―研究論文―
Scientific Paper

Interannual variability in euthecosomatous pteropods (*Limacina* spp.) in the Indian sector of the Southern Ocean during austral summer

Yuko Nishizawa[1*], Hiroshi Sasaki[2] and Kunio T. Takahashi[3,4]

夏季の南極海インド洋区における有殻翼足類（*Limacina* spp.）の経年変化

西澤裕子[1*]・佐々木　洋[2]・高橋邦夫[3,4]

要旨：　海洋酸性化がアラゴナイトの殻を形成する有殻翼足類に及ぼす影響を明らかにするため，1987 年から 2008 年の夏季に，南緯 55–64 度，東経 110 度付近海域において日本南極地域観測隊により採取された有殻翼足類試料を用いてその現存量と殻径の経年変動を調べた．有殻翼足類（*Limacina* 属が優占）の殻径はほとんどが 400 μm 未満で，現存量は最小 2.2 ind. m^{-3} から最大 2767.7 ind. m^{-3} の間で変動していた．55°S, 60°S, 64°S の 3 測点付近の水深 400 m 以浅においては海水 pH の有意な減少は確認されなかった．また有殻翼足類の現存量においても年変動が大きく 1987 年から 2008 年の 20 年間で有意な減少傾向を示さなかった．またそれらのサイズも有意な減少傾向は見いだせなかった．南極海における海洋酸性化は今後徐々に進行すると思われるが，有殻翼足類に対する影響はまだ顕在化していないと考えられる．

Abstract:　Euthecosomatous pteropods with carbonate (aragonite) shells living in polar waters are thought to be very sensitive to ocean acidification resulting from increased anthropogenic CO_2 uptake. To assess the impacts of ocean acidification on pteropods, we investigated pteropod abundance and shell size at three sites located between 55 and 64°S on the 110°E meridian in the Indian sector of the Southern Ocean during austral summer from 1987 to 2008 with the Japanese Antarctic Research Expedition (JARE) monitoring projects. The pteropod community was dominated by *Limacina* spp. with a shell size of ＜400 μm, and there was a distinct interannual variation ranging 2.2–2767.7 ind. m^{-3}. No significant decrease in pH was detected in various water layers above 400 m depth at three sites (55°S, 60°S and 64°S) during the study period, and no marked decrease both in abundance and shell size have been observed in these waters for at least 20 years. Though ocean acidification in the Southern Ocean will be in progress, the effects on shelled pteropods are not yet evident.

[1] 宮城県水産技術総合センター．Miyagi Prefecture Fisheries Technology Institute, 97–6 Watanoha Sodenohama, Ishinomaki, Miyagi 986-2135.
[2] 石巻専修大学．Ishinomaki Senshu University, 1 Minamizakai Shinmito, Ishinomaki, Miyagi 986-8580.
[3] 情報・システム研究機構国立極地研究所．National Institute of Polar Research, Research Organization of Information and Systems, 10–3 Midori-cho, Tachikawa, Tokyo 190-8518.
[4] 総合研究大学院大学複合科学研究科極域科学専攻．Department of Polar Science, School of Multidisciplinary Sciences, SOKENDAI (The Graduate University for Advanced Studies), 10–3 Midori-cho, Tachikawa, Tokyo 190-8518.
[*] Corresponding author. E-mail: nishizawa.t.y@gmail.com

1.　Introduction

The world's oceans have absorbed approximately one third of the total anthropogenic carbon dioxide (CO_2) released into the atmosphere (Sabine *et al*., 2004).　This has caused profound changes in ocean carbonate chemistry including a decrease in seawater pH, carbonate ion (CO_3^{2-}) concentration, and calcium carbonate ($CaCO_3$) saturation state.　The overall process is commonly referred to as "ocean acidification" (Caldeira and Wickett, 2003; Doney *et al*., 2009; Orr *et al*., 2005).　The global mean surface seawater pH has already decreased by approximately 0.1 since the end of the 18th century.　According to model projections, the global mean surface pH will decrease by another 0.3 units by the end of the 21st century (Caldeira and Wickett, 2003).

　　Marine calcifying organisms, including molluscs, corals, echinoderms, and crustaceans, build their skeletons out of two common types of $CaCO_3$, calcite and aragonite; the latter is approximately 50% more soluble than calcite in seawater (Mucci, 1983).　The surface waters of the polar oceans will be the first to become undersaturated with respect to aragonite because cold water absorbs more CO_2 than does warm water.　Model projections have suggested that aragonite undersaturation of the surface waters of the Southern Ocean will begin in 2050 (Orr *et al*., 2005), while in Arctic Ocean surface waters this phenomenon will commence as early as 2016 (Steinacher *et al*., 2009).　Surprisingly, based on direct observations of CO_2 surface waters were undersaturated with respect to aragonite in the Canada Basin (Yamamoto-Kawai *et al*., 2009).　The surface water pH in the Southern Ocean has also been lowered (Midorikawa *et al*., 2012).　Because aragonite undersaturation of seawater impacts the formation of aragonite skeletons and shells by marine organisms (Comeau *et al*., 2009; Fabry *et al*., 2008; Langdon and Atkinson, 2005; Orr *et al*., 2005), there is concern about the future consequences of aragonite undersaturation for marine organisms that possess shells made of aragonite.

　　Euthecosomatous (shelled) pteropods are a major aragonite producing group.　They are holoplanktonic gastropods, and are commonly referred to as "sea butterflies" because of their wing-like parapodia (Lalli and Gilmer, 1989).　They are omnivorous and secrete large mucus webs to collect their prey, which includes phytoplankton, small zooplankton, and juvenile pteropods (Gannefors *et al*., 2005; Gilmer and Harbison, 1991).　They are widely distributed from tropical to polar oceans, although the highest abundances occur at high latitudes (Bé and Gilmer, 1977; Hunt *et al*., 2008; Lalli and Gilmer, 1989).　Four species of shelled pteropod typically occur in the Southern Ocean, including *Limacina helicina antarctica*, *L. retroversa*, *Clio pyramidata*, and *C. piatkowskii* (Hunt *et al*., 2008).　*L. retroversa* is mostly concentrated in the upper 200 m of sub-Antarctic surface waters north of the Antarctic Convergence.　High densities of *L. helicina* occur south of the Antarctic Convergence in Antarctic surface waters extending to depths of 100−250 m (Lalli and Gilmer, 1989).　*L. helicina* is even present in deep layers of ～500 m in the Antarctic Peninsula (Hunt *et al*., 2008).

　　Shelled pteropods can dominate Southern Ocean zooplankton communities, and high *L. helicina* densities (up to 2681 ind. m^{-3}) have been observed at a site around the South

Georgia Islands (Hunt *et al*., 2008). In marine food webs they are important prey organisms for zooplankton and higher predators including herring, salmon, whales, and birds (Hunt *et al*., 2008), and as the main planktonic producers of aragonite in the world's oceans. They also play an important role in the export of carbonate into the deep ocean (Fabry *et al*., 2008). Several investigations on the impacts of aragonite undersaturation on shelled pteropods are available in the literature. Orr *et al*. (2005) reported the dissolution of pteropod shells exposed to aragonite undersaturation, Comeau *et al*. (2009) reported a 28% reduction in calcification in *L. helicina* at the aragonite saturation threshold ($\Omega_a = 1.0$), compared with aragonite oversaturation ($\Omega_a = 1.9$), and Lischka *et al*. (2011) reported a 14% increase in mortality of juvenile *L. helicina* under conditions of aragonite undersaturation ($\Omega_a = 0.7$), compared with aragonite oversaturation ($\Omega_a = 2.4$). However, there has been very little study of the impacts of ocean acidification on pteropods *in situ*. Several studies focused on interannual variability of sediment trap-collected shelled pteropods (Roberts *et al*., 2008) have shown that a decadal trend of decrease in the downward flux and mean shell weight of *L. helicina* occurred with ocean acidification in the sub-Antarctic Ocean. However, Ohman *et al*. (2009) found no clear evidence of a decline in the abundances of *Limacina* spp. and *Clio* spp. from 1951 to 2008 in waters of southern California, even though waters as shallow as 40 m were undersaturated. Thus, it is unclear whether ocean acidification is currently impacting shelled pteropods *in situ*.

A model projection has indicated that seawater is at the aragonite saturation threshold ($\Omega_a = 1.0$) at depths of approximately 500 m in the vicinity of 60°S on the 110°E meridian in the Southern Ocean (Feely *et al*., 2004). *Limacina* shells may dissolve in these waters if there is a shallowing in saturation depth with respect to aragonite. Therefore, long-term observations are needed to establish whether changes in pteropod abundance and their biological properties are occurring. In the Indian sector of the Southern Ocean, plankton sampling has been conducted each austral summer in the vicinity of 60°S on the 110°E meridian, as part of a marine biology monitoring program conducted by the Japanese Antarctic Research Expedition (JARE). Oceanographic data and plankton samples along the line of 110°E in the Southern Ocean have been collected since 1987 (JARE-29). To elucidate any changes in pteropod abundance and mean shell size associated with ocean acidification, in the present study we investigated the interannual variability of pteropods in waters collected from 55–64°S along the 110°E meridian over the 20-year period from 1987 to 2008.

2. Materials and Methods

The zooplankton samples for this study were collected using a NORPAC net (mouth diameter 0.45 m; 110 μm mesh) at 55°S, 60°S, and 64°S along the 110°E meridian in the Indian sector of the Southern Ocean (Fig. 1), during research cruises aboard the JARE icebreaker "*Shirase*" over the austral summers from December 1987 to March 2008. No sampling was performed at the 55°S site in December 1989, 1991, 1995, 1997, and 2001, at the 60°S site in December 1988, 1989, 1990, 1991, 1995, 1997, and 2001, or at the 64°S site in March 1989, 1991, 1992, 2000, and 2001 (Table 1). Sampling was conducted during the daytime on all dates. The net was equipped with a flow meter to estimate the volume of water filtered, and was hauled vertically from 150 m to the surface at a speed of
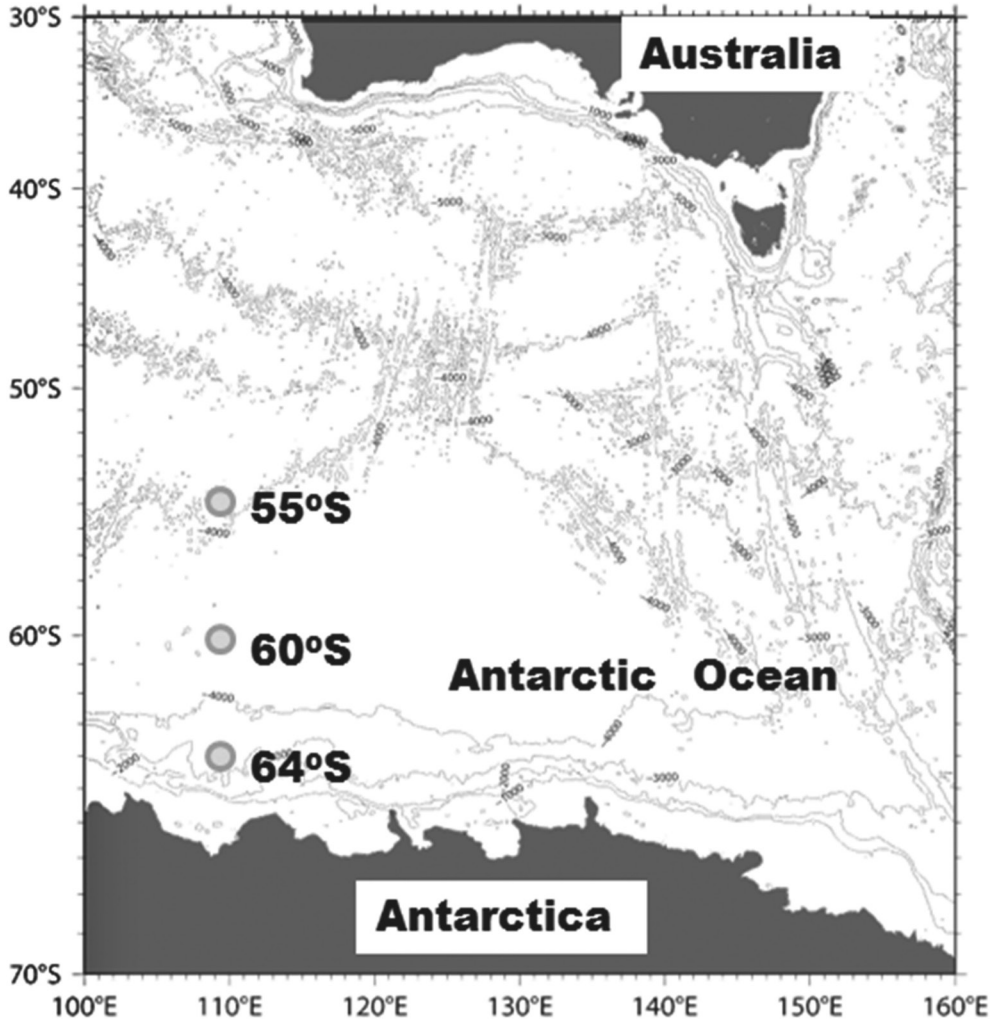
Fig. 1.   Locations of the plankton sampling stations (open circles) at 55°S, 60°S, and 64°S, on the 110°E meridian in the Southern Ocean.

approximately $1 \, \mathrm{m \, s^{-1}}$. After collection, all zooplankton were preserved in 5–10% buffered formalin seawater. All pteropods were counted and the shell size (the maximum shell diameter) was measured using a stereomicroscope; fragmented shells were not included in our analyses. For each station the number of pteropods counted was converted to a density measurement (ind. $\mathrm{m^{-3}}$).

Spearman's rank correlation was performed to assess interannual changes in pteropod abundance, shell size, and seawater pH at the sampling sites. This test was also used to identify correlations between environmental factors (water temperature, pH, and chlorophyll-*a*) and pteropod abundance, and between seawater pH and pteropod shell size. Temporal changes in pteropod abundance, shell size, and seawater pH were tested with the ordinary least-squares regression. The water temperature, salinity, seawater pH, and

Table 1. Dates of plankton sampling made during the JARE cruises. Open circles indicate that the samples were successfully obtained.

Year	Month	Location		
		55°S	60°S	64°S
1987	Mar.	×	×	×
	Dec.	○	×	×
1988	Mar.	×	×	○
	Dec.	×	×	×
1989	Mar.	×	×	×
	Dec.	×	×	×
1990	Mar.	×	○	×
	Dec.	○	×	×
1991	Mar.	×	×	×
	Dec.	×	×	×
1992	Mar.	×	×	×
	Dec.	○	○	×
1993	Mar.	×	×	○
	Dec.	○	○	×
1994	Mar.	×	×	○
	Dec.	○	○	×
1995	Mar.	×	×	○
	Dec.	×	×	×
1996	Mar.	×	×	○
	Dec.	○	○	×
1997	Mar.	×	×	○
	Dec.	×	×	×
1998	Mar.	×	×	○
	Dec.	○	○	×
1999	Mar.	×	×	×
	Dec.	○	○	×
2000	Mar.	×	×	×
	Dec.	○	○	×
2001	Mar.	×	×	×
	Dec.	×	×	○
2002	Mar.	×	×	○
	Dec.	○	○	×
2003	Mar.	×	×	○
	Dec.	○	×	×
2004	Mar.	×	×	○
	Dec.	○	○	×
2005	Mar.	×	×	○
	Dec.	○	○	×
2006	Mar.	×	×	○
	Dec.	○	○	×
2007	Mar.	×	×	○
	Dec.	○	○	×
2008	Mar.	×	×	○
	Dec.	×	×	×

chlorophyll-*a* (Chl-*a*) data used in this study were obtained from JARE data reports (JARE-29–49); oceanography reports were the sources for data on water temperature, salinity, and pH, while marine biology reports were the sources of data on Chl-*a*. The mean pteropod abundance at each site (55°S, 60°S, and 64°S) was analyzed statistically using the Steel-Dwass test.

3. Results

At the 55°S site the water temperature and salinity in the upper 150 m ranged from 0.18 to 2.67℃, and from 33.85 to 34.24 PSU, respectively. The minimum temperature (0℃) was recorded at around 135 m depth, and a halocline was detected at 150 m. At the 60°S site the water temperature and salinity in the upper 150 m ranged from −1.17 to 1.42℃, and from 33.43 to 34.62 PSU, respectively. The minimum temperature, which ranged from −1.5 to −1℃, was recorded at around 75 m depth, and a halocline was detected at around 100 m. At the 64°S site the water temperature and salinity in the upper 150 m ranged from −1.63 to 1.40℃, and from 33.15 to 34.70 PSU, respectively. The minimum temperature, which ranged from −1.5 to −1℃, was recorded at around 75 m depth, and a halocline was detected at around 75 m. This implies that during the observation periods the cold Winter Water (WW; Aoki, 1997) was located at around 135 m depth at the 55°S site, and at around 75 m at the 60°S and 64°S sites in the present observation periods. Although the hydrographic properties differed slightly among sites, the water masses encompassing the three study sites were located south of the polar front (around 52°S).

Because the pH of the seawater changed with depth in the upper 150 m, to assess interannual variability we recalculated the mean seawater pH values for three depth layers; 0–20 m, 20–200 m (dominated by the WW layer), and 200–400 m. No significant trend in pH in the various water layers was detected at any of the three sampling sites during the study period (Table 2a, Fig. 2). Although the surface seawater (0–20 m) pH at the 55°S site

Table 2. *Spearman rank-correlation coefficients for the three sampling sites in the period 1988–2007 between: (a) the seawater pH in the three water layers in the upper 400 m and the time of the year (1988–2007); and (b) between the abundance of* Limacina *spp. and time of year, and between the mean shell sizes and time of year (1987–2008). The ρ and P values indicate the Spearman rank correlation coefficient and the significance level, respectively.*

a	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
0–20 m	0.16	0.6	-0.19	0.57	0.06	0.83
Winter Water layer	-0.15	0.67	-0.16	0.62	-0.11	0.73

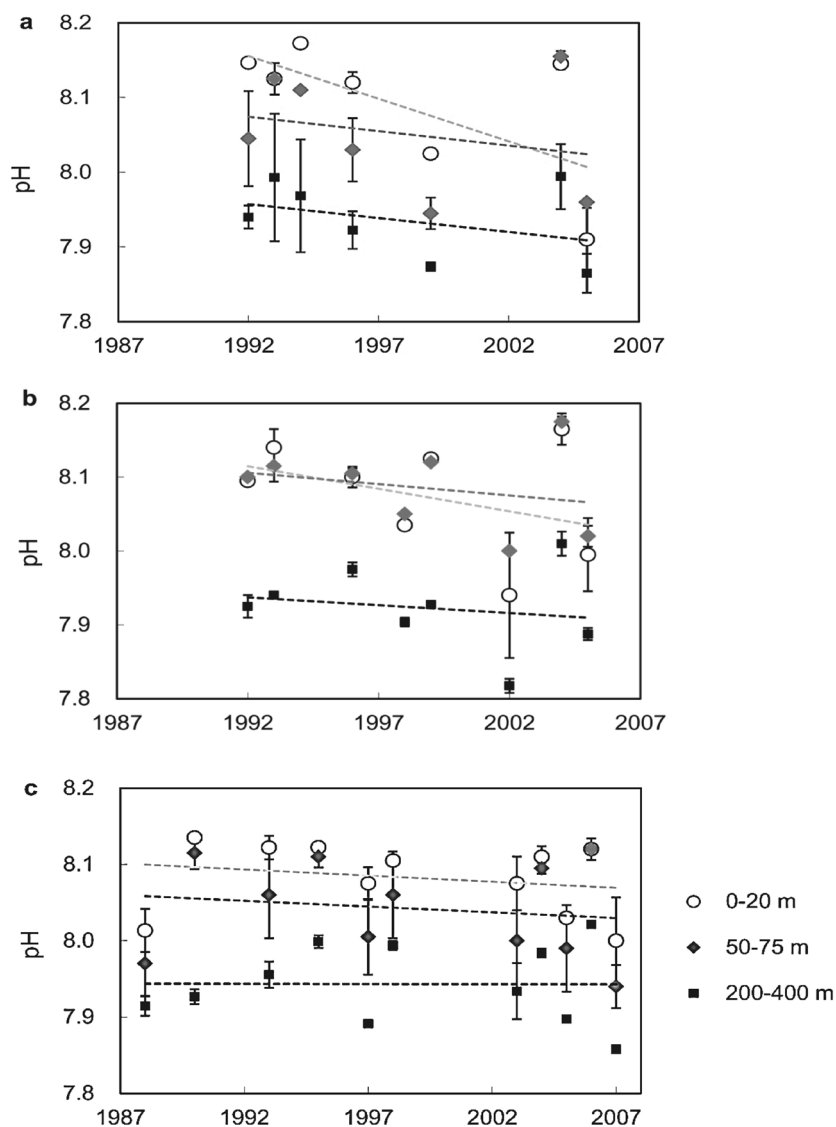| b	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
Pteropod abundance	-0.34	0.2	0.06	0.83	0.16	0.55
Shell size	-0.1	0.72	0.36	0.23	-0.25	0.34

Fig. 2. Interannual variability in seawater pH. (a) Seawater pH at 0–20 m, in the Winter Water layer (50–75 m), and at 200–400 m at 55°S on the 110°E meridian for the period 1992–2005. Linear regressions are indicated by dotted lines (0–20 m: $Y = -0.0114X + 30.9180$, $R^2 = 0.4181$; the Winter Water layer: $Y = -0.0039X + 15.7850$, $R^2 = 0.0625$; 200–400 m: $Y = -0.0037X + 15.4070$, $R^2 = 0.1378$). (b) Seawater pH at 0–20 m, in the Winter Water layer, and at 200–400 m at 60°S on the 110°E meridian for the period 1992–2005. Linear regressions are indicated by dotted lines (0–20 m: $Y = -0.0061X + 20.2930$, $R^2 = 0.1456$; the Winter Water layer: $Y = -0.0031X + 14.2220$, $R^2 = 0.0657$; 200–400 m: $Y = -0.0021X + 12.1660$, $R^2 = 0.0316$). (c) Seawater pH at 0–20 m, in the Winter Water layer, and at 200–400 m at 64°S on the 110°E meridian for the period 1988–2007. Linear regressions are indicated by dotted lines (0–20 m: $Y = -0.0016X + 11.2980$, $R^2 = 0.0503$; the Winter Water layer: $Y = -0.0015X + 11.0440$, $R^2 = 0.0247$; 200–400 m: $Y = -0.00005X + 8.0356$, $R^2 = 0.00004$). Each plot having an error bar shows the mean of 3–4 data points; a single data point is shown with no error bar.

appeared to decrease gradually, based on a weekly significant liner regression coefficient (R^2 = 0.4181; Fig. 2a), the rank correlation coefficient indicated no significant effect (ρ = 0.16, P = 0.60; Table 2a).

The pteropods collected during the study belonged to the genus *Limacina*, which is characterized by the presence of sinistral shells (Lalli and Gilmer, 1989). Two species of *Limacina* (*L. helicina antarctica* and *L. retroversa australis*) are common in the Southern Ocean (Hunt *et al*., 2008). We could not distinguish between the two abovementioned species, primarily because most of the pteropods collected in this study were in the veliger stage of development, when their morphological differences are less obvious. The abundance of *Limacina* spp. ranged from 2.2 ind. m^{-3} (55°S, December 2007) to 2767.7 ind. m^{-3} (64°S, March 1997), and the mean abundance was 341.0 ± 562.3 ind. m^{-3} (Fig. 3). There was regional variability in abundance. The mean abundances were 376.2 ± 386.1 ind. m^{-3} at 55°S (collected in December), 53.9 ± 68.4 ind. m^{-3} at 60°S (December), and 535.6 ± 812.8 ind. m^{-3} at 64°S (March). Based on the Steel-Dwass test, the mean abundances at 55°S and 64°S were significantly higher than at 60°S (p < 0.01). No particular trend in pteropod abundance was observed at any site, based on the linear regression coefficients (55°S: R^2 = 0.1222; 60°S: 0.0197; and 64°S: 0.0234; not shown in Fig. 3), and no marked trend of decrease was found in pteropod abundance at the three sites over the study periods (Table 2a, Fig. 3).

The shell diameter of *Limacina* spp. ranged from 112.0 μm to 825.0 μm, and the mean diameter was 215.4 ± 34.7 μm (n = 4949). The mean shell diameters were 223.6 ± 32.5 μm (n = 2029) for 55°S (December), 212.8 ± 39.1 μm (n = 917) for 60°S (December), and 209.2 ± 33.8 μm (n = 2003) for 64°S (March) (Fig. 4), indicating that there was no distinct size variation between pteropods collected in December and March. No significant decrease in the mean shell size over the 20-year study period was observed. Shells < 400 μm in diameter accounted for 97.6% of the total pteropod abundance at the 55°S site, 99.5% at the
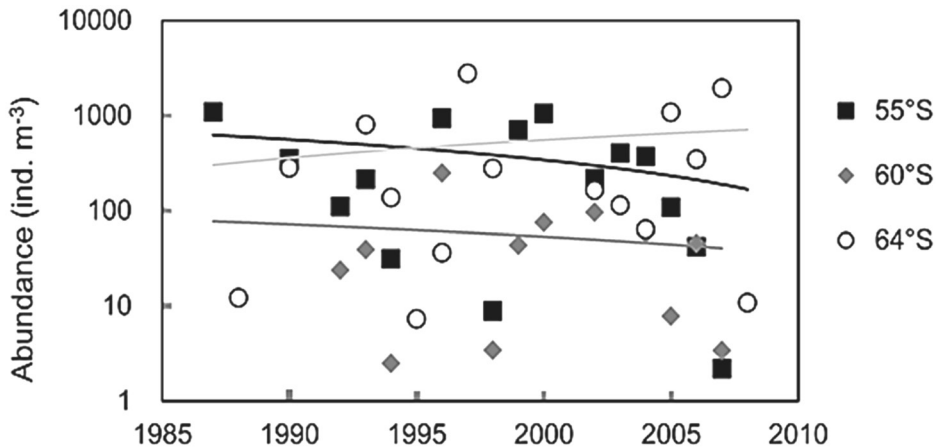

Fig. 3. Interannual variability in Limacina *spp. abundance (ind. m^{-3}) during the period 1987–2008. Linear regressions of pteropod abundance at 55–64°S (110°E meridian) are indicated by solid lines (55°S: Y = − 21.742X + 43825.0, R^2 = 0.122; 60°S: Y = − 1.8413X + 3735.9, R^2 = 0.020; 64S: Y = 19.417X + 38280.0, R^2 = 0.023).*

60°S site, and 98.2% at the 64°S site. There was no significant difference in the mean shell size among the three sampling sites. We found no significant correlations between pH and pteropod abundance (Table 3a), pH and shell size (Table 3b), or water temperature and pteropod abundance (Table 4a). We also found no significant correlation between the Chl-*a* concentration and pteropod abundance at any site (Table 4b).
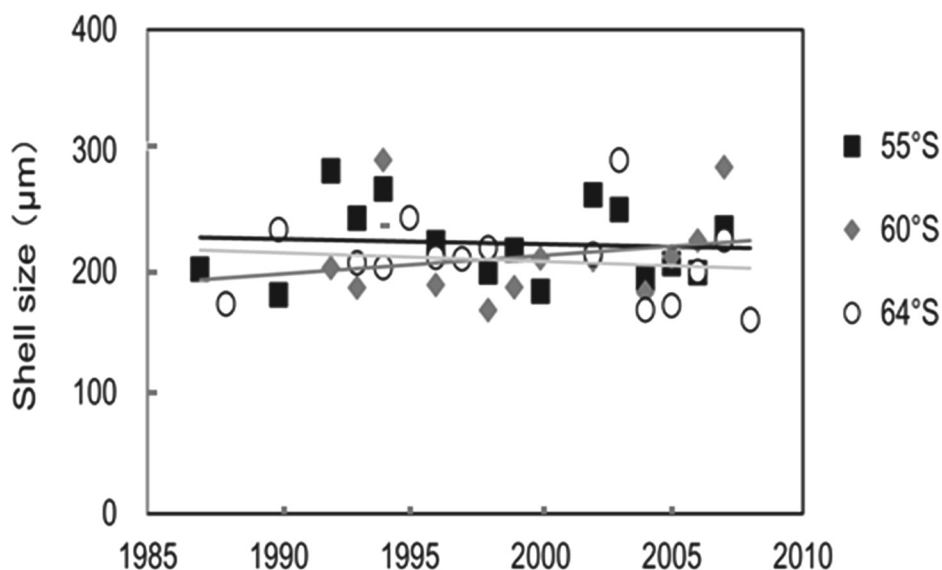

Fig. 4. *Interannual variability in mean pteropod shell size during the period 1987–2008. Linear regressions of the mean pteropod shell size at 55–64°S (110°E meridian) are indicated by solid lines (55°S: $Y = -0.418X + 1058.9$, $R^2 = 0.006$; 60°S: $Y = 1.542X + 2871.6$, $R^2 = 0.042$; 64°S: $Y = -0.712X + 1633.7$, $R^2 = 0.018$).*

Table 3. *Spearman rank-correlation coefficients for the three sampling sites in the period 1988–2007 between: (a) the seawater pH in the three water layers and the abundance of* Limacina *spp. (ind. m^{-3}); and (b) between the seawater pH in the three water layers and the mean shell size (µm). The ρ and P values indicate the Spearman rank correlation coefficient and the significance level, respectively.*

a	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
0–20 m	-0.39	0.34	0.24	0.53	-0.3	0.35
Winter Water layer	-0.18	0.66	0.26	0.49	-0.27	0.4
200–400 m	-0.04	0.93	0.41	0.28	-0.57	0.07

b	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
Pteropod abundance	0.61	0.14	-0.62	0.1	0.23	0.46
Shell size	0.07	0.86	-0.6	0.12	0.07	0.82
200–400 m	0.11	0.79	-0.55	0.15	0.03	0.93

Table 4. Spearman rank-correlation coefficients for the three sampling sites between: (a) the water temperature in the three water layers and the abundance of Limacina *spp. (ind. m^{-3}) in the period 1988–2007; and (b) between the Chl-a concentration in two of the water layers and the abundance of* Limacina *spp. (ind. m^{-3}) in the period 1988–2008. The ρ and P values indicate the Spearman rank correlation coefficient and the significance level, respectively.*

a	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
0–20 m	-0.2	0.47	0.1	0.75	0.36	0.2
Winter Water layer	0.15	0.58	0.36	0.26	0.13	0.64
200–400 m	0.3	0.28	0.39	0.22	0.2	0.48

b	55°S, 110°E		60°S, 110°E		64°S, 110°E	
Depth range	ρ	P	ρ	P	ρ	P
Pteropod abundance	-0.64	0.12	-0.21	0.57	-0.37	0.24
Shell size	-0.29	0.48	-0.14	0.71	-0.13	0.68
200–400 m	-0.36	0.38	-0.24	0.53	-0.03	0.93

4.   Discussion

Based on observations over a period of approximately 20 years, our results reveal no marked trend of decrease in seawater pH in the various water layers at any of the three sampling sites (Fig. 2). However, it is not clear whether a pH decrease at particular sites in the Southern Ocean reflects changes in pH at other locations. Along the 110°E meridian in the Southern Ocean, the $p\mathrm{CO}_2$ in the permanent open ocean zone between the polar front and the northern edge of the winter ice cover (63°S) has increased at a rate of approximately 1.3 µg yr^{-1} (Hashida *et al*., 2010). An increase in anthropogenic CO_2 inputs could cause a decrease in pH in the surface seawater in the vicinity of 55−64°S, along the 110°E meridian. Seawater subject to vertical mixing of deep water with surface water in winter remains in the surface layers when a halocline forms in summer, and this may cause a decrease in surface water pH. However, no marked decrease in pH was observed over the 20-year study period. As the most pronounced decreases in $p\mathrm{CO}_2$ occur during mid-summer (Hashida *et al*., 2010), because of high levels of biological activity, we might miss the appropriate periods for surface water pH observations.

We analyzed the correlations between abiotic factors (pH and water temperature) in the 0–400 m depth range and the pteropod abundance in the range 0–150 m. *L. helicina* and *L. retroversa* undergo diel vertical migrations in summer (Hunt *et al*., 2008; Lalli and Gilmer, 1989). If pteropods migrate downward to layers deeper than 150 m, the abundances calculated are underestimates, and the correlations between abiotic factors and pteropod abundance in the 0–400 m depth range should be recalculated. The results of the present study differ from those of Lischka *et al*. (2011), who reported a 14% increase in mortality of *L. helicina* juveniles at pH 7.6 compared with that at pH 8.2. The average seawater pH (7.86−8.02) found in the 200–400 m depth range at the 64°S site was higher than that reported by Lischka *et al*. (2011), and the present pH level at 64°S did not affect pteropod

abundance. Greater mortality of *L. helicina* juveniles at 5.5 and 8℃ compared with that at 3℃ was also reported by Lischka *et al.* (2011). However, no negative correlation between pteropod abundance and water temperature was observed at our sampling sites (Table 4a). Water temperature in the upper 400 m ranged from 0.08 to 2.70℃ at the 55°S site, −1.27 to 2.14℃ at the 60°S site, and from −1.63 to 2.10℃ at the 64°S site. The water temperature in the upper 400 m did not reach 5℃ at any sampling site during the period from 1988 to 2007, suggesting that pteropod abundance would not have been affected by water temperature.

The Chl-*a* concentration is related to pteropod abundance because diatoms and dinoflagellates dominate the gut contents of *L. helicina* and *L. retroversa* (Hunt *et al.*, 2008). However, no correlation was found between pteropod abundance and the Chl-*a* concentration in this study (Table 4b). This may have been the result of inadequate water sampling frequency. The zooplankton *Clione limacina* is a common predator of *L. helicina* and *L. retroversa* (Hunt *et al.*, 2008). However, *C. limacina* was not found in the study samples. There is very little information on the distribution, abundance, and feeding behavior of predators, including *C. limacina*, at the sampling sites, and therefore the complicated relationships among biotic components remain to be clarified.

The abundance of *Limacina* spp. varied from 2.2 to 2767.7 ind. m^{-3} in the upper 150 m of the water column during the period from 1987 to 2008, indicating a marked heterogeneous spatio-temporal distribution in the water column (Fig. 3). The average abundance of copepods, collected using the same NORPAC net during the same period (1987−2007), was 2814.1 ± 2235.2 ind. m^{-3} (T. Suzuki, Ishinomaki Senshu University; pers. comm.). The contribution of *Limacina* spp. to the total net-collected zooplankton abundance was approximately 10%. The coefficient of variation (s.d./mean abundance) for pteropods (1.99) was larger than that for copepods (0.79), indicating the pronounced heterogeneous distribution of pteropods. It is difficult to pinpoint the factors affecting pteropod abundance, because the abundance estimates in this study were based on sampling performed once at each site in each year. A greater number of observations on *Limacina* spp. abundance and growth characteristics at multiple locations over a long time period will be required to accurately determine the distribution patterns of these organisms.

Shells ＜400 μm in diameter accounted for 98% of the total number of *Limacina* spp. found in our study (Fig. 4). As for *L. helicina* in the Arctic Ocean, specimens ＜300 μm in size are in the veliger stage, those 300−400 μm in size are in the metamorphic stage from veliger to juvenile, those 300−4000 μm in size are in the juvenile stage, those 4000−5000 μm in size are mature males, and specimens ＞5000 μm in size are mature females (Lalli and Gilmer, 1989; Lalli and Wells, 1978). Sub-Arctic *L. retroversa* ＜300 μm in size are in the veliger stage, those 300−1000 μm in size are in the juvenile stage, those 1000−1200 μm in size are mature males, and specimens ＞1300 μm in size are mature females (Lalli and Wells, 1978). Most of the smaller pteropods collected in the present study were probably in the veliger and juvenile stages, although pteropods in the metamorphic stage could not be identified because their morphological features are less distinct. Veliger shells are formed with relatively rapid growth rates (more so than those of developed juveniles and adults), and therefore veliger-stage pteropods are likely be highly sensitive to unfavorable conditions, including ocean acidification. The occurrence of veliger stage pteropods in December, and larger-sized juveniles or adults in March, suggests that the pteropods grew over the period December to March. However, no marked size difference was found

between the pteropods collected in December and May. It is not well explained the differences in life cycle between two pteropod groups mentioned above.

The absence of pteropods ＜400 μm in size indicates a loss of individuals able to produce the next generation. Therefore, a decrease in veliger and juvenile numbers is likely to result in reduced recruitment, and potentially the future extinction of pteropods. Because a decrease in pteropod populations will impact predator populations, the absence of pteropods may be an indicator of drastic changes in the polar marine ecosystem. Previous studies have reported that a decrease in the seawater pH caused a reduction in pteropod calcification rates and shell diameters (Comeau *et al*., 2009; Lischka *et al*., 2011). In these studies the pteropods were cultured in seawater under controlled conditions at low pH (pH 7.63 and 7.78), which is the pH level that some oceans are expected to reach by 2100. However, the mean pteropod shell size did not decrease significantly. The average seawater pH at the 55°S site in the present study was 8.09 ± 0.01 at 0–20 m, 8.05 ± 0.02 in the WW layer, and 7.94 ± 0.03 at 200–400 m. These pH levels are higher than those reported in previous laboratory studies (Comeau *et al*., 2009; Lischka *et al*., 2011). Thus, the present pH levels have not yet affected pteropod shell size. It may be that the effects of ocean acidification on pteropod shell formation will appear when the seawater pH decreases below a certain threshold.

In this study of the interannual and spatial variability in abundance of pteropods in the Southern Ocean, no significant trend was observed for pteropod abundance and shell size, no significant trend was observed for pH in the various water layers, and no significant correlation of pteropod abundance with Chl-*a* or temperature was found across the years of the study. Based on the intensive model simulations and multivariate analyses of Boyd *et al*. (2015), it is difficult to establish how climate change is altering oceanic conditions, and how the changed environmental conditions act as stressors for marine life; this is because regionally distinctive patterns of oceanic change are too complex to distinguish single specific triggering factors. In the Southern Ocean, for example, regionally distinctive multiple environmental stresses (including increased temperature, CO_2, and Fe, and decreased nutrients) have complex effects on phytoplankton. Therefore, Boyd *et al*. (2015) noted that predicting the biological, ecological, and biogeochemical consequences of complex climate change is not yet possible. As grazers of phytoplankton, the shelled pteropods are exposed to multiple stressors other than ocean acidification, and no distinct trend of change in their abundance was clearly evident in this study. Therefore, it is necessary to undertake broad model-based studies, as suggested by Boyd *et al*. (2015). This will require future long-term intensive measurements of pteropod abundance, seawater pH, and oceanographic variables during the period December to March, based on automated instruments not dependent on research vessel involvement.

Acknowledgements

References

Aoki, S. (1997): Trends and interannual variability of surface layer temperature in the Indian sector of the Southern Ocean observed by Japanese Antarctic Research Expeditions. J. Oceanogr., **53**, 623−631.

Bé, A.W.H. and Gilmer, R.G. (1977): A zoogeographic and taxonomic review of euthecosomatous pteropoda. Oceanic Micropalaeontology, Vol. 1, ed. by A.T.S. Ramsay, London, Academic Press, 733−808.

Boyd, P.W., Lennartz, S.T., Glover D.M. and Doney S.C. (2015): Biological ramifications of climate-change-mediated oceanic multi-stressors. Nature Climate Change, **5**, 71−79, doi: 10.1038/nclimate2441.

Caldeira, K. and Wickett, M.E. (2003): Oceanography: Anthropogenic carbon and ocean pH. Nature, **425**, 365, doi: 10.1038/425365.

Comeau, S., Gorsky, G., Jeffree, R., Teyssié, J.L., and Gattuso, J.P. (2009): Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). Biogeosciences, **6**, 1877−1882, doi: 10.5194/bg-6-1877-2009.

Doney, S.C., Fabry, V.J., Feely, R.A. and Kleypas, J.A. (2009): Ocean acidification: The other CO_2 problem. Annu. Rev. Mar. Sci., **1**, 169−192, doi: 10.1146/annurev.marine.010908.163834.

Fabry, V.J., Seibel, B.A., Feely, R.A. and Orr, J.C. (2008): Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J. Mar. Sci., **65**, 414−432.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J. and Millero, f.J. (2004): Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science, **305**, 362−366, doi: 10.1126/science.1097329.

Gannefors, C., Böer, M., Kattner, G., Graeve, M., Eiane, K., Gulliksen, B., Hop, H. and Falk-Petersen, S. (2005): The Arctic sea butterfly *Limacina helicina*: lipids and life strategy. Mar. Biol., **147**, 169−177, doi: 10.1007/s00227-004-1544-y.

Gilmer, R.W. and Harbison, G.R. (1991): Diet of *Limacina helicina* (Gastropoda: Thecosomata) in Arctic waters in midsummer. Mar. Ecol. Prog. Ser., **77**, 125−134.

Hashida, G., Nakaoka, S., Ono, H., Nakazawa, T., Yoshikawa, H., Aoki, S., Morimoto, S., Yamanouchi, T., Odate, T. and Fukuchi, M. (2010): Secular trends and seasonal variations of partial pressure of carbon dioxide in the surface sea water in the Australian sector of the Southern Ocean. Nankyoku Shiryô (Antarctic Rec.), **54**, 438−448 (in Japanese with English abstract).

Hunt, B.P.V., Pakhomov, E.A., Hosie, G.W., Siegel, V., Ward, P. and Bernard, K. (2008): Pteropods in Southern Ocean ecosystems. Prog. Oceanogr., **78**, 193−221, doi: 10.1016/j.pocean.2008.06.001.

Lalli, C.M. and Wells, F.E. (1978): Reproduction in the genus *Limacina* (Opisthobranchia: Thecosomata). J. Zool., **186**, 95−108, doi: 10.1111/j.1469-7998.1978.tb03359.x.

Lalli, C.M. and Gilmer, R.W. (1989): Pelagic snails: the biology of holoplanktonic gastropod mollusks. California. Stanford University Press. 276 p.

Langdon, C. and Atkinson, M.J. (2005): Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res., **110**, C09S07, doi: 10. 1029/2004JC002576.

Lischka, S., Büdenbender, J., Boxhammer, T. and Riebesell, U. (2011): Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth. Biogeosciences, **8**, 919−932, doi: 10.5194/bg-8-919-2011.

Midorikawa, T., Inoue, H. Y., Ishii. M., Sasano, D., Kosugi, N., Hashida, G., Nakaoka, S. and Suzuki, T. (2012): Decreasing pH trend estimated from 35-year time series of carbonate parameters in the Pacific sector of the Southern Ocean in summer, Deep-Sea Res. Part I Oceanogr. Res. Pap, **61**, 131−139, doi: 10.1016/j.dsr.2011.12.003.

Mucci, A. (1983): The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. Am. J. Sci., **283**, 780−799, doi: 10.2475/ajs.283.7.780.

Ohman, M.D., Lavaniegos, B.E. and Townsend, A.W. (2009): Multi-decadal variations in calcareous holozooplankton in the California Current System: Thecosome pteropods, heteropods, and foraminifera. Geophy. Res. Let., **36**, L18608, doi: 10.1029/2009GL039901.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.-K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.-F., Yamanaka, Y. and Yool, A. (2005): Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, **437**, 681−686.

Roberts, D., Howard, W.R., Moy, A.D., Roberts, J.L., Trull, T.W., Bray, S.G. and Hopcrofft, R. (2008): Interannual variability of pteropod shell weights in the high-CO_2 Southern Ocean. Biogeosciences Discuss., **5**, 4453–4480, doi: 10.5194/bgd-5-4453-2008.

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.-H., Kozyr, A., Ono, T. and Rois, A.F. (2004): The oceanic sink for anthropogenic CO_2. Science, **305**, 367–371, doi: 10.1126/science.1097403.

Steinacher, M., Joos, F., Frölicher, T.L., Plattner, G.-K. and Doney, C.S. (2009): Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences, **6**, 515–533, doi: 10.5194/bg-6-515-2009.

Yamamoto-Kawai, M., McLaughlin, F.A., Carmack, E.C., Nishino, S. and Shimada, K. (2009): Aragonite undersaturation in the Arctic Ocean: Effects of ocean acidification and sea ice melt. Science, **326**, 1098–1100, doi: 10.1126/science.1174190.