

The meroplankton communities from the coastal Ross Sea: a latitudinal study

**Ramon Gallego, Dorothea Heimeier,
Shane Lavery & Mary A. Sewell**

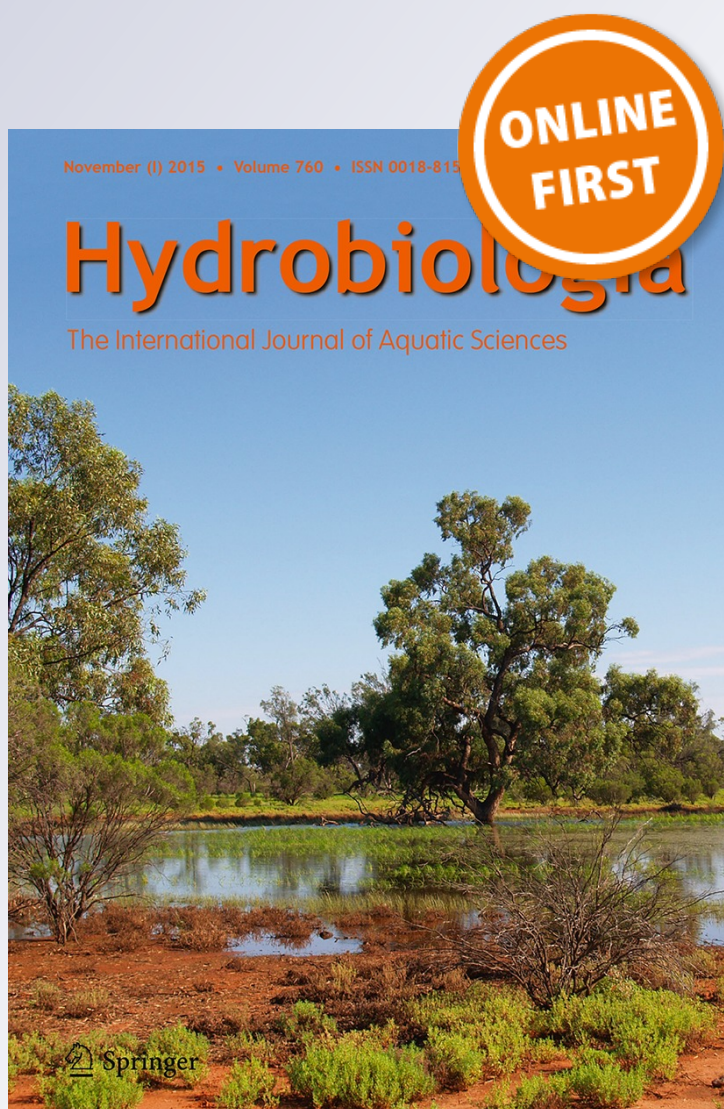
Hydrobiologia

The International Journal of Aquatic
Sciences

ISSN 0018-8158

Hydrobiologia

DOI 10.1007/s10750-015-2487-7



Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

The meroplankton communities from the coastal Ross Sea: a latitudinal study

Ramon Gallego · Dorothea Heimeier · Shane Lavery · Mary A. Sewell

Received: 12 May 2015 / Revised: 24 August 2015 / Accepted: 5 September 2015
© Springer International Publishing Switzerland 2015

Abstract The study of meroplankton communities in the coastal Ross Sea has been difficult due to the logistical challenges associated with sampling in remote areas and with the hurdle of larval identification. As a result, it has not been possible to estimate the latitudinal changes in reproductive strategies of marine invertebrates and to investigate Thorson's rule—that planktotrophy is less favoured than lecithotrophy and brooding at high latitudes—along the southernmost coastline on Earth. As part of the Latitudinal Gradient Project (Antarctica New Zealand), we studied the meroplankton communities at three sites on the Victoria Land Coast and identified—using morphological and genetic information—11,117 larvae from 52 molecular Operational Taxonomical Units. This allowed for the analysis of patterns in larval diversity and abundance

with latitude. Genetic identification permitted the usage of multivariate techniques to study changes in the meroplankton community and to further investigate the latitudinal variation in larval mode of nutrition. Our results showed distinct larval communities at each site, with species dominating nearby benthos being crucial in site discrimination, but with no latitudinal gradients in abundance or diversity. Planktotrophy was predominant in all three sites reflecting the minimal effect of Thorson's rule within the Ross Sea.

Keywords Ross Sea · Meroplankton · Larvae · Thorson's rule · LGP

Introduction

Antarctic marine benthic invertebrates often reproduce through planktonic larvae, which are their main vehicle for dispersion, colonization of new habitats, and maintenance of population connectivity (Hunter & Halanych, 2008; Heimeier et al., 2010a; Hoffman et al., 2011). Larval stages present in the water column—collectively known as meroplankton—represent an important percentage of the zooplankton community; although their actual species diversity has been historically difficult to estimate given the hurdle of identifying larvae to the species-level. Since the implementation of DNA identification techniques, it is possible to obtain species-level larval IDs (Olson et al., 1991; Sewell et al., 2006; Webb et al., 2006; Grant &

Guest editors: Diego Fontaneto & Stefano Schiaparelli /
Biology of the Ross Sea and Surrounding Areas in Antarctica

Electronic supplementary material The online version of this article (doi:10.1007/s10750-015-2487-7) contains supplementary material, which is available to authorized users.

R. Gallego (✉) · D. Heimeier · S. Lavery · M. A. Sewell
School of Biological Sciences, University of Auckland,
Auckland Mail Centre,
Private Bag 92019, Auckland 1142, New Zealand
e-mail: ramon.gallego-simon@auckland.ac.nz

S. Lavery
Institute of Marine Science, University of Auckland,
Private Bag 92019, Auckland 1142, New Zealand

Linse, 2009), although few studies on coastal meroplankton have broadly employed this technique to the entire meroplankton community (Heimeier et al., 2010b).

Study of the reproductive biology of benthic marine invertebrates in Antarctica began prior to the “Heroic” era (approx. 1900–1920), when the lack of pelagic larval stages (Murray, 1895) and the preponderance of brooding strategies (Thomson, 1876) was noted. These observations, along with Thorson’s work on gastropods in the Arctic (1950) led to the formulation of the Thorson’s rule (Mileikovsky, 1971) which stated that planktotrophic strategies (those in which larvae are broadcast into the water column and feed on other plankton) are less favoured in deep waters and at high latitudes, where strategies in which larvae are brooded or rely on maternal reserves are dominant. The validity of Thorson’s rule has been the focus of scientific debate for more than half a century (Vance, 1973; Levin & Bridges, 2001; Laptikhovsky, 2006), and a recent meta-analysis of reproductive strategies has found a significant negative relationship between latitude and the proportion of planktotrophy, supporting Thorson’s rule (Marshall et al., 2012). However, the ecological implications of such a pattern—e.g. potential for dispersal, exposure to pelagic predators—are still unclear, as other studies have questioned the previously assumed relationship that planktotrophic larvae have a longer pelagic larval duration (Mercier et al., 2013).

The influence of latitude on reproductive strategy has rarely been studied within Antarctic waters. However, studies considering such differences have reported results contrary to Thorson’s rule, with greater planktonic larval diversity at higher latitudes—a pattern seen in annelid polychaetes (Bhaud et al., 1999); and in meroplankton communities from the southern waters of the oceanic Ross Sea (Gallego et al., 2014). However, the greater benthic diversity in the more southerly shelf environments (Clarke, 2008) may contribute to such patterns, and a comparative study between areas of similar benthic diversity is needed to clarify the influence of latitude on the reproductive strategies of Antarctic marine invertebrates.

The multidisciplinary Latitudinal Gradient Project (LGP, www.lgp.aq), launched in 2002 by the Antarctic programmes of New Zealand, Italy and the United States of America investigated the extent to which latitudinal change is a good surrogate for environmental gradients that shape biological and geological

phenomena (Berkman et al., 2005; Howard-Williams et al., 2006, 2010). As a main hypothesis, the LGP stated that “ice-driven dynamics control the structure and function of biological systems (marine, terrestrial and freshwater) near the limits of life at high latitudes” (Howard-Williams et al., 2006). The Victoria Land coast exhibits strong latitudinal variation in sunlight input and its seasonality, sea water temperature and sea ice extent. The effects on the marine realm are dominated by ice dynamics: sea ice extension, ice disruption on the sea floor and ice sheet extension follow a latitudinal pattern in their variation and greatly affect marine ecosystems (Berkman et al., 2005). However, sea ice conditions do not always follow a latitudinal gradient, and the formation of polynyas—open areas surrounded by sea ice—begins at different locations in the Ross Sea such as the Drygalski Ice Tongue enhancing the formation of the Terra Nova Bay polynya (Martin et al. 2007). Polynyas expand northward, driven by the Ross Ice Shelf air stream (Smith et al., 2007), and disrupt simple latitudinal gradients by creating sea ice-free waters, increasing the depth-penetrance of solar radiation and allowing for a higher primary production in the water column even at high latitudes (Arrigo & van Dijken, 2003).

As part of the LGP our research team conducted detailed meroplankton sampling in the early spring at three sites along the Victoria Land Coast covering a latitude from 72°S to 77°S. Companion work by other LGP participants has shown that benthic communities along this coast have similar biological diversity (Cummings et al., 2010; Schiaparelli et al., 2014), and thus provided the potential for studying the validity of Thorson’s rule within the Southern Ocean. Here we address two specific questions: (1) to what extent do meroplankton communities change with latitude, and (2) does Thorson’s rule apply within the latitudinal span of the Ross Sea, i.e. is there a change in the dominant reproductive strategy of planktonic larvae along the Victoria Land Coast?

Methods

Sample collection and site description

Meroplankton samples were collected during the austral spring at three sites in the Western Ross Sea, shown in Fig. 1. At each site, a hole was drilled

through the sea ice, and plankton tows were collected using a collapsible net as described in Sewell (2005). Samples consisted of three vertical tows covering from 50 m depth to near surface—at the bottom of the sea ice—filtering an estimated 3.92 m³ of seawater in each tow. A fourth tow was also collected, fixed in 4% Steedman's solution (Steedman, 1976) and kept as an unsorted reference sample.

Six sampling campaigns were performed from mid-November to early or mid-December between 2003 and 2009 at the three LGP sites—no samples were collected in 2005. Sampling campaigns consisted of 14–25 samples collected on consecutive days, weather permitting. Each site was visited in two consecutive years; a summary of the sampling scheme can be found in Table 1. Conductivity, temperature and depth (CTD) casts were performed daily, before the first plankton tow using two different devices, an RBR Instrument with an attached chlorophyll *a* fluorometer

at Cape Hallett and Terra Nova Bay (courtesy Ken Ryan, Victoria University of Wellington) and a Minipack CTD-F (Chelsea Technologies Group) in Granite Harbour.

Remote sensing data, output from the NASA Ocean Biogeochemical Model (<http://oceancolor.gsfc.nasa.gov/>), was used to characterize the sampling sites, including the depth of the mixed layer, the total chlorophyll *a* and the percentage of sea ice coverage for the sampling weeks (Table 1) between 2003 and 2007 (no data available after 2007).

Larval identification

Specimens collected in the plankton tows were freshly sorted under dissecting microscopes by a research team of two to six. The first level of larval identification was into morphological operational taxonomic units (OTUs), using zooplankton and meroplankton

Fig. 1 Location of the sampling sites (noted in *bold*) on the Victoria Land coast and western Ross Sea. Cape Hallett samples were collected during the 2003 and 2004 campaigns, Terra Nova Bay in 2006 and 2007 and Granite Harbour in 2008 and 2009. Bathymetry is represented in *greyscale*, with lighter tones for shallow waters

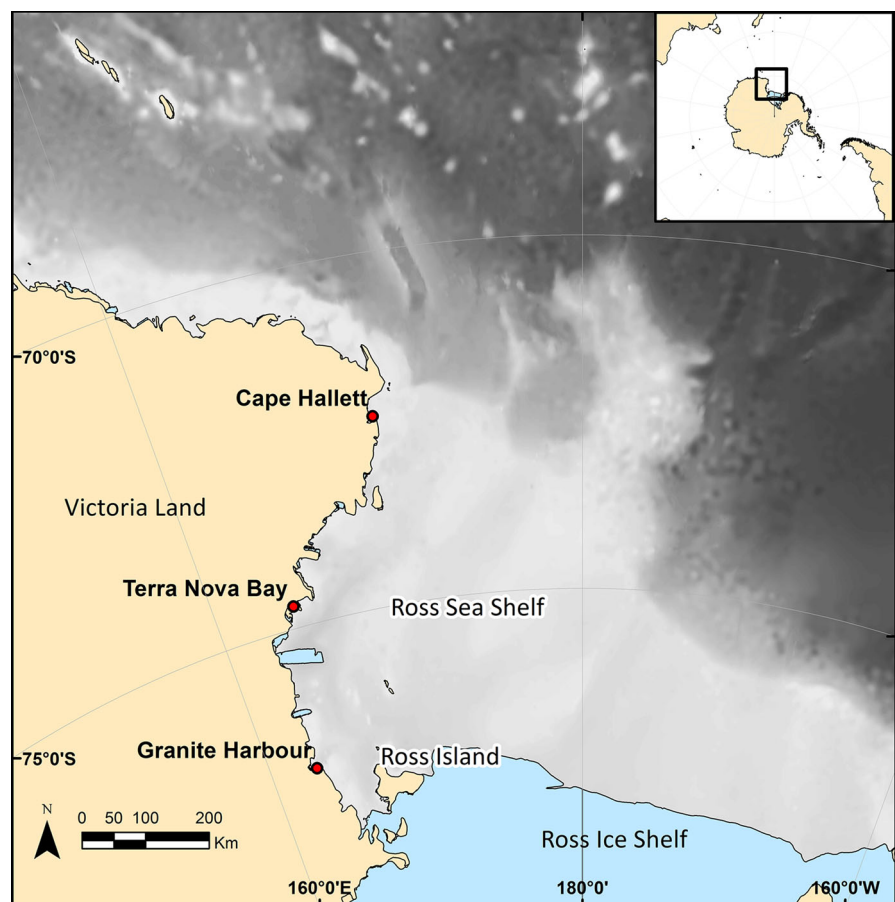


Table 1 Summary of the sampling campaigns performed as part of the Latitudinal Gradient Project along the Victoria Land coast

Site	Location	Campaign	Sampling days per week				
			Week 1 14-Nov	Week 2 21-Nov	Week 3 28-Nov	Week 4 5-Dec	Week 5 12-Dec
Cape Hallett	72.31°S	2003		5	4	6	2
	170.19°E	2004	6	6	6	1	
Terra Nova Bay	74.64°S	2006	7	7	7	2	
	164.21°E	2007	6	7	7	2	
Granite Harbour	77.02°S	2008	2	7	7	2	
	162.89°E	2009	3	7	5		

Individual samples were combined and averaged by week for the latitudinal comparison. Dates below weeks (1–5) represent the limits of each week

identification guides (Smith & Johnson, 1977; Todd et al., 1996; Stanwell-Smith et al., 1997). Every larva was classified into an OTU (e.g. Elephant trunk pilidia, orange trochophores and barnacle nauplii) and representatives for each OTU were isolated and preserved in ethanol in 96-well sampling plates for later molecular identification. Only a small number of specimens were sequenced for highly abundant OTUs or those that appeared in many samples.

Protocols for DNA extraction, PCR identification and sequencing of the 16S, 18S and COI loci were as in Heimeier et al. (2010b) and Gallego et al. (2014). DNA sequences obtained from the amplified fragments were analysed in Geneious v6.1.6 (Drummond et al., 2011), where sequences were trimmed by deleting PCR primers and positions with a 5% probability of base call error. An initial quality control of the sequences obtained was made by performing a BLAST (Basic Local Alignment Search Tool) search against the National Center for Biotechnology Information (NCBI) nucleotide database, looking for consistency between the amplicon obtained and the original taxonomic description of the specimen based on morphology. Discrepancies between the amplicon and the genetic identification may have occurred either due to initial mis-identification, or PCR amplification of other plankton contaminants. Sequences that passed this initial screening were assigned to molecular OTUs (mOTUs) following the procedures detailed in Gallego et al. (2014) and Heimeier et al. (2010b). In brief, the process included the generation of phylogenetic trees of reference and query sequences for each genetic marker. Final identification (down to the lowest taxonomic level possible) was based on the

strong bootstrap support for query and reference sequences belonging to the same clade. The taxonomic level of identification was conservative, taking into consideration the proportion of that taxon represented in the tree by reference sequences, and the agreement among different loci analysed for that taxon.

Once larvae were identified to the lowest taxonomic level possible, we undertook a literature search on the known feeding mechanisms for the larvae of that group, assigning, if possible, each larval species to either a planktotrophic or lecithotrophic category.

Statistical analysis

Exploratory analysis

The abundance of each mOTU was tallied for each sampling day across different years and sampling sites. For each site, an exploratory analysis included: (1) the generation of a species accumulation curve (*specaccum* function from *vegan* package implemented in R) to assess whether our sampling recovered a good representation of the meroplankton community; (2) variation of alpha diversity—number of mOTUs and Shannon–Wiener diversity index (Shannon, 1948)—and larval abundance across weeks and years with significance testing of their variation with two-way ANOVA, and (3) the variation of the meroplankton community composition was estimated with a Bray–Curtis matrix over a square-root transformed dataset. These similarities were visualized using a Multi Dimensional Scaling (MDS) plot, also implemented in the *vegan* package in R.

Comparisons among sites

To overcome the potential problem of short-term temporal changes in meroplankton composition, and to allow comparison of larval abundance, diversity, and community composition between years and sites, daily tow samples were collapsed into weekly samples, and the mean weekly abundance of each mOTU was calculated. These values were used in the subsequent analysis to account for missing sampling days due to poor weather conditions.

Using these weekly averaged samples, we analysed the variation in alpha diversity and larval abundance among years and sites using a two-way ANOVA. We used the Bray–Curtis index over a square-root transformed dataset to estimate the similarities in community composition and a two-way PERMANOVA (Anderson, 2005) to test the influence of site and year on meroplankton composition. To assess the distinctiveness of the meroplankton community from each site, a canonical analysis of principal components (CAP) was performed, followed by a leave-one-out allocation procedure, in which the ability of the CAP to assign each sample to its group is tested. PERMANOVA and CAP analyses included 9999 permutations and were performed in PRIMER 6 with the PERMANOVA + add-on (Plymouth Marine Laboratory).

Two approaches were used to gain insight into which species were having a greater contribution to the differences between sites: we calculated Pearson correlations between each of the species present in the meroplankton and the CAP axes and plotted those showing a correlation >0.6 . We also ran the similarity percentages (SIMPER) routine in PRIMER 6 to show which species contributed more to the similarities within sites and the dissimilarities between them.

Results

Environmental changes with latitude

At the time of sampling, the three LGP sites were sea ice covered and had constant temperature and salinity in the first 50 m of the water column (Supplementary Fig. 1). We additionally used remote sensing data to quantify sea ice coverage, depth of the mixed layer, and total chlorophyll *a* to obtain a “characteristic”

environmental profile for each site during the weeks of sampling. Box plots of these parameters (Fig. 2) reveal the broad environmental differences between sites: Cape Hallett has a mid-range mixed layer (20–35 m), high sea ice coverage ($>40\%$), and the lowest total chlorophyll (max $\sim 0.5 \text{ mg/m}^3$); Terra Nova Bay shows the influence of the Terra Nova Bay polynya, with lower sea ice coverage (max $< 20\%$), a shallower mixed layer ($\sim 10 \text{ m}$) and the highest total chlorophyll (max $> 1.0 \text{ mg/m}^3$); Granite Harbour has the highest sea ice coverage ($>50\%$), deep mixed layer ($>30 \text{ m}$) and intermediate total chlorophyll (max $\sim 0.75 \text{ mg/m}^3$). Overall, the Terra Nova Bay polynya disrupts a simple latitudinal gradient in these parameters (Fig. 2) and emphasizes the role of ice dynamics in the control of ecological processes in the Ross Sea (Howard-Williams et al., 2006).

Meroplankton communities from the coastal Ross Sea

One hundred and sixteen plankton tows were collected during the austral spring campaigns of the LGP on the Victoria Land coast, and the 11,117 larvae found were classified into 52 mOTUs, summarized in Table 2. These mOTUs belong to 11 phyla and the majority have been previously characterized in Heimeier et al. (2010b) or in Gallego et al. (2014). The newly found larvae were one annelid polychaete (Ampharetidae), one crustacean (Facetotecta), two cnidarians (Alcyonacea and Hydrozoa), one Entoprocta (Loxosomatidae), one Platyhelminth (Turbellaria), one Echiura (Echiura) and four Porifera (Demospongiae). The sequences and phylogenetic trees generated to identify them are available from the authors or on GenBank (accession nos. KT383394–430).

Species accumulation curves showed that our sampling obtained a fair representation of the meroplankton community at each site, with the curves all showing an asymptotic tendency (Fig. 3). The number of mOTUs per sample at each site was found to be almost constant across weeks and years, with some significant variation in Cape Hallett, driven by the only two samples collected in week 5 in 2003 (Fig. 4). Alpha diversity showed variation only in Terra Nova Bay, where the Shannon–Wiener diversity index dropped once larvae from the Antarctic silverfish emerged from just below the sea ice into the water column. Larval abundance was the most variable

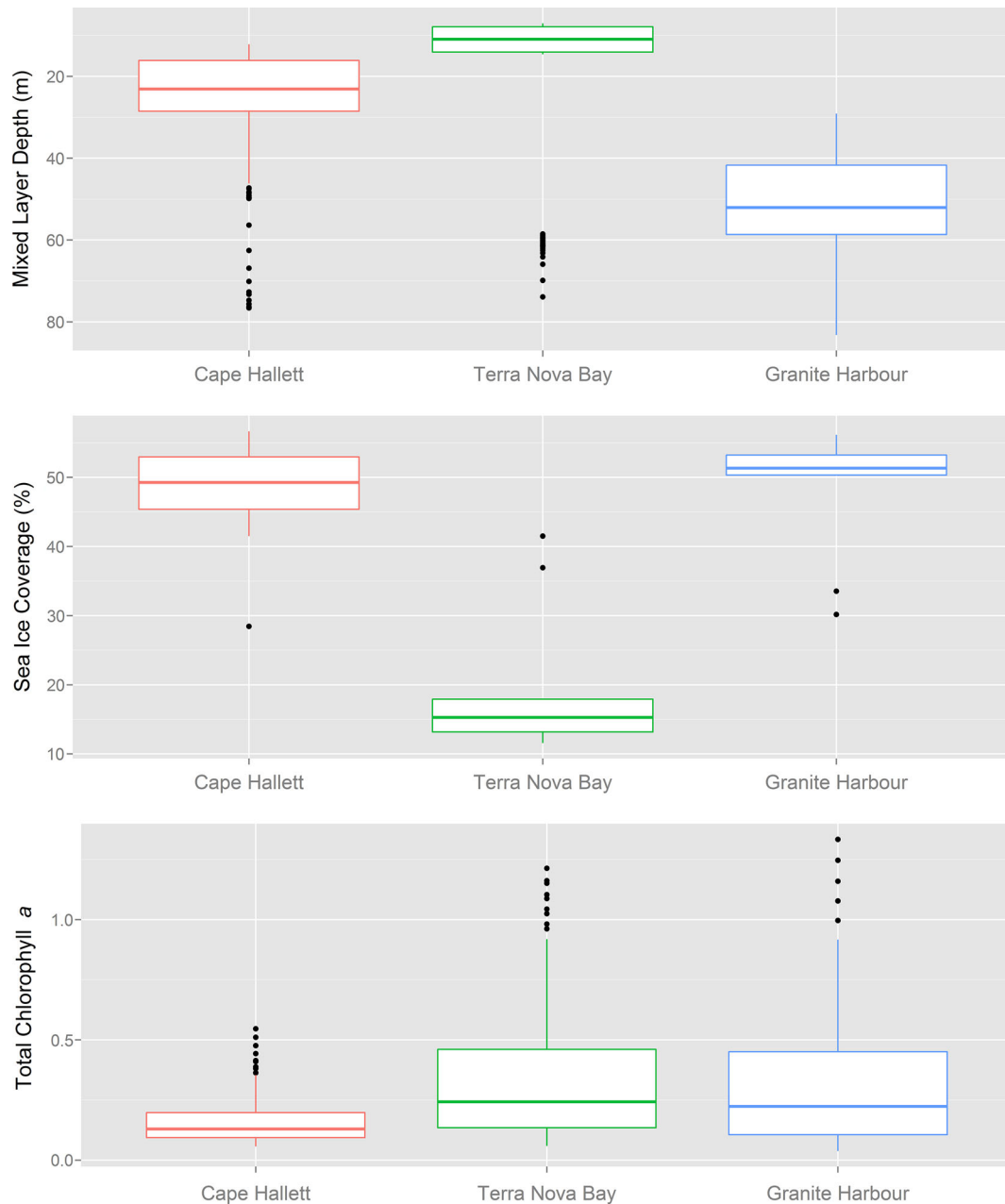


Fig. 2 Environmental variation across the three study sites, showing the mixed layer depth, percentage of sea ice coverage and total chlorophyll *a* for the sampling weeks between 2003

and 2007. Data obtained from the NOBM program—Assimilated Daily Data (doi:10.5067/WWVPAVQDEHF3). Full dataset available in the Supplementary material

parameter studied, with significant differences at each site between weeks (Cape Hallett, $F_{4,28} = 5.68$, $P < 0.0001$), years (Granite Harbour, $F_{1,26} = 6.07$, $P = 0.02$) or both (Terra Nova Bay, $F_{1,39} = 16.75$, $P < 0.0001$; $F_{3,39} = 14.03$, $P < 0.0001$). The MOTU composition showed greater similarity between

samples from the same year, and this pattern was consistent at each site (Fig. 5).

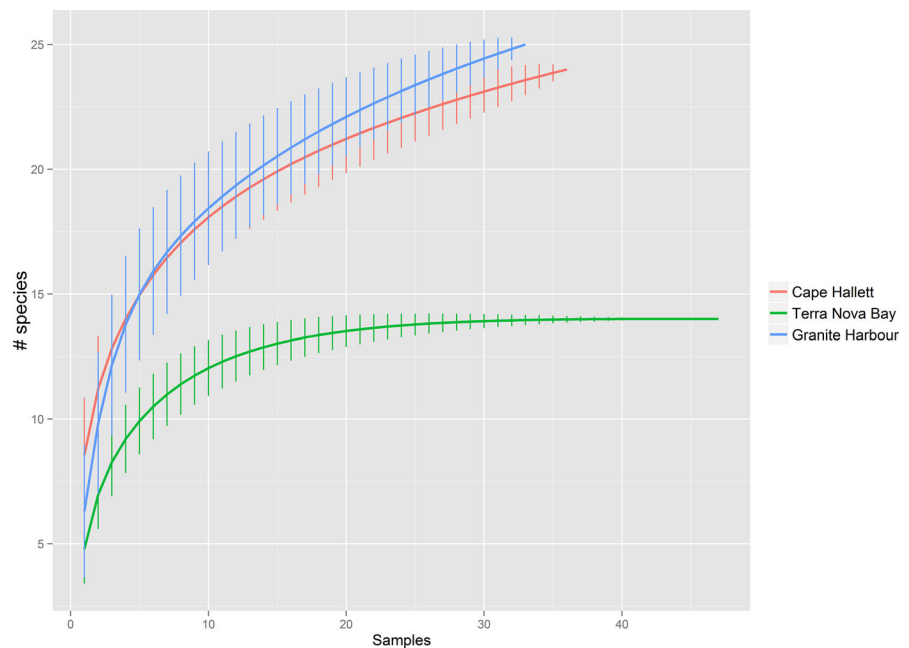
The latitudinal comparison between meroplankton assemblages was conducted with pooled samples collected in the same week and year to ensure independence of the observations, as required for

Table 2 Summary of the mOTUs found on the Victoria Land coast during the LGP, noting their putative feeding strategy (*L* lecithotrophic, *P* Planktotrophic)

Phylum	mOTU	Sampling campaign					
		Cape Hallett		Terra Nova Bay		Granite Harbour	
		2003	2004	2006	2007	2008	2009
Annelida	<i>Laonice</i> spp. (2) (P)	.		.	.	*	*
	Nephtyidae sp. (P)			.			.
	Polynoidae spp. (3) (P)	***	**	.	*	.	.
	<i>Scolecopsis eltaninae</i> (P)	.	**				*
	<i>Spiophanes</i> sp. (P)		.				
	Syllidae sp.	.					
	Ampharetidae sp.						.
	Chaetopteridae sp. (P)	**	**	*	*		.
Echinodermata	<i>Odontaster</i> spp.(3) (P)	
	<i>Lophaster</i> sp. (L)	.					
	<i>Acodontaster</i> sp. (L)	.					
	<i>Ophiacantha antarctica</i> (L)				*	.	
	<i>Ophiolima antarctica</i> (L)	.					
	<i>Ophiocten megaloplax</i> (P)	.	*	.		***	*
	<i>Astrota agassizii</i> (L)	.	*				.
	Amphiuridae sp.	***					
	<i>Sterechinus neumayeri</i> (P)	**	***		.	.	***
	<i>Adamussium colbecki</i> (P)				.	.	.
Mollusca	<i>Conus</i> sp. (P)		.				
	<i>Niveria</i> spp. (2) (P)	*	*
	<i>Trichotropis</i> spp. (2) (P)	.		.			.
	<i>Tergipes antarcticus</i>	***	***	*	***	**	***
	Littorinimorpha sp. (P)		
	Hoplonemertea spp. (2) (L)
Nemertea	<i>Parborlasia corrugatus</i> (P)	*					.
	<i>Cerebratulus</i> sp. (P)		.	.			.
	<i>Lineus</i> sp. (P)
	<i>Parvicirrus</i> spp. (2) (P)	**	**	*	*	.	*
	Heteronemertea sp. (P)		.				.
	<i>Cephalothrix</i> sp.			***	*	.	*
	Demospongiae spp. (4) (L)						.
Arthropoda	<i>Bathylasma corolliforme</i> (P)	***	***
	Facetotecta spp. (2) (P)		
Platyhelminthes	Turbellaria sp. (L)					.	.
Entoprocta	Loxosomatidae sp. (P)						.
Cnidaria	Hydrozoa sp.						.
	Alcyonacea sp.						.
Chordata	<i>Pleuragramma antarctica</i> (P)			***	***		
Echiura	Echiura sp. (P)						.

When mOTUs were collapsed for the comparison between sites, their numbers are noted in parentheses. Abundance of mOTUs are shown here per 5 m³ to facilitate their comparison with previous meroplankton studies. They are averaged across the sampling season and summarized as follows: ***, more than 4.25 specimens per 5 m³; **, between 2.125 and 4.25; *, between 0.425 and 2.125; ., less than 0.425 larvae per 5 m³

Fig. 3 Species accumulation curves of the meroplankton samples at each site in the Victoria Land Coast



hypothesis testing for multivariate techniques (Anderson, 2005).

Week of sampling was found to be a significant source of variation for the multivariate study in the exploratory analysis (Fig. 5), hence we limited the latitudinal comparisons to weeks 2 and 3, which were sampled every year. Neither latitudinal trend, nor site differences were apparent in number of mOTUs, Shannon–Wiener index and larval abundance (Fig. 4); findings which were supported by the ANOVA test (Full results in Supplementary Table 1). However, the species composition of the meroplankton community was found to be different among sites, with the MDS plot discriminating samples from different sites (Fig. 6) and the PERMANOVA test finding these differences significant (Table 3).

The CAP analysis returned a clear distinction between sites; the Pearson correlation between mOTU abundance and the CAP axes revealed the importance of particular species in discriminating among sites (Fig. 7). The crustacean *B. corolliforme*, the sea urchin *S. neumayeri*, the gastropod *Niveria* spp., and several polynoid species characterized Cape Hallett samples; the Antarctic silverfish *Pleuragramma antarctica* and the nemertean *Cephalothrix* sp. discriminated Terra Nova Bay; and many mOTUs were correlated with Granite Harbour samples: polychaetes, echinoderms, nemerteans, molluscs and Facetotecta

crustaceans. These findings were supported by the SIMPER procedure, which also showed the ubiquitous presence of *Tergipes antarcticus* and *Parvicirrus* spp. in the meroplankton samples from all sites making these species less important for discriminating sites (Table 2, Supplementary Table 1).

Thorson's rule within the Ross Sea

The taxonomic identification achieved with a combined molecular and morphological identification allowed us to assign a putative feeding type to the majority of the mOTUs collected (Table 4). The literature sources used to determine the mode of nutrition of each mOTU are provided in Supplementary Table 2. We did not assign a mode of nutrition to seven mOTUs due to the lack of information in the literature regarding their larval feeding (i.e. *Tergipes antarcticus*, *Cephalothrix* sp.) or the high taxonomic level of identification achieved (i.e. Ampharetidae, Hydrozoa, Amphuiriidae, Asteriidae, Nemertea spp.). Our results showed that Terra Nova Bay samples had a high proportion of planktotrophic larvae and Granite Harbour had the highest number of lecithotrophic larvae, but more importantly, there was no pattern of a decreasing proportion of planktotrophic species with latitude as predicted by Thorson's rule (Table 4).

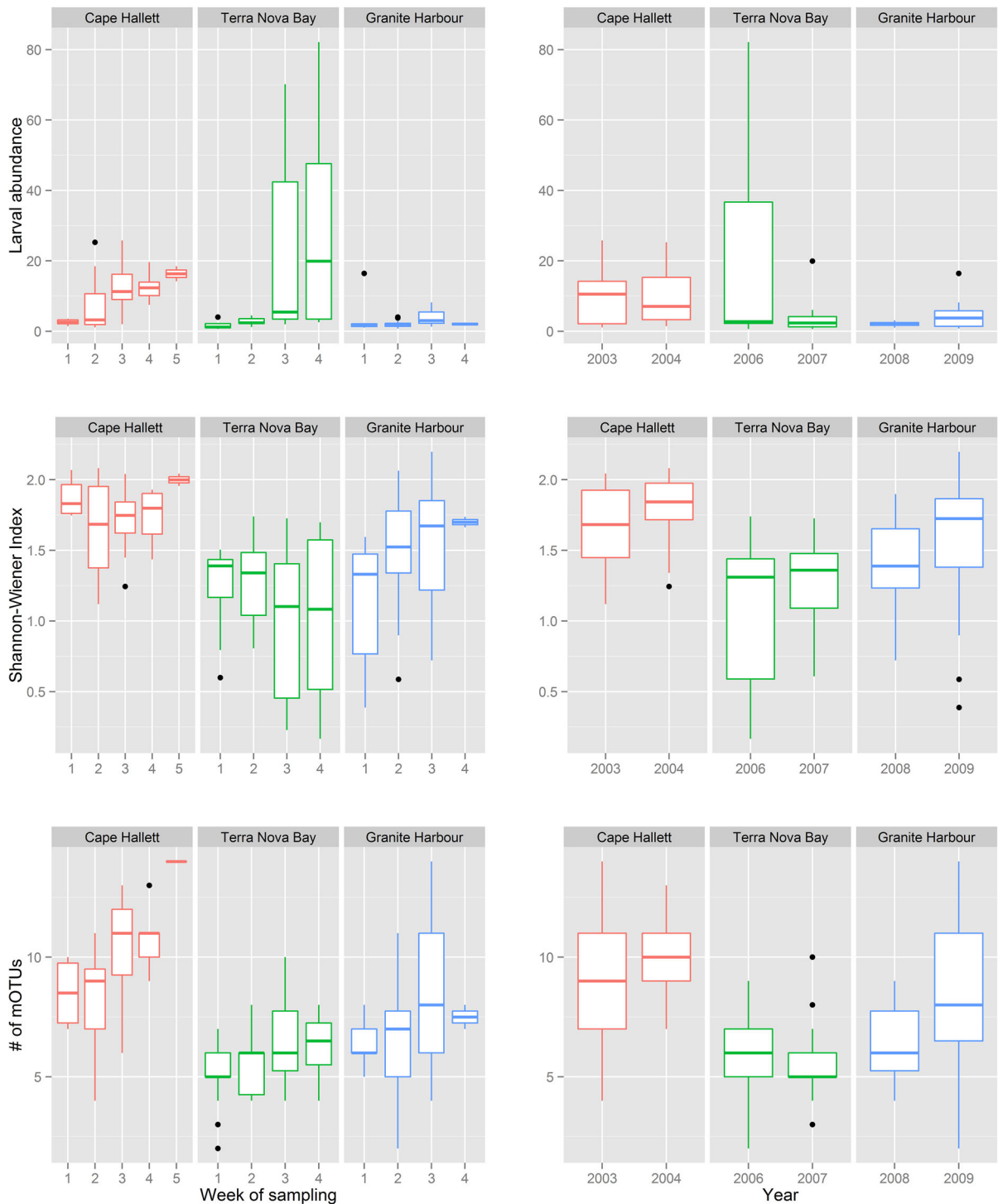


Fig. 4 Weekly (left) and yearly (right) variation in the number of mOTUs (top), Shannon–Wiener index (centre) and larval abundance (bottom) per meroplankton sample

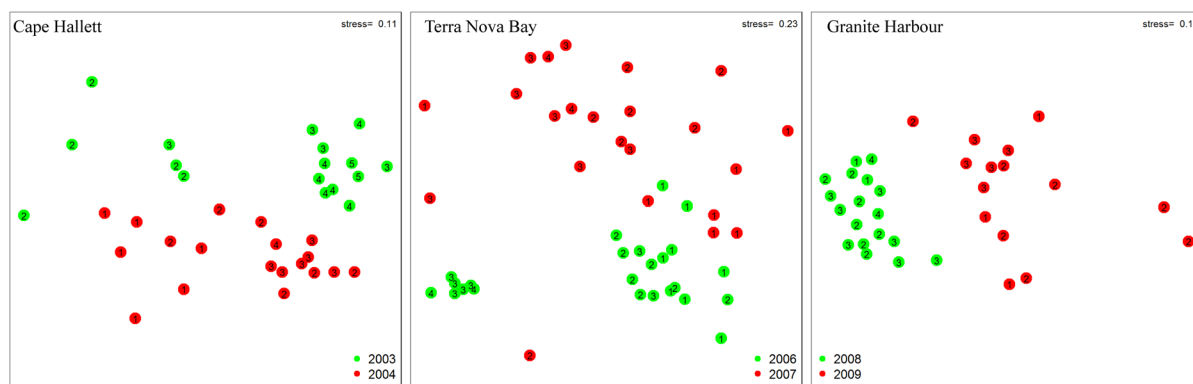


Fig. 5 Multidimensional scaling plot of the Bray–Curtis similarities over square-root transformed dataset between meroplankton samples from Cape Hallett (*left*), Terra Nova Bay (*centre*) and Granite Harbour (*right*)

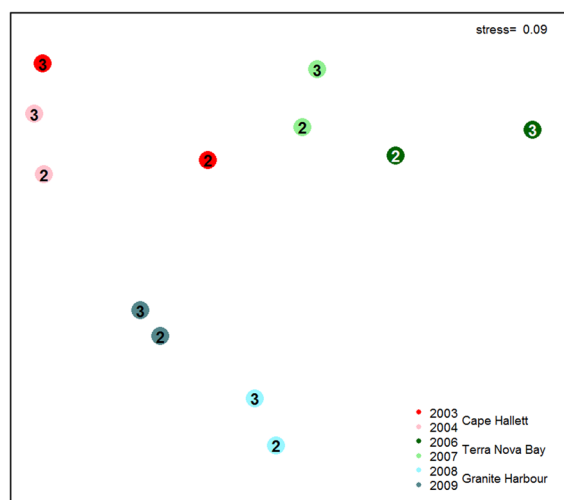


Fig. 6 Multidimensional scaling plot of the Bray–Curtis similarities over a square-root transformed dataset between weekly pooled meroplankton samples from the coastal Ross Sea. Colour codes represent the year and location of sampling while the number within the circle notes the week of sampling

Discussion

The meroplankton community from the Victoria Land coast is diverse, and the results presented here have shown high larval abundances, new species and distinct communities at each site. The morphological and molecular identification approach has allowed for the first accurate estimation of differences in larval diversity among localities along the Victoria Land coast, and the establishment of similarities among those communities based on their species composition.

Latitudinal variation

The marine component of the LGP examined three sites along the Victoria Land Coast where existing facilities were available (Gondwana Station, Terra Nova Bay) or where it was logistically possible to safely set-up and maintain tent-based field camps, which were then used for two consecutive years. Although the sites were separated by almost 550 km, latitude did not prove to be an effective surrogate for marine environmental conditions, as the Terra Nova Bay polynya resulted in a mid-latitude minimum in sea ice coverage, and associated changes to mixed layer depth and total chlorophyll *a* (Fig. 2), and there were no significant changes between sites in temperature and salinity of the upper 50 m. Comparison of sites along the Victoria Land Coast also, by necessity, confounded site with time (Sewell, 2006). While recognizing these limitations, as one of the largest mOTU-based data sets in coastal Antarctica, we believe that we can never-the-less obtain important information on spatial patterns in the meroplankton community along the Victoria Land Coast.

Firstly, we found no latitudinal differences in alpha diversity or abundance: Terra Nova Bay showed—although not significantly—the highest abundance and lowest diversity, driven by larvae of the Antarctic silverfish *Pleuragramma antarctica*, which dominates the local ichthyoplankton in November and December (Vacchi et al., 2004, 2012). Terra Nova Bay has significantly higher primary production and organic matter content in the water column than at Cape Hallett (Povero et al., 2006), which is especially

Table 3 PERMANOVA test between samples from different sites and weeks, based on the Bray–Curtis similarities of their square-root transformed mOTU abundances

Term	df	SS	MS	Pseudo-F	P	Unique perms
Site	2	10,650	5324.8	4.57	0.0015	9648
Week	1	1196.7	1196.7	1.03	0.3974	9462
Site:Week	2	1389.8	694.88	0.60	0.8424	9852
Residuals	6	6986.8	1164.5			
Total	11	20,223				

PERMANOVA consisted of 9999 permutations of residuals using Type III sum of squares

df degrees of freedom, SS sum of squares, MS mean sum of squares. Probabilities in bold denote significant differences ($P < 0.05$)

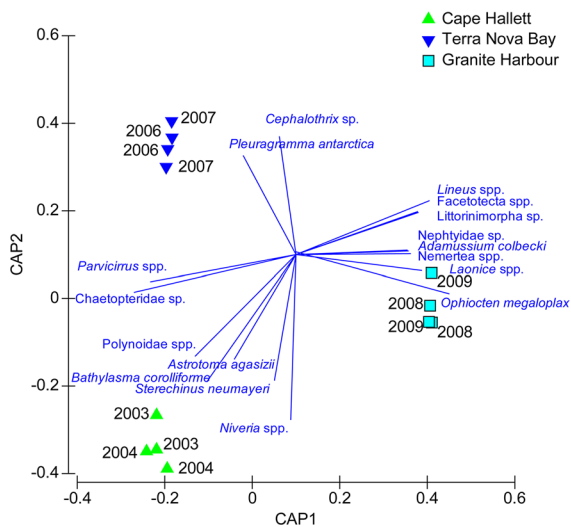


Fig. 7 Constrained ordination of the meroplankton samples from the coastal Ross Sea obtained with the CAP routine. Vectors show the Pearson correlations (>0.6) between meroplankton inhabitants and the CAP axes

notable in early December (Supplementary Fig. 1). Data available from Cape Roberts, in close proximity to Granite Harbour, show high levels of chlorophyll *a* under fast ice during austral spring (Ryan et al., 2006), but not to a level seen in the polynya in Terra Nova Bay (Supplementary Fig. 1). The presence of the polynya and its associated shallower mixed layer could be a key factor shaping meroplankton communities, through changes to the phytoplankton abundance and composition, particularly the relative dominance of diatoms and *Phaeocystis antarctica* (Smith et al., 2014). Future work would usefully consider simultaneous changes in the phytoplankton and meroplankton composition over time, particularly

given the dominance of planktotrophy as a feeding mode in larvae from all sites.

Secondly, the meroplankton community across the coastal Ross Sea did show significant differences in community composition between Cape Hallett, Terra Nova Bay and Granite Harbour with the multivariate analyses (CAP, SIMPER) revealing key mOTUs that discriminated each site. These key discriminating taxa also appear to be consistent over time. Sewell (2005) sampled at Cape Roberts, a few kilometres away from the Granite Harbour site, for 5 consecutive days in 2001 and using less-discriminatory morphological IDs showed a significant difference in the meroplankton community over the same 5-day period at Cape Hallett (Sewell, 2006). SIMPER analysis revealed the morphological larval types that discriminated between Cape Roberts and Cape Hallett (Sewell, 2006) are directly comparable to the mOTUS revealed here between Granite Harbour and Cape Hallett in the 2008 and 2009 sampling (Supplementary Table 2). Discriminating morphological larval types from Cape Roberts (e.g. echinoid, barnacle, polychaete egg and <2-setiger) have now been more precisely identified using molecular markers, and allow us to show that the taxa *Stereochinus neumayeri*, *Bathylasma corolliforme* and polynoid annelids shape the Cape Hallett community. Similarly, several species of nemertean pilidia (*Cephalothrix* sp.) and early stages of the Antarctic silverfish *Pleuragramma antarcticum* are important in Terra Nova Bay and some species such as *Tergipes antarcticus* are ubiquitous. The advantages of the molecular approach in larval identification exceed the mere increased accuracy, allowing also for a better understanding of the coupling of pelagic and benthic stages of each species: this offers new tools to assess

Table 4 Summary of the number of mOTUs with lecithotrophic and planktotrophic development in the coastal meroplankton from Victoria Land coast and the proportion of planktotrophic mOTUs at each site

Feeding mode	Phylum	Cape Hallett	Terra Nova Bay	Granite Harbour
Lecithotrophy	Echinodermata	5	1	2
	Entoprocta			1
	Porifera			4
	Nemertea	1		
	Platyhelminthes			2
	Cnidaria			1
	Total	6	1	10
Planktotrophy	Echinodermata	3	3	3
	Mollusca	3	5	5
	Annelida	4	5	7
	Nemertea	5	3	5
	Arthropoda	1	1	2
	Entoprocta			1
	Chordata		1	
	Total	16	18	23
Proportion of planktotrophic mOTUs		0.73	0.95	0.7

the distribution limits of benthic species and the potential changes in their distribution with changing climate conditions (Gallego et al. in review).

Thirdly, within sites there were no significant differences between years in larval abundance and alpha diversity (Fig. 4). In contrast, when considering the entire meroplankton community (composition and abundance of each mOTU) using Bray–Curtis similarities there was a clear year effect—illustrated by the MDS plots (Fig. 5)—in addition to the site effect. This result was not unexpected, as relatively small fluctuations in the time of spawning of those species with seasonal reproduction (Brethes et al., 1994; Stanwell-Smith & Clarke, 1998; Tyler et al., 2003; Grange et al., 2004, 2011; Brockington et al., 2007; Kang et al., 2009) may impact between-year similarities. Strong year effects have also been found in King George Island, where mollusc larval abundances were orders of magnitude higher in 2002 than in 2001 (Absher et al., 2003), with possible causes of these pattern in local environmental conditions and the influx of oceanic waters into coastal systems (Pruszek, 1980). Latitude does not appear to be a driver of the observed patterns in the meroplankton, and we hypothesize that the nature of the benthic assemblages (Gallego et al., 2014), hydrographical conditions (e.g. Vázquez et al., 2007) and/or levels of primary production (Ameneiro et al., 2012) may be of more importance. Companion studies on variation of benthic assemblages along the

Victoria Land Coast (Cummings et al., 2006, 2010; Rehm et al., 2006; Thrush et al., 2006; Rehm et al., 2011) have shown that most of the benthic variation occurs on the local scale—metres to hundreds of metres within sites (Cummings et al., 2006)—and that latitude is not a main driver of change in community composition (Cummings et al., 2010). Diversity changes among sites are not consistent across taxa: Cape Hallett sites showed higher species richness in molluscs (Schiaparelli et al., 2006) but lower alpha diversity in echinoderms (De Domenico et al., 2006) than Terra Nova Bay. This benthic variation has an impact in meroplankton communities: for example, nauplii from the acorn barnacle *Bathylasma corolliforme* were numerous in Cape Hallett (>700 specimens), near the shelf break where benthic populations are predominant (Dayton et al., 1982), whereas they were extremely rare in Terra Nova Bay (7 specimens) and Granite Harbour (1).

The LGP was restricted to just three coastal sites on the Victoria Land Coast and can only be considered a first step to understanding patterns in the Antarctic meroplankton community with latitude. Ideally, we could with a ship-based sampling programme extend the latitudinal gradient northwards to the Balleny Islands, where there is a rich benthic community but with higher influence of the Antarctic Circumpolar Current (Schiaparelli et al., 2006), include intermediate sites northward and southward of Terra Nova Bay

beyond the influence of the polynya, and avoid the space/time confound that was an unavoidable constraint on the current research.

Thorson's rule within the Ross Sea

Differences in primary production could be expected to have an impact on the relative abundance of planktotrophic and lecithotrophic larvae, and therefore on the species composition of the meroplankton assemblages at each site. Thanks to the molecular identification of the larvae and to their morphological description, it is possible to quantify how many of the species present in the water column are planktotrophic or lecithotrophic. We are aware that this is a first approximation, as assignment of a mOTU to planktotrophy or lecithotrophy is based on larval morphology and partially on the feeding type of non-Antarctic forms, and we acknowledge that there may be some inaccuracies.

Notwithstanding, our data does not support a latitudinal variation within the Ross Sea in terms of diversity of planktotrophic larvae, and does not find evidence in favour of Thorson's Rule along the Victoria Land coast. This suggests that, even with primary production concentrated within just a few months (Smith et al., 2014) and long developmental times associated with low temperatures (Pearse et al., 1991), planktotrophy is still ecologically successful in the high Antarctic. Granite Harbour is located at the northern end of McMurdo Sound, the southernmost body of ice-free water, and the number of species with pelagic larvae is higher than expected under the Thorson's rule (Sewell & Jury, 2011). The presence of planktotrophic larvae in all sites sampled, and the fact that 12 of the 13 mOTUs present in all sites are planktotrophic, suggests that this reproductive strategy is not limiting larval distribution, nor it is likely to be the limiting factor of their benthic populations.

A broader latitudinal test of this hypothesis would require sampling on northern islands, both in the Antarctic (Balleny Islands, 66°S) and in the Subantarctic (e.g. Auckland Islands, 50°S). While it is undeniable that differences in reproductive strategy do exist on a broader geographical scale (i.e. between polar, temperate and tropical seas), with a strong combined effect of water temperature and primary production on the proportion of benthic species without a planktonic stage (Marshall et al., 2012), it

is still debated whether the lower larval diversity in the Antarctic is caused by the ecological pressures seen today (as suggested by Thorson's rule) or reflects the speciation of the Antarctica fauna before ocean cooling, isolation by the establishment of the Antarctic Counter Current and the cyclic niche reductions during the Glacial Maxima (Poulin et al., 2002).

In conclusion, the study of three sites along the Victoria Land coast covering ~5° of latitude has revealed a highly diverse meroplankton community, with clear differences among sites, and with larvae from locally abundant taxa being critical to the distinctiveness of each site. Latitude was not a major driver of the differences in the meroplankton composition, nor of its abundance or diversity, although it would be valuable if future research expanded the latitudinal scope outside the Ross Sea, and investigated seasonal patterns across that range.

Acknowledgements The authors wish to thank Antarctica New Zealand and, in particular, S. Gordon for making the Latitudinal Gradient Project possible. Special thanks to the field team: J. Jury, K. Ruggiero, S. Van Dijken, L. Suberg, M. Hudson, M. Anderson, R. Millar, I. McLeod, R. Ingley and A. Fowler for assistance with sample collection and morphological identification of the specimens. The authors want to thank Dr Brian McArdle for statistical advice and the comments of the editors and reviewers which greatly improved the manuscript. Research was funded by a series of grants from the University of Auckland Research Committee and the Faculty Research Development Fund.

References

- Absher, T. M., G. Boehs, A. R. Feijó & A. C. Da Cruz, 2003. Pelagic larvae of benthic gastropods from shallow Antarctic waters of Admiralty Bay, King George Island. *Polar Biology* 26(6): 359–364.
- Ameneiro, J., B. Mouriño-Carballido, J. Parapar & E. Vázquez, 2012. Abundance and distribution of invertebrate larvae in the Bellingshausen Sea (West Antarctica). *Polar Biology* 35(9): 1–15.
- Anderson, M. J., 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Permutational multivariate analysis of variance, a computer program. Department of Statistics, University of Auckland, New Zealand: 24.
- Arrigo, K. R. & G. L. van Dijken, 2003. Phytoplankton dynamics within 37 Antarctic coastal polynya systems. *Journal of Geophysical Research: Oceans* 108(C8): 3271.
- Berkman, P. A., R. Cattaneo-Vietti, M. Chiantore, C. Howard-Williams, V. Cummings & R. Kvitek, 2005. Marine research in the Latitudinal Gradient Project along Victoria Land, Antarctica. *Scientia Marina* 69(Suppl. 2): 57–63.

- Bhaud, M., P. Koubbi, S. Razouls, O. Tachon & A. Accornero, 1999. Description of planktonic polychaete larvae from Terre Adelie and the Ross Sea (Antarctica). *Polar Biology* 22(5): 329–340.
- Brethes, J. C., G. Ferreyra & S. Delavega, 1994. Distribution, growth and reproduction of the limpet *Nacella (Patinigera) concinna* (Strebel 1908) in relation to potential food availability, in Esperanza Bay (Antarctic Peninsula). *Polar Biology* 14(3): 161–170.
- Brockington, S., L. S. Peck & P. A. Tyler, 2007. Gametogenesis and gonad mass cycles in the common circumpolar Antarctic echinoid *Sterechinus neumayeri*. *Marine Ecology Progress Series* 330: 139–147.
- Clarke, A., 2008. Antarctic marine benthic diversity: patterns and processes. *Journal of Experimental Marine Biology and Ecology* 366(1–2): 48–55.
- Cummings, V. J., S. Thrush, A. Norkko, N. Andrew, J. Hewitt, G. Funnell & A. M. Schwarz, 2006. Accounting for local scale variability in benthos: implications for future assessments of latitudinal trends in the coastal Ross Sea. *Antarctic Science* 18(4): 633–644.
- Cummings, V. J., S. F. Thrush, M. Chiantore, J. E. Hewitt & R. Cattaneo-Vietti, 2010. Macrobenthic communities of the north-western Ross Sea shelf: links to depth, sediment characteristics and latitude. *Antarctic Science* 22(6): 793–804.
- Dayton, P. K., W. A. Newman & J. Oliver, 1982. The vertical zonation of the deep-sea Antarctic acorn barnacle, *Bathylasma corolliforme* (Hoek): experimental transplants from the shelf into shallow water. *Journal of Biogeography* 9(2): 95–109.
- De Domenico, F., M. Chiantore, S. Buongiovanni, M. P. Ferranti, S. Ghione, S. Thrush, V. Cummings, J. Hewitt, K. Kroeger & R. Cattaneo-Vietti, 2006. Latitude versus local effects on echinoderm assemblages along the Victoria Land coast, Ross Sea. *Antarctica. Antarctic Science* 18(4): 655–662.
- Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer & A. Wilson, 2011. Geneious v6.1. Available from <http://www.geneious.com/>.
- Gallego, R., S. Lavery & M. A. Sewell, 2014. The meroplankton community of the oceanic Ross Sea during late summer. *Antarctic Science* 26(4): 345–360.
- Grange, L. J., P. A. Tyler, L. S. Peck & N. Cornelius, 2004. Long-term interannual cycles of the gametogenic ecology of the Antarctic brittle star *Ophionotus victoriae*. *Marine Ecology Progress Series* 278: 141–155.
- Grange, L. J., L. S. Peck & P. A. Tyler, 2011. Reproductive ecology of the circumpolar Antarctic nemertean *Parborlasia corrugatus*: no evidence for inter-annual variation. *Journal of Experimental Marine Biology and Ecology* 404(1–2): 98–107.
- Grant, R. A. & K. Linse, 2009. Barcoding Antarctic biodiversity: current status and the CAML initiative, a case study of Marine invertebrates. *Polar Biology* 32(11): 1629–1637.
- Heimeier, D., S. Lavery & M. A. Sewell, 2010a. Molecular species identification of *Astrotoma agassizii* from planktonic embryos: further evidence for a cryptic species complex. *Journal of Heredity* 101(6): 775–779.
- Heimeier, D., S. Lavery & M. A. Sewell, 2010b. Using DNA barcoding and phylogenetics to identify Antarctic invertebrate larvae: lessons from a large scale study. *Marine Genomics* 3(3–4): 165–177.
- Hoffman, J. I., L. S. Peck, K. Linse & A. Clarke, 2011. Strong population genetic structure in a broadcast-spawning antarctic marine invertebrate. *Journal of Heredity* 102(1): 55–66.
- Howard-Williams, C., D. Peterson, W. B. Lyons, R. Cattaneo-Vietti & S. Gordon, 2006. Measuring ecosystem response in a rapidly changing environment: the Latitudinal Gradient Project. *Antarctic Science* 18(4): 465–471.
- Howard-Williams, C., I. Hawes & S. Gordon, 2010. The environmental basis of ecosystem variability in Antarctica: research in the Latitudinal Gradient Project. *Antarctic Science* 22 (Special Issue 06): 591–602.
- Hunter, R. L. & K. M. Halanych, 2008. Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. *Journal of Heredity* 99(2): 137–148.
- Kang, D. H., I. Y. Ahn & K. S. Choi, 2009. The annual reproductive pattern of the Antarctic clam, *Laternula elliptica* from Marian Cove. King George Island. *Polar Biology* 32(4): 517–528.
- Laptikhovsky, V., 2006. Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. *Marine Ecology* 27(1): 7–14.
- Levin, L. A. & T. S. Bridges, 2001. Pattern and diversity in the reproduction and development. In McEdward, L. R. (ed.), *Ecology of Marine Invertebrate Larvae*. CRC Press, London: 2–48.
- Marshall, D. J., P. J. Krug, E. K. Kupriyana, M. Byrne & R. B. Emler, 2012. The biogeography of marine invertebrate life histories. *Annual Review of Ecology, Evolution, and Systematics* 43(1): 97–114.
- Martin, S., R. S. Drucker & R. Kwok, 2007. The areas and ice production of the western and central Ross Sea polynyas, 1992–2002, and their relation to the B-15 and C-19 iceberg events of 2000 and 2002. *Journal of Marine Systems* 68(1–2): 201–214.
- Mercier, A., M. A. Sewell & J.-F. Hamel, 2013. Pelagic propagule duration and developmental mode: reassessment of a fading link. *Global Ecology and Biogeography* 22(5): 517–530.
- Mileikovsky, S. A., 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Marine Biology* 10(3): 193–213.
- Murray, J., 1895. General observations on the distribution of marine organisms. Report scientific research, voyage of the HMS challenger, a summary of the scientific results, second part: 1431–1462.
- Olson, R. R., J. A. Runstadler & T. D. Kocher, 1991. Whose larvae? *Nature* 351(6325): 357–358.
- Pearse, J. S., J. B. McClintock & I. Bosch, 1991. Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. *American Zoologist* 31(1): 65–80.
- Poulin, É., A. T. Palma & J. P. Féral, 2002. Evolutionary versus ecological success in Antarctic benthic invertebrates. *Trends in Ecology and Evolution* 17(5): 218–222.
- Povero, P., M. Castellano, N. Ruggieri, L. S. Monticelli, V. Saggiomo, M. Chiantore, M. Guidetti & R. Cattaneo-

- Vietti, 2006. Water column features and their relationship with sediments and benthic communities along the Victoria Land coast, Ross Sea, summer 2004. *Antarctic Science* 18(4): 603–613.
- Pruszek, Z., 1980. Currents circulation in the waters of Admiralty Bay (region of Arctowski Station on King George Island). *Polish Polar Research* 1(1): 55–74.
- Rehm, P., S. Thatje, W. E. Arntz, A. Brandt & O. Heilmayer, 2006. Distribution and composition of macrozoobenthic communities along a Victoria-Land transect (Ross Sea, Antarctica). *Polar Biology* 29(9): 782–790.
- Rehm, P., R. A. Hooke & S. Thatje, 2011. Macrofaunal communities on the continental shelf off Victoria Land, Ross Sea. *Antarctica. Antarctic Science* 23(05): 449–455.
- Ryan, K. G., E. N. Hegseth, A. Martin, S. K. Davy, R. O'Toole, P. J. Ralph, A. McMinn & C. J. Thorn, 2006. Comparison of the microalgal community within fast ice at two sites along the Ross Sea coast. *Antarctica. Antarctic Science* 18(4): 583–594.
- Schiaparelli, S., A. N. Lörz & R. Cattaneo-Vietti, 2006. Diversity and distribution of mollusc assemblages on the Victoria Land coast and the Balleny Islands, Ross Sea. *Antarctica. Antarctic Science* 18(4): 615–631.
- Schiaparelli, S., C. Ghiglione, M. Alvaro, H. Griffiths & K. Linse, 2014. Diversity, abundance and composition in macrofaunal molluscs from the Ross Sea (Antarctica): results of fine-mesh sampling along a latitudinal gradient. *Polar Biology* 37(6): 859–877.
- Sewell, M. A., 2005. Examination of the meroplankton community in the south-western Ross Sea, Antarctica, using a collapsible plankton net. *Polar Biology* 28(2): 119–131.
- Sewell, M. A., 2006. The meroplankton community of the northern Ross Sea: a preliminary comparison with the McMurdo Sound region. *Antarctic Science* 18(4): 595–602.
- Sewell, M. A. & J. A. Jury, 2011. Seasonal patterns in diversity and abundance of the high antarctic meroplankton: plankton sampling using a Ross Sea desalination plant. *Limnology and Oceanography* 56(5): 1667–1681.
- Sewell, M. A., S. Lavery & C. S. Baker, 2006. Whose larva is that? Molecular identification of planktonic larvae of the Ross Sea. *New Zealand Aquatic Environment and Biodiversity Report No. 3*: 57.
- Shannon, C. E., 1948. A mathematical theory of communication. *Bell System Technical Journal* 27: 379–423.
- Smith, D. L. & K. B. Johnson, 1977. A guide to marine coastal plankton and marine invertebrate larvae. Kendall/Hunt Publishing Company, Dubuque, Iowa.
- Smith Jr., W. O., D. G. Ainley & R. Cattaneo-Vietti, 2007. Trophic interactions within the Ross Sea continental shelf ecosystem. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362(1477): 95–111.
- Smith Jr., W. O., D. G. Ainley, K. R. Arrigo & M. S. Dinniman, 2014. The oceanography and ecology of the Ross Sea. *Annual Review of Marine Science* 6: 469–487.
- Stanwell-Smith, D. & A. Clarke, 1998. Seasonality of reproduction in the cushion star *Odontaster validus* at Signy Island. *Antarctica. Marine Biology* 131(3): 479–487.
- Stanwell-Smith, D., A. Hood & L. S. Peck, 1997. A field guide to the pelagic invertebrate larvae of the maritime Antarctic. British Antarctic Survey, Cambridge.
- Steedman, F. H., 1976. Zooplankton fixation and preservation, Vol. 4. UNESCO, Paris.
- Thomson, C. W., 1876. Notice of some peculiarities in the mode of propagation of certain echinoderms of the Southern Sea. *Journal of the Linnean Society of London, Zoology* 13(66): 55–79.
- Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews* 25(1): 1–45.
- Thrush, S., P. Dayton, R. Cattaneo-Vietti, M. Chiantore, V. Cummings, N. Andrew, I. Hawes, S. Kim, R. Kvitek & A.-M. Schwarz, 2006. Broad-scale factors influencing the biodiversity of coastal benthic communities of the Ross Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 53(8): 959–971.
- Todd, C. D., M. S. Laverack & G. A. Boxshall, 1996. Coastal Marine Zooplankton, 2nd ed. Cambridge University Press, Cambridge.
- Tyler, P. A., S. Reeves, L. Peck, A. Clarke & D. Powell, 2003. Seasonal variation in the gametogenic ecology of the Antarctic scallop *Adamussium colbecki*. *Polar Biology* 26(11): 727–733.
- Vacchi, M., M. La Mesa, M. Dalu & J. Macdonald, 2004. Early life stages in the life cycle of Antarctic silverfish, *Pleurogramma antarcticum* in Terra Nova Bay, Ross Sea. *Antarctic Science* 16(03): 299–305.
- Vacchi, M., A. L. DeVries, C. W. Evans, M. Bottaro, L. Ghigliotti, L. Cutroneo & E. Pisano, 2012. A nursery area for the Antarctic silverfish *Pleurogramma antarcticum* at Terra Nova Bay (Ross Sea): first estimate of distribution and abundance of eggs and larvae under the seasonal sea-ice. *Polar biology* 35(10): 1573–1585.
- Vance, R. R., 1973. On reproductive strategies in marine benthic invertebrates. *American Naturalist*:339-352.
- Vázquez, E., J. Ameneiro, S. Putzeys, C. Gordo & P. Sangrà, 2007. Distribution of meroplankton communities in the Bransfield Strait, Antarctica. *Marine Ecology Progress Series* 338: 119–129.
- Webb, K. E., D. K. A. Barnes, M. S. Clark & D. A. Bowden, 2006. DNA barcoding: a molecular tool to identify Antarctic marine larvae. *Deep-Sea Research Part II: Topical Studies in Oceanography* 53(8–10): 1053–1060.