were obtained from the same locations in the tanks (i.e. at 0.5 m, 2 m and 6 m depths) on both voyages using a self-priming petrol-driven diaphragm pump (ASM DE 50C1). Suction hose (internal diameter 52 mm) carried water in steady pulses from the ballast tank to an outlet suspended over a 100 µm-mesh plankton net. Sample volumes were 400 l and 250 l for voyages 1 and 2 respectively.

Two replicate zooplankton samples were taken through the open manhole in succession at 6 m, 2 m and 0.5 m depths. An opaque cover was fitted over the manhole



**Fig. 1.** MV 'Iron Sturt' trade route in Southeastern Australia; dotted line represents voyage 1, dashed line represents voyage 2.



**Fig. 2.** Cross-sectional diagram of the starboard 4-ft ballast tank of the MV 'Iron Sturt' illustrating the connected top and bottom wing tank configuration. Sampling points at 0.5 m, 2 m and 6 m below the manhole are identified (•). Note that the port 4-ft ballast tank is a mirror image.

while sampling to reduce external illumination and decrease the tendency for phototactic organisms to accumulate at the surface. The 6 m depth samples were obtained through permanently installed lengths of hose, while the 0.5 m and 2 m samples were obtained by lowering the appropriate length of hose through an elasticized hole in the manhole cover.

Initial samples were taken at the beginning of the voyage (26 h and 10 h after ballasting for voyages 1 and 2 respectively) on what was considered for both voyages to represent 'Day 1'. Thereafter, the tanks were sampled in the middle ('Day 3') and at the end ('Day 5') of each voyage. In total, 36 pump samples were obtained on each voyage (3 depths  $\times$  2 tanks  $\times$  3 days  $\times$  2 replicates). Sampling was conducted during daylight hours (08:00 to 20:00 h) with sampling of both tanks completed within 4 h. Samples were fixed in 4% formaldehyde solution at the end of each sampling session.

Patterns in zooplankton vertical distributions were inferred from the plankton densities in samples taken from the 0.5 m, 2 m and 6 m depths. We include in our analysis taxa common to both voyages and in sufficient

densities to allow statistical comparisons. We grouped taxa according to similar morphological characteristics (chaetognaths, bivalve larvae, gastropod larvae, polychaete larvae, mysids, brachyuran zoea, brachyuran megalopae, *Lucifer* sp., *Palaemon* sp., alpheid shrimp). Fine taxonomic resolution was prevented by the absence of taxonomic experts and keys for many larval planktonic species. We do not consider our inability to distinguish between different species a reason to invalidate our results, since if statistically significant distribution patterns are detected for combinations of species, these differences are likely to be real for one or more of the species comprising the group. While several species of crab zoea were present in the samples, we present here the results for a single easily recognizable species with short dorsal and lateral spines, hereafter referred to as 'Crab Zoea'. Copepods, although abundant, were excluded from the study in order to concentrate effort on known high-risk groups (e.g. bivalves, crabs) that have had documented economic and environmental impacts following invasion (Strayer *et al.*, 1998; Grosholz *et al.*, 2000) yet have received less attention in previous ballast water studies, usually because of their comparatively low occurrence in ballast water samples (eg Gollasch *et al.*, 2000).

Most taxa exhibited low ( $< 50$  individuals  $m^{-3}$ ) and patchy zooplankton densities, necessitating the use of non-parametric analyses. Wilcoxon tests revealed two depth-related distribution patterns: non-stratified and vertically stratified. Chaetognaths, bivalve larvae, polychaete larvae and *Lucifer* sp. were captured in similar numbers from all three depths (Table I). In contrast, most crustacean groups (alpheid shrimp, *Palaemon* sp., brachyuran crab zoea and brachyuran crab megalope sp. 1) were caught more often from depths of 0.5 m or 2 m than from a depth of 6 m. Gastropods were caught more often from a depth of 0.5 m than from the other two depths. The total number of taxa (taxon richness, R) was significantly lower in samples obtained from a depth of 6 m compared with samples obtained from either 0.5 m or 2 m (Table I).

Two groups were captured in high densities: bivalve larvae (up to 700 individuals  $m^{-3}$ ) and Crab Zoea (up to 1000 individuals  $m^{-3}$ ), facilitating parametric analyses for these taxa. Data were log transformed to remove the positive correlation of variances with means (Sokal and Rohlf, 1981). Assumptions of normality and homoscedasticity were examined and were not violated. Four-way blocked ANOVAs: VOYAGE (blocked), DEPTH, TANK and DAY, were carried out using the SYSTAT(version 5.1) statistical software with DEPTH, TANK and DAY assigned as fixed factors.

We found no evidence for depth-related distribution of bivalves at any time on either voyage (Table II; Figure 3). Conversely, estimates of whole-of-tank densities of Crab

*Table I: Summary of the results of Wilcoxon signed-rank tests on the mean abundances of ten relatively common taxa at 0.5 m, 2 m and 6 m depths on voyages 1 and 2, and on total Taxan richness (n = 12 at each depth)*

Taxon	0.5 m </> 2.0 m	2.0 m </> 6.0 m	0.5 m </> 6.0 m
<b>Chaetognatha</b>			
<i>Sagitta</i> sp.	-	-	-
<b>Decapoda</b>			
Alpheid shrimp	-	>*	>*
Brachyuran crab zoea	>*	>**	>**
Brachyuran crab megalope sp. 1	-	>*	>**
<i>Lucifer</i> sp.	-	-	-
Mysids	-	-	-
<i>Palaemon</i> sp.	-	>*	>*
<b>Mollusca</b>			
Bivalve larvae	-	-	-
Gastropod larvae	-	-	>*
<b>Polychaeta</b>			
Polychaete larvae	-	-	-
Species richness	-	>*	>**

Non-significant differences for pairs of depths are indicated by -. Significant differences in abundances are indicated in direction (< = less than, > = greater than) with associated probabilities shown at the \**P* < 0.05 and \*\**P* < 0.01 levels.

Zoea, if based solely upon surface samples, were as much as 12-fold higher than estimates accommodating the vertical density gradient. Crab Zoea densities generally decreased with depth ( $F_{[2, 62]} = 23.73$ ; *P* < 0.0001), although depth-related density variation depended upon the day of sampling ( $F_{[4, 62]} = 3.22$ ; *P* < 0.05) (Figure 4).

We speculate that the absence of normal light cues for diel vertical migration may encourage some cyclically migrating species to remain in a ‘night time’ near-surface distribution (Forward, 1988; Ward and Blaber, 1994). This may also be accentuated by low ambient food concentrations (Cronin and Forward, 1980; Young, 1995), as are reported to prevail in ballast tanks (Carlton, 1982;

Wonham *et al.*, 2001). Temperature changes have been documented to induce vertical migrations of zooplankton (Gerritsen, 1982; Forward, 1990), though it is unclear whether the relatively small temperature change in this study would have been sufficient to cue a measurable response. While our sampling protocol deliberately minimized light introduction, it should be noted that most ballast water sampling techniques necessitate the removal of manhole covers, creating temporary light fluctuations that may be capable of influencing the distribution of species in the collected sample, particularly by attracting phototactic organisms.

We noted that mysid abundances appeared particularly

*Table II: ANOVA table for bivalve abundance at three depths on two voyages*

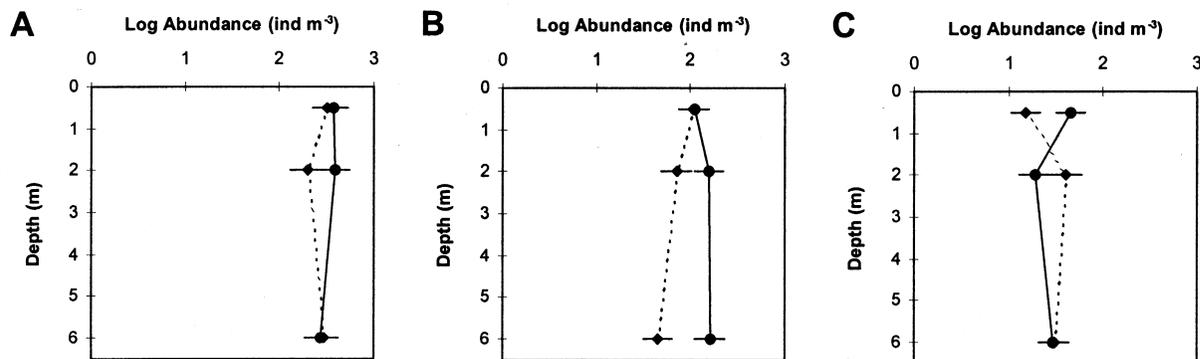
Source	df	SS	F	<i>P</i>
Voyage	1	0.443	4.258	0.043*
Day	2	6.362	61.111	<0.001***
Depth	2	0.013	0.129	0.879
Day * Depth	4	0.020	0.194	0.941
Error	60	0.104		

Probabilities are indicated at the \**P* < 0.05 and \*\*\* *P* < 0.001 levels.

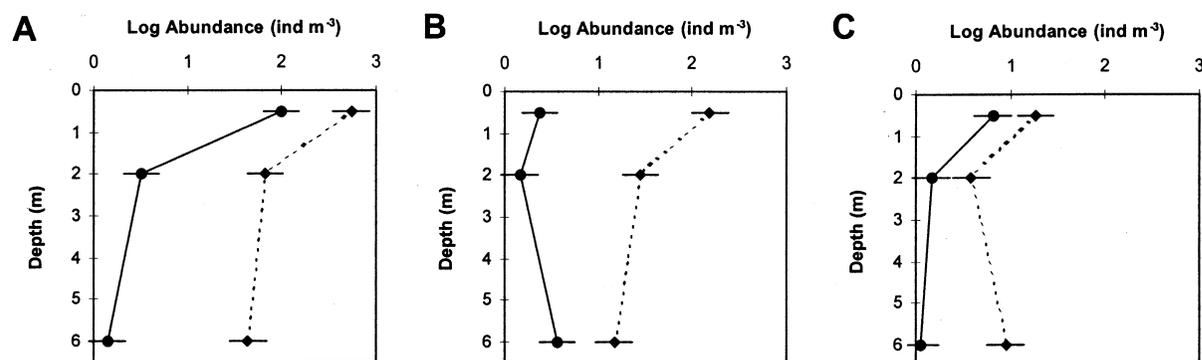
*Table III: anova table for Crab Zoea abundance at three depths on two voyages*

Source	df	SS	F	<i>P</i>
Voyage	1	18.113	85.12	<0.001***
Day	2	4.268	20.058	<0.001***
Depth	2	5.049	23.729	<0.001***
Day * Depth	4	0.686	3.224	0.018*
Error	62	0.213		

Probabilities are indicated at the \**P* < 0.05 and \*\*\* *P* < 0.001 levels.



**Fig. 3.** Bivalve larval abundance (mean and standard errors) at the beginning (**A**, Day 1), middle (**B**, Day 3) and end (**C**, Day 5) of voyage 1 (♦) and voyage 2 (•) at three depths.



**Fig. 4.** Crab Zoea abundance (mean and standard errors) at the beginning (**A**, Day 1), middle (**B**, Day 3) and end (**C**, Day 5) of voyage 1 (♦) and voyage 2 (•) at three depths.

variable among replicate samples, possibly indicating that they retained some swarming behaviour despite low ambient light (O'Brien, 1988). We attribute the marginally elevated gastropod densities at 0.5 m to chance and patchiness; since gastropod larvae settle to a benthic existence, we would expect increased abundances of older individuals of this and other benthic groups near the bottom of the tanks, a region not sampled during this study.

The apparent lack of vertical stratification among bivalves, mysids, chaetognaths, polychaetes and the crustacean *Lucifer* sp. may reflect their actual distributions or the low statistical power of the non-parametric comparisons, possibly reduced further by the grouping together of dissimilar species. The dramatic zooplankton mortality over the course of these voyages also meant that it was easier to detect differences in initial samples than in those taken at the end of a voyage. This effect may have contributed to the apparent weakening of depth zonation over the course of both voyages in this study.

This study suggests that plankton behaviour is an important consideration for individuals measuring the densities of organisms in ships' ballast water. The observed over-representation of crustaceans near the tank surface implies that a bottom to surface net tow [e.g. (Carlton and Geller, 1993)] if adjusted for filtration effects (McQueen and Yan, 1993) may generate more representative measurements of whole-tank plankton densities than samples obtained over a restricted depth [e.g. (Hay *et al.*, 1997; Smith *et al.*, 2000; Gollasch *et al.*, 2000)]. Conversely, the lack of significant 'tank' differences in this study suggest that horizontal variation may pose less of a problem for representative sampling.

## ACKNOWLEDGEMENTS

We would like to thank the master and crew of the MV 'Iron Sturt' for their aid throughout. We especially thank Alan Taylor of BHP Transport for his help in facilitating access to the vessel. This work is in partial fulfilment of

K.R.M.'s honours thesis for the Department of Zoology, University of Tasmania. This paper is funded in part by the Australian Quarantine and Inspection Service under Project #8/96 through the Australian Ballast Water Management Advisory Council's Strategic Research Plan [C.L.H.]. This is publication CS0X from the Centre for Research on Introduced Marine Pests, CSIRO.

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Received on 28 July, 2001; accepted on 22 February, 2002