Acta Oceanologica Sinica

Vol. 37   No. 8   August   2018

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Analyzing biases of nitrogen contents and δ\textsuperscript{15}N values arising from acidified marine sediments with different CaCO\textsubscript{3} concentrations

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Received 26 May 2017; accepted 30 August 2017

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Abstract

In some studies, the researchers pretreated and measured organic carbon, nitrogen and their isotopes (δ\textsuperscript{13}C and δ\textsuperscript{15}N) of marine sediment together, to save costs and resources of analysis. However, the procedure of acidification to remove inorganic carbon for analysing δ\textsuperscript{13}C can affect the values of nitrogen and δ\textsuperscript{15}N, and the biases vary a lot depending on the CaCO\textsubscript{3} contents of sediments. In this study, the biases of total nitrogen (TN) and δ\textsuperscript{15}N values arising from acidified sediments were compared between the CaCO\textsubscript{3}-poor (1%–16%) and CaCO\textsubscript{3}-rich (20%–40%) samples. TN and δ\textsuperscript{15}N values were altered during acid treatment (without centrifugation) that possibly led to N-containing compounds volatilization. For CaCO\textsubscript{3}-poor samples, acidification led to a range of 0%–40% TN losses and 0‰–2‰ shift in δ\textsuperscript{15}N values; and 10%–60% TN losses and 1‰–14‰ shift in δ\textsuperscript{15}N values for CaCO\textsubscript{3}-rich samples. The biases from most samples exceeded the precision of the instrument (0.02‰ for TN and 0.08‰ for δ\textsuperscript{15}N), and high biases could mislead our judgment for the environmental implication of the data. Thus, avoiding co-analysis of organic carbon, nitrogen and their isotopes (δ\textsuperscript{13}C and δ\textsuperscript{15}N) in sediments, even for CaCO\textsubscript{3}-poor marine sediments, is necessary.

Key words: stable isotope nitrogen, nitrogen, CaCO\textsubscript{3}, acidification, marine sediment

Citation: Peng Yajun, Liu Dongyan, Wang Yujue, Richard Pierre, Keesing John K. 2018. Analyzing biases of nitrogen contents and δ\textsuperscript{15}N values arising from acidified marine sediments with different CaCO\textsubscript{3} concentrations. Acta Oceanologica Sinica, 37(8): 1–5, doi: 10.1007/s13131-018-1188-2

1 Introduction

Organic matter contents (e.g., total carbon and nitrogen) and their isotopic fingerprints (δ\textsuperscript{13}C and δ\textsuperscript{15}N) in the sediments are important environmental proxies to evaluate the sediment quality and trace the material sources in coastal and estuarine ecosystems (Tucker et al., 1999; Voss et al., 2006; Savage, 2005). For example, anthropogenically enriched organic nitrogen (e.g., human sewage, livestock runoff) can significantly elevate the values of δ\textsuperscript{15}N. However, the procedure of acidification varied greatly depending on the species of acid reagent (e.g., HCl, H\textsubscript{2}SO\textsubscript{4}, H\textsubscript{3}PO\textsubscript{4} or HF) and carbonate contents in the sediments (Schubert and Nielsen, 2000; Lorrain et al., 2003; Kennedy et al., 2005; Schmidt and Gleixner, 2005; Fernandes and Krull, 2008). For example, Harris et al. (2001) only detected a slight increase in δ\textsuperscript{15}N values (0.04‰–0.14‰) after acidification using HCl fumigation; Fernandes and Krull (2008) also found 1‰–2‰ deviation of δ\textsuperscript{15}N values between the acidified (using HCl washing) and non-acidified samples. For the biases of nitrogen content, Lobse et al. (2000) reported there were approximately 28%–47% losses in nitrogen compounds for CaCO\textsubscript{3}-rich (55%–61%) samples after acidification using H\textsubscript{2}SO\textsubscript{4}. Kennedy et al. (2005) however only found about 0.3% nitrogen losses in the acidified samples (30% CaCO\textsubscript{3}) using HCl. Therefore, there is uncertainty

Foundation item: The National Natural Science Foundation of China under contract No. 41376121; the Strategic Priority Research Program of the Chinese Academy of Sciences under contract No. XDA11020405; the Natural Science Foundation of Shandong Province under contract No. JQ201414; the Program of Advanced Talents of The Guizhou University of Finance and Economics, 2016.

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on the biases in TN and δ15N values after acidification, and it is necessary to evaluate the biases according to the CaCO3 contents of the sediments.

Global mean CaCO3 content in marine surface sediments is approximately 34.8%, with a wide range of approximately 0%–80% (Ridgwell and Hargreaves, 2007). For example, the samples from north and west Pacific Ocean contain approximately 0%–20% CaCO3, but CaCO3 contents in the samples from Atlantic Ocean can reach to 80% (Ridgwell and Hargreaves, 2007). In this study, TN and δ15N values in 236 marine sediment samples with higher CaCO3 contents (20%–40%) from the coastal waters in the western Australia and 273 samples with lower CaCO3 contents (0%–16%) from the coastal waters in China’s seas were measured, respectively, under acidified and non-acidified conditions. The biases of TN and δ15N values were evaluated and the correlation between the biases and CaCO3 contents in the sediments were examined to understand the impact of CaCO3 contents.

2 Materials and methods

2.1 Sample collection

Two sediment cores were collected from the coast of King George River (KGR) in the northwestern Australia, and 236 sediment samples were obtained by slicing at 1-cm interval. Two one sediment cores were collected from the Bohai Sea and Yellow Sea in China, respectively, and 221 sediment samples were obtained by slicing at 1-cm interval. In addition, 52 surface marine sediment samples were collected from the South Yellow Sea, China (Table 1). Totally, 509 marine sediment samples were stored in a freezer at −20°C preparing for the analysis of TOC, TN, δ15N values and CaCO3 contents.

| Samples identifier | Location Description | Number of samples | TOC(1)/% | TN(2)% | C/N(1) | δ15N(2)/‰ | CaCO3(1)/% |
|-------------------|---------------------|------------------|----------|--------|--------|----------|------------|
| KGR6              | Down-core marine sediment from King George River, northwestern Australia (11.8 m depth) | 114       | 0.40–0.75 | 0.03–0.11 | 8–19   | 3.6–5.7  | 20–31     |
| KGR10             | Down-core marine sediment from King George River, northwestern Australia (11.2 m depth) | 122       | 0.38–0.83 | 0.04–0.12 | 7–22   | 4.0–7.8  | 23–40     |
| B62-2             | Down-core marine sediment from Bohai Sea, China (24 m depth) | 76        | 0.46–0.92 | 0.06–0.12 | 6–12   | 4.9–6.4  | 1–7       |
| B24-1             | Down-core marine sediment from North Yellow Sea, China (45 m depth) | 63        | 0.68–1.14 | 0.09–0.18 | 7–12   | 4.5–5.6  | 1–5       |
| H10-1             | Down-core marine sediment from South Yellow Sea, China (78 m depth) | 82        | 0.20–1.09 | 0.03–0.14 | 7–14   | 3.8–6.2  | 1–7       |
| SMS               | Surface marine sediment from South Yellow Sea, China | 52        | 0.14–1.18 | 0.02–0.19 | 6–21   | 3.7–6.0  | 1–16      |

Note: 1) Measurement on acidified sample; 2) measurement on unacidified whole sample.

2.2 Analytical procedures

TOC, TN and δ15N values were measured using a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash EA 1112, Thermo Scientific, Italy) in Littoral Environment et Sociétés (LIENSs)-UMR7266, France. Firstly, all samples were freeze-dried and homogenized by grinding. Each sample was divided into two shares, using for the pretreatment with and without acidification, respectively. One share was acidified by adding 1 mL of 0.5 mol/L HCl (or 1 mol/L HCl) to every 100 mg of samples. The acidified samples were dried at <60°C, then mixed with 1 mL Milli-Q water and freeze-dried again. The freeze-dried samples were weighed into 5 mm×8 mm tin capsules, and the prepared capsules were used for the measurement of total organic carbon (TOC), total nitrogen (TNacidified) and stable nitrogen isotope (δ15Nacidified). The corrections for TOC and TNacidified values were performed because the weight of samples had increased after decarbonation (formation of CaCl2). The other sample share, using for non-acidification treatment, was weighed directly and placed in 5 mm×8 mm tin capsules for the measurement of total carbon (TC), total nitrogen (TNacidified) and stable nitrogen isotope (δ15Nacidified). Based on the results of TOC and TC, the CaCO3 contents were estimated using CaCO3(TC-TOC)x8.33 (Stein et al., 1994; Schubert and Calvert, 2001).

The isotopic composition results are expressed relative to atmospheric N2 for δ15N. Replicate measurements of an acetanilide standard (Thermo Scientific) indicated that the δ15N analytical errors were less than 0.1‰. The total organic matter and total nitrogen weight data obtained as part of the stable isotope analyses. Replicate measurements of a certified reference material (Low Organic Content Soil, Elemental MicroAnalysis, UK) indicated relative errors of 0.025% and 0.002% for total organic carbon and total nitrogen, respectively.

2.3 Statistical analysis

We tested the differences of TN and δ15N values between non-acidified and acidified samples, using software SPSS 16.0 (t-test). The correlation between these differences and CaCO3 contents were analyzed respectively using SigmaPlot 12.0. The biases in TN and δ15N values after acidification were calculated, respectively, using the following equations:

\[ \Delta N = \frac{TN_{nonacidified} - TN_{acidified}}{TN_{acidified}} \times 100 \]  

(1)

\[ \Delta \delta^{15}N = \delta^{15}N_{nonacidified} - \delta^{15}N_{acidified} \]  

(2)

3 Results and discussion

3.1 CaCO3 contents of all samples

The CaCO3 contents were approximately 20%–40% and 1%–16% for the sediments collected from the northwestern Australia and China’s seas, respectively (Table 1). Based on the CaCO3 contents of global marine sediments (Ridgwell and Hargreaves, 2007), the samples were divided into CaCO3-rich samples (CaCO3 contents >20%) and CaCO3-poor samples (CaCO3 contents >20%), respectively, for a comparison.
3.2 Effects of acid treatment on TN

The results of paired-sample t-test indicated a significant difference in TN contents (P=0.000<0.01, 2-tailed) between the samples measured with and without acid treatment. For the non-acidified samples, TN contents ranged from 0.02%–0.19%, while this range reduced to 0.01%–0.16% for the acidified samples. About 99% non-acidified vs. acidified TN values are located below the ideal 1:1 relation, indicating that acidification resulted in the losses of nitrogen contents from the samples (Fig. 1a). In addition, losses of nitrogen contents were on average higher in CaCO₃-rich samples compared to CaCO₃-poor samples after acidification. About 0%–40% and 10%–60% of nitrogen contents were lost from non-acidified CaCO₃-poor samples and CaCO₃-rich samples, respectively (Fig. 2a). Moreover, our data suggest a statistically significant positive correlation between the losses of nitrogen contents and the CaCO₃ contents (Fig. 2a). These findings partially agree with Lohse et al. (2000) and Fernandes and Krull (2008), who found a particularly high losses of nitrogen

![Fig. 1. Scatter plots of unacidified vs. acidified values for TN and δ¹⁵N. The ideal relation (1:1) is described by the solid line. The dashed lines indicate the statistic relation by regression analysis.](image1)

![Fig. 2. Correlation between CaCO₃ contents and the biases in TN and δ¹⁵N values after acidification.](image2)

| Table 2. Comparison of differences caused by acidification in sedimentary TN and δ¹⁵N values with references |
| --- |
| Sample type | CaCO₃/% | ΔN/% | Δ¹⁵N/% | Acidification method | Possible derives | Analysis mode | Reference |
| --- | --- | --- | --- | --- | --- | --- | --- |
| DSS<sup>4</sup> | 55–61 | 28–47 | – | in situ H₂SO₄ | H₂SO₄ volatisilises nitrogenous organic matter | single<sup>6</sup> | Lohse et al. (2000) |
| 7–17 | 2–4 | – | | | | | |
| SMS<sup>5</sup> | – | 11–29 | – | direct HCl | nitrogen appears to be liberated during direct acidification | single | Byba and Burgess (2002) |
| MPOM<sup>5</sup> | <5 | 0 | 0 | HCl fumes for 4 h | HCl fume treatment did not alter δ¹⁵N and N values for low carbonate contents | dual<sup>7</sup> | Lorrain et al. (2003) |
| DSS<sup>4</sup> | 30 | 0.3 | 0.005 | in situ 2 mol/L HCl | weak HCl (2 mol/L) did not significantly affect δ¹⁵N and TN values | dual | Kennedy et al. (2005) |
| SMS<sup>5</sup> | >30 | 30–64 | 1–2 | HCl washing | losses through volatilization or solubilization (acid-soluble compounds) | single | Fernandes and Krull (2008) |
| SES<sup>5</sup> | <10 | 19–40 | 0–1 | | | | |
| SOILB<sup>5</sup> | – | 15 | 0.7 | in situ 5% HCl | loss of soluble organic and/or inorganic N species, e.g., δ¹⁵N-rich species (e.g., protein, ammonium) | single | Brodie et al. (2011a, b) |

Note: Single or dual indicate separate or simultaneous C and N analyses; <sup>1</sup> deep-sea sediment; <sup>2</sup> surface marine sediment; <sup>3</sup> marine suspended particulate organic matter; <sup>4</sup> surface estuarine sediment; <sup>5</sup> international soil standard; <sup>6</sup> measurement on unacidified whole sample for TN and δ¹⁵N; <sup>7</sup> measurement on acidified sample for TN and δ¹⁵N.
contents in CaCO₃-rich (>30%) samples, up to 64% after acid treatment (Table 2), but no significant correlation between the loss of nitrogen contents and the CaCO₃ contents.

The loss of nitrogen contents from acidified samples was observed in the present and previous studies, although the acidification methods were different (Table 2). In our study, we used an acid treatment with 0.5 mol/L or 1 mol/L HCl in glass tubes (Peng et al., 2015), which avoided the loss of acid-soluble organic matter and/or fine particles by rinsing process (rinse method) and intensive effervescence (in situ acidification). This indicates that volatilisation of nitrogen compounds seems to be the most reasonable explanation for our results, and further investigation is still needed for the explanation of the mechanism. Previous studies have demonstrated that in the CaCO₃-rich samples the fraction of proteinaceous compounds absorbed on carbonate surface layers became unstable with the increasing of acid addition, and this acid-soluble component was lost during carbonate dissolution (Fernandes and Krull, 2008). Meanwhile, this fraction of acid-soluble organic matter is greater in more calcareous sediments (Froelich, 1980). This may explain the higher losses observed in the CaCO₃-rich samples compared to the CaCO₃-poor samples.

3.3 Effects of acid treatment on δ¹⁵N

Nitrogen isotope (δ¹⁵N) values of samples measured with and without acidification are shown in Fig. 1b. There have been significant shifts in δ¹⁵N values between acidified and non-acidified samples, and these shifts have statistically significant positive correlation with the CaCO₃ contents, indicating that losses of acid-soluble nitrogen compounds with different δ¹⁵N values (Fig. 2b). For CaCO₃-poor sediments the measured δ¹⁵N values after acid treatment varied in the range of -1‰ to 6‰ (Fig. 1b). The shift of δ¹⁵N values of these samples after acidification (up to 3‰) is similar to the deviation (0‰–2‰) observed by Fernandes and Krull (2008) (Table 2). For CaCO₃-rich samples about 40% of the measured δ¹⁵N values were significant negative after acidification (down to -9‰, Fig. 1b), and bias in δ¹⁵N values (up to 14‰) were remarkable compared to non-acidified samples (Fig. 2b). Schmidt and Gleixner (2005) also found some samples with unusual negative δ¹⁵N values (e.g., -4.24‰ for a podzol Sample 6) after acid treatment due to the accumulation of coal-derived products. However, it is really difficult to understand what cause such depletion for part of CaCO₃-rich samples, since there is no rinse of the samples, which could trigger a loss of δ¹⁵N-enriched compounds. Volatilisation of nitrogenous compounds like chloramine should lead to an enrichment of δ¹⁵N values not depletion.

In addition, our data suggest that isotopic shifts were not consistent for all samples (Fig. 2b). The depletion of δ¹⁵N values was observed in 95% samples after acid treatment reflecting the loss of δ¹⁵N-enriched compounds such as protein and ammonium (Brodie et al., 2011a, b) (Fig. 2b). The loss of heavy isotope during acid treatment had previously been reported by other studies (Table 2). However, about 5% acidified samples (originating in CaCO₃-poor sediments collected from the Bohai Sea) detected a 0‰–1‰ increase signature in δ¹⁵N values, suggesting the loss of δ¹⁵N-enriched compounds. Harris et al. (2001) observed a small increase (0.04‰–0.11‰) in δ¹⁵N values of acidified soil samples following HCl fumigation. This contrasting shifts (depletion or enrichment) in δ¹⁵N values probably influenced by the organic matter composition and clay mineralogy of samples (Baldock and Skjemstad, 2000; Schubert and Calvert, 2001; Fernandes and Krull, 2008), but more work need to do to identify differences in acid-soluble compounds, inorganic nitrogen and mineralogy of samples containing different CaCO₃ contents during acid treatment.

4 Conclusions

Our results suggest that the analysis of nitrogen contents and its isotopic composition should be measured on non-acidified whole sample, because acidification to remove carbonates in marine sediments leads to significant nitrogen losses through volatilisation and isotopic shifts, particularly for samples with high CaCO₃ contents (>20%). Meanwhile, there was a statistically significant positive correlation between the CaCO₃ contents and the biases in nitrogen contents and its isotopic composition after acid treatment (Fig. 2), indicating that the CaCO₃ contents in the sample play an important role in determining what extent the nitrogen fraction may have been affected by acid treatment.

References

Baldock J A, Skjemstad J O. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry, 31(7–8): 697–710

Brodie C R, Heaton T H E, Leng M J, et al. 2011a. Evidence for bias in measured δ¹⁵N values of terrestrial and aquatic organic materials due to pre-analysis acid treatment methods. Rapid Communications in Mass Spectrometry, 25(8): 1089–1099

Brodie C R, Leng M J, Casford J S L, et al. 2011b. Evidence for bias in C and N concentrations and δ¹³C composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. Chemical Geology, 282(3–4): 67–83

Bunn S E, Loneragam N R, Kempest M A. 1995. Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: Implications for food-web studies using multiple stable isotopes. Limnology and Oceanography, 40(3): 622–625

Fernandes M, Krull E. 2008. How does acid treatment to remove carbonates affect the isotopic and elemental composition of soils and sediments?. Environmental Chemistry, 5(1): 33–39

Froelich P N. 1980. Analysis of organic carbon in marine sediment. Limnology and Oceanography, 25(3): 564–572

Harris D, Horwáth W R, Van Kessel C. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America, 65(6): 1853–1856

Hu Jianfang, Peng Ping’an, Jia Guodong, et al. 2006. Distribution and stable isotope ratios of low-carbonated suspended particulate matter (SPM) in the Bohai Sea. Estuarine, Coastal and Estuarine Science, 1063–1068

Jacob U, Mintenbeck K, Brey T, et al. 2005. Stable isotope food web studies: a case for standardized sample treatment. Marine Ecology Progress Series, 287: 251–253

Jaschinski S, Hansen T, Sommer U. 2008. Effects of acidification in multiple stable isotope analyses. Limnology and Oceanography: Methods, 6(1): 12–15

Kennedy P, Kennedy H, Papadimitriou S. 2005. The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. Rapid Communications in Mass Spectrometry, 19(8): 1063–1068

Lohse L, Kloosterhuis R T, De Stigter H C, et al. 2000. Carbonate removal by acidification causes loss of nitrogenous compounds in continental margin sediments. Marine Chemistry, 69(3–4): 193–201

Lorrain A, Savoye N, Chauvaud L, et al. 2003. Decarbonation and stable isotope ratios of low-carbonated suspended particulate material. Analytica Chimica Acta, 491(2): 125–133

Middelburg J J, Nieuwenhuize J. 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Scheldt Estuary. Marine Chemistry, 60(3–4): 217–225

Peng Yajun, Wang Yujue, Liu Dongyan, et al. 2015. Acid treatment effects on the carbon stable isotope values of marine sediments.
Ridgwell A, Hargreaves J C. 2007. Regulation of atmospheric CO$_2$ by deep-sea sediments in an Earth system model. Global Biogeochemical Cycles, 21(2): GB2008, doi: 10.1029/2006GB002764

Ryba S A, Burgess R M. 2002. Effects of sample preparation on the measurement of organic carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments. Chemosphere, 48(1): 139–147

Savage C. 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. AMBIO: A Journal of the Human Environment, 34(2): 145–150

Schlacher T A, Connolly R M. 2014. Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. Methods in Ecology and Evolution, 5(6): 541–550

Schmidt M W I, Gleixner G. 2005. Carbon and nitrogen isotope composition of bulk soils, particle-size fractions and organic material after treatment with hydrofluoric acid. European Journal of Soil Science, 56(3): 407–416

Schubert C J, Calvert S E. 2001. Nitrogen and carbon isotopic composition of marine and terrestrial organic matter in Arctic Ocean sediments: implications for nutrient utilization and organic matter composition. Deep Sea Research Part I: Oceanographic Research Papers, 48(3): 789–810

Schubert C J, Nielsen B. 2000. Effects of decarbonation treatments on $\delta^{13}$C values in marine sediments. Marine Chemistry, 72(1): 55–59

Serrano O, Serrano L, Mateo M A, et al. 2008. Acid washing effect on elemental and isotopic composition of whole beach arthropods: implications for food web studies using stable isotopes. Acta Oecologica, 34(1): 89–96

Stein R, Grobe H, Wahlen M. 1994. Organic carbon, carbonate, and clay mineral distributions in eastern central Arctic Ocean surface sediments. Marine Geology, 119(3–4): 269–285

Sweeney R E, Kaplan I R. 1980. Natural abundances of $^{15}$N as a source indicator for near-shore marine sedimentary and dissolved nitrogen. Marine Chemistry, 9(2): 81–94

Tucker J, Sheats N, Giblin A E, et al. 1999. Using stable isotopes to trace sewage-derived material through Boston harbor and Massachusetts bay. Marine Environmental Research, 48(4–5): 353–375

Voss M, Larsen B, Leivuori M, et al. 2000. Stable isotope signals of eutrophication in Baltic Sea sediments. Journal of Marine Systems, 25(3–4): 287–298

Wang Yujue, Liu Dongyan, Richard P, et al. 2013. A geochemical record of environmental changes in sediments from Sishili Bay, northern Yellow Sea, China: Anthropogenic influence on organic matter sources and composition over the last 100 years. Marine Pollution Bulletin, 77(1–2): 227–236
Seasonal variations and distributions of dissolved free and total carbohydrates at the İzmir Bay, Aegean Sea

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Received 30 November 2017; accepted 24 February 2018

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Abstract
Seasonal variations and distributions of dissolved carbohydrate concentrations at the İzmir Bay were investigated with salinity, chlorophyll a (Chl a), and dissolved organic carbon (DOC) levels to understand their relationships. Samples were collected from surface, subsurface and bottom depths at seven stations. DOC concentrations ranged from 32.2 to 244.2 μmol/L, and in general, DOC levels increased from winter to summer, then slightly decreased in autumn. Monosaccharide (MCHO), polysaccharide (PCHO) and total dissolved carbohydrate (TDCHO) levels were found between 0.7–8.3, 0.7–19.5, and 2.6–24.6 μmol/L. DOC, MCHO, PCHO and TDCHO levels were found higher in middle-inner bays, under the influence of anthropogenic inputs, compared to outer bay. Seasonal changes of MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios were statistically significant (p<0.05) and the ratios showed decrease trends from winter to summer-autumn seasons. Distributions of TDCHO/DOC ratios at wide ranges (2.5%–42.3%) indicated the presence of newly forming and degrading fractions of DOM. According to results of factor analysis, Chl a, MCHO and TDCHO were explained in the same factor groups. In conclusion, the results showed that dissolved carbohydrate levels in the İzmir Bay might be influenced by biological processes and terrestrial/anthropogenic inputs.

Keywords: monosaccharides, polysaccharides, carbohydrates, DOC, Chl a, İzmir Bay

Citation: Alyuruk Hakan, Kontas Aynur. 2018. Seasonal variations and distributions of dissolved free and total carbohydrates at the İzmir Bay, Aegean Sea. Acta Oceanologica Sinica, 37(8): 6–14, doi: 10.1007/s13131-018-1229-x

1 Introduction
Dissolved organic matter (DOM) is one of the most important components in seawater and it is under constant circulation and conversion within the marine environment. DOM could be originated from terrestrial processes (called as allochthonous) or in situ marine (called as autochthonous) sources (Hedges, 2002; Libes, 2009). Terrestrial organic matter including degradation products of plants and other organisms could be transported to the marine environments by rivers, rain runoff or winds. On the other hand, a great amount of DOM in marine environments is produced in situ by primary producers like phytoplankton, macroalgae and bacteria found in seawater. Chemical and physical characterization of DOM pool is difficult since it includes vast amounts of biomolecules at different chemical structures and molecular sizes. Among these biomolecules, most abundant ones found in DOM are amino acids, carbohydrates, lipids, fatty acids, sterols, humic acids, fulvic acids, and lignins. Carbohydrates are one of the major groups that are commonly utilized by the microorganisms (Rich et al., 1997; Kirchman et al., 2001; Khodse et al., 2010). They are also the products of photosynthesis process that takes place within phytoplankton and marine algae. Qualitative and quantitative studies have been performed for understanding the biogeochemical cycling of dissolved carbohydrates including its molecular and polymeric forms. Dissolved carbohydrates could be divided into groups as monosaccharides (MCHO) and polysaccharides (PCHO) or neutral and acidic sugars based on their chemical structures (Hedges et al., 1994; Myklestad et al., 1997; Benner and Olsahl, 2001; Chanudet and Filella, 2006; Lin and Guo, 2015). Glucose, fucose, galactose, mannose, and xylose are reported as dominant monosaccharides in DOM from different regions (McCarthy et al., 1996; Borch and Kirchman, 1997). Polysaccharides, such as starch and cellulose, are polymeric forms of monosaccharides linked to each other with glycosidic bonds. Total dissolved carbohydrates (TDCHO) are one of the well identified components of DOM that constitutes up to 3%–30% of bulk DOM (Pakulska and Benner, 1994; Benner, 2002; Hung et al., 2003; Wang et al., 2006). This fraction contains mono-, oligo- and polysaccharides, the latter one is used as storage material, cell wall components and extracellular exudates (Myklestad and Borsheim, 2007; He et al., 2015). Polysaccharides have been studied extensively due to their tendency for aggregation and colloidal properties. It has been reported that they were responsible from biofilm production, mucilage events (Baldi et al., 1997; Leppard, 1997; Pettine et al., 1999; Penna et al., 2003, 2009), complexation with trace metals (Jang et al., 1990, 1995), and marine snow formation (Alldredge et al., 1993; Passow et al., 1994; Skog et al., 2008; He et al., 2015). In the water column, carbohydrate concentrations are changed both vertically and horizontally based on their production and uptake rates by the organisms, and they are also influenced by terrestrial inputs (Zhang, 2010; He et al., 2015). In estuaries, gulfs and bays, carbohydrate concentrations were reported at high levels, whereas, they were observed at intermediate and low levels in coastal waters and in oligotrophic ocean waters, respectively (Handa, 1966, 1967; Pakulska and Benner, 1994; Amon and Benner, 2003; Hung et al., 2003; Wang et al., 2006; Khodse et

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The study area in this study, the Izmir Bay, is located at the western coast of Anatolia. Its structure is L-shape which is oriented to north with its longer part and connected to the Aegean Sea. According to Sayin (2003), the hydrography of the Izmir Bay is influenced by several factors such as freshwater inputs that carry anthropogenic loads to the bay, atmospheric transport of low molecular weight molecules, exchange of water between the Aegean Sea and the bay, topography of the bay, the sea level changes, movement of waters directed by wind-driven circulation and winter convection. Under the influences of these factors, the Izmir Bay could be divided into three parts as outer, middle and inner bays since each part have different water mass characteristics. While inner bay water has been anthropogenically polluted, outer bay water is influenced by the Gediz River and Aegean Sea, the upwelling water at the Gulpahçe Bay, and the water mass located at salt production area (Sayin, 2003). The third water mass at middle bay connects outer bay to inner bay. Due to the different physical and chemical characteristics of the water masses, remarkable differences have been reported for dissolved organic carbon (DOC), chlorophyll a (Chl a), dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) levels at outer, middle and inner bay stations in the previous studies (Kontas et al., 2004; Kucuksezgin et al., 2005; Sunlu et al., 2012). Also, eutrophication has been reported for inner part of the Izmir Bay (Kontas et al., 2004). On the other hand, outer bay has oligotrophic character. Studies on the fractions of DOM in the Izmir Bay are very limited and this will be the first study on the dissolved carbohydrates (MCHO, PCHO and TDCHO). The aim of this study was to investigate seasonal variations and distributions of dissolved carbohydrates in the bay and their relationships with salinity, Chl a and DOC levels.

2 Materials and methods

2.1 Seawater sampling

Seawater samples were collected from seven stations located in the Izmir Bay (Fig. 1). Sampling stations were selected according to physical and chemical properties of water masses in the bay, shortly explained at the introduction, based on the previous studies (Sayin, 2003; Kontas et al., 2004; Kucuksezgin et al., 2005). Samples were collected from surface (0–0.5 m), subsurface (5 m) and bottom depths. Water depths at sampling stations from 1 to 7 were as follows: 9, 22, 52, 36, 27, 66, and 44 m, respectively. The samplings were performed by seasonal cruises (February, April, September and December 2015) with the R/V K. Piri Reis. Seawater samples were collected with 10 L Go Flo Rosette bottles (General Oceanic) and a CTD system (SBE911plus, Sea-Bird). DOC and carbohydrate samples were immediately filtered from 47 mm Whatman GF/F (0.7 μm) glass fiber filters (precombusted at 450°C for 4 h) and stored at –20°C until the analyses in the laboratory. Chl a samples were pre-filtered with 210 μm nylon mesh in order to remove the larger particles. Then, the samples collected on GF/F filters and fixed with saturated MgCO3 just before the end of filtration. The filters stored at –20°C within 15 mL screw capped centrifuge tubes with Teflon liners.

2.2 Chlorophyll a analysis

Chl a was extracted with 10 mL of 90:10 acetone:water (v/v) solution for 24 h using a vortex mixer at 6 h of intervals and kept within refrigerator at 4°C. Following the extraction, the samples were centrifuged at 3 000 r/min for 10 min. Absorbances of the samples were recorded at 630, 647, 664 and 750 nm on a spectrophotometer (DR5000, Hach). Chl a concentrations (μg/L) were calculated according to APHA (1998).

2.3 Dissolved organic carbon analysis

DOC analysis is based on the discoloration of buffered phenolphthalein solution proportional to the CO2 concentration. The analysis was performed on a continuous flow nutrient analyzer (San Plus, Skalar) according to instructions of the manufacturer (Cat.No: 311–412). First, 0.06 mol/L sulfuric acid was added to the sample. Then, the digestion reagent (12 g K2SO4 and 34 g Na2B4O7·10H2O per liter of distilled water) was added and UV digestion was applied. Following the digestion, hydroxylammonium chloride solution (includes 10% Triton X-100 and sulfuric acid) was added and CO2 was separated from reaction mixture with a gas dialysis membrane. Then, CO2 was reacted with the 1% phenolphthalein buffer solution (prepared in ethanol and sodium carbonate buffer). The colorimetric reading performed at 550 nm. Potassium hydrogen phthalate was used as organic carbon standard. Accuracy of the method was checked using potassium hydrogen phthalate at every 10 sample readings. Synthetic seawater including NaCl, MgSO4 and Milli-Q water was used as blank. The system was washed with Milli-Q water until the low and stable instrumental blank.

2.4 Carbohydrate analysis

The carbohydrate analyses were performed according to the method proposed by Myklestad et al. (1997). TDCHO were ini-
tially hydrolyzed and analyzed according to the standard procedures given below following neutralization. For hydrolysis, 4 mL of sample and 0.4 mL of 1 mol/L HCl were added into 20 mL amber hydrolysis vials and the vials were tightly closed with Teflon lined screw caps. The vials were kept at 150°C for 1 h on a block heater. Then, the vials allowed to cool at room temperature and neutralized with 0.4 mL of 1 mol/L NaOH. For analysis of dissolved free carbohydrates (MCHO), 1 mL of sample was mixed in 20 mL amber vial. A total of 1 mL of 0.7 mmol/L potassium ferricyanide solution was added to sample and kept on the block heater at 100°C for 10 min. Then, 1 mL of 2 mmol/L ferric chloride and 2 mL of 2.5 mmol/L TPTZ solutions were added and thoroughly mixed on a vortex mixer. The absorbance was read at 595 nm after 30 min in a 50 mm cuvette against distilled water. The absorbance of a reagent blank prepared in Milli-Q water subtracted before further calculations. The working standards were prepared at 3.3, 8.3, 16.7, 33.3, 66.7, and 133.3 μmol/L (glucose-C) concentrations by dissolving neat D-glucose (47829, Supelco) analytical standard in Milli-Q water. Dissolved polysaccharide (PCHO) concentrations were calculated by subtracting the concentrations of MCHO from TDCHO. Precision of the method was between 3%–10% for high to low concentration samples and the detection limit was 0.4 μmol/L (Myklestad et al., 1997; Engel and Händel, 2011).

In the hydrolysis step of carbohydrates, 1 mol/L HCl was used. According to Myklestad et al. (1997), the hydrolysis works sufficiently but, in some studies, equal or two to four fold higher hydrolysis yields were reported by using two step hydrolysis (12 mol/L H₃PO₄ at prehydrolysis and 1.2 mol/L H₂SO₄ at post hydrolysis) (Pakulski and Benner, 1992; Myklestad et al., 1997). However, it is not almost possible to achieve 100% yield during hydrolysis of polysaccharides (Myklestad et al., 1997).

2.5 Statistical analyses

Statistical analyses were performed with R Statistical Computing Software, v3.2.5 (R Core Team, 2016). In all statistical tests, significance level was α=0.05. Seasonal changes of salinity, Chl a, DOC, MCHO, PCHO and TDCHO were evaluated with One Way ANOVA tests. Prior to ANOVA, test assumptions were checked using Software, v3.2.5 (R Core Team, 2016). In all statistical tests, significance level was α=0.05. Seasonal changes of salinity, Chl a, DOC, MCHO, PCHO and TDCHO were evaluated with linear regression analyses. Factor analysis was used to determine how the independent variables (salinity, Chl a, DOC, MCHO and TDCHO) were effective on the variation of data. Factor analysis was performed by using psych package in R (Revelle, 2016). Both Horn’s parallel analysis (Horn, 1965) and Very Simple Structure (VSS) (Revelle and Rocklin, 1979) methods were used to determine the correct number of factors. Prior to factor analysis, the data were divided into two subsets as middle-inner and outer bay. Factors were estimated with minimum residual (minres) method (Harman and Jones, 1966) and the data were not rotated.

3 Results

3.1 Seasonal variations

Seasonal and vertical salinity, Chl a, DOC, MCHO, TDCHO and PCHO levels were given in Table 1. According to One Way ANOVA test results, there were no significant vertical changes for salinity, Chl a, DOC, MCHO, TDCHO and PCHO (p>0.05). Sea-water salinity was found between 36.9 and 40.8 in all seasons. Salinity was remarkably higher in summer at middle-inner and outer bays (p<0.05, Table 2). Salinity was nearly constant with increasing depth.

Chl a concentrations were between 0.1–2.6 μg/L and 1.0–25.4 μg/L at outer and middle-inner bays, respectively. In middle-inner bays, maximum Chl a levels were found at surface waters and decreased at 5 m and bottom depths. Chl a levels were observed higher at Stas 1 and 2 in summer and autumn. Minimum Chl a levels were observed in autumn at middle-inner bays. Seasonal changes of Chl a levels were significant at middle-inner and outer bays (p<0.05, Table 2).

DOC concentrations ranged between 32.2–96.3 and 41.4–244.2 μmol/L at outer and middle-inner bays, respectively. Highest DOC concentration was observed in summer at Sta. 1. DOC levels increased from winter to summer and slightly decreased in autumn. Vertical variations of DOC levels were not remarkable, but highest concentrations were observed at surface waters. Seasonal changes of DOC concentrations were significant at middle-inner and outer bays (p<0.05, Table 2).

MCHO levels were found between 0.7–8.3 μmol/L (1.3–8.3 μmol/L for middle-inner bay, 0.7–5.6 μmol/L for outer bay) and...
maximum MCHO levels were observed in spring. MCHO levels were increased from winter to spring and decreased to minimum levels in autumn. Seasonal changes of MCHO levels were significant at middle-inner and outer bays (p<0.05, Table 2).

PCHO levels were found between 0.7–19.5 μmol/L (0.7–19.5 μmol/L for middle-inner bay, 1.8–10.8 μmol/L for outer bay). PCHO levels were decreased from winter to autumn and highest PCHO concentrations were observed at surface waters. Seasonal variations of PCHO levels were significant at middle-inner and outer bays (p<0.05, Table 2).

TDCHO concentrations were ranged between 2.6–14.4 μmol/L and 3.6–24.6 μmol/L at outer and middle-inner bays, respectively. Similar to PCHO, TDCHO levels were decreased from winter to autumn. Seasonal changes of TDCHO levels were significant at middle-inner and outer bays (p<0.05, Table 2).

Seasonal and vertical MCHO/TDCCHO, PCHO/TDCCHO, MCHO/DOC, PCHO/DOC, TDCHO/DOC and PCHO/MCHO ratios were given in Table 3. MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios showed significant decreasing trends from winter to summer-autumn seasons (p<0.05, Table 2). Maximum MCHO/DOC ratio was found as 11% at outer bay. Maximum PCHO/DOC (34%) and TDCHO/DOC (42%) ratios were observed at middle-inner bays. TDCHO/DOC ratios for middle-inner and outer bays were found in the range of 2.5%–42.3% (x±s=15.6, s²=129.2) and 4.4%–37.1% (x±s=72.9), respectively. MCHO/TDCCHO ratios were increased from winter to autumn, especially at middle-inner bays, and MCHO comprised up to 85% of TDCHO in autumn. MCHO/TDCCHO ratios were observed between 21%–42% in winter, spring and summer. PCHO/TDCCHO ratios were found lower at middle-inner bays compared to outer bay in autumn. PCHO/MCHO ratio was significantly lower at autumn compared to other seasons at middle-inner bays (p<0.05). PCHO/MCHO ratios were not changed significantly at outer bay (p>0.05).

### 3.2 Spatial distribution

Spatial and vertical distributions of MCHO, PCHO, TDCHO and DOC were given in Fig. 2. Carbohydrate concentrations were not changed significantly with depth. In summer, all carbohydrate species at Stas 1 and 2 (especially at surface and subsurface) were found remarkably higher than outer bay stations. MCHO, PCHO and TDCHO levels at Sta. 1 were higher than other stations at all seasons, except for autumn.

DOC concentrations were changed consistently with carbohydrate concentrations. In winter and spring, DOC levels were generally below 100 μmol/L except for Sta. 1. Maximum DOC concentration was found as 244.2 μmol/L at surface waters of Sta. 1 in summer. DOC levels were also found higher at 5 m and bottom depths of Stas 1 and 2 compared to the other stations in summer. DOC was slightly decreased from summer to autumn at all depths of Sta. 1, but DOC levels in autumn were higher than winter and spring seasons.

### 3.3 Correlation analysis

Correlations between MCHO, TDCHO, DOC, Chl a, and salinity were investigated with Spearman’s rank correlation test (Table 4). MCHO and TDCHO were positively correlated in all seasons (p<0.05) and a strong positive correlation was observed in summer (p=0.822, p<0.001). DOC was positively correlated with MCHO and Chl a in winter and summer (p<0.01). There was

### Table 2. Results of One Way ANOVA and Tukey’s HSD tests for seasonal changes of salinity, Chl a, DOC, MCHO, TDCHO and PCHO

| Middle-inner bays | Winter | Spring | Summer | Autumn |
|-------------------|--------|--------|--------|--------|
| Salinity          | 38.4±0.4<sup>a</sup> | 38.0±0.4<sup>b</sup> | 40.0±0.6<sup>b</sup> | 38.3±0.5<sup>b</sup> |
| Chl a/μg·L<sup>–1</sup> | 8.5±1.1<sup>ab</sup> | 11.0±2.2<sup>ab</sup> | 15.8±1.6<sup>a</sup> | 1.7±0.4<sup>b</sup> |
| DOC/μmol·L<sup>–1</sup> | 52.5±8.2<sup>a</sup> | 84.7±13.8<sup>ab</sup> | 163.0±67.1<sup>a</sup> | 104.5±50.8<sup>b</sup> |
| MCHO/μmol·L<sup>–1</sup> | 4.5±1.2<sup>b</sup> | 5.1±2.3<sup>b</sup> | 4.0±1.6<sup>b</sup> | 2.5±0.9<sup>b</sup> |
| PCHO/μmol·L<sup>–1</sup> | 12.4±4.4<sup>a</sup> | 10.4±2.3<sup>b</sup> | 8.6±3.1<sup>a</sup> | 1.7±1.0<sup>b</sup> |
| TDCHO/μmol·L<sup>–1</sup> | 17.0±5.4<sup>a</sup> | 15.4±4.3<sup>b</sup> | 12.6±4.5<sup>b</sup> | 4.0±0.4<sup>b</sup> |
| (MCHO/DOC)%       | 8.6±1.2<sup>a</sup> | 5.8±1.8<sup>b</sup> | 2.4±0.4<sup>a</sup> | 2.5±0.5<sup>b</sup> |
| (PCHO/DOC)%       | 23.4±6.0<sup>a</sup> | 12.2±1.2<sup>b</sup> | 5.5±1.0<sup>a</sup> | 2.2±1.9<sup>b</sup> |
| (TDCHO/DOC)%      | 31.9±6.6<sup>a</sup> | 17.9±2.4<sup>b</sup> | 7.9±1.2<sup>a</sup> | 4.8±2.2<sup>b</sup> |
| (MCHO/TDCCHO)%    | 27.4±4.4<sup>a</sup> | 31.7±6.7<sup>b</sup> | 30.9±4.9<sup>b</sup> | 60.3±21.0<sup>b</sup> |
| (PCHO/TDCCHO)%    | 72.6±4.4<sup>a</sup> | 68.3±6.7<sup>b</sup> | 69.1±4.9<sup>b</sup> | 39.7±21.0<sup>b</sup> |
| (PCHO/MCHO)%      | 2.7±0.6<sup>a</sup> | 2.3±0.7<sup>b</sup> | 2.3±0.6<sup>b</sup> | 0.9±0.7<sup>b</sup> |

| Outer bay         | Winter | Spring | Summer | Autumn |
|-------------------|--------|--------|--------|--------|
| Salinity          | 38.7±0.5<sup>a</sup> | 38.9±0.3<sup>b</sup> | 39.3±1.0<sup>a</sup> | 38.7±0.3<sup>b</sup> |
| Chl a/μg·L<sup>–1</sup> | 1.1±0.7<sup>a</sup> | 0.7±0.4<sup>b</sup> | 0.4±0.3<sup>b</sup> | 0.4±0.2<sup>b</sup> |
| DOC/μmol·L<sup>–1</sup> | 48.1±10.8<sup>a</sup> | 75.1±12.6<sup>a</sup> | 46.0±9.0<sup>b</sup> | 62.3±9.6<sup>b</sup> |
| MCHO/μmol·L<sup>–1</sup> | 2.9±0.6<sup>a</sup> | 3.2±1.2<sup>b</sup> | 0.9±0.2<sup>b</sup> | 1.2±0.4<sup>b</sup> |
| PCHO/μmol·L<sup>–1</sup> | 7.5±1.5<sup>a</sup> | 7.0±2.1<sup>b</sup> | 2.7±0.7<sup>b</sup> | 2.6±0.6<sup>b</sup> |
| TDCHO/μmol·L<sup>–1</sup> | 10.4±1.7<sup>a</sup> | 10.2±2.7<sup>b</sup> | 3.6±0.8<sup>b</sup> | 3.8±0.5<sup>b</sup> |
| (MCHO/DOC)%       | 6.6±1.9<sup>a</sup> | 4.4±2.0<sup>b</sup> | 2.0±0.5<sup>b</sup> | 2.0±0.8<sup>b</sup> |
| (PCHO/DOC)%       | 17.5±6.8<sup>a</sup> | 9.7±3.6<sup>b</sup> | 6.0±1.5<sup>a</sup> | 4.3±0.6<sup>b</sup> |
| (TDCHO/DOC)%      | 24.1±8.2<sup>a</sup> | 14.1±5.0<sup>b</sup> | 8.0±1.8<sup>b</sup> | 6.3±1.2<sup>a</sup> |
| (MCHO/TDCCHO)%    | 28.7±6.1<sup>ab</sup> | 31.2±7.2<sup>ab</sup> | 25.3±5.1<sup>b</sup> | 31.6±7.3<sup>b</sup> |
| (PCHO/TDCCHO)%    | 71.3±6.1<sup>b</sup> | 68.8±7.2<sup>ab</sup> | 74.7±5.1<sup>b</sup> | 68.4±7.3<sup>b</sup> |
| (PCHO/MCHO)%      | 2.7±1.0 | 2.4±1.0 | 3.1±0.8 | 2.4±0.9 |

Note: Values represent mean±SD; n=6 for inner bay; n=15 for outer bay; p<0.05; Tukey’s HSD test results are given with superscript letters.
a strong positive correlation between DOC and TDCHO in summer ($r^2=0.798$, $p<0.001$). Chl a and MCHO was positively correlated in all seasons ($p<0.01$). Chl a was positively correlated with TDCHO in spring and summer ($p<0.01$). There were strong negative correlations between salinity and other variables (MCHO, TDCHO and Chl a) in spring ($p<0.001$).

As shown in Fig. 3, linear relationships were observed between MCHO and TDCHO/DOC ratio in middle-inner (p=0.05) and outer (p=0.001) bays, respectively. Strong linear relationships observed between PCHO and TDCHO/DOC ratio at both parts of the bay (middle-inner bays: $R^2=0.621$, $p<0.001$, outer bay: $R^2=0.684$, $p<0.001$). The ratio of TDCHO/DOC was linearly related with Chl a at outer bay ($p<0.05$). However, linear relationship between Chl a and TDCHO/DOC ratio was not significant at middle-inner bays ($p=0.426$).

### 4 Discussion

In the literature, it has been reported that PCHO levels were changed on a seasonal basis and high PCHO values were observed in winter and summer (Myklestad and Børshøim, 2007). Nutrient transport from seabed to surface waters might also support phytoplankton activity in winter following the breakdown of stratification at water column (Scoullos et al., 2006). In some studies, higher dissolved carbohydrate levels have been reported in spring and summer than in winter and autumn (Myklestad and Børshøim, 2007).

Table 3. Seasonal variations for MCHO/TDCHO, PCHO/TDCHO, MCHO/DOC, PCHO/DOC and TDCHO/DOC ratios in the water column of the İzmit Bay (n=7 for each range)

| Season/Depth | (MCHO/TDCHO)% | (PCHO/TDCHO)% | (MCHO/DOC)% | (PCHO/DOC)% | (TDCHO/DOC)% |
|--------------|---------------|---------------|-------------|-------------|-------------|
| Winter       | Outer bay     | Middle-inner bay | Outer bay | Middle-inner bay | Outer bay | Middle-inner bay | Outer bay | Middle-inner bay |
| Surface      | 23–39         | 26–29         | 61–77      | 71–74       | 5–11        | 7–10        | 8–26        | 20–26        | 12–37        | 27–36        |
| Subsurface   | 23–39         | 21–33         | 67–77      | 67–79       | 4–9         | 8–9         | 6–25        | 16–34        | 10–33        | 23–42        |
| Bottom       | 15–33         | 26–30         | 67–85      | 70–74       | 4–8         | 8–9         | 9–28        | 22–24        | 13–32        | 31–32        |
| Spring       | Outer bay     | Middle-inner bay | 61–80      | 64–66       | 3–9         | 6–7         | 6–14        | 12–12.3      | 9–23         | 18–19        |
| Subsurface   | 18–41         | 28–42         | 59–82      | 58–72       | 3–9         | 4–9         | 6–14        | 11–12        | 10–23        | 15–21        |
| Bottom       | 26–39         | 24–27         | 61–74      | 73–76       | 2–5         | 4–5         | 5–15        | 12–14        | 7–20         | 15–20        |
| Summer       | Surface       | 28.8–28.9     | 68–83      | 71.1–71.2   | 1–3         | 2–3         | 4–7         | 6–7          | 6–9          | 8–9          |
| Subsurface   | 19–35         | 31–38         | 65–81      | 62–69       | 2–3         | 2.6–2.9     | 6–10        | 4–7          | 8–13         | 7–9          |
| Bottom       | 24–29         | 24–35         | 71–76      | 65–76       | 1–2         | 1.7–2.4     | 4–7         | 4–6          | 5–9          | 6.8–7.2      |
| Autumn       | Surface       | 46–67         | 58–75      | 33–54       | 2–4         | 2–3         | 3.7–5.0     | 0.8–3.7      | 6–9          | 3–7          |
| Subsurface   | 24–41         | 46–83         | 59–76      | 17–54       | 1–3         | 2.5–2.9     | 3.3–4.8     | 0.5–3.3      | 4–8          | 3–6          |
| Bottom       | 19–38         | 35–85         | 63–81      | 15–65       | 1–3         | 2.4–2.5     | 3.8–5.3     | 0.4–4.7      | 5–8          | 3–7          |
spring. The highest PCHO and TDCHO levels were observed in winter and spring.

Seasonal changes of PCHO and TDCHO were showed similar trends with Chl \(a\) levels. According to Table 4, PCHO and TDCHO were highly correlated with Chl \(a\) levels at spring and summer. PCHO, TDCHO and Chl \(a\) concentrations were decreased in autumn. These results indicated that PCHO and TDCHO levels might be influenced by biological processes especially in spring, summer and autumn. Similar significant correlations between carbohydrate and Chl \(a\) concentrations were reported in the literature (Hung et al., 2001, 2003; Khodse et al., 2007). However, carbohydrate concentrations are also influenced by other factors like bacterial utilization and grazing activities (Strom et al., 1997; Hopkinson et al., 2002; Guo et al., 2004; Wang et al., 2006). In winter, PCHO and TDCHO levels might be affected by rain runoffs, weathering and terrestrial inputs (Wang et al., 2003; Shin et al., 2003; He et al., 2015). DOC and carbohydrate levels (MCHO, PCHO and TDCHO) were found higher in middle-inner bays, under the influence of anthropogenic inputs, compared to outer bay.

Vertical variations of Chl \(a\), DOC and dissolved carbohydrate concentrations were not significant in this study \((p<0.05)\). This
might be resulted from shallow water depths in the Izmir Bay. Depths of stations at middle-inner and outer bays range between 9–22 m and 27–66 m, respectively. In the literature, significant vertical variations in Chl \(a\), DOC and dissolved carbohydrate concentrations were observed between upper water column ( euphotic zone) and deep waters where light penetration is very low (Hung et al., 2009; Wang et al., 2006; Lin and Guo, 2015).

The ratio of MCHO/TDCHO increased from winter to autumn, especially at middle-inner bays, and similar results were reported in the literature (Wang et al., 2006; He et al., 2015; Lin and Guo, 2015). The ratios of MCHO/DOC and TDCHO/DOC decreased from winter to autumn and similar ratios were also observed in the literatures (Wang et al., 2006; Myklestad and Børsgaard, 2007; He et al., 2015; Lin and Guo, 2015). Increasing MCHO/TDCHO and decreasing TDCHO/DOC ratios indicated production of significant MCHO fraction or breaking down of significant PCHO fraction from winter to autumn in the bay. A similar case was also reported by Wang et al. (2006).

Carbohydrate concentrations in bulk DOC pool (i.e., TDCHO/DOC ratio) were used as a tool to investigate the degradation and diagenetic status of bulk DOM and its conversion rate in aquatic environments (Skoog and Benner, 1997; Hung et al., 2009; Kaiser and Benner, 2009; Khodse et al., 2010; Lin and Guo, 2015). Lin and Guo (2015) have reported TDCHO/DOC ratios in the ranges of 11%–71% and 14%–52% at surface waters of shelf and basin regions, respectively. TDCHO/DOC ratios were distributed at wide ranges and it might be related to the presence of newly forming and degrading fractions of DOM in the bay. Higher carbohydrate fractions were observed in freshly produced DOM and lower TDCHO/DOC ratios were found during the degradation process of DOM (Opsahl and Benner, 1999; Lin and Guo, 2015).

According to factor analysis between salinity, Chl \(a\), DOC, MCHO and TDCHO (Fig. 4a), two factors were extracted and the factors were statistically sufficient to model the variations in the data of middle-inner bays. Factors 1 and 2 sufficiently explained 68% and 32% of the variability in the data, respectively. While the MCHO, TDCHO, DOC and Chl \(a\) were explained by Factor 1, salinity and also DOC identified by Factor 2 for middle-inner bays. Factor analysis showed that variability of the data could be explained by at least two factors at outer bay (Fig. 4b). Factors 1 and 2 were accounted for 83% and 17% of the variability in the data, respectively. DOC, MCHO, TDCHO and Chl \(a\) were explained by Factor 1. Salinity and Chl \(a\) were explained by Factor 2 at outer bay. According to results of factor analyses, Chl \(a\) and dissolved carbohydrate species (MCHO and TDCHO) were explained in the same factor groups and phytoplankton activities could have an important role on the dissolved carbohydrate concentrations in the Izmir Bay.

DOC, MCHO and TDCHO levels determined in this study were similar to levels reported from other bays and gulfs (Table 5). DOC concentrations in the present study were very close to DOC concentrations in the present study (Hung and Santschi, 2001). DOC, MCHO and TDCHO levels determined in this study were similar to levels reported from other bays and gulfs (Table 5). DOC, MCHO and TDCHO concentrations in the present study were very close to DOC concentrations in the present study (Hung and Santschi, 2001).

Fig. 4. Biplots of factor loadings indicating the explained proportions of variances for salinity, Chl \(a\), DOC, MCHO, and TDCHO in the bay: middle-inner bay (a) and outer bay (b).

Table 5. Comparison of DOC, MCHO and TDCHO levels of the Izmir Bay with different parts of the world

| Location                     | DOC/μmol·L\(^{-1}\) | MCHO/μmol·L\(^{-1}\) | TDCHO/μmol·L\(^{-1}\) | Reference                  |
|------------------------------|--------------------|-----------------------|------------------------|-----------------------------|
| Atlantic Ocean and Pacific Ocean | 47–119             | 2.4–6.2               | 7–33                   | Pakulski and Benner (1994)   |
| Black Sea                    | 148–270            | –                     | 12–20                  | Cauvet et al. (2002)         |
| Northern Adriatic Sea        | –                  | 5–54                  | 5–95                   | Ahel et al. (2005)           |
| Beaufort Sea                 | 30–202             | –                     | 0.6–1.3                | Panagiotopoulos et al. (2014) |
| Bay of Bengal                | –                  | 0.9–2.9               | 4.5–7.9                | Blosle et al. (1998)         |
| Trieste Gulf                 | 108–200            | 2–13                  | 11–126                 | Terzić et al. (1998)         |
| San Francisco Bay            | 52–172             | 0.2–1.3               | 1–4                    | Murrell and Hollibaugh (2000) |
| Gulf of Mexico               | 205                | –                     | 28.8                   | Hung and Santschi (2001)     |
| Galveston Bay                | 300–363            | –                     | 27.1–83.3              | Hung and Santschi (2001)     |
| Izmir Bay                    | 32–244             | 0.7–8.3               | 2.6–24.6               | this study                  |


levels observed at the Beaufort Sea (Panagiotopoulos et al., 2014), Black Sea (Cauwet et al., 2002) and Gulf of Mexico (Hung and Santschi, 2001). DOC levels at the Galveston Bay (Hung and Santschi, 2001) were greatly higher than the DOC levels in this study. MCHO levels in this study were only lower than those reported from the northern Adriatic Sea (Ahel et al., 2005). Maximum MCHO concentration in this study was higher than the maximum levels in the Atlantic Ocean, Pacific Ocean, Beaufort Sea, Trieste Gulf, San Francisco Bay and Gulf of Mexico (Pakulski and Benner, 1994; Terzic et al., 1998; Murrell and Hollibaugh, 2000; Hung and Santschi, 2001; Panagiotopoulos et al., 2014). TDCHO levels in this study were close to the TDCHO levels at the Black Sea, Bay of Bengal, Gulf of Mexico and San Francisco Bay (Bhosle et al., 1998; Murrell and Hollibaugh, 2000; Hung and Santschi, 2001; Cauwet et al., 2002). On the other hand, maximum TDCHO levels at the northern Adriatic Sea and Trieste Gulf were much higher than the TDCHO levels in this study (Terzic et al., 1998; Ahel et al., 2005).

5 Conclusions
Seasonal variations and distributions of dissolved carbohydrates, salinity, Chl a and DOC levels were studied in the Izmir Bay. According to the results, the highest MCHO levels were found in spring and the MCHO levels decreased from summer to autumn. Maximum PCHO and TDCHO levels were observed in winter and the PCHO and TDCHO levels decreased from winter to autumn. Mean dissolved carbohydrate, DOC and Chl a levels were decreased from middle-inner to outer bay. Increasing MCHO/ TDCHO and decreasing TDCHO/DOC ratios indicated production of significant MCHO fraction or breaking down of significant PCHO fraction from winter to autumn. TDCHO/DOC ratio distributed at a wide range that might be linked with the presence of newly forming and degrading fractions of DOM. According to results of factor analysis, Chl a and dissolved carbohydrate species (MCHO and TDCHO) were explained in the same factor group and phytoplankton activities could have an important role on the dissolved carbohydrate concentrations in the Izmir Bay. As indicators of biological and physical processes in seawater, seasonal and vertical variations of DOC, Chl a, MCHO, PCHO and TDCHO were useful in investigation of organic matter distribution. The effects of other environmental factors (i.e., primary production, bacterial abundance, extracellular enzyme activities) on dissolved carbohydrate levels should be investigated in further studies.

References
Ahel M, Tepic N, Terzic S. 2005. Spatial and temporal variability of carbohydrates in the northern Adriatic—a possible link to mucilage events. Science of the Total Environment, 355(1–3): 139–150
Allredge A L, Passow U, Logan B E. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep Sea Research Part I: Oceanographic Research Papers, 40(6): 1131–1140
Amon R M W, Benner R. 2003. Combined neutral sugars as indicators of the diagenetic state of dissolved organic matter in the Arctic Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 50(1): 151–169
APHA. 1998. Chlorophyll, Section: 10200 H. In: Clesceri LS, Greenberg AE, Eaton AD, eds. Standard Methods for the Examination of Water and Wastewater. 20th ed. Baltimore, Maryland: United Book Press
Baldi F, Minacci A, Sallot A, et al. 1997. Cell lysis and release of particulate polysaccharides in extensive marine mucilage assessed by lipid biomarkers and molecular probes. Marine Ecology Progress Series, 153: 45–57
Benner R. 2002. Chemical composition and reactivity. In: Hansell D A, Carlson C A, eds. Biogeochemistry of Marine Dissolved Organic Matter. San Diego: Academic Press, 59–90
Benner R, Opsahl S. 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi river plume. Organic Geochemistry, 32(4): 597–611
Bhosle N B, Bhaskar P V, Ramachandran S. 1998. Abundance of dissolved polysaccharides in the oxygen minimum layer of the Northern Indian Ocean. Marine Chemistry, 63(1–2): 171–182
Borch N H, Kirchman D L. 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Marine Chemistry, 57(1–2): 85–95
Cauwet G, Déliat G, Krastev A, et al. 2002. Seasonal DOC accumulation in the Black Sea: a regional explanation for a general mechanism. Marine Chemistry, 79(3–4): 193–205
Chanudet V, Filella M. 2006. The application of the MBTH method for carbohydrate determination in freshwaters revisited. International Journal of Environmental Analytical Chemistry, 86(9): 693–712
Engel A, Händel N. 2011. A novel protocol for determining the concentration and composition of sugars in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater. Marine Chemistry, 127(1–4): 180–191
Guo J D, Tanaka T, Wang D L, et al. 2004. Distributions, speciation and stable isotope composition of organic matter in the southeastern Bering Sea. Marine Chemistry, 91(1–4): 211–226
Handa N. 1966. Distribution of dissolved carbohydrate in the Indian Ocean. Journal of the Oceanographical Society of Japan, 22(2): 50–55
Handa N. 1967. The distribution of the dissolved and the particulate carbohydrates in the Kuroshio and its adjacent areas. Journal of the Oceanographical Society of Japan, 23(3): 115–123
Harman H H, Jones W H. 1966. Factor analysis by minimizing residuals (minres). Psychometrika, 31(3): 351–368
He Zhen, Wang Qi, Yang Guipeng, et al. 2015. Spatiotemporal variation characteristics and related affecting factors of dissolved carbohydrates in the East China Sea. Continental Shelf Research, 108: 12–24
Hedges J I. 2002. Why dissolved organics matter. In: Hansell D A, Carlson C A, eds. Biogeochemistry of Marine Dissolved Organic Matter. San Diego: Academic Press, 1–33
Hedges J I, Cowie G L, Richey J E, et al. 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. Limnology and Oceanography, 39(4): 743–761
Hopkinson C S Jr, Vallino J J, Nolín A. 2002. Decomposition of dissolved organic matter from the continental margin. Deep Sea Research Part II: Topical Studies in Oceanography, 49(20): 4461–4478
Horn J L. 1965. A rationale and test for the number of factors in factor analysis. Psychometrika, 30(2): 179–185
Hung C C, Guo J D, Santschi P H, et al. 2003. Distributions of carbohydrate species in the Gulf of Mexico. Marine Chemistry, 81(3–4): 119–135
Hung C C, Gong G C, Chiang K P, et al. 2009. Particulate carbohydrates and uronic acids in the northern East China Sea. Estuarine, Coastal and Shelf Science, 84(4): 565–572
Hung C C, Santschi P H. 2001. Spectrophotometric determination of total uronic acids in seawater using cation-exchange separation and pre-concentration by lyophilization. Analytica Chimica Acta, 427(1): 111–117
Hung C C, Tang D, Warnken K W, et al. 2001. Distributions of carbohydrates, including uronic acids, in estuarine waters of Galveston Bay. Marine Chemistry, 73(3–4): 305–318
Jang J K, Harpt N, Grasmick D, et al. 1990. A two-phase model for determining the stability constants for interactions between copper and alginic acid. Journal of Physical Chemistry, 94(1): 482–488
Jang J K, Nguyen D, Geesey G G. 1995. Selectivity of alginate gel for lipid biomarkers and molecular probes. Marine Ecology Progress Series, 50(1): 151–169

Cu vs Co. Water Research, 29(1): 307–313
Kaiser K, Benner R. 2009. Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. Marine Chemistry, 113(1–2): 63–77
Khodse V B, Bhosle N B, Matondkar S G P. 2010. Distribution of dissolved carbohydrates and uronic acids in a tropical estuary, India. Journal of Earth System Science, 119(4): 519–530
Khodse V B, Fernandes L, Gopalkrishna V V, et al. 2007. Distribution and seasonal variation of concentrations of particulate carbohydrates and uronic acids in the northern Indian Ocean. Marine Chemistry, 103(3–4): 327–346
Kirchman D L, Moon B, Ducklow H W, et al. 2001. Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. Deep-Sea Research II: Topical Studies in Oceanography, 48(19–20): 4179–4197
Kontas A, Kucuksezgin F, Altay O, et al. 2004. Monitoring of eutrophication and nutrient limitation in the Izmir Bay (Turkey) before and after wastewater treatment plant. Environment International, 29(6): 1057–1062
Kucuksezgin F, Kontas A, Altay O, et al. 2005. Elemental composition of particulate matter and nutrient dynamics in the Izmir Bay (Eastern Aegean). Journal of Marine Systems, 56(1–2): 67–84
Lee J H, Lee D, Kang J J, et al. 2017. The effects of different environmental factors on the biochemical composition of particulate organic matter in Gwangyang Bay, South Korea. Biogeoosciences, 14(7): 1903–1917
Leppard G G. 1997. Colloidal organic fibrils of acid polysaccharides in surface waters: electron-optical characteristics, activities and chemical estimates of abundance. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 120(1–3): 1–15
Libes S. 2009. Introduction to Marine Biogeochemistry. 2nd ed. California: Academic Press
Lin Peng, Guo Laodong. 2015. Spatial and vertical variability of dissolved combined neutral sugars in the northern Gulf of Mexico following the Deepwater Horizon oil spill, 2010–2011. Marine Chemistry, 174: 13–25
McCarthy M, Hedges J, Benner R. 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. Marine Chemistry, 55(3–4): 281–297
Murrell M C, Hollibaugh J T. 2000. Distribution and composition of particulate matter and nutrient dynamics in the northern Gulf of Mexico following the Deepwater Horizon oil spill, 2010–2011. Marine Chemistry, 174: 13–25
Myklestad S M, Børshheim K Y. 2007. Dynamics of carbohydrates in the Norwegian Sea inferred from monthly profiles collected during 3 years at 66°N, 2°E. Marine Chemistry, 107(4): 475–485
Myklestad S M, Skánøy E, Hestmann S. 2007. A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater. Marine Chemistry, 103(3–4): 279–286
Opsahl S, Benner R. 1999. Characterization of carbohydrates during early diagenesis of five vascular plant tissues. Organic Geochemistry, 30(1): 83–94
Pakulski J D, Benner R. 1992. An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Marine Chemistry, 40: 143–160
Pakulski J D, Benner R. 1994. Abundance and distribution of carbohydrates in the ocean. Limnology and Oceanography, 39(4): 930–940
Panagiopoulou C, Sempère R, Jacq V, et al. 2014. Composition and distribution of dissolved carbohydrates in the Beaufort Sea Mackenzie margin (Arctic Ocean). Marine Chemistry, 166: 92–102
Passow U, Allerdige A L, Logan B E. 1994. The role of particulate carbohydrate exudates in the flocculation of diatom blooms. Deep-Sea Research Part I: Oceanographic Research Papers, 41(2): 335–357
Penna N, Capellacci S, Ricci F, et al. 2003. Characterization of carbohydrates in mucilage samples from the northern Adriatic Sea. Analytical and Bioanalytical Chemistry, 376(4): 436–439
Penna N, Kovacic N, Ricci F, et al. 2009. The role of dissolved carbohydrates in the northern Adriatic macroaggregation formation. Acta Chimica Slovenica, 56(2): 305–314
Petitm M, Patrocello L, Manganelli M, et al. 1999. Seasonal variations of dissolved organic matter in the northern Adriatic Sea. Marine Chemistry, 64(3): 153–169
R Core Team. 2016. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/
Revelle W. 2016. Psych: Procedures for Psychological, Psychometric, and Personality Research. Illinois, USA: Northwestern University, http://cran.r-project.org/package=psych
Revelle W, Rocklin T. 1979. Very simple structure: alternative procedure for estimating the optimal number of interpretable factors. Multivariate Behavioral Research, 14(4): 403–414
Rich J M, Gosselin E, Sherr B, et al. 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the central Arctic Ocean. Deep Sea Research Part II: Topical Studies in Oceanography, 44(8): 1645–1663
Sunlu F S, Sunlu U, Buyukiskik B, et al. 2012. Nutrient and chlorophyll a trends after wastewater treatment plant in Izmir bay (Eastern Aegean Sea). Journal of Animal and Veterinary Advances, 11(1): 113–123
Sayin E. 2003. Physical features of the Izmir Bay. Continental Shelf Research, 23(10): 957–970
Scoullos M, Plavšič M, Karavoltsos S, et al. 2006. Partitioning and distribution of dissolved copper, cadmium and organic matter in Mediterranean marine coastal areas: The case of a mucilage event. Estuarine, Coastal and Shelf Science, 67(3): 484–490
Shin K H, Hama T, Handa N. 2003. Effect of nutrient conditions on the composition of photosynthetic products in the East China Sea and surrounding waters. Deep Sea Research Part II: Topical Studies in Oceanography, 50(2): 389–401
Skog Å, Allerdige A, Passow U, et al. 2008. Neutral aldoses as source indicators for marine snow. Marine Chemistry, 108(3–4): 195–206
Skog Å, Benner R. 1997. Aldoses in various size fractions of marine organic matter: implications for carbon cycling. Limnology and Oceanography, 42(8): 1803–1813
Strom S L, Benner R, Ziegler S, et al. 1997. Planktonic grazers are a potentially important source of marine dissolved organic carbon. Limnology and Oceanography, 42(6): 1364–1374
Terzic S, Ahel M, Cauwet G, et al. 1998. Group-specific phytoplankton biomass/dissolved carbohydrate relationships in the Gulf of Trieste (Northern Adriatic). Hydrobiologia, 363(1–3): 191–205
Wang Bengdong, Wang Xiaolin, Zhan Run. 2003. Nutrient conditions in the Yellow Sea and the East China Sea. Estuarine, Coastal and Shelf Science, 58(1): 127–136
Wang Deli, Henrichs S M, Guo Laodong. 2006. Distributions of nutrients, dissolved organic carbon and carbohydrates in the western Arctic Ocean. Continental Shelf Research, 26(14): 1654–1667
Yang Guipeng, Zhang Yanping, Lu Xiaolan, et al. 2010. Distributions and seasonal variations of dissolved carbohydrates in the Jiaozhou Bay, China. Estuarine, Coastal and Shelf Science, 88(1): 12–20
Zhang Yanping. 2010. Distributions and influencing factors of dissolved carbohydrates in the coastal waters of China [dissertation] (in Chinese). Qingdao: Ocean University of China
Fish diversity and molecular taxonomy in the Prydz Bay during the 29th CHINARE

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Received 19 July 2017; accepted 18 May 2018

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Abstract

In 2013, the 29th Chinese National Antarctic Research Expedition (CHINARE) prospected the Prydz Bay on the Antarctic continental shelf, and the Chinese R/V Xuelong icebreaker sampled all of the examined locations. The nature of Antarctic fish diversity in the high-latitude Prydz Bay is virtually unknown, and the accuracy of relevant estimates has not been established. Thus, it is necessary to evaluate this diversity and propose protective measures. In total, ninety-nine specimens were collected from various locations. To overcome uncertainties associated with identifying species based on morphology, DNA barcoding (COI gene) was employed to reconstruct phylogenetic relationships with delimited references from NCBI. Twenty-two species representing six families were unambiguously identified from a neighbor-joining (NJ) tree and barcoding gaps. With the development of ameliorations are still underway; however, these approaches seem promising for numerous taxa and developmental stages.

Antarctic waters are home to a largely benthic and highly endemic ichthyofauna, dominated by actinopterygian members that arose through nested adaptive radiations within the isolated Southern Ocean (Eastman, 2005; Lecointre et al., 2013; Fallon et al., 2016). Five families—Artedidraconidae, Bathydraconidae, Chanichthyidae, Harpagiferidae, and Nototheniidae—are traditionally recognized, encompassing predominantly endemic Antarctic species (Gon, 1990; Eastman and Eakin, 2014). The major-
tion in the Southern Ocean. The Prydz Bay is an important source of bottom water in Antarctica and is a focus of research on physical oceanography, marine geology, marine geophysics, and marine ecology (Van de Putte et al., 2010; Gao et al., 2013; Yin et al., 2014). Unfortunately, no dedicated studies of fish diversity and molecular taxonomy in the Prydz Bay have been performed. In contrast, several studies of high-latitude fish barcoding and molecular taxonomy have been conducted further east in the Dumont d’Urville Sea and McMurdo Sound (Dettai et al., 2011a, b; Murphy et al., 2017).

The ecological community is greatly impacted by global warming. With changes already visible in the Antarctic, a biodiversity baseline inventory is necessary to monitor these changes. A large amount of taxonomic work, especially for fish, is still needed in the Antarctic (Dettai et al., 2011b). We employed a DNA barcoding approach as a molecular tool for the identification of fish species collected from the Prydz Bay using available data from NCBI as a reference. We reconstructed phylogenetic relationships to assign species identity and evaluated the identification success rates based on the Kimura’s two-parameter (K2P) model. Furthermore, the present study of fish diversity and molecular taxonomy can provide important information regarding biodiversity, biogeography and conservation in Antarctic coastal waters.

2 Materials and methods

2.1 Specimen collection and morphological identification

All specimens from 15 stations were collected from the Prydz Bay based on a triangular bottom trawl net (2.2 m wide, 0.65 m high, and 6.5 m long; 20 mm mesh size) during the 29th Chinese National Antarctic Research Expedition (CHINARE) in 2013 (Table 1), which was surveyed on the R/V Xuelong icebreaker. Every net was operated for 10–60 min, with variations due to differences among the seabeds at a speed of 3–4 kn. A few specimens were obtained from each station, and all specimens from the same station were packaged together and sorted again in the laboratory. Morphological identification was performed by visual inspection, and the fish were taxonomically classified by taxonomic specialists (Fischer and Hureau, 1985). Photographs of as many fresh specimens as possible were taken on board. Muscle samples were obtained and preserved in 95% ethanol or frozen for DNA extraction after specimen identification and morphological characterization. Subsequently, the whole fish were fixed in a 10% formaldehyde solution and stored as voucher samples in the Third Institute of Oceanography, State Oceanic Administration.

2.2 DNA extraction, amplification and sequencing

Genomic DNA was isolated from muscle tissue by proteinase

| ID | Scientific name from morphological identification | NCBI accession Nos | Sort by identification /% | Species name from NCBI | NCBI accession Nos |
|----|--------------------------------------------------|--------------------|---------------------------|------------------------|--------------------|
| S1 | Bathyrja sp.                                      | 99                 | Bathyrja spinicauda       | FI164384, IF895081     |
| S2 | Trematomus scotti                                | 100, 100           | Trematomus scotti         | HQ713283, JN641171     |
| S4 | Artedidraco lonnbergi                           | 99, 100            | Artedidraco lonnbergi     | HQ712811, HQ712827     |
| S6 | Dolloidraco sp.                                  | 100                | Histiodraco velfer        | HQ713027, JN640978     |
| S7 | Chionodraco hamatus                              | 100, 100           | Chionodraco hamatus       | HQ712912, JN640841, KT912182 |
| S8 | Pogonophryne sp.                                 | 99, 100            | Pogonophryne scotti       | HQ713108, JN641119     |
| S11| Chaeonodraco wilsonii                           | 100, 100           | Chaeonodraco wilsonii     | EJ316416, HQ713203, JN641128 |
| S12| Trematomus sp.                                   | 99                 | Trematomus eulepidotus    | HQ712920, JN640813     |
| S13| Dieidolycus sp.                                  | 100                | Lycodichthys antarcticus  | HQ713053, HQ713056     |
| S14| Cryodraco antarcticus                           | 100, 100           | Cryodraco antarcticus     | HQ712949, JN640867     |
| S15| Gerlachea australis                              | 100                | Gerlachea australis       | HQ713006, JN640928     |
| S16| Pachycara brachycephalum                        | 100                | Pachycara brachycephalum  | HQ713113, JN641050     |
| S17| Pachycara brachycephalum                        | HQ713113, JN641050 | Ophiobolmophycus amherensis | HQ713104, HQ713105, HQ713106 |
| S18| Dolloidraco longedorsalis                       | 99                 | Dolloidraco longedorsalis | HQ712976, JN640907     |
| S19| Lycodichthys antarcticus                        | 99                 | Lycodichthys antarcticus  | HQ713053, HQ713056     |
| S20| Bathyrjawera macrolepis                         | EU326324, JN640779, JN640780 | 99 | Akarotaxis nudiceps       | HQ712805, HQ712806     |
| S21| Racovitza glacialis                              | 100                | Racovitza glacialis       | HQ713223, JN641132     |
| S22| Trematomus pennellii                            | 99, 99             | Trematomus cf, lepidorhina/leoniernergi | HQ713319, HQ713348, HQ713349 |
| S24| Artedidraco sp. 1                               | 100                | Artedidraco shackleboni   | HQ712858, HQ712859, HQ712860 |
| S27| Chionobathyscus dewitti                         | 99, 100            | Chionobathyscus dewitti   | HQ712909, JN640826     |
| S29| Dacodraco hunteri                                | 100, 100           | Dacodraco hunteri         | HQ712963, JN640896     |
K digestion and purified by standard phenol-chloroform extraction (Sambrook et al., 1989). The extracted DNA was evaluated using 1.5% agarose gel electrophoresis and was then stored at −20°C before PCR amplification. A partial fragment of the COI gene was amplified using universal barcoding primers, F1: 5′-TCAACCAACACAAAGACATTGGAC-3′ (forward) and R1: 5′-TAGACTTCTGGTGCCAAAAGATCTA-3′ (reverse) (Ward et al., 2005). The PCR reactions were carried out in a 25 μL reaction mixture containing 17.25 μL of ultrapure water, 2.5 μL of 10×PCR buffer (including MgCl2) (Takara), 2 μL of dNTPs (Takara), 1 μL of each primer (5 μmol/L), 0.25 μL of Taq polymerase (Takara), and 1 μL of DNA template. The thermal cycling program consisted of an initial step for 4 min at 95°C, 35 cycles of 0.5 min at 94°C, 0.5 min at 52°C, and 0.5 min at 72°C; and a final step for 10 min at 72°C. Negative controls were included in all PCR reactions to confirm the absence of contaminants. Successful amplifications were purified, and both strands were sequenced by Personal Biotechnology Co., Ltd.

2.3 Data analysis

Sequences were edited and aligned using the DNASTAR Lasergene software package (Madison, WI, USA) and refined manually. All of the aligned sequences were translated into amino acids to confirm the accuracy of the sequences and to detect the presence of nuclear DNA pseudogenes. Other genetic indices, such as polymorphic sites, transitions, transversions, insertions/deletions, and nucleotide composition, were calculated with ARLEQUIN 3.0 (Excoffier et al., 2005). Pairwise genetic distance calculations and NJ tree analysis were implemented using MEGA 5.0 (Tamura et al., 2011) based on the K2P model with 1000 bootstrap replicates. The DNA “barcoding gap”, which is the maximum intraspecific distance of each species compared with its minimum distance to the nearest neighbor and is defined as the average interspecific distance at least 10-fold greater than the average intraspecific genetic distance, was calculated for all species (Hebert et al., 2004). All specimen sequences were aligned through a BLAST search in NCBI to evaluate the accuracy of morphological identification. Almost all currently barcoded Antarctic fish were collected during the CEAMARC survey in the winter of 2007–2008 (Dettai et al., 2011a, b). We used a general rule that defined a sequence similarity of at least 97% as a top-matched species and 3% sequence similarity as a relatively loose criterion for matched species (Wong and Hanner, 2008).

3 Results

Ninety-nine specimens were collected during the 29th CHINARE. Most of them were adults and could be easily sorted, but there were also some juvenile stages. Some specimens were damaged and could not be identified morphologically; these were termed “sp.”, for example, Dolloidraco sp., Pogonophyrne sp., Bathyraja sp., Trematomus sp., Diodon macrolepis sp. and Arte- ddraco sp. Thirty-three specimens were used for molecular analysis; the number of species per specimen used for the molecular analysis ranged from one to three, but most species were represented by only one specimen.

3.1 Amplification and sequencing

Low-quality sequences (double peaks, short fragments, and background noise), which may represent pseudogenes, were not detected. The aligned sequences contained no insertions, deletions or stop codons, indicating that all amplified sequences were functional mitochondrial COI sequences. A 652 bp fragment was successfully amplified and sequenced in this study, and the alignment revealed 256 polymorphic sites (including 239 parsimony-informative sites and 6 singleton sites), with 256 transitions and 119 transversions. The content of A, T, G, and C was 21.22%, 30.18%, 19.04% and 29.56% on average, respectively, with a slight base against G and C.

3.2 Species identification by phylogenetic analysis of COI sequences

Because there are limited Antarctic fish taxonomic references, mistakes were unavoidable during morphological identification. Our results indicated that six families were correctly identified: Channichthyidae (5 species, 15 specimens), Arthedracoidae (6 species, 26 specimens), Bathydraconidae (4 species, 17 specimens), Zoarcidae (3 species, 3 specimens), Nototheniidae (3 species, 37 specimens) and Rajidae (1 species, 1 specimen) (Table 1). The consensus strength of all sequences was determined by alignment through a BLAST search in NCBI. Most morphological identification results matched the BLAST annotations of the NCBI databases with at least 97% similarity, supporting that they were the same species (Murphy et al., 2017), except for five species (H. velifer, L. antarcticus, O. amberensis, A. nudiceps, and Trematomus cf. lepidorhinus/loennbergi) that matched with their sister species.

Based on our species sequences and vouched data from NCBI as a reference, an NJ tree was constructed to assign species identity (Fig. 1), which depicted relationships between morphologically identified species and vouched references. Twenty-two species from six families (Channichthyidae, Arthedracoideae, Bathydraconidae, Zoarcidae, Nototheniidae and Rajidae) were characterized by DNA barcoding. Based on the NJ tree, all species formed distinct clusters, and all individuals were associated with their conspecifics in the monophyletic clades with high bootstrap support values. However, all species from the same family clustered together, except for Bathydraconidae. In total, five Arthedracoideae species, five Channichthyidae species, four Nototheniidae species, three Zoarcidae species and one Rajidae species clustered together in the NJ tree. The NJ analysis also recovered the family Bathydraconidae as paraphyletic, consistent with recent molecular phylogenies (Dettai et al., 2012; Murphy et al., 2017), while the other families were monophyletic. Gerlachea was sister to other genera in Bathydraconidae.

The results of the NJ tree showed that not all of the morphologically identified species were in agreement with the vouched references (Fig. 1); however, the majority was identified successfully, with at least 99% similarity (Table 1). S17 was distinctly different from S16, both of which were identified as P. brachyccephalum morphologically but shared 99% nucleotide sequence similarity with the reference O. amberensis. S20 was identified as B. macrolepis morphologically but was 99% similar to the reference A. nudiceps. S22 and S23 were distinctly different from T. pennelli references but shared 99% identity with the reference Trematomus cf. lepidorhinus/loennbergi. Some damaged individuals and juvenile fish were also identified accurately based on barcoding but had been previously unidentified and considered as “sp.” S1 (Bathyraja sp.) shared 99% nucleotide sequence similarity with the reference B. spinicauda, indicating that it was B. spinicauda. S6 (Dolloidraco sp.) was identified as H. velifer with 100% nucleotide sequence similarity; thus, this species was distinctly different from genus Dolloidraco species. S8 and S8-1 (Pogonophyrne sp.) shared 99% and 100% nucleotide sequence identities with the reference P. scotti, respectively. S12 (Trematomus sp.) shared 99% nucleotide sequence identity with the reference T. eulepidotus. S13 (Diodon macrolepis sp.) was identified as L. antarctic-
Both Artedidraco sp. 1 (S24) and Artedidraco sp. 2 (S25, S26) were identified as A. shackletoni with 100% nucleotide sequence similarity, suggesting that they were the same species. The majority of the problematic species were, in fact, due to erroneous primary identification with poor references.

### 3.3 Barcoding gaps

Intraspecific variability was generally similar across species, and the range of interspecific differences was much more variable depending on the group (Dettai et al., 2011b). The use of the means for intraspecific and interspecific divergence comparison does not allow the detection of problematic cases (Meier et al., 2008); therefore, we instead compared minima for interspecific diversions to maxima for intraspecific diversions. If the entire dataset was considered, there would be a clear overlap between intraspecific and interspecific variabilities, as the smallest interspecific diversions were well below 2%, but the largest intraspecific diversions exceeded 2% (Hebert et al., 2003a; Dettai et al., 2011b; Shen et al., 2016).

The intraspecific K2P distances exhibited considerable heterogeneity and ranged from 0% to 1.24%, with a mean value of 0.29%. The minimum interspecific distances of all species were greater than 2%. The species discrimination power of DNA barcoding was demonstrated by the barcoding gaps that were drawn for all species on the basis of the K2P distances shown in Fig. 2. Because the latter value was always higher than the former, overlaps were not detected in all species.
Fig. 2. DNA barcoding gaps for all of the species based on the K2P model. Median interspecific distances with maximum and minimum values are represented by the upper and lower bars, respectively. Red cross: maximum intraspecific distance; blue cross: mean intraspecific distance.

4 Discussion

Our study represents the first comprehensive molecular assessment of fish in the Prydz Bay. In the current study, DNA barcoding was effective for identifying species and provided a straightforward identification system when a perfect match existed between the morphology-based taxonomy and genetic divergence (Chen et al., 2015). A few specimens that could not be identified at the species level could therefore be placed with confidence within species clusters by the molecular analysis. The results showed nearly unanimous (99%–100%) species group support for the unknown species with their vouched references in the NJ tree (Fig. 1), providing strong confidence for the accuracy of the species assignment.

Of the twenty-two species investigated in this analysis, only thirteen species were identified correctly, five species were identified correctly at the genus level, and unfortunately, four species were only identified to their close sister species based on morphological characteristics. However, all problematic species were entirely resolved with COI sequences. Identifications were successfully made due to reference sequences of vouchered species and the selection of COI as the gold standard barcode gene. Furthermore, a 10-fold sequence divergence between the average interspecific and the average intraspecific difference was detected; this divergence was suggested to be the standard COI threshold for species identification (Hebert et al., 2003a, b). Unfortunately, there was slight uncertainty regarding T. lepidorhinus and T. loenbergi, which did not form distinct clusters according to the morphologically identified species in the NJ tree. Sillago analis, S. ciliata (Krück et al., 2013), Thryssa mystax and T. vitrirostris (Ma et al., 2015) had different morphological characteristics but also clustered together in the phylogenetic tree. However, Krück et al. (2013) previously presented a multigene barcoding approach to successfully discriminate the two Sillago species.

Recent molecular phylogenies indicated that the family Nototheniidae was paraphyletic (Dettai et al., 2012; Murphy et al., 2017), but it was monophyletic in this study. Because all individuals of Nototheniidae were represented by only one genus, the congeners were closely related and easily formed a monophyletic clade. In Antarctic coastal waters, notothenioids constitute over 70% of the species diversity and 91% of catch biomass (Eastman and Hubold, 1999; Murphy et al., 2017), and five families (Arteidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae, and Nototheniidae) are traditionally recognized (Gon, 1990). Moreover, notothenioid Trematomus fishes (family Nototheniidae) were overwhelmingly dominant, as reported by previous studies (Eastman and DeVries, 1982; Murphy et al., 2017), accounting for 100% of all collected specimens. In contrast, the dominance of Nototheniidae species was not evident in the Prydz Bay and was less than that reported in previous studies; species from the other five families were also caught at higher frequencies than notothenioids. The low species diversity and catch biomass in this survey were caused by the severe constraints of the limited fishing methods and localized sites, which led to a biased underestimation.

This study demonstrated that DNA barcoding is a useful and effective tool and can provide further insight into the identification of species in addition to morphological characterization. The identification and distribution of Antarctic species should be an integral component in understanding Antarctic fish biodiversity and biogeography. The overall Prydz Bay diversity (21 species) represents only a small subset of Antarctic fauna. Therefore, further morphological characterization and multigene barcoding of specimens from around the Antarctic Continent is necessary.

5 Conclusions

This study elucidates fish species and their phylogenetic relationships in the Prydz Bay on the Antarctic continental shelf based on the 29th CHINARE. All of the study results show that DNA barcoding is an effective tool for accurate species identification and could play a supporting role in species diversity surveys.
Twenty-two species from six families were characterized; only thirteen species were identified correctly, five species were identified correctly at the genus level, and four species were merely identified at the close sister species level. This work is important; in particular, the partial list of fish species in the prospected waters provided in this paper represents a resource relevant to the structure of the Antarctic community. More specimens should be collected from the Prydz Bay in the future. In subsequent studies, morphological identification should be combined with DNA barcoding to identify species because morphological identification alone may not be sufficiently robust.

Acknowledgements
The authors thank Huang Dingyong and He Xuebao for their efforts in collecting specimens.

References
Chen Weitao, Ma Xiuhui, Shen Yanjun, et al. 2015. The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. Scientific Reports, 5(1): 17437, doi: 10.1038/srep17437
Dettai A, Adamowicz S J, Allcock L, et al. 2011a. DNA barcoding and molecular systematics of the bentic and demersal organisms of the CEAMARC survey. Polar Science, 5(2): 298–312
Dettai A, Berkani M, Lautredou A C, et al. 2012. Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Marine Genomics, 8(4): 49–58
Dettai A, Lautredou A C, Bonillo C, et al. 2011b. The actinopterygian diversity of the CEAMARC cruises: barcoding and molecular taxonomy as a multi-level tool for new findings. Deep Sea Research Part II: Topical Studies in Oceanography, 58(1–2): 250–263
Eastman J T. 2005. The nature of the diversity of Antarctic fishes. Polar Biology, 28(2): 93–107
Eastman J T, DeVries A L. 1982. Buoyancy studies of nototheniid fishes in McMurdo Sound, Antarctica. Copeia, 1982(2): 385–393
Eastman J T, Eakin R R. 2014. Nototheniid classification and list of species. Ohio University. http://www.oucom.ohiou.edu/dbms-eastman/index.htm [2014-04-14/2016-10-29]
Eastman J T, Hubold G. 1999. The fish fauna of the Ross Sea, Antarctica. Antarctica. Antarctic Science, 11(3): 293–304
Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1: 47–50
Fallon N G, Collins M A, Marshall C T, et al. 2016. Assessing consistency of fish survey data: uncertainties in the estimation of mackerel icefish (Champsospechus gymnari) abundance at South Georgia. Polar Biology, 39(4): 593–603
Fischer W, Hureau J C. 1985. FAO species identification sheets for the fishery purposes. Southern Ocean (Fishing areas 48, 58 and 88), vol. I and II. Rome: FAO
Gao Guoping, Dong Zhaoqian, Shi Maochong, et al. 2013. Advances of physical oceanographic study on Prydz Bay and adjacent region, Antarctica. Journal of Shanghai Ocean University (in Chinese), 22(2): 313–320
Gon O. 1990. Teleostei. In: Gon O, Heemstra P C, eds. Fishes of the Southern Ocean. Grahamstown: J L B Smith Institute of Ichthyology, 364–380
Hebert P D N, Cywinski A, Ball S L, et al. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences, 270(1512): 313–321
Hebert P D N, Ratnasingham S, de Waard J R. 2003b. Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proceedings of the Royal Society B: Biological Sciences, 270(Sup 1): S96–S99
Hebert P D N, Stoeckle M Y, Zemlak T S, et al. 2004. Identification of birds through DNA barcodes. PLoS Biology, 2(10): e312, doi: 10.1371/journal.pbio.0020312
Keskin E, Ağdamar S, Tarkan A S. 2013. DNA barcoding common non-native freshwater fish species in Turkey: Low genetic diversity but high population structuring. Mitochondrial DNA, 24(3): 276–287
Krück N C, Tibbetts I R, Ward R D, et al. 2013. Multi-gene barcoding to discriminate sibling species within a morphologically difficult fish genus (Silago). Fisheries Research, 143: 39–46
Lecointre G, Amézia N, Ransouer M C, et al. 2013. Is the species flock concept operational? The Antarctic shelf case. PLoS One, 8(6): e68787, doi: 10.1371/journal.pone.0068787
Loh W K W, Bond P, Ashton K J, et al. 2014. DNA barcoding of freshwater fishes and the development of a quantitative qPCR assay for the species–specific detection and quantification of fish larvae from plankton samples. Journal of Fish Biology, 85(2): 307–328
Ma Chunyan, Ma Hongyu, Ni Yong, et al. 2015. Molecular identification of the genus Thysya based on DNA barcoding. Genetics and Molecular Research, 14(4): 18530–18536
Meier R, Zhang Guanyang, Ali F. 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. Systematic Biology, 57(5): 809–813
Miyamoto Y, Tanimura A. 1999. Behavior of the Antarctic fish Trematomus bernacchii (Pisces, Notototheniidae) beneath the sea ice near the Antarctic station Syowa using acoustic biotelemetry. Fisheries Science, 65(2): 315–316
Murphy K R, Kalmanek E A, Cheng C H C. 2017. Diversity and biogeography of larval and juvenile nototheniid fishes in McMurdo Sound, Antarctica. Polar Biology, 40(1): 161–173
Sambrook J, Fritsch E F, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory Press
Shen Yanjun, Guan Lihong, Wang Dengqiang, et al. 2016. DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. Ecology and Evolution, 6(9): 2702–2713
Srivastava A, Meier R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics, 28(2): 190–194
Tamura K, Peterson D, Peterson N, et al. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731–2739
Van de Putte A P, Jackson G D, Pavlovom E, et al. 2010. Distribution of squid and fish in the pelagic zone of the Cosmonaut Sea and Prydz Bay region during the BROKE-West campaign. Deep Sea Research Part II: Topical Studies in Oceanography, 57(9–10): 956–967
Ward R D, Zemlak T S, Innes B H, et al. 2005. DNA barcoding Australia’s fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462): 1847–1857
Wong E H K, Hanner R H. 2008. DNA barcoding detects market substitution in North American seafood. Food Research International, 41(8): 828–837
Yin Xijie, Li Yunhai, Qiao Lei, et al. 2014. Source and spatial distributions of particulate organic carbon and its isotope in surface waters of Prydz Bay, Antarctica, during summer. Advances in Polar Science, 25(3): 175–182
The impact of natural mortality variations on the performance of management procedures for Spanish mackerel (Scomberomorus niphonius) in the Yellow Sea, China

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Received 18 December 2017; accepted 1 February 2018

Abstract

Natural mortality rate (M) is one of the essential parameters in fishery stock assessment, however, the estimation of M is commonly rough and the changes of M due to natural and anthropogenic impacts have long been ignored. The simplification of M estimation and the influence of M variations on the assessment and management of fisheries stocks have been less well understood. This study evaluated the impacts of the changes in natural mortality of Spanish mackerel (Scomberomorus niphonius) on their management strategies with data-limited methods. We tested the performances of a variety of management procedures (MPS) with the variations of M in mackerel stock using diverse estimation methods. The results of management strategies evaluation showed that four management procedures DCAC, SPM SY, curE75 and minlenLopt1 were more robust to the changes of M than others; however, their performance were substantially influenced by the significant decrease of M from the 1970s to 2017. Relative population biomass (measure as the probability of B>0.3BMSY) increased significantly with the decrease of M, whereas the possibility of overfishing showed remarkable variations across MPS. The decrease of M had minor effects on the long-term yield of curE75 and minlenLopt1, and reduced the fluctuation of yield (measure as the probability of AAVY<15%) for DCAC, SPS SY. In general, the different methods for M estimation showed minor effects on the performance of MPS, whereas the temporal changes of M showed substantial influences. Considering the fishery status of Spanish mackerel in China, we recommended that curE75 has the best trade-off between fishery resources exploitation and conservation, and we also proposed the potentials and issues in their implementations.

Key words: fishery management, uncertainty, management strategy evaluation (MSE), data limited method, DLMool

Citation: Chen Ning, Zhang Chongliang, Sun Ming, Xu Binduo, Xue Ying, Ren Yiping, Chen Yong. 2018. The impact of natural mortality variations on the performance of management procedures for Spanish mackerel (Scomberomorus niphonius) in the Yellow Sea, China. Acta Oceanologica Sinica, 37(8): 21–30, doi: 10.1007/s13131-018-1234-0

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1 Introduction

Natural mortality rate (M) is one of the most important parameters in fishery stock assessment. The magnitude of natural mortality rate directly relate to fish productivity, sustainable yields, optimal exploitation rates, and management reference points (Brodziak et al., 2011). Meanwhile, it is also one of the parameters that difficult to quantify (Lee et al., 2011), and most estimation methods make certain compromise due to the limitation of available data. For example, integrated assessment models may provide relatively precious estimation of M but have a high requirement for the time series of data (Brodziak et al., 2011), which make it unfeasible or impractical for less-well studied stocks. On the other hand, methods that do not rely on time series data, such as Pauly and other empirical formula may be useful for data-limited stocks and are actually commonly used for estimating natural mortality rate when other methods are unavailable; however, their reliability and applicability are not systematically tested (Kenchington, 2014). Other specific methods also show their limitation in use, e.g., the catch curve analysis method is applicable to the undeveloped population and unsuitable for the highly exploited fish populations (Windsland, 2015). Despite of the limit of estimation methods, most stock assessment assume constant M for the whole population (Dero ba and Schueller, 2013), whereas in fact M is age-specific and generally decreases with body size (Power, 2014). In addition, many environmental and anthropogenic factors can influence natural mortality rates, such as the climate change, marine currents, and biotic interactions (Hamel, 2015). In general, we concluded that the estimation of natural mortality has substantial uncertainties in fishery stock assessment, resulting from data quality, estimated methods and time-variation, etc. These uncertainties will affect the results of stock assessment by biasing the estimate for fishing and total mortality, blurring the interspecific relationships and predation (Suda et al., 2005), and reducing/increasing...
the estimate of total population production (Dutil and Lambert, 2000). Therefore, the variation and uncertainty of \( M \) should be explicitly considered in stock assessment and management (Whitlock et al., 2012), and in this study, we mainly studied the impact of two sources: data sources and estimated methods.

The uncertainty of fish natural mortality has been evaluated in many studies. For instance, Quirioz et al. (2010) used two sources of uncertainty including error in the life history traits estimates and variability of the equations coefficients to incorporate error on \( M \) estimates, and they suggested that Pauly’s method is the most appropriate one in terms of trade-off between parameter needed and CV of \( M \) estimates incorporating all sources of uncertainty. Gaertner (2015) estimated \( M \) based on life history parameters with different estimators for the entire population and by length size classes, respectively, suggesting that the estimation of natural mortality was associated with considerable uncertainty. However, the impact of \( M \) uncertainty on the assessment and management of fisheries stock have been less well understood. Furthermore, most existing fisheries lack survey data and formal assessments (Costello et al., 2012), which makes such evaluations unfeasible. In particular, the situation is prevalent in China where most fisheries are not well assessed due to the lack of historical documents, imposing significant challenges for the sustainable restoration and management.

The recent development of data-limited methods (DLM) shows the potential to provide reasonable management advices with scarce data. The reliability of those methods should be extensively evaluated before the actual implementation to fisheries management (Kokkalis et al., 2017). Regarding the significant importance and large uncertainty of natural mortality, the sensitivity of DLM to this parameter should be evaluated with priority. In this study, we used Spanish mackerel (Scomberomorus Niphognus) in China’s seas as an example to evaluate the robustness of a range of management procedures (MPs) on the uncertainty of natural mortality. Spanish mackerel is a long-lived pelagic fish with high commercial values. The annual commercial and recreational catch for Spanish mackerel in China is about 45 000 t, contributing significantly to regional economy (Cheng and Wei, 1987). In recent years, this species is subject to increasing fishing effort, diverse fishing gears, and expanded fishing grounds, all of which implied a high risk of overexploitation, although the fishery status have not been formally assessed in literature. Remarkable changes have been observed in the life history traits of Spanish mackerel, such as higher growth rate, earlier maturation, and changes of population structure (Qiu and Ye, 1996). A preliminary analysis using Beverton-Holt life history invariants method (Prince et al., 2015) suggested that the natural mortality rate of this species decreased significantly in last decades, i.e., from 0.80 in the 1970s (Liu et al., 1982) to 0.59 in 2001–2013 (You, 2014).

The aim of this study is to take into account the uncertainty of natural mortality in the management of Spanish mackerel fishery to achieve sustainability improvement. In this study, we estimated the changes in natural mortality of Spanish mackerel in recent years, and attributed the uncertainty of natural mortality into temporal and methodological variations. We compared the performances of a range of management procedures for Spanish mackerel fisheries using an approach of management strategy evaluation, and evaluated the effect of the uncertainty and variation of natural mortality on stock management. In addition, we proposed the potentials and issues in the practical application of those MPs in China to improve the effective fishery management of Spanish mackerel.

2 Materials and methods

2.1 Data-limited methods (DLM)

In the present study, we adopted a DLMtool method to simulate the dynamics of mackerel fishery and test the performance of a variety of management procedures. The DLMtool (Data-limited methods Toolkit) is an R package (R Development Core Team, 2016) developed by the University of British Columbia’s Institute for Oceans and Fisheries and the Natural Resources Defense Council (Carruthers et al., 2015). The implementation of DLMtool can be divided into four steps as other data-limited methods (Honey et al., 2010): (1) determining data richness of specified fishery, (2) selecting the appropriate assessment methods, (3) assessing stock status, and (4) management strategy evaluation (MSE). The core process of DLMtool, i.e., the MSE step is generally introduced below.

MSE is a fisheries management paradigm that evaluates the performance of management procedures and the trade-off among different management objectives as the basis for management decisions (Butterworth and Punt, 1999; Butterworth, 2007). MSE predict the status of fish stocks in terms of specific management objectives and provide management recommendations with explicit consideration of the uncertainty and sensitivity of management strategies. MSE involves three components of DLMtool, operating model, management procedure and performance criteria (Fromentin et al., 2014).

1) Operating model (OM) is used to simulate the dynamics of fishery stocks and fleets, and includes the error processes of monitoring and observation. An OM is composed of three sub-models in DLMtool, including Stock, Fleet and Observation, in which the Stock model contains biological parameters of the population; Fleet model includes gear selectivity and fishing effort; and Observation model contains parameters of observation error and bias.

2) Management procedure (MP) is a general approach to fisheries management, including both input and output control methods. It provides management advices in the form of TAC or limit gear size and fishing effort. MPs of Output control provide TAC as a management suggestion while MPs of input control adjust the catchable size and the relative fishing effort in management. DLMtool now includes 89 MPs and the new method is still being added. Depending on data availability, there are 22 MPs in DLMtool that can be applied on Spanish mackerel fishery. They were 11 output control MPs including Avc, CC1, CC4, DD, DD4010, GB_slope, SPmod, SPMSY, NPref, DCAC and Fratio, and 11 input control MPs including curE, curE75, DDe, DDe75, EtargtLopt, matlenlim, matlenlim2, minlenLopt1, MRnoreal, MRnoreal, MRreal and slodlim (Table 1).

3) Performance criteria are used for evaluating the performance of MPs. The evaluation criteria for MSE in DLMtool include relative spawning population biomass, long and short term yield, probability of overfishing and so on. We used the following six criteria for the evaluation of MPs considering the necessary trade-off among management objectives: (i) Median yield (last 5 years) relative to current; (ii) Median biomass (last 5 years) relative to current; (iii) B50%: likelihood of biomass dropping below 50 percent \( B_{\text{SSY}} \); (iv) PNOF: probability of not overfishing; (v) LTY: fraction of simulations getting over half \( B_{\text{SSY}} \) yield in the last ten years of the projection; (vi) AAVY: fraction of simulations where average annual variability in yield is less than 10 percent.

2.2 Fishery data

The samples of Spanish mackerel were collected from five
Table 1. The description of MPs in this study, including 11 output control MPs and 11 input control MPs.

| Type          | MPs              | Description                                                                                       | Reference |
|---------------|------------------|---------------------------------------------------------------------------------------------------|-----------|
| Output control| DCAC             | depletion-corrected average catch, which calculated as the sum of catches divided by the number of years; relevant formulas are $W=0.5R_y$, $W = \frac{1}{M}$, where $W$ is Harvest ratio, $R_y$ is unfished biomass, $Y_{pot}$ is potential yield, and $M$ is natural mortality. | Maccall (2009) |
|               | NRef             | $TAC=0$                                                                                           | Carruthers and Hordyk (2016) |
|               | SPMSY            | an MP for estimating MSY to determine the OFL (optimal fishing limit); $OFL = D(1-D)\times K_x \times 2$, where $K_x$ is the carrying capacity, $r$ is the maximum rate of population increase, and $D$ is depletion. | Martell and Froese (2013) |
|               | AvC              | $TAC=$average catch                                                                               |           |
|               | CC1              | $TAC=$recent catch level                                                                          | Geromont and Butterworth (2015) |
|               | CC4              | $TAC=70\%$ of recent catch level                                                                  |           |
|               | Fratio           | $TAC=$fixed ration of nature mortality rate $\times$ current absolute stock biomass               | Gulland (1971), Martell and Froese (2013) |
|               | DD               | a delay difference stock assessment                                                                | Carruthers et al. (2012), Carruthers and Hordyk (2016) |
|               | DDD4010          | a delay difference stock assessment with a 40–10 harvest control rule superimposed               |           |
|               | SMod             | $\frac{4}{5} C_y, \Delta B < \frac{4}{5}$ $\Delta B > \frac{6}{5}$ $TA_C = 4 \frac{6}{5}$ $\frac{4}{5}$ $S_y, \Delta B > \frac{6}{5}$ $\Delta B = \frac{B_y}{B_y-1}$ $S_y = B_y - B_{y-1} + C_{y-1}$. | Carruthers and Hordyk (2016), Maunder (2014) |
|               | GB_slope         | the incremental changes of TAC made to maintain a constant relative abundance index                | Geromont and Butterworth (2015) |
| Input control | TargetLopt       | adjust effort up/down if mean length above/below $L_{target}$                                     |           |
|               | curE             | using the fishing effort of the final year in historical simulations as the management target     |           |
|               | curE75           | using 75 percent of the fishing effort of the final year in historical simulations as the management target |           |
|               | Mrnoreal         | a spatial control, requiring no fishing in Area 1 and not reallocating this fishing effort to Area 2 |           |
|               | MRreal           | a spatial control, requiring no fishing in Area 1 and reallocating this fishing effort to Area 2 |           |
|               | minlenLopt1      | rebuilding the stock biomass towards the optimal length by restricting the catch of small fish; $L_{opt} = b/(M/K+b)$, where $b$ is length-weight parameter beta, approximately equal to 3, $M$ is natural mortality rate, $K$ is von Bertalanffy growth rate, $M/K$ is Beverton-Holt life-history invariant, and $M$ and $K$ should come from the same research time and sea area | Hordyk et al. (2015) |
|               | matlenlim        | selectivity-at-length set equivalent to maturity-at-length                                          | Carruthers and Hordyk (2016) |
|               | matlenlim2       | selectivity-at-length set slightly higher than the maturity-at-length                             | Carruthers and Hordyk (2016) |
|               | slotlim          | selectivity-at-length set using a slot limit. The maximum limit is the 75th percentile between the new minimum legal length and the estimated asymptotic length. | Carruthers and Hordyk (2016) |
|               | DDe              | a delay-difference assessment, using a time-series of catches and a relative abundance index to estimate and recommend FMSY | method based on equations of Carl Walters |
|               | DDe75            | a delay-difference assessment, using a time-series of catches and a relative abundance index to estimate and recommend 75 percent FMSY. |           |

Docks around the Yellow Sea from 2016 to 2017, including Yantai, Weihai, Qingdao, Nantong in China and Jeju in Korea (Fig. 1a). A range of biological parameters such as weight-length relationship and von-Bertalanffy growth rate were estimated from the samples using ELEFAN method (Gayanilo, 1988). The parameters that are not precisely estimated from the survey were obtained from relevant studies (reference values in Table 2). In addition, annual averaged CPUE data was collected by the East China Sea Fisheries Research Institute (unpublished data), and annual catch data was obtained from Chinese Fisheries Statistical yearbook (the Ministry of Agriculture Fishery and Fishery Administration, 2016) (Fig. 1b). The parameters were used to simulate the dynamics and the associated uncertainty of mackerel stock. We evaluated $M$ with a variety of estimation methods and from different periods of time. Four empirical methods were used in this study considering data availability, including $M/K$ invariants, Hoenig, Pauly and Then’s empirical formula.

1. $M/K$ (Prince et al., 2015): the method is based on Beverton-Holt life history invariants, $M/K=1.5$, where $K$ is the von Bertalanffy growth rate.

2. Hoenig empirical formula (Hoenig, 1983): $lnM=1.48-1.01ln_{max}$ where $t_{max}$ is the maximum age. We use the maximum age of 30 according to the growth equation.
3.1 MP selection

Management strategy evaluation (MSE) showed that 22 MPs had different trade-offs with respect to fishery yield, population biomass and the risk of overfishing, whereas none of these MPs could simultaneously optimize all the demanding properties. AvC, MRreal could maintain the level of production but lead to uncertainty of M as well as other biological parameters that cannot be estimated accurately using MSE approach, in which OM was specified with a range of possible parameter values (Carruthers and Hordyk, 2016), and the specific parameter settings of our simulations can be seen in Table 4. The values of M were drawn from the preassigned uniform distribution in each simulation run. The scenarios were repeated for 1 000 times for each MP, and the MSE processes were repeated for 100 times. The number of years used for implementing MPs was set to 20 years and the interval of stock assessment was every 2 years in MSE. The performance of different MPs was compared using a range of criteria to select suitable MPs for the mackerel fishery. In addition, we distinguished the influence of M variation on fishery management resulting from two sources of uncertainty, for which the performance of the selected MPs was compared across different time periods and estimation methods.

3 Results

We first tested the robustness of available MPs on the uncertainty of M as well as other biological parameters that cannot be estimated accurately using MSE approach, in which OM was specified with a range of possible parameter values (Carruthers and Hordyk, 2016). (3) Pauly empirical formula (Pauly, 1980): \( \ln M = -0.0152 - 0.279t \ln \alpha + 0.6543nK + 0.4634nT \), where \( L_\infty \) is maximum length, \( K \) is von Bertalanffy growth rate, and \( T \) is annual average temperature. The average annual water temperature was 14°C according to the average temperature of the Yellow Sea and Bohai Sea (Sun, 2009).

(4) Then empirical formula (Then et al., 2015): \( M = 4.889t_{max}^{-0.906} + 7.5 \times 10^3L_{\infty}^{-0.35} \).

It should be noted that the accuracy of these methods have not been explicitly examined for Spanish mackerel, however, as the aim of this study is to evaluate the uncertainty of M and its effect on fisheries management, a wide range of M in simulation scenarios could relieve the requirement of accurate estimation. In addition, the changes of M in four different periods from 1974 to 2017 were estimated using \( M/K \), Pauly and Then’s empirical approach (Table 3). A remarkable decline of M was illustrated from all the three methods, and we used the result of Pauly’s equation in the following simulation study. We assume the changes of M following \( M_e = M_0(1 + r) \), and fitted a linear regression analysis between lnM and survey time to estimate the declining rate (r) and annual variation of M (Msd). The variation, declining rate (r=−0.019) and annual variation (Msd=0.19) of M was used in the simulation of DLMtool (Carruthers and Hordyk, 2016).

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Table 3. The variation of nature mortality estimated from different methods and time periods (four time periods were chose and four empirical methods were used to estimate the natural mortality rate of Spanish mackerel)

| Sub-model          | Name                  | Type of stock-recruit relationship | Comment |
|--------------------|-----------------------|------------------------------------|---------|
| Stock              | Name                  | Scomberomorus niphonius            |         |
|                    | maxage                |                                    | Value 1 |
|                    |                       | the maximum age of individuals that is simulated |         |
|                    | R0                   | the magnitude of unished recruitment |         |
|                    | M                    | natural mortality rate (uniform distribution) |         |
|                    | Msd                  | inter-annual variability in natural mortality rate expressed as a coefficient of variation (uniform distribution) |         |
|                    | Mgrad                | mean temporal trend in natural mortality rate, expressed as a percentage change in M per year (uniform distribution) |         |
|                    | h                    | steepness of the stock recruit relationship (uniform distribution) |         |
|                    | Srrel                | type of stock-recruit relationship: (1) Beverton-Holt; (2) Ricker |         |
|                    | Linf                 | maximum length (uniform distribution) |         |
|                    | K                    | von Bertalanffy growth rate K (uniform distribution) |         |
|                    | t0                  | von Bertalanffy theoretical age at length zero (uniform distribution) |         |
|                    | Ksd                  | inter-annual variability in growth parameter K (uniform distribution) |         |
|                    | kgrad                | mean temporal trend in growth parameter K, expressed as a percentage change in K per year (uniform distribution) |         |
|                    | LinfSD               | inter-annual variability in maximum length-uniform distribution |         |
|                    | Linfgrad             | mean temporal trend in maximum length, expressed as a percentage change in Linf per year (uniform distribution) |         |
|                    | regrad               | mean temporal trend in log-normal recruitment deviations (uniform distribution) |         |
|                    | a                   | length-weight parameter alpha (uniform distribution) |         |
|                    | b                   | length-weight parameter beta (uniform distribution) |         |
|                    | L50                 | length-at-50 percent maturity (uniform distribution) |         |
|                    | AC                  | autocorrelation in recruitment deviations rec(t)=AC×rec(t−1)+(1−AC)×sigma (t) (uniform distribution) |         |
|                    | D                   | current level of stock depletion (Bcurrent/Bunfished) (uniform distribution) |         |
|                    | L50,95               | length increment from 50 percent to 95 percent maturity |         |
|                    | Period               | period for cyclical recruitment pattern in years (uniform distribution) |         |
|                    | Perr                 | process error, the CV of lognormal recruitment deviations (uniform distribution) |         |
|                    | Size_area_1          | the size of Area 1 relative to Area 2 (uniform distribution) |         |
|                    | Frac_area_1          | the fraction of the unished biomass in Stock 1 (uniform distribution) |         |
|                    | Prob_staying         | the probability of individuals in Area 1 remaining in Area 1 over the course of one year |         |
|                    | Source               | reference to a website or article for which parameters were taken to define the operating model |         |
| Fleet              | Name                 | myFleet                            |         |
|                    | years               | the number of years for the historical simulation |         |
|                    | Spat_targ           | distribution of fishing in relation to spatial biomass: F is proportional to B^Spat_targ (uniform distribution) |         |
|                    | Fsd                 | inter-annual variability in fishing mortality rate |         |
|                    | L5                  | shortest length corresponding ot 5 percent vulnerability (uniform distribution) |         |
|                    | LFS                 | shortest length that is fully vulnerable to fishing (uniform distribution) |         |
|                    | EffYears            | years at which to simulate varying relative effort |         |
|                    | EffLower            | lower bound on relative effort corresponding to EffYears (uniform distribution) |         |
|                    | EffUpper            | Upper bound on relative effort corresponding to EffYears (uniform distribution) |         |
|                    | Vimaxden            | the vulnerability of the longest (oldest) fish (uniform distribution) |         |
|                    | qinc                | average percentage change in fishing efficiency (uniform distribution) |         |

Table 4. The description of Operating model (OM)

| Slot names | Value 1 | Value 2 | Comment |
|------------|---------|---------|---------|
| Name       | Scomberomorus niphonius |         |         |
| F0         | 100 000 000 |         |         |
| M          | 0.1     | 0.22    |         |
| Msd        | 0.18    | 0.2     |         |
| Mgrad      | –0.02   | –0.02   |         |
| h          | 0.3     | 0.8     |         |
| Srrel      | 1       |         |         |
| Linf       | 777     | 983     |         |
| K          | 0.08    | 0.1     |         |
| t0         | –1.6    | –0.7    |         |
| Ksd        | 0       | 0.05    |         |
| kgrad      | –0.25   | 0.25    |         |
| LinfSD     | 0       | 0.05    |         |
| Linfgrad   | –0.25   | 0.25    |         |
| regrad     | –10     | 10      |         |
| a          | 0.000 023 | 0.000 1 |         |
| b          | 2.4     | 2.94    |         |
| L50        | 357     | 403     |         |
| AC         | 0.1     | 0.9     |         |
| D          | 0.05    | 0.6     |         |
| L50,95     | 30      | 50      |         |
| Period     | NA      | NA      |         |
| Perr       | 0.15    | 0.25    |         |
| Size_area_1 | 0.095   | 0.105   |         |
| Frac_area_1 | 0.095  | 0.105   |         |
| Prob_staying | 0.8     | 0.9     |         |
| Source     | None    |         |         |

to be continued
declined biomass. EutgerLopt, minlenLopt1, curE, matlenlim, matlenlim2, DD, DCAC, Fratio, SPMSY, slotlim, CC4 and GB_slope had satisfactory performances on keeping biomass, but yield relatively low catch. CurE75 and MRnoreal showed better performance on balancing production and biomass. DDe, DDe75, DD4010, CC1 and SPmod failed to maintain either yield or population biomass (Fig. 2a).

We then evaluated the trade-off between long-term yield (LTY) and the risk of overfishing (Fig. 2b). CC4, SPMSY, GB_slope, DCAC and curE75 were the most effective MPs to avoid overfishing. In particular, the possibilities of not-overfishing using CC4 and SPMSY were 75.5% and 76.0% respectively. MinlenLopt1 provided the highest yield with LTY>80% MSY, followed by curE, MRnoreal. Regarding the trade-off between population biomass and the variation of yield, DCAC and SPMSY were able to reduce yield fluctuation effectively and maintain population biomass.

The MSE results suggested that curE75, DCAC, minlenLopt1 and SPMSY were more robust to the parameter uncertainty than others for the management of Spanish mackerel. Specifically, minlenLopt1 and DCAC performed best in terms of providing yield and reducing yield fluctuation, and curE75 and SPMSY had a balanced performance in the aspects of population status. It should be noted that the four methods covered both output control (DCAC and SPMSY) and input control (curE75 and minlenLopt1) methods. Their responses to the variation of M were further evaluated in the following section.

3.2 Effect of M variation

The dots size represent four different time periods from 1974

![Fig. 2](image-url)  
**Fig. 2.** Performance of 22 different management procedures for Spanish mackerel. a. The short-term trade-off for fisheries management, and b. the long-term trade-off for fisheries management.
to 2017 in the upper panels in Fig. 3, and regarding the different temporal periods (Table 3), the performance of each MP showed minor responses to the variation of $M$. MinlenLopt1 and curE75 could keep relatively stable yield, for which both long-term yield and the fluctuation of yield (AAVY<15%) showed limited response and the changes in the fluctuation of yield less than 5%. The long-term yield of DCAC and SPMSY also showed minor variations, whereas the fluctuation of yield changed substantially which was more than 5%, indicating unstable status of mackerel fishery (Fig. 3a). Regarding population status, the relative population biomass (measure as the probability of $B>0.5B_{MSY}$) of mackerel increased significantly with the decrease of $M$ from 1970s to 2017 using all MPs. The trends in the risk of overfishing of DCAC were different between other three MPs. DCAC showed a slight increase, while the other methods showed a downward trend (Fig. 3b).

The size of triangles denote the relative $M$ values estimated from different methods, ranging from 0.1 to 0.22, and the variation of $M$ from four estimation methods showed different effects on the performance of MPs with respect to the yield and population status. The changes of overfishing possibility and the relative population biomass showed minor responses to different estimation methods, with less than 5% value changes, whereas the long-term yield and the fluctuation of yield of Spanish mackerel fishery showed remarkable differences in all MPs, especially for SPMSY (Fig. 3c). In particular, the possibility of overfishing under the management of curE75 tended to increase with the decreasing $M$ (Fig. 3d).

The management advices were derived from the MPs of Spanish mackerel fishery on the basis of our survey data. Among the most robust MPs, SPMSY suggested a total yield of 601.77 thousand tons; minlenLopt1 required the current fishing effort unchanged while the catchable size limited to 46.61 cm. Otherwise, the fishing effort could be reduced to 75% of current level to ensure stock biomass as suggested by curE75.

4 Discussion

The natural mortality rate of Spanish mackerel is determined by its ecological niches in marine ecosystems and its relationship with related species, thus that may fluctuate with the alteration of the marine ecosystem structure. In the data-limited situation, the estimation methods may impose additional uncertainty to $M$. We evaluated the performance of a variety of MPs and showed that the uncertainty and variation of $M$ has substantial effects on both input control and output control MPs, although the influences varied among different aspects of the fishery. Specifically, $M$ uncertainty had minor effects on the production of input control MPs but great influence on the production of output control MPs. The same uncertainty had large effects on population biomass and overfishing possibility for all MPs. From a mechanistic prospect, the uncertainty of $M$ may influence MPs in two approaches, by affecting control rules directly or affecting operating model indirectly. For example, the changes of $M$ will affects the recommended harvest relative to potential yield for DCAC \( W \left( \frac{W}{\text{pot}} - 1 \right) \) (MacCall, 2009), and the lower value of $M$ leads to the overestimated biomass level. For minlenLopt1, $M$ influences the optimal trap length, and the lower $M$ lead to the underestimated risk of overfishing (Hordyk et al., 2015). We emphasize the importance of improving the precision of $M$ estima-
estimation for stock assessment and fisheries management.

MSE revealed that none of the management procedures could provide satisfactory population biomass, yield, overfishing possibility and fluctuation of yield simultaneously. The choice of MP should thusly take into account practical management objectives and trade-off, such as maintaining production, long-term restoration and short-term production. The general conclusion is consistent with the results of Carruther et al. (2015). Additionally, some results of this study may be useful in the application of many MPs when $M$ is in large uncertainty. For instance, when the range of $M$ changed from 0.35 to 0.11 in this study, the changes in the risk of overfishing was the most obvious for some common methods such as curE75. This implied that risk of overfishing should be taken with priory with a large uncertainty of $M$. The population biomass may also be significantly affected by $M$ for some MPs, e.g., the probability of B-0.5M is increased by 8% as natural mortality declined using MinlenLopt1, suggesting a strong effect of population conservation. According to fisheries statistics, the production of Spanish mackerel fishery in China is in a relatively stable state in recent years, with the catch stabilizing at 450,000 t per year (Zheng et al., 2014). We therefore suggest that it is a superior challenge to sustain the population biomass and avoid overfishing rather than pursuing high yields for Spanish mackerel fishery, for which conservative MPs such as SPMSY are more desirable. However, it should be noticed that the result of MSE can be affected by not only $M$, but also other biological parameters such as length composition, growth rate, recruitment and depletion. In particular, the quality of data is pivotal for parameter estimation thus more critical for fisheries management, indicating that time-series survey observations is needed and the simulation should be revisited periodically according the gradually accumulated data (Uriarte et al., 2016). In addition, there are some limitation of DLMtools which need to be needs further research and development. For example, although the operating model can simulate the temporal trend in natural mortality rate, it only represents the overall trend of change and currently simulation assumes constant $M$ with age. OM also using a Beverton-Holt stock recruitment model, in which all stocks are assumed to have density-dependent recruitment that does not decrease with increasing stock size, however, not all of the recruitment pattern in the fish community are this one. Besides, the observation model used in DLMtool is unrealistically well-behaved, and it simulates catch-composition data from the true simulated catch composition data via a multinomial distribution and some effective sample size, which will favor the simulation (Carruthers and Hordyk, 2016).

When the uncertainty of natural mortality comes mainly from data sources, curE75 can provide satisfactory yields while maintaining the stock biomass and reducing the risk of overfishing (Fig. 3). Therefore, our results promoted this MP for the management of Spanish mackerel fishery in data-limited situation. However, it is important to note that accurate data of historical fishing effort are needed in the application of curE75 to actual Spanish mackerel fishery (Carruthers and Hordyk, 2016). Due to the current challenge of the large number of fishing vessels, complicated practitioners of fisheries, decentralized production and the fisheries management system in China (Sun and Lu, 2016), the control of current fishing effort in management is surely difficult. On the contrary, if the uncertainty of natural mortality comes mainly from different methods, SPMSY can keep the biomass at a satisfactory level while reducing the fluctuation of production and the possibility of overfishing. This MP requires accurate estimation of the maximum age of the population, individual size at 50% sexual maturity, growth parameter $K$ and catch data. It should also be noted that SPMSY is based on the maximum sustainable yield (MSY) to determine overfishing limits (OFL) (Martell and Froese, 2013), so the uncertainty of catch data has a great impact on this MP. At present, due to the large uncertainty of catch data in China, it is actually challenging to apply SPMSY to the Spanish mackerel fishery without more detailed data.

Generally, the estimation of natural mortality is challenging, and the estimation of $M$ based on empirical equations could not ensure the accuracy of $M$ estimation for Spanish mackerel; however, there is a wide acknowledge of the decrease in mackerel natural mortality (Sun, 2009; You, 2014; Liu et al., 1982). The decrease of $M$ could be attributed to a variety of biological changes in China’s seas. Specifically, the predator species of mackerel, such as dolphins, whales and catshark have been decreasing along with the high fishing intensity in China since 1980. The relief of predation mortality could be one of the major factors that reduce the total natural morality. Secondly, many other species of similar feeding habitat are enduring high fishing pressure in this area, such as small yellow croaker (Yan et al., 2014). It has been widely reported that the intensive fishing in China’s coast caused the reduction of population size and simplification of the age structure in fish population, such as yellow snout sea bass (Lin et al., 2016). As Spanish mackerel is carnivorous since early life stage, such changes of size composition in marine ecosystems might suppress the competition between juvenile mackerel and other predatory species thus benefit their survival. However, as the $M$ has not been accurately evaluated for this species, further studies would be needed to validate this assumption. In addition, the early development of Spanish mackerel was significantly affected by changes in water temperature and salinity (Song et al., 2016), thus the habitat quality will also affect the survival rate of Spanish mackerel. It is reported that when water temperature increases from 15 to 21°C, the development rate of embryonic mackerel will gradually accelerate (Jiang et al., 2016). This study explicitly simulated the changes of $M$ value in different time periods; however, the future changes of $M$ due to climate changes are less well understood. In addition, we assumed that $M$ was fixed in each simulation but $M$ is actually size-dependent and age-dependent in the life history (Johnson et al., 2015). These aspects are also needed to be taken into account in further study.

In summary, the changes of natural mortality rate of Spanish mackerel should be particularly concerned in the management of Spanish mackerel fisheries. Particularly, in order to improve the utilization of marine resources, China has adopted a series of “double control” actions to fishing vessel system in recent years (Zheng et al., 2014), and evaluating the uncertainty of different management strategies would be helpful for Chinese fisheries, especially in a data-limited situation. However, although some MPs showed satisfactory performances in our evaluation, the implementation of them would remain to be challenging in practice. Most output control methods provide an advice of TAC, however, it is difficult to assign the total TAC to the different marine regions as the mackerel is wide-distributed in China’s seas and there is no traditional quota. Moreover, as this species has a wide range of migration and long life history (Shui et al., 2009), the country-wise cooperation for managing Spanish mackerel would be necessary, which, to the best of our knowledge, is far from routine. More research efforts of natural mortality are needed to build a solid foundation for the fisheries management in China, and the uncertainty of fishery statistics and other biological parameters should also be taken into account in stock as-
Acknowledgements

The authors thank Ocean University of China for their generous modeling support, ideas and perspectives. The authors also thank the other members of the Ecosystem Assessment and Evaluation Laboratory for their help and the East China Sea Fisheries Research Institute for their data support.

References

Brodzik J, Janelli J, Lorenzen K, et al. 2011. Estimating natural mortality in stock assessment applications. NOAA Technical Memorandum NMFS-F/SPO-119. Washington, DC: US Department of Commerce, 38

Butterworth D S, 2007. Why a management procedure approach? Some positives and negatives. ICES Journal of Marine Science, 64(4): 613–617

Butterworth D S, Punt A E. 1999. Experiences in the evaluation and implementation of management procedures. ICES Journal of Marine Science, 56(6): 985–998

Carruthers T R, Hordyk A R. 2016. DLMtool: data-limited methods toolkit. https://cran.r-project.org/web/packages/DLMtool/index.html [2016-12-27/2017-03-11]

Carruthers T R, Kell L T, Butterworth D D S, et al. 2015. Performance review of simple management procedures. ICES Journal of Marine Science, 72(2): 464–482

Carruthers T R, Walters C J, McAulister M K. 2012. Evaluating methods that classify fisheries stock status using only fisheries catch data. Fisheries Research, 119–120: 66–79

Cheng Qintai, Wei Baoshan. 1987. Systematic Synopsis of Chinese Fishes (in Chinese). Beijing: Science Press

Costello C, Ovando D, Hillborn R, et al. 2012. Status and solutions for the world’s unassessed fisheries. Science, 338(6106): 517–520

Deroba J J, Schueller A M. 2013. Performance of stock assessments with misspecified age- and time-varying natural mortality. Fisheries Research, 146: 27-40

Dutil J D, Lambert Y. 2000. Natural mortality from poor condition in Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences, 57(4): 826–836

Fromentin J M, Bocheneau S, Arrizabalaga H, et al. 2014. The spectre of uncertainty in management of exploited fish stocks: the illustrative case of Atlantic Bluefin tuna. Marine Policy, 47: 8–14

Gaetner D. 2015. Indirect estimates of natural mortality rates for Atlantic skipjack (Katsuwonus pelamis), using life history parameters. Collect Vol Sci Pap ICCAT, 71(1): 189–204

Gayanilo F C Jr, Soriano M, Pauly D. 1988. A draft guide to the complete ELEFAN. In: ICLARM Software 2. Manila, Philippines: International Center for Living Aquatic Resources Management

Geromon H F, Butterworth D S. 2015. Generic management procedures for data-poor fisheries: forecasting with few data. ICES Journal of Marine Science, 72(1): 251–261

Gulland J A. 1971. The Fish Resources of the Ocean. West Byfleet, UK: Fishing News Books

Hamel O S. 2015. A method for calculating a meta-analytical prior for the natural mortality rate using multiple life history correlates. ICES Journal of Marine Science, 72(1): 62–69

Hoenig J M. 1983. Empirical use of longevity data to estimate mortality rates. Fish Bull, 82: 898–903

Honey K T, Moxley J H, Fujita R M. 2010. From rags to fishes: data-poor methods for fishery managers. Managing Data-Poor Fisheries: Case Studies, Models & Solutions, 1: 159–184

Hordyk A, Oso K, Sainsbury K, et al. 2015. Some explorations of the life history tables to describe length composition, spawning-per-recruit, and the spawning potential ratio. ICES Journal of Marine Science, 72(1): 204–216

Jiang Yiqian, Fan Yannan, Zheng Chunjing, et al. 2016. The effect of temperature on embryonic development of Scomberomorus niphoniis. Journal of Zhejiang Ocean University (Natural Science), 35(4): 271–275

Johnson K F, Monnahann C C, Mc Gilliard C R, et al. 2015. Time-vari ing natural mortality in fisheries stock assessment models: identifying a default approach. ICES Journal of Marine Science, 72(1): 137–150

Kenchington T J. 2014. Natural mortality estimators for information-limited fisheries. Fish and Fisheries, 15(4): 533–562

Kokkalis A, Eike set A M, Thygesen U H, et al. 2017. Estimating uncertainty of data limited stock assessments. ICES Journal of Marine Science, 74(1): 69–77

Lee H H, Maun der M N, Piner K R, et al. 2011. Estimating natural mortality within a fisheries stock assessment model: an evaluation using simulation analysis based on twelve stock assessments. Fisheries Research, 109(1): 89–94

Lin Qun, Wang Jun, Yuan Wei, et al. 2016. Effects of fishing and environmental change on the ecosystem of the Bohai Sea. Journal of Fishery Sciences of China, 23(3): 619–629

Liu Chaxin, Zhang Xu, Yang Kaiwen. 1982. Studies on the growth of Spanish mackerel, Scomberomorus niphoniis in the Huang hai sea and Bohai sea. Oceanologia et Limnologia Sinica, 13(2): 179–178

MacCall A D. 2009. Depletion-corrected average catch: a simple formula for estimating sustainable yields in data-poor situations. ICES Journal of Marine Science, 66(10): 2267–2271

Martell S, Froese R. 2013. A simple method for estimating MSY from catch and resilience. Fish and Fisheries, 14(4): 304–314

Maun der M N. 2014. Management strategy evaluation (MSE) implementation in stock synthesis: application to Pacific Bluefin tuna. IATTC Stock Assessment Report 15. La Jolla: Inter-American Tropical Tuna Commission, 100–117

Pauly D. 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. ICES Journal of Marine Science, 39(2): 175–192

Powers J E. 2014. Age-specific natural mortality rates in stock assessments: size-based vs. density-dependent. ICES Journal of Marine Science, 71(7): 1629–1637

Prince J, Hordyk A, Valencia S R, et al. 2015. Revisiting the concept of Beverton-Holt life-history invariants with the aim of informing data-poor fisheries assessment. ICES Journal of Marine Science, 72(1): 194–203

Qiu Shengyao, Ye Maozhong. 1996. Studies on the reproductive biology of Scomberomorus niphoniis in the Yellow sea and Bohai sea. Oceanologia et Limnologia Sinica, 27(5): 463–470

Quiroz J C, Wiff R, Caneco B. 2010. Incorporating uncertainty into estimation of natural mortality for two species of Rajidae fished in Chile. Fisheries Research, 102(3): 297–304

R Development Core Team. 2016. R: a language and environment for statistical computing. Vienna, Austria: The R Foundation for Statistical Computing, http://www.R-project.org [2016-06-21/2016-09-25]

Shui Bonian, Han Zhiqiang, Gao Tianxiang, et al. 2009. Mitochondrial DNA variation in the East China sea and Yellow sea populations of Japanese Spanish mackerel Scomberomorus niphoniis. Fisheries Science, 73(3): 593–600

Song Chao, Wang Yutan, Lian Zulei, et al. 2016. Relationship between environmental factors and distribution of Scomberomorus niphoniis eggs, larvae, and juveniles in Xiangshan Bay. Journal of Fishery Sciences of China, 23(5): 1197–1204

Suda M, Akamine T, Kishida T. 2005. Influence of environment factors and distribution of Scomberomorus niphoniis in the Yellow sea and Bohai bay (in Chinese)[dissertation]. Beijing: Chinese Academy of Agricultural Sciences

Sun Jiting, Lu Kun. 2016. Effect evaluation and implementation adjustment of “Double Control” system of Chinese marine fishing. Fujian Tribune, (11): 49–55

The Ministry of Agriculture Fishery and Fishery Administration. 2016. Chinese Fisheries Statistical Yearbook (in Chinese). Beijing:
Then A Y, Hoenig J M, Hall N G, et al. 2015. Evaluating the predictive performance of empirical estimators of natural mortality rate using information on over 200 fish species. ICES Journal of Marine Science, 72(1): 82–92

Uriarte A, Ibaibarriaga L, Pawlowski L, et al. 2016. Assessing natural mortality of bay of biscay anchovy from survey population and biomass estimates. Canadian Journal of Fisheries and Aquatic Sciences, 73(2): 216–234

Whitlock R E, McAllister M K, Block B A. 2012. Estimating fishing and natural mortality rates for Pacific Bluefin tuna (Thunnus orientalis) using electronic tagging data. Fisheries Research, 119–120: 115–127

Windsland K. 2015. Total and natural mortality of red king crab (Paralithodes camtschaticus) in Norwegian waters: catch-curve analysis and indirect estimation methods. ICES Journal of Marine Science, 72(2): 642–650

Yan Liping, Liu Zunlei, Zhang Hui, et al. 2014. On the evolution of biological characteristics and resources of small yellow croaker. Marine Fisheries, 36(6): 481–488

You Zongbo. 2014. The selectivity of the big mesh gillnet for Scomberomorus niphonius (in Chinese) [dissertation]. Shanghai: Shanghai Ocean University

Zheng Yuanjia, Li Jiasheng, Zhang Qiyong, et al. 2014. Research progresses of resource biology of important marine pelagic food fishes in China. Journal of Fisheries of China, 38(1): 149–160
Projecting distributions of Argentine shortfin squid (Illex argentinus) in the Southwest Atlantic using a complex integrated model

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Received 7 March 2018; accepted 24 April 2018

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Abstract
We developed an approach that integrates generalized additive model (GAM) and neural network model (NNM) for projecting the distribution of Argentine shortfin squid (Illex argentinus). The data for this paper was based on commercial fishery data and relevant remote sensing environmental data including sea surface temperature (SST), sea surface height (SSH) and chlorophyll a (Chl a) from January to June during 2003 to 2011. The GAM was used to identify the significant oceanographic variables and establish their relationships with the fishery catch per unit effort (CPUE). The NNM with the GAM identified significant variables as input vectors was used for predicting spatial distribution of CPUE. The GAM was found to explain 53.8% variances for CPUE. The spatial variables (longitude and latitude) and environmental variables (SST, SSH and Chl a) were significant. The CPUE had nonlinear relationship with SST and SSH but a linear relationship with Chl a. The NNM was found to be effective and robust in the projection with low mean square errors (MSE) and average relative variances (ARV). The integrated approach can predict the spatial distribution and explain the migration pattern of Illex argentinus in the Southwest Atlantic Ocean.

Key words: Illex argentinus, abundance index, remote sensing environmental data, Southwest Atlantic Ocean

Citation: Wang Jintao, Chen Xinjun, Chen Yong. 2018. Projecting distributions of Argentine shortfin squid (Illex argentinus) in the Southwest Atlantic using a complex integrated model. Acta Oceanologica Sinica, 37(8): 31–37, doi: 10.1007/s13131-018-1231-3

1 Introduction

The Argentine shortfin squid, Illex argentinus, is a common neritic species occurring in waters off Brazil, Uruguay, Argentina, and the Falkland/Malvinas Islands in the Southwest Atlantic (Haimovici et al., 1998). It is the most economically important cephalopod species for China and many other countries (Chen et al., 2008). Illex argentinus is an opportunistic feeder (Ivanovic and Brunetti, 1994; Brunetti et al., 1998a) growing rapidly with a short life cycle and a high degree of intra-population differentiation (Arkhipkin, 1993, 2000). Meanwhile Illex argentinus is migratory species, and their concentrations are usually found at 45°–46°S in January or February, while growing rapidly this squid subsequently migrate southward towards the Falkland Islands. Peak concentrations are found around the Falkland Islands between March and May. Toward the end of this period, this squid starts to migrate northward, ultimately to spawn and die in the shelf and slope waters off northern Argentina, Uruguay and Brazil around July or August (Basson et al., 1996; Brunetti et al., 1998b). The population of Illex argentinus has been separated into four stocks based on their lengths at maturity, areas and timing of spawning, and the distribution of early juveniles and adults life stages: South Patagonic Stock (SPS), Bonaerensis-Northpatagonic Stock (BNS), Summer Spawning Stock (SSS) and Southern Brazil Stock (SBS) (Hatanaka, 1986). Of the four stocks, the SPS is the dominant commercial stock and is mainly exploited by some areas in China’s mainland (Lu and Chen, 2012), Chinese Taipei (Chen and Chiu, 2009), and Falkland (Waluda et al., 1999).

The oceanographic environment for Illex argentinus is mainly influenced by joint Brazil and Malvinas/Falkland Currents around 33°–39°S, causing an important thermohaline front that separates the subtropical waters from the subantarctic waters (Legeckis and Gordon, 1982; Olson et al., 1988; Gordon, 1989). Strong thermal and saline gradients can be observed in this convergence area, due to interactions of the two currents and the influence of the Patagonian Current and the discharge of the Río de la Plata, which provides nutrients for high levels of primary production (Haimovici et al., 1988).

Previous studies suggest that Illex argentinus tends to be highly susceptible to environmental changes during all its life history. For example, Waluda et al. (2001) suggested that large-scale oceanographic variability in the location of the spawning/hatching grounds during the early life stage of Illex argentinus was im-
important in determining recruitment to the fishery. Bazzino et al. (2005) identified environmental associations (depth, bottom temperature and bottom salinity) of shortfin squid in the Northern Patagonian Shelf, and found that squid distribution showed significant associations mostly with depth and bottom temperature. Sacau et al. (2005) developed generalized additive models (GAMs) of squid abundance in relation to physical and environmental conditions including sea surface temperature (SST), latitude, longitude, month, average fishing depth and year to be applied to fishery forecasting, and predictors retained in the optimal model. However, other environmental variables, such as sea surface height (SSH) and chlorophyll $a$ (Chl $a$) are also important indicators of physical oceanographic processes, and their impacts on the squid distributions received little attention (Chen et al., 2012).

In this study, we evaluated the influence of SST, SSH and Chl $a$ on the spatial distribution of $I. argentinus$ in the Southwest Atlantic, and developed a neural network model for forecasting spatial CPUE distribution. We then linked spatio-temporal variability in these oceanographic variables with the migration pattern of $I. argentinus$. The framework developed in the study provides an approach for forecasting spatial CPUE distribution and migration pattern of $I. argentinus$ in the Southwest Atlantic, which can also be used for the other oceanic squid species.

2 Materials and methods

2.1 Fishery data

Daily $I. argentinus$ fishery data were obtained from the Chinese Squid-Jigging Technology Group of Shanghai Ocean University from January to June during 2003–2011. The Chinese squid-jigging vessels were all equipped with a main engine power of 120 kW×2, squid-attracting lamp power of 112 kW, and 16 squid-jigging machines. These vessels were similar in size and nighttime fishing operation and protocol. The data were digitized from fishing logbook of the Chinese commercial squid fishery operating on the fishing ground between 40°−50°S and 55°−70°W in the Southwest Atlantic Ocean. The catch in this area accounted for 90% in each year. The fishery data comprised fishing dates (year and month), fishing locations (latitude and longitude), daily catch (tonnes), and effort (days fished).

Most of the catches were from the South Patagonic stock of $I. argentinus$ and there was no bycatch in the squid fishery (Chen et al., 2008). Fishing vessels and their operations were almost identical. Thus, the CPUE tends to be an approximate indicator of local stock abundance (Chen et al., 2008). In this study, we defined one unit of fishing area as 0.25° latitude by 0.25° longitude. The monthly nominal CPUE in one fishing unit of 0.25°×0.25° was calculated as follows:

$$CPUE_{ymi} = \frac{C_{ymi}}{F_{ymi}},$$

where $CPUE_{ymi}$, $C_{ymi}$ and $F_{ymi}$ are the monthly nominal CPUE, the total catch for all the fishing vessels in a given fishing grid, and the days fished in a given fishing grid at grid $i$ in month $m$ and year $y$, respectively.

2.2 Remotely sensed environmental data

Monthly remotely sensed data, comprising SST, SSH and Chl $a$ concentration for the fishing ground between 40°−50°S and 50°−70°W were downloaded from the Live Access Server of the National Oceanic and Atmospheric Administration Ocean Watch from 2003 to 2011 (http://oceanwatch.pifsc.noaa.gov/las/servlets/dataset). The method of converting spatial resolutions of remotely sensed data to those for the fishery data were discussed by Wang et al. (2015). Monthly fishery and oceanographic data were plotted allowing for overlaying and displaying of the distribution of $I. argentinus$ from January to June during 2003–2011.

2.3 Statistical method and forecasting model

The generalized additive model (GAM) has been used for confirming the importance of oceanic environmental data and analysis its relationships with the $I. argentinus$ distribution (Portela et al., 2005; Sacau et al., 2005). The GAMs were first proposed by Hastie and Tibshirani (1990). The model can deal with non-linear relationships between independent variable and response variable. We developed a GAM to quantify the relationship between the squid abundance (CPUE) and environmental variables. The variables built into the model include year, month, longitude, latitude, SST, SSH and Chl $a$. To deal with zero catches in log transformation, we added a constant of 10% of mean CPUE (Maunder and Punt, 2004). Thus, the GAM can be written as

$$\ln(\text{CPUE} + \text{mean (CPUE)} \times 10%) = \text{factor (year)} + \text{factor (month)} + s (\text{longitude}) + s (\text{latitude}) + s (\text{SST}) + s (\text{SSH}) + s (\text{Chl} a) + \varepsilon,$$

where $s$ is a spline smoother function, $\varepsilon$ is the residual error, $\varepsilon \sim N(0, \sigma^2)$ and $E(\varepsilon)=0$.

We conducted a preliminary analysis to evaluate the significance of variables with no interaction terms being considered. Seven variables were included in the GAM by forward selection, and the most significant terms were selected based on correlation analysis, Chi-square statistical significance and AIC ($\alpha=0.05$; Jensen et al., 2005; Chang et al., 2010).

After selecting significant variables in the GAM, we developed neural network models to predict the spatial distribution of squid abundance on the fishing ground of $I. argentinus$. The neural network models with functions of self-learning, good generalizations, and fault tolerance provides an approach to evaluate and predict complex non-linear relationships (Weigend et al., 1990). The neural network models are basically composed of input layer, hidden layer and output layer. Input and output layers consist of explanatory and response variables, respectively. In most cases, only one hidden layer is enough effective and satisfactory (Funahashi, 1989; Lek et al., 1996). However, how to confirm the number of nodes in the hidden layer is quite important and difficult. We utilized back-propagation algorithm in neural network models and added the number of nodes one by one in the hidden layer to search the optimal structure of model. For each different number of nodes in the hidden layer, 70% of data samples during 2003 to 2010 were randomly assigned to train, whereas the remaining 30% were used to validate the model, and the data samples in 2011 were used to test the model. We calculated mean square error (MSE) and average relative variance (ARV) (Nowlan and Hinton, 1992) to quantify the comparison results and confirm the best structure of neural network model. If ARV=1.0 implies that the model has come to a result of prediction average value, ARV=0.0 implies that the model achieves the desired result.

$$\text{MSE} = \frac{1}{n} \sum_{i=1}^{n} (\hat{Y}_i - Y_i)^2,$$
where $Y_i$ is observed CPUE in fishery data, $\bar{Y}_i$ is average CPUE in fishery data, and $\hat{Y}_i$ is estimated CPUE in neural network models. The MSE value measures the accuracy of models. The ARV value reflects the stability of models; the smaller is the ARV values, the more robust is the model. We used R package including “mgcv”, “neuralnet” to select significant variables and establish neural network models respectively.

3 Results

3.1 Spatio-temporal distribution of squid abundance index

The monthly distributions of squid abundance index (CPUE) from January to June in 2003–2011 were plotted in Fig. 1. It is found that the high catch locations for *I. argentinus* occurred on the Patagonian Shelf of the north of 50°S during January to June with the peak values higher than 10 t/d mainly located within 42°–46°S and 50°S.

From the January to June, there was a clear “route” of high CPUE for Argentine shortfin squid (Fig. 1). High CPUEs are found at 46°S in January or February and then the vessels moved southward towards the Falkland Islands gradually. The peak concentrations were found around the Falkland Islands between March and May. At the end of this period, the vessels started migrating northward, and then the Chinese jigging fishing was stopped. In some years (such as 2003, 2009 and 2010) there was no fishing in June because of low CPUE in end of fishing season.

3.2 Environmental factors affecting CPUE

The final model included effects of year, month, latitude, longitude, SST, SSH and Chl $a$ after significance tests. The GAM with the seven variables explained 54.8% variances for CPUE (Table 1). The GAM confirmed the non-linearity of the relationships between squid CPUE and variables (Fig. 2). *Ilex argentinus* CPUE appeared to decrease from shelf to the high sea (Fig. 2a), and the relationship between CPUE and latitude was a curve of “V” shape, with peak values at about 50°S and 42°S (Fig. 2b).

The highest squid CPUEs were associated with a range of SST (10–16°C) (Fig. 2c), concentrating at the range of –60 to –20 cm of SSH (Fig. 2d). In the range of 0–2 mg/m$^3$ of Chl $a$, the squid CPUE showed an increasing trend (Fig. 2e).

3.3 Predicting model of squid CPUE

The neural network models were constructed using month, longitude, latitude, SST, SSH and Chl $a$, which were significant in the GAM, as the input vector and CPUE as the output vector. The MSE and ARV values showed that the model had higher accuracy and more robustness (Fig. 3) when the count of nodes in the hidden layer was equal to 9 (the structure of neural network model is 6:9:1, Fig. 4). In the best neural network model, the importance of the input variables including month, longitude, latitude, SST and Chl $a$ were the more important variables than month, longitude, latitude, SST and Chl $a$ in the prediction model was 5, 9, 27, 26, 15 and 18%, respectively. The latitude, longitude, latitude, SST and Chl $a$ were the more important variables than month, longitude and SSH (Fig. 5).

3.4 Forecasting migration pattern of squid

The monthly CPUE gravities were calculated for the predicted CPUE and observed CPUE to show the gravity shifts from Fig. 1. Monthly catch distribution for *Illex argentinus* during 2003–2011: January (a), February (b), March (c), April (d), May (e), and June (f).

MSEs of training, validation and testing are 6.98, 7.77 and 7.79, respectively, the ARVs of training, validation and testing are 0.2, 0.4 and 0.45, respectively.

Based on the best neural network model, the predicted neural interpretation diagram also showed for complex relationships between the input variables and the squid CPUEs (Fig. 4). The weights of each node in the input and hidden layers showed the positive and negative impact synchronously on the squid abundances. The importance of the input variables including month, longitude, latitude, SST, SSH and Chl $a$ in the prediction model was 5%, 9%, 27%, 26%, 15% and 18%, respectively. The latitude, longitude, latitude, SST and Chl $a$ were the more important variables than month, longitude and SSH (Fig. 5).

Table 1. Model selection and performance for the GAMs

| Model formula | $P$ | $R^2$ adj | GVC | Dev.Exp | AIC |
|---------------|-----|-----------|-----|---------|-----|
| $Y+M+s(Lo)+s(La)+s(SST)$ | $<0.001$, $<0.001$, $<0.001$ | 0.508 | 0.605 | 51.6% | 4609.765 |
| $Y+M+s(Lo)+s(La)+s(SST)+s(SSH)$ | $<0.001$, $<0.001$, $<0.001$, $<0.001$ | 0.517 | 0.596 | 52.6% | 4577.056 |
| $Y+M+Lo+La+s(SST)+s(SSH)+s(Chl a)$ | $<0.001$, $<0.001$, $<0.05$, $<0.001$, $<0.01$ | 0.538 | 0.554 | 54.8% | 4102.478 |

Note: $M$ represents month, $Y$ year, $Lo$ longitude, $La$ latitude, $R^2$ adj adjusted $R^2$, GVC global cross validation, and Dev.Exp deviance explained (%).
January to June for *I. argentinus* in 2005 (Fig. 6a) (The method of calculating CPUE gravities was stated by Wang et al. (2016)). The longitudinal change of CPUE gravity was more stable than latitudinal change of CPUE gravity, either forecasting gravity shift or observed gravity shift could better represent the “south-north” migration pattern of *I. argentinus* in the Southwest Atlantic Ocean.

### 4 Discussion

Understanding and projecting how fish species react to climate change and variability in the regional/local oceanographic environmental is essential for the effective management of marine resources (Waluda et al., 2001). *Illex argentinus* abundance and distribution are found to be significantly influenced by surrounding environmental conditions (Waluda et al., 2001; Bazzino et al., 2005; Chen et al., 2012; Sacau et al., 2005). In this study, the integrated approach which using GAM to select significant variables and using NNM to establish relationships may overcome disadvantages associated with the use of sub-models alone. The GAM can interpret the relationships between results and factors,
Fig. 5. The importance of input vector of neural network model. But its inference ability may be somewhat problematical, such as hypothesis test or obtaining confidence intervals for the fitted values caused by fitting process (Venables and Dichmont, 2004); neural network model is a "black box" model of obtaining a good understanding of the underlying mechanisms difficulty, but has strong ability of fitting non-linear relationships (Paruelo and Tomasel, 1997). The aim of combining the GAM and the ANN is to obtain more accurate prediction with better explanatory between independent and dependent variables, though the ANN is more capable in dealing with nonlinear relationships than GAM.

In the GAM analysis, all the environmental variables (SST, SSH and Chl \(a\)) had significant impacts on squid abundance (Table 1). The highest CPUE was found in temperature between 10–16°C, this was similar to the conclusion obtained by different methods based on different fishery data in other studies (e.g., Sacau et al., 2005). The other two environmental variables (SSH and Chl \(a\)) are rarely utilized for analyzing the distribution of \(I. argentinus\), but have been used to other species, such as neon flying squid \(Ommastrephes bartramii\) in the Northwest Pacific Ocean (Wang et al., 2015). The Chl \(a\) concentration maybe a good indicator of the food availability for squid with high Chl \(a\) concentration yielding good feeding environments (Nishikawa et al., 2014). The relationship between Chl \(a\) concentration and squid abundance is possibly linear in the GAM. The SSH filed may be effective for predicting a water mass front, which is a potential aggregation mechanism for planktons as well as their predators, such as squid (Polito et al., 2000). The relationship between SSH and squid abundance demonstrated non-linearity being the highest CPUE found at SSH between –60 and –20 cm.

For the NNM, one of the greatest advantages is predictive ability. Though there are some methods to try interpret model mechanism (Özesmi and Özesmi, 1999), it is insufficiently specific in our research. For example, we just knew the relationship between squid abundance and environmental variables is nonlinear in neural interpretation diagram, but did not know the exact form of non-linear. Thus, we just took full advantage of NNM to predict spatial CPUE of \(I. argentinus\). In fact, we also did try to predict spatial CPUE distribution using GAM model fitted on the same data samples, it was found that the outcomes were not better than NNM’s. Additionally, the relationships between squid and environmental variables may be varied among different life-history stages. However, the current neural networks developed in this paper were general models those mixed the effect of environmental gradients (spatial effects) with life-history changes (temporal effects), it tends to be risky.

In this study, the annual maps of monthly CPUE distribution (Fig. 1), especially in monthly CPUE gravity shift (Fig. 6a), exhibit the migration pattern of \(I. argentinus\) during January to June. The results were consistent with the conclusion reported by Sacau et al. (2005) and Waluda et al. (2001). Previous work suggests that the life-cycle of \(I. argentinus\) is associated with the subtropical confluence of the Brazil and Falkland Currents during reproduction and the early life stages (Brunetti and Ivanovic, 1992; Hatanaka, 1988) and with the Falkland Current over the Southern Patagonian shelf during maturation, feeding and growth (Rodhouse et al., 1995). In order to evaluate this mechanism with environmental variables, we plotted maps of monthly catch distribution in 2005 overlapped it with SST, SSH and Chl \(a\) concentration, because the catch in 2005 was relatively steady (Figs 6, 7 and 8). From the maps, the catch locations were basically distributed in warm-cold (Brazil-Falkland) confluence in the SST map.

Fig. 6. Monthly CPUE distribution overlapping SST map for \(I. argentinus\) in 2005: January (a), February (b), March (c), April (d), May (e), and June (f); and monthly CPUE gravity shift of \(I. argentinus\) from January to June (a). The gray line with arrows represents the gravity shift of operation and the black line with arrows the gravity shift of forecasting.
between high and low surface layer especially along low surface layer (about –80 cm) in the SSH map (Fig. 7), on the road of high Chl a concentration that interweaved with dark environment where almost no Chl a concentration in the Chl a concentration map (Fig. 8). The south-north migration pattern for *I. argentinus* was consistent with the change of warm-cold (Brazil-Falkland) confluence from January to June. However, the migration pattern was not obvious in SSH and Chl a maps, because the catch locations were looked like constant in a fixed environmental surrounding. This suggested that *I. argentinus* lives in special habitat from SSH and Chl a concentration perspective.

In summary, the integrated model was well developed for representing and predicting the spatial distribution of *I. argentinus* squid with approximately 82% average accuracy, the model could be further used for spatial habitat reconstructing. But more work should be done in further. For example, if the neural network models of *I. argentinus* were developed by monthly or other environmental variables, such as sea surface salinity and sea depth temperature (Yu et al., 2015), can be obtained, the better model would be developed.

**Acknowledgements**

The authors thank the Chinese Squid-Jigging Technology Group of Shanghai Ocean University for providing the catch data and the National Oceanic and Atmospheric Administration for providing the environmental data.

**References**

Arkhipkin A. 1993. Age, growth, stock structure and migratory rate of pre-spawning short-finned squid *Illex argentinus* based on...
statolith ageing investigations. Fisheries Research, 16(4): 313–338

Arkhipkin A. I. 2000. Intropopulation structure of winter-spawned Argentine shortfin squid, Illex argentinus (Cephalopoda: Ommastrephidae), during its feeding period over the Patagonian Shelf. Fisheries Research, 98(1–13).

Basson M, Beddington J R, Crombie I A, et al. 1996. Assessment and management techniques for migratory annual squid stocks: the Illex argentinus fishery in the Southwest Atlantic as an example. Fisheries Research, 28(1): 3–27.

Bazzino G, Quinones R A, Norbis W. 2005. Environmental associations of shortfin squid Illex argentinus (Cephalopoda: Ommastrephidae) in the Northern Patagonian Shelf. Fisheries Research, 76(3): 401–416.

Brunetti N E, Elena B, Rossi G R, et al. 1998a. Summer distribution, abundance and population structure of Illex argentinus on the Argentine shelf in relation to environmental features. South African Journal of Marine Science, 20(1): 175–186.

Brunetti N E, Ivanovic M L. 1992. Distribution and abundance of early life stages of squid (Illex argentinus) in the south-west Atlantic. ICES Journal of Marine Science, 49(2): 175–183.

Brunetti N E, Ivanovic M L, Rossi G, et al. 1998b. Fishery biology and life history of Illex argentinus. In: Okutani T, Ed. Contributed papers to International Symposium on Large Pelagic Squids. Tokyo: Japan Marine Fishery Research Center, 217–231.

Chang J H, Chen Yong, Holland D, et al. 2010. Estimating spatial distribution of American lobster Homarus americanus using habitat variables. Marine Ecology Progress Series, 420: 145–156.

Chen C S, Chiu T S. 2009. Standardising the CPUE for Illex argentinus fishery in the Southwest Atlantic. Fisheries Science, 75(2): 265–272.

Chen Xinjun, Liu Bilin, Chen Yong. 2008. A review of the development of Chinese distant-water squid jiggings fisheries. Fisheries Research, 96(12): 217–221.

Chen Xinjun, Lu Huajie, Liu Bilin, et al. 2012. Forecasting fishing ground of Illex argentinus by using habitat suitability model in the southwest Atlantic. Journal of Shanghai Ocean University (in Chinese), 21(3): 431–438.

Funahashi K I. 1989. On the approximate realization of continuous mappings by neural networks. Neural Networks, 2(3): 183–192.

Gordon A L. 1989. Brazil-Malvinas confluence-1984. Deep Sea Research Part A: Oceanographic Research Papers, 36(3): 359–384.

Haimovic M, Brunetti N E, Rodhouse P G, et al. 1998. Illex argentinus. In: Rodhouse P G, Dave E G, O’Don P K, eds. Squid Research Papers to International Symposium on Large Pelagic Squids. Rome: FAO, 27–58.

Hastie T J, Tibshirani R. I. 1990. Generalized additive models. In: Cox D R, Hinkley D V, Rubin D, et al., eds. Monographs on Statistics and Applied Probability. London: Chapman and Hall, 136–173.

Hatanaka H. 1986. Growth and life span of short-finned squid Illex argentinus in the waters off Argentina. Nippon Suisan Gakkaishi, 52(1): 11–17.

Hatanaka H. 1988. Feeding migration of short-finned squid Illex argentinus in the waters off Argentina. Nippon Suisan Gakkaishi, 54(8): 1343–1349.

Ivanovic M L, Brunetti N E. 1994. Food and feeding of Illex argentinus. Antarctic Science, 6(2): 185–193.

Jensen O P, Seppelt R, Miller T J, et al. 2005. Winter distribution of blue crab Callinectes sapidus in Chesapeake Bay: application and cross-validation of a two-stage generalized additive model. Marine Ecology Progress Series, 299: 239–255.

Legeckis R, Gordon A L. 1982. Satellite observations of the Brazil and Falkland currents—1975 1976 and 1978. Deep Sea Research Part A: Oceanographic Research Papers, 29(3): 375–401.

Lek S, Delacoste M, Baran P, et al. 1996. Application of neural network models to modelling nonlinear relationships in ecology. Ecological Modelling, 90(1): 39–52.

Lu Huajie, Chen Xinjun. 2012. Age, growth and population structure of Illex argentinus based on statolith microstructure in Southwest Atlantic Ocean. Journal of Fisheries of China (in Chinese), 36(7): 1049–1056.

Maund M N, Punt A E. 2004. Standardizing catch and effort data: a review of recent approaches. Fisheries Research, 70(2–3): 141–159.

Nishikawa H, Igarashi H, Ishikawa Y, et al. 2014. Impact of paralarvae and juveniles feeding environment on the neon flying squid (Ommastrephes bartramii) winter-spring cohort stock. Fisheries Oceanography, 23(4): 289–303.

Nowlan S J, Hinton G E. 1992. Simplifying neural networks by soft weight-sharing. Neural Computation, 4(4): 473–493.

Olson D B, Podestá G P, Evans R H, et al. 1988. Temporal variations in the separation of the Brazil and Malvinas Currents. Deep Sea Research Part A: Oceanographic Research Papers, 35(12): 1971–1990.

Özesmi S L, Özesmi U. 1999. An artificial neural network approach to spatial habitat modelling with interspecific interaction. Ecological Modelling, 116(1): 15–31.

Paruelo J, Tomasel F. 1997. Prediction of functional characteristics of ecosystems: a comparison of artificial neural networks and regression models. Ecological Modelling, 98(2–3): 173–186.

Polito P S, Sato O T, Liu W T. 2000. Characterization and validation of the heat storage variability from TOPEX/Poseidon at four oceanographic sites. Journal of Geophysical Research: Oceans, 105(C7): 16911–16921.

Portela J, Sacau M, Wang J, et al. 2005. Analysis of the variability in the abundance of shortfin squid Illex argentinus in the Southwest Atlantic fisheries during the period 1999–2004. ICES CM 2005/O: 16.

Rodhouse P G, Barton J, Hatfield E M C, et al. 1995. Illex argentinus: life cycle, population structure, and fishery. ICES Marine Science, 199: 425–432.

Sacau M M, Pierce G J, Wang Jianjun, et al. 2005. The spatio-temporal pattern of Argentine shortfin squid Illex argentinus abundance in the southwest Atlantic. Aquatic Living Resources, 18(4): 361–372.

Venables W N, Dichmont C M. 2004. GLMs, GAMs and GLMMs: an overview of theory for applications in fisheries research. Fisheries Research, 70(2–3): 319–337.

Waluda C M, Rodhouse P G, Trathan P N, et al. 2001. Remotely sensed mesoscale oceanography and the distribution of Illex argentinus in the South Atlantic. Fisheries Oceanography, 10(2): 207–216.

Waluda C M, Trathan P N, Rodhouse P G. 1999. Influence of oceanographic variability on recruitment in the Illex argentinus (Cephalopoda: Ommastrephidae) fishery in the South Atlantic. Marine Ecology Progress Series, 183: 159–167.

Wang Jintao, Chen Xinjun, Chen Yong. 2016. Spatio-temporal distribution of skipjack in relation to oceanographic conditions in the west-central Pacific Ocean. International Journal of Remote Sensing, 37(24): 6149–6164.

Wang Jintao, Yu Wei, Chen Xinjun, et al. 2015. Detection of potential fishing zones for neon flying squid based on remote-sensing data in the Northwest Pacific Ocean using an artificial neural network. International Journal of Remote Sensing, 36(13): 3317–3330.

Weigend A S, Huberman B A, Rumelhart D E. 1990. Predicting the future: a connectionist approach. International Journal of Neural Systems, 1(3): 193–209.

Yu Wei, Chen Xinjun, Yi Qian, et al. 2015. Variability of suitable habitat of western winter-spring cohort for neon flying squid in the Northwestern Pacific under anomalous environments. PLoS One, 10(4): e0122997.
Transcriptome assembly of *Modiolus modiolus* and comparative analysis with *Bathymodiolus platifrons*

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Received 18 August 2017; accepted 13 March 2018

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Abstract

The genetic basis for bivalves’ adaptation and evolution is not well understood. Even few studies have focused on the mechanism of molluscan molecular evolution between the coastal intertidal zone and deep-sea environment. In our studies, we first conducted the transcriptome assembly of *Modiolus modiolus* mussels living in coastal intertidal zones. Also, we conducted transcriptome comparison analyses between *M. modiolus* and *Bathymodiolus platifrons* living in hydrothermal vents and cold methane/sulfide-hydrocarbon seeps. De novo assemblies of the clean reads yielded a total of 182,476 and 156,261 transcripts with N50 values of 1,769 and 1,545 for *M. modiolus* and *B. platifrons*. A total of 27,868 and 23,588 unigenes were identified, which also displayed the similar GO representation patterns. Among the 10,245 pairs of putative orthologs, we identified 26 protein-coding genes under strong positive selection (Ka/Ks > 1) and 12 genes showing moderate positive selection (0.5 < Ka/Ks < 1). Most of those genes are predicted to be involved in stress resistance. Overall, our study first provides the transcriptomic database for *M. modiolus*. Transcriptome comparison illustrates the genome evolution between *M. modiolus* and *B. platifrons*, and provides an important foundation for future studies on these two species.

Key words: mollusc, transcriptome comparison, positive selection, stress adaptation

Citation: Meng Jie, Yang Mei, Xu Fei, Li Xinzheng, Li Li. 2018. Transcriptome assembly of *Modiolus modiolus* and comparative analysis with *Bathymodiolus platifrons*. Acta Oceanologica Sinica, 37(8): 38–45, doi: 10.1007/s13131-018-1232-2

1 Introduction

Bivalves, which were comprised of 30,000 extant species, are an important component of the ecosystem and biodiversity (Saavedra and Bachere, 2006). They were widely spread from the intertidal coastal areas to hydrothermal vents and cold seeps (Egas et al., 2012; Li et al., 2013). However, the genetic basis for their different adaptations is not well understood (Dame, 2011). *Modiolus modiolus* is a benthic marine organism, which filter feeds in near-shore habitats. As the important intertidal coastal habitat shellfish, its transcriptome sequence has not been conducted. *Bathymodiolus platifrons* are phylogenetically close to *M. modiolus* and belonged to the same family—Mytilidae. *Bathymodiolus platifrons* is a highly specialized animal inhabiting hydrothermal vent and cold seep ecosystems (Barry et al., 2002) and its genome sequences were also completed. These results have provided good data sets for the further genome comparison analysis. In our study, we first conduct the transcriptome analysis of *M. modiolus* which provided good data sources for further analysis. Also, we conducted the transcriptome comparison between *M. modiolus* and *B. platifrons* living in different environments, which provide valuable information to understand their different environmental adaptation mechanism.

In previous studies of marine invertebrate, the adaptive research has been conducted using single markers or candidate genes (Riesgo et al., 2012). Next-generation sequencing technology enabled analysis of large quantities of sequence data efficiently and cost-effectively (Schuster, 2008; Wang et al., 2009), which provided an efficient way to identify adaptive genes and
explain the adaptive evolution process. Recently, marine bi-valves’ genomic databases have been obtained, including Crassostrea gigas (Zhang et al., 2012), B. platifrons, M. philippinarum (Sun et al., 2017) and Patinopecten yessoensis (Wang et al., 2017), etc. Most of these publications mainly focused on the responses to multiple stresses including periodic hypoxia, hypersalinity, temperature fluctuations, and pollution. For evolutionary analysis, Zhao et al. (2014) conducted a comparative transcriptome analysis of two oysters, C. gigas and C. hongkongensis, and explained their adaptations and evolutionary mechanisms for dealing with hypo-osmotic conditions (Zhao et al., 2014). Wang et al. (2013) performed the first large-scale transcriptome comparison between the two scallop species, Chlamys farreri and P. yessoensis, and identified fast evolving genes, which played an important role in their speciation and local adaptation (Wang et al., 2013). For the evolutionary mechanisms of molluscs living in coastal intertidal zones and deep sea environments, there are also some studies. For example, Zheng et al. (2017) have conducted transcriptome comparison among B. platifrons, B. manussenis, M. kurilensis and Perna viridis. The results indicated that some immune responsive genes were positively selected and more highly expressed in the deep-sea mussels, which may be related with their endosymbiosis (Wang and Sun, 2017).

In this study, we first performed de novo transcriptome sequencing of M. modiolus using the Illumina sequence platform. Also, according to transcriptome comparison between M. modiolus and B. platifrons, 38 putative fast-evolving genes were identified, which may explain their different evolutionary mechanisms. This is the first time that the transcriptome of M. modiolus has been sequenced and will provide transcriptome resources for this mollusk. Additionally, in comparison with B. platifrons, we may use this transcriptome to find orthologous genes under potential positive selection between two species. This will help us to explain the different mechanisms for adaptation to hydrothermal vents and cold seep ecosystems versus coastal intertidal environments.

2 Materials and methods

2.1 Sample collection and RNA isolation for Illumina sequencing

Liquid nitrogen-frozen samples B. platifrons were provided by Li Xinzhen from the Institute of Oceanology, Chinese Academy of Science. These were originally sampled from a cold seep located at a depth of 996.9 m (27°47′44″N, 126°53.802′9″E). Modiolus modiolus specimens were collected in Dalian, Liaoning Province, China. These samples were collected and immediately frozen in liquid nitrogen and then transferred and stored at −80°C. For these two species, various tissues (including gills, mantles and adductor muscle) were mixed equally and ground in liquid nitrogen. Total RNA was isolated using Trizol reagent (Invitrogen). RNA purity, concentration, and integrity were checked using a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), Qubit® RNA Assay Kit (Life Technologies, CA, USA), and Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

2.2 Illumina sequencing

RNA (3 µg) per sample was used for RNA sample preparation. Sequencing libraries were generated using Illumina TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, USA) and index codes were added to each sample. The mRNA was purified from total RNA and the fragmentation was carried out using divalent cations under elevated temperature. First strand cDNA was synthesized using random oligonucleotides and the second strand cDNA synthesis was performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and enzymes were removed. After adenylation of the 3’ ends of DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. Illumina PCR Primer Cocktail in a 10-cycle PCR was conducted to obtain DNA fragments with ligated adaptor molecules. Products were purified and quantified on the Agilent Bioanalyzer 2100 system. The clustering of the index-coded samples was performed using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) on a cBot Cluster Generation System. After cluster generation, the library preparations were sequenced.

2.3 Quality control and de novo assembly

Clean data were obtained by removing reads from raw data, containing adapter sequences, or with more than 10% known nucleotides, or with low quality reads (more than 50% base with quality Qphred ≤5) using NGS QC toolkit package (Version 2.3). The Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. Transcriptome assembly was accomplished using Trinity software (2.4.2609) and the parameters were set as “seqType=fq, min_contig_length=100, min_kmer_cov=2”, with the rest being default parameter (Grabherr et al., 2011). All the sequences from two transcriptomes were then taken into further process of redundancy removing using CD-HIT-EST v4.6 62 with a sequence identity threshold of 99% in every 1 000 bp.

2.4 Gene functional annotation

Gene function annotation was conducted based on these databases: Pfam (protein family); Nt (NCBI non-redundant nucleotide sequences); Swiss-Prot (a manually annotated and reviewed protein sequence database); KOG/COG (clusters of orthologous groups of proteins); KO (KEGG ortholog database); and GO (gene ontology). Gene expression levels were estimated by RSEM for each sample. Clean data were mapped back onto the assembled transcriptome, and the read count for each gene was obtained (Li and Dewey, 2011). We applied a sensitive HMM scanning method on known Pfam functional protein domains to classify the gene families (Sun et al., 2017). Heatmap analysis was conducted with R script.

2.5 GO and KEGG enrichment analysis

GO enrichment analysis DEGs was implemented by the GOseq R packages based Wallenius non-central hyper-geometric distribution (Young et al., 2010). KOBAS software 2.0 was used for KEGG enrichment analysis (Mao et al., 2005). KEGG is a database resource for understanding high-level functions produced by genome sequencing and other high-throughput experimental technologies (Kanehisa et al., 2008).

2.6 Ka/Ks analysis

We used the BLAST-base (OrthoMCL) method (Li et al., 2003) to identify putative orthologs between the two species. We retained only those ortholog pairs that matched the same proteins to avoid the inclusion of paralogs. The CDSs of orthologous were aligned for further analysis. The ratio of the number of nonsynonymous substitutions per nonsynonymous site (Ka) to the number of synonymous substitutions per synonymous site (Ks) was used to test for positive selection using PAML-CODEML method (Yang, 2007). The rates of Ka to Ks between putatively orthologous coding regions were calculated based on the maximum-likelihood method using KaKs_Calculator 2.0. The orthologs with a Ka rate less than 0.1 were excluded from further analysis.
3 Results and discussion

3.1 Overall de novo assembly of the transcriptome for M. modiolus and B. platifrons

The cDNA libraries representing the different tissues (gills, mantles, and adductor) of M. modiolus and B. platifrons were constructed and then pooled for sequences. Sequencing of the tissue transcriptomes using the Illumina HiSeq 2000 platform in paired-end mode with a read length of 125 bp resulted in a total of 7.04 Gb and 5.97 Gb clean data in M. modiolus and B. platifrons (Table 1), respectively. After filtration, 46 904 896 and 39 817 358 clean reads were obtained and over 90% and 85% of them exceeded Q20 and Q30, indicating high quality of the sequencing data. De novo assemblies of the clean reads yielded a total of 182 476 and 156 261 transcripts with N50 values of 1 769 and 1 545 in M. modiolus and B. platifrons. ESTscan and BLAST search of the protein databases also resulted in the prediction of 137 763 and 119 880 coding transcripts (Table 1). The raw sequencing data have been submitted to NCBI under accession number SRR5043294. The statistics for the de novo assemblies and functional annotations are displayed in Table 1 and Fig. 1.

Modiolus modiolus has not been sequenced, which may be a bottleneck for further research into its ecology (Dinesen and Morton, 2014). In our study, we sequenced M. modiolus using transcriptome methods and more than two-thirds of the annotated unique sequences were matched to the known species. These results provided abundant sequence information for further studies of M. modiolus. Bathymodiolus platifrons transcriptome has already been published in previous studies (Wong et al., 2015). However, in our study, we obtained more than 1.6-fold numbers of transcripts for the mixture of different tissues used for sequencing, though we did not obtain more annotated transcripts. This may be because of the poor genomics database for marine bivalves. Finally, it should be noted that in hydrothermal vent and cold seep ecosystems, many bacteria are parasitic on B. platifrons (Nakamura-Kusakabe et al., 2016). In order to obtain clean sequences for B. platifrons, we also performed strict raw data quality control to remove contamination by pathogen genomes.

3.2 Functional analysis comparison of dominant transcripts in two species

We constructed functional dominant transcripts according to

| Name                        | B. platifrons | M. modiolus |
|-----------------------------|--------------|-------------|
| De novo assembly by Trinity | Clean base   | 5.97 Gb     | 7.04 Gb     |
|                             | Clean reads  | 39 817 358  | 46 904 896  |
|                             | Q20/Q30/%    | 97.12/92.88 | 97.78/94.02 |
|                             | Total number of transcripts | 156 261 | 182 476 |
|                             | Mean length of transcripts/bp | 832 | 885 |
|                             | Transcripts size range/bp | 201–41 720 | 201–32 099 |
|                             | Transcripts N50/bp | 1 545 | 1 769 |
|                             | Mean length of unigene/bp | 672 | 693 |
|                             | Unigene size range/bp | 201–41 720 | 201–32 099 |
|                             | Unigene N50/bp | 1 097 | 1 236 |
| Functional annotation       | Total number of transcripts annotated by public databases | 31 418 (26.2%) | 37 371 (27.12%) |
|                             | Total CDS predicted | 119 880 | 137 763 |
|                             | NCBI non-redundant database (e-value<10^{-5}) | 23 823 (19.87%) | 28 218 (20.48%) |
|                             | SwissProt (e-value<10^{-5}) | 16 336 (13.62%) | 18 438 (13.38%) |
|                             | Annotated in PFAM | 23 525 (19.62%) | 28 218 (20.22%) |
|                             | Annotated in GO | 23 588 (19.67%) | 27 868 (20.22%) |
|                             | Annotated in KEGG | 11 627 (9.69%) | 13 078 (9.49%) |

Fig. 1. The length distribution of contigs and coding sequences (CDSs) of M. modiolus and B. platifrons. Contigs were generated from de novo assembly of Illumina sequencing reads.
seven databases. Only 27.12% and 26.20% unique genes in *M. modiolus* and *B. platifrons* database were annotated in at least one database. A total of 27,868 and 23,588 transcripts were assigned with at least one GO term (Level 4) for 464 and 538 GO assignments in *M. modiolus* and *B. platifrons* (Figs S1 and S2). GO classification at Level 2 is shown in Figs S1 and S2. This wide distribution of GO terms further indicates that the transcripts represent a diverse range of functional classes. The top ten enriched GO terms are shown in Fig. 2a. From GO analysis, we can see that the most enriched GO terms in *B. platifrons* are related to the metabolism pathway, including the cellular macromolecule metabolic process, organic cyclic compound metabolic process, cellular nitrogen compound metabolic process, and cellular aromatic compound metabolic process. These enriched terms may be directly related with its methane/sulfide-hydrocarbon seeps and organic enrichment living environments. The same distribution was also observed in *M. modiolus*, which may be related with the increasingly polluted coastal environment.

Further, KEGG enrichment analysis was conducted with all annotated sequences. The analysis shows that 9,040 and 8,239 sequences were enriched in *M. modiolus* and *B. platifrons*, respectively. 

**Fig. 2.** The GO and KEGG annotation of the transcripts in *B. platifrons* and *M. modiolus*. a. The top ten enriched GO terms in these two species, b. the genes distributed in five KEGG metabolic processes, and c. the top ten enriched KEGG metabolism pathways in these two species.
quences are mapped to 32 metabolic pathways (Hierarchy2) in *M. modiolus* and *B. platifrons* (Figs S3 and S4). Among these, cellular processes (*B. platifrons* 27%, *M. modiolus* 29%) and metabolic processes (*B. platifrons* 28%, *M. modiolus* 28%), had the most unigenes (Fig. 2b). Moreover, in both two species, the “signal transduction pathway” (967 genes in *B. platifrons*, 1084 genes in *M. modiolus*) was most significantly enriched. We propose that these pathways may be developed to deal with complicated environmental pressures (Fig. 2c). However, the “immune system” metabolic pathways varied between the two species, which may be related with their different living environments. *Bathymodiolus platifrons* is capable of acquiring chemo autotrophic bacteria as its major nutritional food source (Wong et al., 2015). It remains unclear how *Bathymodiolus* mussels distinguish pathogens from symbionts and how pathogens trigger immune responses (Bettencourt et al., 2007). However, *M. modiolus* living in coastal areas are exposed to constant challenge by invasive and pathogenic microbes. It has an open circulatory immune system with hemolymph serum containing diverse immune proteins, including soluble lectins, lysosomal enzymes and various antimicrobial peptides (Canesi et al., 2002). Overall, these annotations are useful to identify functional genes and specific biological processes in these two species.

### 3.3 Identification of putative orthologs and analysis of Ka/Ks

Assessing the ratio of substitution rates at nonsynonymous and synonymous sites can help to identify genes under positive selection (Vitti et al., 2013). In our results, we searched for orthologs between these two species and found 10,245 putative orthologous genes according to OrthoMCL method (Li et al., 2003). Only 38 protein showed positive selection (dn/dS>0.5). A total of 26 genes (0.12%) had dn/dS>1 suggestive of signs of strong positive selection and 12 (0.079%) genes had 0.5<dn/dS<1 representing signatures of moderate positive selection (Kavembe et al., 2015) (Table 2). When Ka/Ks<0.1, the 8,661 orthologous genes are likely to be experiencing selection constraints.

#### 3.4 Analysis of candidate genes under positive and conserved selection

The two species *B. platifrons* and *M. modiolus* have different inhabitations and possess specific adaptations to variable environmental factors between coastal intertidal area and deep sea, such as salinity, temperature, pH, heavy metals, and bacteria (Jones et al., 2006; Duperron et al., 2011). GO analysis was used to analyze the gene categories. The 38 genes were distributed among 15 different GO terms, most of which have physiological functions related to stress response (Fig. 3a). The enrichment GO terms included compound metabolic processes (nitrogen compound metabolic process, cellular aromatic compound metabolic process, and organic substance metabolic process), signal transduction processes (intracellular signal transduction and signal transduction process), and response-to-stimulus processes (pathogenesis and oxidation-reduction process). *Bathymodiolus platifrons* lives in the deep sea and experiences detrimental chemical pollutions, including heavy metal and methane. As a result, this species may have evolved abilities to adapt to the highly toxic chemical environment. Additionally, *B. platifrons* is capable of acquiring chemoautotrophic bacteria as its major nutritional food source and were involved in different immune responses (Fujiwara et al., 2000). The enriched GO terms may indicate that the different living environments have driven the evolution of these two species.

The orthologous genes with Ka/Ks<0.1 was considered to be conserved, and 8,661 orthologous pairs were identified between

| Table 2. Genes showing signs of positive selection between *B. platifrons* and *M. modiolus* |
|---------------------------------|---------------------------------|
| **Gene ID**                     | **Description**                 | **dn/dS**    |
| OG03779                         | probable 60S ribosomal protein L37-A | 999.00       |
| OG17598                         | putative fungistatic metabolite  | 999.00       |
| OG17046                         | EF-hand calcium-binding domain-containing protein 1 | 36.03       |
| OG16945                         | 60S ribosomal protein            | 12.35        |
| OG10206                         | protein PIF                      | 1.99         |
| OG05168                         | serum response factor-binding protein 1 | 1.74         |
| OG18841                         | basement membrane-specific heparan sulfate proteoglycan core protein | 1.39       |
| OG05072                         | PR domain zinc finger protein 14 | 1.34         |
| OG18790                         | cystatin C                       | 1.00         |
| OG17599                         | putative fungistatic metabolite  | 1.00         |
| OG16869                         | heterogeneous nuclear ribonucleoprotein 27C | 1.00       |
| OG18737                         | clusterin                        | 1.00         |
| OG02089                         | nucleoplasmin-like protein ANO39 | 1.00         |
| OG1629                          | low-density lipoprotein receptor-related protein 2 | 1.00       |
| OG16709                         | myelin basic protein             | 1.00         |
| OG16956                         | no annotation                    | 1.00         |
| OG18823                         | perlucin-like protein            | 1.00         |
| OG30927                         | transcription factor jun-D       | 1.00         |
| OG16782                         | cerebellin-2                     | 1.00         |
| OG16732                         | glial fibrillary acidic protein  | 1.00         |
| OG16776                         | no annotation                    | 1.00         |
| OG16787                         | EF-hand calcium-binding domain-containing protein 1 | 1.00       |
| OG18891                         | myelin proteolipid protein       | 1.00         |
| OG18863                         | collagen-2                       | 1.00         |
| OG18718                         | 40S ribosomal protein S11        | 1.00         |
| OG16693                         | myelin proteolipid protein       | 1.00         |
the two species in our results. GO enrichment analysis revealed that 11, 10 and 26 terms were enriched in biological processes, cellular components and molecular function processes, respectively ($P\text{-value} \leq 0.05$) (Fig. 3b). Among the biological processes, carbohydrate metabolism, catabolism, and biosynthetic processes were enriched. Among the molecular function process, nucleotide binding, ribonucleotide binding, and purine nucleotide binding processes were enriched. Among the cellular components, organelles and membrane bound-organelles were enriched. These metabolic pathways are primary processes in many species. For example, carbohydrate metabolism provides energy in nearly all known organisms, and the purine nucleotide binding process is related to nucleotide metabolism. These conserved metabolic pathways showed relative lower Ka/Ks values, indicating that they are subject to strong selection constraints.

### 3.5 Immune responsive genes analysis

One of the most extraordinary adaptation trait of Bathymodiolin mussels is their endosymbiosis (Jones et al., 2006). In previous studies, it has been revealed that *B. platifrons* has expanded and specie-specific immune responsive genes, which was the important genome basis for their adaptation under deep sea environment (Sun et al., 2017). However, for few species to be analyzed, it is still unknown whether this adaptation mechanism is species lineage-specific or is broadly conserved in other species. In our result, we conducted the transcriptome comparison analysis using four species, including two deep sea mussels, *B. platifrons* and *B. manusensis*, and two shallow-water mussels, *M. kurileensis* and *M. modiolus*. The transcriptome data of *B. manusensis* and *M. kurileensis* were obtained from previous studies (Zheng et al., 2017). We mainly focused on immune recognition receptors, which played important roles in initiation the immune responses (Toubiana et al., 2013). All these molecules were found in these four species, which were identified with previous studies (Fig. 4) (Zheng et al., 2017). Heatmap analysis of genes numbers revealed that two deep-sea bathymodiolin mussels and two shallow-water mussels clustered into two branches respectively. This further confirms the different immune systems between deep-sea and shallow water mussels may be related with their different living environments.

### 4 Conclusions

Our study represents the first transcriptome profile in *M. modiolus*. According to comparative transcriptome analysis with *B. platifrons*, our results provide new insights into the molecular mechanisms underpinning unique adaptations to coastal inter-
tidal environments or deep sea hydrothermal vent and cold seep environments. Selection analysis revealed that strong positive selection in genes is related to stress responses, indicating that the different living environments have driven the evolution of these two species. Our study provides transcriptomic resources for future genetic or genomic studies on *M. modiolus* and *B. platifrons*.

**Acknowledgements**

The authors thank the scientists and crew of the R/V *Kexue* for their assistance in specimen collecting.

**Data Accessibility**

The transcriptome data were submitted to NCBI database under SRR5043294 (PRJNA353979). All data underlying the findings are fully available without restriction.

**References**

Barry J P, Buck K R, Kochevar R K, et al. 2002. Methane-based symbiosis in a mussel, *Bathymodiolus platifrons*, from cold seeps in Sagami Bay, Japan. Invertebrate Biology, 121(1): 47–54.

Bettencourt R, Roch P, Stefanni S, et al. 2007. Deep sea immunity: unveiling immune constituents from the hydrothermal vent mussel *Bathymodiolus azoricus*. Marine Environmental Research, 64(2): 108–127.

Canesi L, Gallo G, Gavioli M, et al. 2002. Bacteria-hemocyte interactions and phagocytosis in marine bivalves. Microscopy Research and Technique, 57(6): 469–476.

Dame R F. 2011. Ecology of Marine Bivalves: An Ecosystem Approach. 2nd ed. Boca Raton: CRC Press.

Dinesen G E, Morton B. 2014. Review of the functional morphology, biology and perturbation impacts on the borial, habitat-forming horse mussel *Modiolus modiolus* (Bivalvia: Mytilidae: Modiolinae). Marine Biology Research, 10(5): 845–870.

Duperron S, Guezi H, Gaudron S M, et al. 2011. Relative abundances of methane- and sulphur-oxidising symbionts in the gills of a cold seep mussel and link to their potential energy sources. Geobiology, 9(6): 481–91.

Egas C, Pinheiro M, Gomes P, et al. 2012. The Transcriptome of *Bathymodiolus azoricus* gill reveals expression of genes from endosymbionts and free-living deep-sea bacteria. Marine Drugs, 10(8): 1765–1783.

Fujiiyama Y, Takai K, Uematsu K, et al. 2000. Phylogenetic characterization of endosymbionts in three hydrothermal vent mussels: influence on host distributions. Marine Ecology Progress Series, 208: 147–155.

Grabherr M G, Haas B J, Yassour M, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology, 29(7): 644–652.

Jones W J, Won Y J, Maas P A Y, et al. 2006. Evolution of habitat use by deep-sea mussels. Marine Biology, 148(4): 841–851.

Kanelisa M, Araki M, Goto S, et al. 2008. KEGG for linking genomes to life and the environment. Nucleic Acids Research, 36: D480–D484.

Kavembe G D, Franchini P, Iriasiri I, et al. 2015. Genomics of adaptation to multiple concurrent stresses: insights from comparative transcriptomics of a cichlid fish from one of earth’s most extreme environments, the Hypersaline Soda Lake Magadi in Kenya, East Africa. Journal of Molecular Evolution, 81(3–4): 90–109.

Li Bo, Dewey C N. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics, 12: 323.

Li Li, Stoeckert C J Jr, Roos D S. 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Research, 13(19): 2178–2189.

Li Qi, Zhao Xuelin, Kong Lingfeng, et al. 2013. Transcriptomic response to stress in marine bivalves. ISJ-Invertebrate Survival Journal, 10(1): 84–93.

Mao Xizeng, Cai Tao, Olyarchuk J G, et al. 2005. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics, 21(19): 3787–3793.

Nakamura-Kusakabe I, Nagasaki T, Kinjo A, et al. 2016. Effect of sulfide, osmotic, and thermal stresses on taurine transporter mRNA levels in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 191: 74–79.

Nieserg A, Andrade S C S, Sharma P P, et al. 2012. Comparative description of ten transcriptomes of newly sequenced invertebrates and efficiency estimation of genomic sampling in non-model taxa. Frontiers in Zoology, 9: 33.

Saavedra C, Bachère E. 2006. Bivalve genomics. Aquaculture, 256(1–4): 1–14.

Schuster S C. 2008. Next-generation sequencing transforms today’s biology. Nature Methods, 5(1): 16–18.

Sun Jin, Zhang Yu, Xu Ting, et al. 2017. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nature Ecology & Evolution, 1(5): 0121.
Toubiana M, Gerdol M, Rosani U, et al. 2013. Toll-like receptors and MyD88 adaptors in Mytilus: complete cds and gene expression levels. Developmental & Comparative Immunology, 40(2): 158–166
Vitti J J, Grossman S R, Sabeti P C. 2013. Detecting natural selection in genomic Data. Annual Review of Genetics, 47(1): 97–120
Wang Zhong, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics, 10(1): 57–63
Wang Shan, Hou Rui, Bao Zhenmin, et al. 2013. Transcriptome sequencing of Zhikong scallop (Chlamys farreri) and comparative transcriptomic analysis with Yesso scallop (Patinopecten yessoensis). PLoS One, 8(5): e63927
Wong Y H, Sun Jin, He Lisheng, et al. 2015. High-throughput transcriptome sequencing of the cold seep mussel Bathymodiolus platifrons. Scientific Reports, 5: 16597
Wong Y, Sun Jin, He Lisheng, et al. 2015. High-throughput transcriptome sequencing of the cold seep mussel Bathymodiolus platifrons. Scientific Reports, 5: 16597
Yang Ziheng. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution, 24(6): 1586–1591
Young M D, Wakefield M J, Smyth G K, et al. 2010. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biology, 11(2): R14
Zhang Guofang, Fang Xiaodong, Guo Ximing, et al. 2012. The oyster genome reveals stress adaptation and complexity of shell formation. Nature, 490(7418): 49–54
Zhao Xuelin, Yu Hong, Kong Lingfeng, et al. 2014. Comparative transcriptome analysis of two oysters, Crassostrea gigas and Crassostrea hongkongensis provides insights into adaptation to hypo-osmotic conditions. PLoS One, 9(11): e111915
Zheng Ping, Wang Minxiao, Li Chaolun, et al. 2017. Insights into deep-sea adaptations and host-symbiont interactions: a comparative transcriptome study on Bathymodiolus mussels and their coastal relatives. Molecular Ecology, 26(19): 5133–5148

Supplementary information:

Fig. S1. GO enrichment analysis of annotated genes in M. modiolus (GO level 2).
Fig. S2. GO enrichment analysis of annotated genes in B. platifrons (GO level 2).
Fig. S3. KEGG enrichment analysis of annotated genes in M. modiolus. A represents “cellular processes”, B “environmental information processing”, C “genetic information processing”, D “metabolism processing”, and E “organismal systems”.
Fig. S4. KEGG enrichment analysis of annotated genes in B. platifrons. A represents “cellular processes”, B “environmental information processing”, C “genetic information processing”, D “metabolism processing”, and E “organismal systems”.
Table S1. GO enrichment analysis of annotated genes in M. modiolus.
Table S2. GO enrichment analysis of annotated genes in B. platifrons.

The supplementary information is available online at www.hyxb.org.cn/aosen/ch/index.aspx. The supplementary information is published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.
Transcriptomic analysis reveals the effect of the exopolysaccharide of *Psychrobacter* sp. B-3 on gene expression in RAW264.7 macrophage cells

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Received 1 December 2017; accepted 12 April 2018  

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Abstract  
B-3 exopolysaccharide is extracted from the Antarctic psychrophilic bacterium *Psychrobacter* sp. B-3. We have previously shown that it activates macrophages and affects their immunoregulatory activities. To determine what genes are affected during this process, we detected the genes differentially expressed in cells of RAW264.7 macrophages treated with B-3 exopolysaccharide by transcriptomic analysis. B-3 exopolysaccharide treatment caused differential expression of 420 genes, of which 178 were up-regulated and 242 were down-regulated. These genes were shown to be involved in many aspects of cell function, mainly metabolism and immunity. Genes were enriched in multiple immune-related pathways, and the most significantly enriched genes were involved in antigen processing and presentation pathways. The pathway in which differentially expressed genes were the most significantly enriched was the metabolic pathway; specifically, the expression of many metabolic enzyme genes was altered by B-3 exopolysaccharide treatment. Additionally, the genes involved in metabolisms of amino acids, carbohydrates, lipids and nucleotides, varied to certain degrees. B-3 exopolysaccharide, therefore, appears to directly affect the immune function of RAW264.7 macrophages as an immunostimulant, or to indirectly change intracellular metabolism. This is the first study to determine the effect of an Antarctic psychrophilic bacterial exopolysaccharide on RAW264.7 macrophages. Our findings provide an important reference for research into the regulation of macrophage immune function by different polysaccharides.

Key words: *Psychrobacter* sp. B-3, B-3 exopolysaccharide, RAW264.7 macrophage, differentially expressed genes, immunological pathway, metabolic pathway

Citation: Zhang Pingping, Li Jiang, Yu Leiye, Wei Jingfang, Xu Tong, Sun Guojie. 2018. Transcriptomic analysis reveals the effect of the exopolysaccharide of *Psychrobacter* sp. B-3 on gene expression in RAW264.7 macrophage cells. Acta Oceanologica Sinica, 37(8): 46–53, doi: 10.1007/s13131-018-1227-z

1 Introduction  
Polysaccharides from fungi, plants, bacteria, and animals have been shown to exhibit a variety of biological activities, including antitumor (Wasser, 2002; Lemieszek and Rzeski, 2012), immunoregulatory (Wakabayashi et al., 1997; Wasser, 2002; Schepetkin and Quinn, 2006; Won et al., 2011), and antiviral functions (Arena et al., 2009; Wang et al., 2012). The immunoregulatory activity of polysaccharides, such as β-glucans from fungi and higher plants, has been an important topic for research, both in vitro and in vivo. Studies have shown that polysaccharides affect the production of cytokines and other molecules, thereby activating different immune cells, such as macrophages (Stier et al., 2014) and T cells (Inatsuka et al., 2013; Stier et al., 2014). Many in vitro studies have determined the effects of polysaccharides on macrophage activation (Schepetkin and Quinn, 2006; Tseng et al., 2012; Li et al., 2017), and RAW264.7 macrophages have been widely used as a model system. Most in vitro studies regarding macrophage regulation (Leung et al., 2006; Li et al., 2007; Won et al., 2011; Reiber et al., 2017; Lee et al., 2018) have tested the following effects: secretion of cytokines and small molecules, phagocytic ability, differentiation state, and activation of signaling pathways and polysaccharide receptors on cell membranes. However, it is not clear whether polysaccharides solely affect the expression of specific immune-related molecules and signaling pathways in macrophages, or whether other genes are affected. A comprehensive understanding of polysaccharide-induced gene expression changes in activated macrophages would thus contribute to a better understanding of the underlying molecular mechanisms.  

In recent years, high-throughput sequencing technology has
been used to study the entire genome (Zhu et al., 2014; Han et al., 2011). As a form of deep sequencing technology, RNA sequencing has been widely applied to research at the transcriptional level (Wang et al., 2009; Landolt et al., 2016). For example, transcriptomic analysis has been used for research into quantitative gene expression regarding the biological processes of cells and tissues.

B-3 exopolysaccharide was extracted from an Antarctic psychrophilic bacterium, Psychrobacter sp. B-3, which was isolated from ice samples collected at the Uruguay Station (62°11'50.52”S, 58°55'50.4"W) during the 24th Chinese National Antarctic Research Expedition. We previously showed that B-3 exopolysaccharide is composed of two monosaccharides, including mannose and glucose, and has a molecular weight of 5,400 Da. An immunomodulation assay showed that B-3 exopolysaccharide activates RAW264.7 macrophages and affects their immunomodulatory activity, for example, by increasing the production of tumor necrosis factor-α and nitric oxide (NO) (Yu et al., 2016). Based on our previous work, the present study used transcriptomic analysis to analyze the effects of B-3 exopolysaccharide on the gene expression of RAW264.7 cells. This is the first time that the effect of an exopolysaccharide from an Antarctic bacterium on RAW264.7 macrophage global gene expression has been determined. These results will provide an important reference for in-depth and comprehensive studies regarding activated macrophages and the changes to their immunomodulatory activity that are induced by polysaccharides from various sources.

2 Materials and methods

2.1 Sample handling

RAW264.7 cells were purchased from KeyGEN BioTECH (Nanjing, China), and cultured in DMEM medium (Gibco, Waltham, MA, USA) containing 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO₂. Cells were seeded into six-well plates at a density of 2×10⁴ cell/mL in 1 mL medium per well. Next, 3.3 μL of 3 mg/mL Polyomixin B (20 μg) was added to each well and incubated for 30 min. Then, 1 mL of diluted B-3 exopolysaccharide was added to two wells at a final concentration of 2 μg/mL, while 1 mL of complete medium was added to each of two other wells as the control group. The plates were incubated for 24 h because we previously showed that 0.02–20 μg/mL B-3 exopolysaccharide enhanced cell proliferation, phagocytosis, and NO production, and NO production after stimulation for 24 h or 48 h (Yu et al., 2016).

2.2 RNA extraction and quality determination

Total RNA of four samples was extracted using an ultrapure RNA extraction kit (Kangwei Shiji Biological Technology Co., Ltd., Beijing, China) and stored at −80°C for later use. Before freezing, a sample was separated via 1% agarose gel electrophoresis at 180 V for 16 min. Concentration was measured by a NanoDrop spectrophotometer; integrity and purity were tested using the Agilent 2100 bioanalyzer and NanoDrop spectrophotometer, respectively.

2.3 cDNA library construction and sequencing

Oligo (dT) magnetic beads were used for mRNA enrichment. mRNA was broken into short pieces and used as a template. First-strand cDNA was synthesized from this using random hexamer primers; other components of PCR amplification were added for second-strand cDNA synthesis. Double-stranded cDNA was purified by AMPure XP beads, then ends were repaired, a poly-A tail was added, and sequencing joints were connected. AMPure XP beads were used to select the correct fragment sizes, and PCR was performed to enrich the cDNA library.

The Qubit2.0 fluorometer, Agilent 2100 bioanalyzer, and quantitative PCR were used respectively for initial quantitative detection, insert size detection, and accurate concentration quantification (effective concentration of the library ≥2 nmol/L). The SE50 strategy was used for sequencing. Sequencing and subsequent data analysis were performed by Novogene (Beijing, China).

2.4 Data analysis

The Illumina HiSeq™2500 sequencing platform was used for high-throughput sequencing. The original raw image data file was converted by base calling into sequenced reads, using CASAVA base recognition. Clean reads were obtained by removing belt joints and low-quality reads, and then used for subsequent analysis. Filtered clean sequences were aligned with the reference sequence using TopHat software for genome positioning analysis. The positioning number (total mapped reads) and clean reads percentage were obtained. Reads per kilobase of transcript per million mapped reads (RPKM), which considers both the depth and length of the gene sequence, was used to estimate the gene expression level (Mortazavi et al., 2008).

We adopted DESeq (Anders and Huber, 2010) for the analysis of differentially expressed genes. The screening standard for differentially expressed genes was \( p_{adj}<0.05 \). Gene Ontology (GO) and Kyoko Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed for differentially expressed genes.

2.5 Gene function annotation

The following databases were used for gene function annotation: Ensembl (http://www.asia.ensembl.org), GO (http://www.geneontology.org), and KEGG (http://www.genome.jp/kegg/pathway.html).

2.6 Real-time PCR

RAW264.7 cells were stimulated by 2 μg/mL B-3 exopolysaccharide for 24 h. A total of 3 μg RNA per sample was reverse transcribed into cDNA using Invitrogen MMLV retrovirus kits, following manufacturer’s instructions. Quantitative PCR was performed using the IQ5 real-time multiple fluorescent quantitative PCR system (Bio-Rad, Hercules, CA, USA). Primer sequences are listed in Table A1. Reaction mixtures (15 μL) contained 4.5 μL double-distilled water, 7.5 μL 2X QuantiFast SYBR Green PCR Master Mix (Qiagen, Beijing, China), 1 μL upstream and downstream primers (6 μmol/L), and 1 μL cDNA. PCR conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 10 s, and 60°C for 30 s. Each experiment was performed in triplicate. GAPDH was chosen as a reference gene. The 2⁻^ΔΔCt method was used for relative quantitation.

2.7 Statistical analysis

SPSS17.0 software was used for statistical analysis, and significant differences between means were analyzed using Student’s t-test. Values of \( p<0.05 \) were regarded as statistically significant.

3 Results and discussion

3.1 Evaluation and analysis of sequencing data

To study the effect of B-3 exopolysaccharide on RAW264.7 cell gene expression, cells were treated for 24 h with 2 μg/mL B-3 exopolysaccharide. Sequencing results are summarized in Table 1.
Table 1. Sequencing data output quality list

| Sample name | Raw reads | Clean reads | Clean bases | Error rate/% | Q20/% | Q30/% | GC content/% |
|-------------|-----------|-------------|-------------|--------------|-------|-------|--------------|
| MRCN2       | 11564799  | 11558830    | 0.58G       | 0.01         | 98.48 | 97.05 | 50.64        |
| MRCN3       | 12618276  | 12611092    | 0.63G       | 0.01         | 98.50 | 97.08 | 50.79        |
| MRCP2       | 13197215  | 13189345    | 0.66G       | 0.01         | 98.53 | 97.15 | 51.27        |
| MRCP3       | 11969746  | 11962711    | 0.66G       | 0.01         | 98.52 | 97.13 | 50.82        |

Note: MRCN2, MRCN3, MRCP2 and MRCP3 indicate the samples of Control group 1, Control group 2, Experimental group 1 and Experimental group 2, respectively.

The original data received from the control and experimental groups were 11564799, 12618276 and 13197215, 11969746, respectively, including clean sequences of 11558830, 12611092 and 13189345, 12611092, respectively. Each accounted for 99.9% of the original data, and the sequencing error rate was only 0.01%.

The percentages of total sequence positioning to the genome for MRCN2, MRCN3, MRCP2, and MRCP3 were 94.35%, 94.22%, 93.68%, and 94.35%, respectively, while sequences with the sole position comprised 81.22%, 81.22% and 80.8%, 80.96%, respectively. Only a small number of sequences were found in multiple alignments. Comparing the distribution of reads with the genome, most sequences matched to exons, followed by introns and intergenic regions. Clean reads were used for subsequent GO and KEGG analyses of gene expression.

3.2 Quantitative analysis of gene expression and differentially expressed genes

RPKM was used to evaluate gene expression levels (Mortazavi et al., 2008) via the standard RPKM>1. HTSeq software was used to analyze gene expression levels of various samples via the union model. A Venn diagram of gene expression was drawn to depict the quantitative results of gene expression, as shown in Fig. 1. A total of 209 genes were only expressed in the B-3 exopolysaccharide-treated group (MRCP), while 214 were only expressed in the control group (MRCN); 10 567 genes were expressed in both groups. Differentially expressed genes were screened using the standard of genetic variations, \( p_{adj} < 0.05 \), as a filter. A total of 420 genes were differentially expressed between MRCP and MRCN, of which 178 were up-regulated while 242 were down-regulated, as shown in Fig. 2. These genes were involved in diverse functions within the cell.

3.3 Real-time quantitative PCR validation

To verify our preliminary results that B-3 polysaccharide treatment changed the expression of an extensive proportion of RAW264.7 macrophage genes involved in different biochemical pathways, we selected four differentially expressed genes that are representative of different biochemical pathways for quantitative PCR analysis: \( Ace1 \) (encoding aminoaacylase 1), \( Nos2 \), \( Pgm5 \) (encoding phosphoglucomutase 5), and \( Pik3c2b \) (encoding phosphoinositide-3-kinase, class 2, beta polypeptide). \( Ace1 \) and \( Pgm5 \) are mainly involved in substance metabolism, while \( Nos2 \) and \( Pik3c2b \) have roles in immune-related pathways. Our quantitative PCR results were consistent with differential expression findings. \( Nos2 \) and \( Ace1 \) were upregulated, while \( Pgm5 \) and \( Pik3c2b \) were downregulated after treatment with B-3 exopolysaccharide, as shown in Fig. 3.

3.4 GO enrichment analysis

GO is the international standard classification system of gene function that is mainly divided into the three categories of Cellular Component, Molecular Function, and Biological Process.
**Selected genes expression level relative to control**

- **a** Nos2 Acy1 Pik3c2b Pgm5
- **b** control

* 2 μg/mL B-3

**Fig. 3.** Histogram of selected differentially expressed genes, constructed using GraphPad Prism 6. a. Quantitative PCR results using the 2−△△CT method and b. differential gene expression. Gene reads per kilobase of transcript per million mapped reads (RPKM) ratios of experimental groups relative to control groups. Student’s t-test was used for statistical analysis with SPSS17.0 software. * p<0.05.

**enrichment analysis of differentially expressed genes identified in the present study was performed via GOseq software (Young et al., 2010), which annotated 319 differentially expressed genes. We selected the 30 most significantly enriched GO terms, as shown in Fig. 4. Among them, the immune response (GO: 0006955) and immune system process (GO: 0002376) were the most significantly enriched within the Biological Process category. Eleven genes in these two GO terms were differentially expressed, of which six were up-regulated and five were down-regulated. Dephosphorylation (GO: 0016311) was another significantly enriched GO term within Biological Process, while catalytic activity (GO: 0003824) was the most enriched GO term within Molecular Function. A total of 154 genes in this GO term were differentially expressed, of which 60 were up-regulated and 94 were down-regulated. Enzyme activities (GO: 00030234) was another significantly enriched GO term within Molecular Function, which revealed 16 GO differentially expressed genes (seven up-regulated and nine down-regulated), as well as GTP combination (GO: 0005523), which showed 16 GO differentially expressed genes (six up-regulated and 10 down-regulated), and amidine base nucleotide (GO: 0032561), which revealed 16 GO differentially expressed genes (six up-regulated and 10 down-regulated). Cell migration regulation, cell adhesion regulation, phosphatase activity, nicotinamide adenine dinucleotide (NAD) layer synthesis, metabolism, protein complex, and tumor necrosis factor receptor were also significantly enriched GO terms.

To determine which catalytic activities of enzymes were involved in the GO term catalytic activity (0003824), this term was further characterized. The differentially expressed gene enrichment numbers of this GO term are listed in Table 2. The three most enriched categories were hydrolase activity, transferase activity, and oxidoreductase activity. As examples, Nos2 encodes an oxidoreductase, Acy1 encodes a hydrolase, and Pik3c2b encodes a hydrolase/transferase. Genes with enzyme catalytic activity involved in this GO term participated in the synthesis, processing, and metabolism of a variety of cellular substances. This suggests that B-3 exopolysaccharide greatly changed the metabolism of RAW264.7 cells. This may be because B-3 exopoly-
Among all differentially expressed genes, 154 were involved in the catalytic activity (0003824) GO term, and 43 were involved in metabolic pathways (mmu01100) of the KEGG analysis. This indicates that stimulation by B-3 exopolysaccharide affected multiple immune-related pathways of macrophages, such as antigen processing and presentation, the Toll-like receptor signaling pathway, and leukocyte transendothelial migration. The Jak-STAT signaling pathway and NF-kappa B signaling pathway were also significantly enriched by B-3 stimulation. These two pathways affect macrophage proliferation, differentiation, and immune regulation (Rawlings et al., 2004; Hayden and Ghoosh, 2011).

Among all differentially expressed genes, 154 were involved in the catalytic activity (0003824) GO term, and 43 were involved in metabolic pathways (mmu01100) of the KEGG analysis. This indicated that B-3 exopolysaccharide has a significant effect on RAW264.7 cell metabolism. Macrophage metabolism is known to be closely associated with immune function (Ghesquière et al., 2011).

### Table 2. Enzyme catalytic activity involved in GO enrichment

| GO: accession | Description                  | DEG_item (up:down) |
|--------------|------------------------------|--------------------|
| GO: 0003824  | catalytic activity           | 154 (60:94)        |
| GO: 0008987  | quinolinate synthetase A activity | 1 (1:0)            |
| GO: 0009975  | cyclase activity             | 1 (0:1)            |
| GO: 0016491  | oxidoreductase activity      | 29 (11:18)         |
| GO: 0016740  | transferase activity         | 49 (15:34)         |
| GO: 0016787  | hydrolase activity           | 66 (27:39)         |
| GO: 0016829  | lyase activity               | 7 (4:3)            |
| GO: 0016853  | isomerase activity           | 7 (2:5)            |
| GO: 0016874  | ligase activity              | 7 (4:3)            |

Note: DEG_item represents the number of differentially expressed genes related to the GO term, up up-regulated genes, and down down-regulated genes.

### Table 3. Immune-related signaling pathways in KEGG enrichment

| Term                              | ID       | Input number | Background number |
|-----------------------------------|----------|--------------|-------------------|
| Antigen processing and presentation| mmu04612 | 10           | 81                |
| Fc gamma R-mediated phagocytosis   | mmu04666 | 9            | 88                |
| Natural killer cell mediated cytotoxicity | mmu04650 | 10           | 119               |
| B cell receptor signaling pathway  | mmu04662 | 7            | 73                |
| T cell receptor signaling pathway  | mmu04660 | 8            | 105               |
| Cytosolic DNA-sensing pathway      | mmu04623 | 5            | 63                |
| RIG-I-like receptor signaling pathway | mmu04622 | 5            | 68                |
| Toll-like receptor signaling pathway | mmu04620 | 6            | 101               |
| Fc epsilon RI signaling pathway    | mmu04664 | 4            | 70                |
| Leukocyte transendothelial migration | mmu04670 | 6            | 121               |
| Chemokine signaling pathway        | mmu04062 | 8            | 196               |
| Complement and coagulation cascades | mmu04610 | 3            | 77                |
| NOD-like receptor signaling pathway | mmu04621 | 2            | 58                |
| Intestinal immune network for IgA production | mmu04672 | 1            | 42                |

Note: ID represents unique serial number of pathway in KEGG database; input number number of differentially expressed genes related to this pathway, and background number total number of genes related to this pathway.
For example, metabolites and metabolic enzymes regulate immune cell plasticity by affecting gene transcription, signaling pathways, or epigenetics (Ghesquière et al., 2014). Nos2 is a type of oxidoreductase and inducible NO synthase that catalyzes substrates to generate NO, while Acy1 is a hydrolase that catalyzes the hydrolysis of acylated L-amino acids into L-amino acids and an acyl group (https://en.wikipedia.org/wiki/ACY1). Acy1 also participates in the synthesis of L-arginine, which, together with NADPH, is a precursor molecule of NO (Aktan, 2004). NO and Nos2 play complex roles in innate and adaptive immunity (Bogdan, 2015), and immune cell metabolic activity is known to be closely linked to its immune function.

Our sequencing and quantitative PCR results showed that B-3 exopolysaccharide stimulation upregulated Nos2 and Acy1 in RAW264.7 cells. The resulting increased NO synthesis would further adjust the immune reactivity of RAW264.7 cells.

### 4 Conclusions

B-3 exopolysaccharide stimulation was found to alter the expression of a large array of genes in RAW264.7 macrophages. It also dramatically changed the metabolism of various substances and affected multiple signaling pathways, especially immune-related pathways. These results reveal that changes in metabolic activity caused by B-3 exopolysaccharide are closely linked with its immune function. This study provides a reference for research into the regulation of macrophage immune function by polysaccharides.

### References

Aktan F. 2004. iNOS-mediated nitric oxide production and its regulation. Life Sci, 75(6): 639–653

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. Genome Biol, 11: R106

Arena A, Gugliandolo C, Stassi G, et al. 2009. An exopolysaccharide produced by Geobacillus thermodenitrificans strain B3-72: anti-viral activity on immunocompetent cells. Immunol Lett, 123(2): 132–137

Blum J S, Wearsch P A, Cresswell P. 2013. Pathways of antigen processing. Annu Rev Immunol, 31(1): 443–473

Bogdan C. 2015. Nitric oxide synthase in innate and adaptive immunity: an update. Trends Immunol, 36(3): 161–178

Ghesquière B, Wong B W, Kuchnio A, et al. 2014. Metabolism of stromal and immune cells in health and disease. Nature, 511(7508): 167–176

Han Hong, Nutiu R, Moffat J, et al. 2011. SnapShot: high-throughput sequencing applications. Cell, 146(6): 1044–1044.e2

Hayden M S, Ghosh S. 2011. NF-kB in immunobiology. Cell Res, 21: 223–244

Inatsuka C, Yang Y, Gad E, et al. 2013. Gamma delta T cells are activated by polysaccharide K (PSK) and contribute to the anti-tumor effect of PSK. Cancer Immunol Immunother, 62(8): 1335–1345

Kanehisa M, Araki M, Goto S, et al. 2008. KEGG for linking genomes to life and the environment. Nucleic Acids Res, 36: D480–D484

Kelly B, O’Neill L A J. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res, 25(7):
Landolt L, Marti H P, Beisland C, et al. 2016. RNA extraction for RNA sequencing of archival renal tissues. Scandinavian Journal of Clinical and Laboratory Investigation, 76(5): 426–434

Lee J W, Seo K H, Won Ryu H, et al. 2018. Anti-inflammatory effect of stem bark of *Paulownia tomentosa* Steud. in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and LPS-induced murine model of acute lung injury. Journal of Ethnopharmacology, 210: 23–30

Lemieszek M, Rzeski W. 2012. Anticancer properties of polysaccharides isolated from fungi of the Basidiomycetes class. Contemp Oncol (Pozn), 16(4): 285–289

Leung M Y K, Liu C, Koon J C M, et al. 2006. Polysaccharide biological response modifiers. Immunol Lett, 105(2): 101–114

Li Bing, Cramer D, Wagner S, et al. 2007. Yeast glucan particles activate murine resident macrophages to secrete proinflammatory cytokines via MyD88- and Syk kinase-dependent pathways. Clin Immunol, 124(2): 170–181

Li Min, Yan Yixi, Yu Qingtiao, et al. 2017. Comparison of immunomodulatory effects of fresh garlic and black garlic polysaccharides on RAW 264.7 macrophages. Journal of Food Science, 82(3): 765–771

Mortazavi A, Williams B A, McCue K, et al. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods, 5(7): 621–628

Rawlings J S, Rosler K M, Harrison D A. 2004. The JAK/STAT signaling pathway. Journal of Cell Science, 117(8): 1281–1283

Reilh C, Brieger A, Engelhardt G, et al. 2017. Zinc chelation decreases IFN-β-induced STAT1 upregulation and iNOS expression in RAW 264.7 macrophages. Journal of Trace Elements in Medicine and Biology, 44: 76–82

Schepetkin I A, Quinn M T. 2006. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. Int Immunopharmacol, 6(3): 317–333

Stier H, Ebbeskotte V, Gruenwald J. 2014. Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. Nutr J, 13: 38

Tseng K C, Fang T J, Chiang S S, et al. 2012. Immunomodulatory activities and antioxidant properties of polysaccharides from *Monascus*-fermented products *in vitro*. Journal of the Science of Food and Agriculture, 92(7): 1483–1489

Vyas J M, Van der Veen A G, Ploegh H L. 2008. The known unknowns of antigen processing and presentation. Nat Rev Immunol, 8(8): 607–618

Wakabayashi T, Hirokawa S, Yamauchi N, et al. 1997. Immunomodulating activities of polysaccharide fractions from dried safflower petals. Cytotechnology, 25: 205–211

Wang Zhong, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet, 10(1): 57–63

Wang Wei, Wang Shixin, Guan Huashi. 2012. The antiviral activities and mechanisms of marine polysaccharides: an overview. Mar Drugs, 10: 2795–2816

Wasser S P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol, 60(3): 258–274

Won D P, Lee J S, Kwon D S, et al. 2011. Immunostimulating activity by polysaccharides isolated from fruiting body of *Inonotus obliquus*. Mol Cells, 31(2): 165–173

Young M D, Wakefield M J, Smyth G K, et al. 2010. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol, 11(2): R14

Yu Leije, Sun Guojie, Wei Jingfang, et al. 2016. Activation of macrophages by an exopolysaccharide isolated from Antarctic *Psychrobacter sp.* B-3. Chinese Journal of Oceanology and Limnology, 34(5): 1064–1071

Zhu Qianglong, Liu Shi, Gao Peng, et al. 2014. High-throughput sequencing technology and its application. Journal of Northeast Agricultural University, 21(3): 84–96
Appendix:

**Table A1.** Genes primers sequences

| Gene ID               | Gene | Forward primer (5’ to 3’)         | Reverse primer (5’ to 3’)       |
|-----------------------|------|-----------------------------------|---------------------------------|
| ENSMUSG000000023262   | Acy1 | AAGGATTCCGAGGGCTAC                | TCGTCAGGCACAAAGGTC              |
| ENSMUSG000000020826   | Nos2 | CAGGGACGAGACGGATG                 | CATGCAAGGAAGGAACCT              |
| ENSMUSG000000026447   | Pik3c2b | GCAACACCTGGCAATAAC              | ACGCAGCACTTTCTCCTCAT            |
| ENSMUSG000000041731   | Pgm5 | CCCAGGAAGATACCAGC                | CCCAGGAGAATACCCAGC             |
Phylogenetically diverse, acetaldehyde-degrading bacterial community in the deep sea water of the West Pacific Ocean

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Received 14 December 2017; accepted 13 March 2018

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Abstract
As a major aldehyde pollutant widely existing in industry and our daily life, acetaldehyde is more and more harmful to human health. As characteristic habitat niche, bacteria from deep sea environments are abundant and distinctive in heredity, physiology and ecological functions. Thus, the development of acetaldehyde-degrading bacteria from deep sea provides a new method to harness acetaldehyde pollutant. Firstly, in this study, acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean were enriched in situ and in the laboratory respectively, and then the diversity of uncultured bacteria was studied by using 16S rRNA genes. Then acetaldehyde-degrading strains were isolated from two samples, including enrichment in situ and enrichment in laboratory samples of deep sea water from the West Pacific Ocean using acetaldehyde as the sole carbon source, and then the ability of acetaldehyde degradation was detected. Our results showed that the main uncultured bacteria of two samples with different enrichment approaches were similar, including Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria, but the structure of bacterial community were significant different. Four subgroups, α, γ, δ and ε, were found in Proteobacteria group. The γ-Proteobacteria was dominant (63.5% in laboratory enriched sample, 75% clones in situ enriched sample). The species belonged to γ-Proteobacteria and their proportion was nearly identical between the two enrichment samples, and Vibrio was the predominant genus (45% in laboratory enriched sample, 48.5% in situ enriched sample), followed by Halomonas (9% in situ enriched sample) and Streptococcus (6% in laboratory enriched sample). A total of 12 acetaldehyde-degrading strains were isolated from the two samples, which belonged to Vibrio, Halomonas, Pseudomonas, Pseudoalteromonas and Bacillus of γ-Proteobacteria. Strains ACH-L-5, ACH-L-8 and ACH-S-12, belonging to Vibrio and Halomonas, have strong ability of acetaldehyde degradation, which could tolerate 1.5 g/L acetaldehyde and degrade 350 mg/L acetaldehyde within 24 hours. Our results indicated that bacteria of γ-Proteobacteria may play an important role in carbon cycle of deep sea environments, especial the bacteria belonging to Vibrio and Halomonas and these strains was suggested for their potentials in government of aldehyde pollutants.

Key words: acetaldehyde-degrading bacteria, ALDH, deep sea, in situ

Citation: Gao Boliang, Shang Xiezie, Li Li, Di Wenjie, Zeng Runying. 2018. Phylogenetically diverse, acetaldehyde-degrading bacterial community in the deep sea water of the West Pacific Ocean. Acta Oceanologica Sinica, 37(8): 54–64, doi: 10.1007/s13131-018-1235-z

1 Introduction
Deep sea is the biggest Gene Bank on the earth, which contains abundant marine microorganisms. Previous studies paid great attentions to the marine microorganisms in shallow sea, and many marine microorganisms have been reported (Okami et al., 1976; Okazaki et al., 1975; Ding and Valentine, 2008). Compared with a lot of research attentions on marine microorganisms in shallow sea, the investigation of marine microorganisms in deep sea is relatively lacking. Deep sea has extremely heavy climate and environment, for example strong acid and base, high or low temperature, high pressure. Therefore, marine microorganisms of deep sea are significantly different with microorganisms from land including genetic constitution, metabolism regulation, metabolite, chemical defense, and many microorganisms which are acidophilic, basophilic, haloduric and so on (Horikoshi, 1998; Ista et al., 2004; Koyama and Yoshida, 2016). The refractory organisms which come from upper layer of the ocean and bottom sediments are the main nutrient source of microorganisms in deep sea (Horikoshi, 1998; Gao et al., 2017). Thus the microorganisms in deep sea have potential of environment government, for example apply in industrial wastewater treatment. Meanwhile, the potential application of microorganisms of deep sea in biomedicine, new biomaterial was noticed. As exhaustion of land resources, the marine microorganisms, especially the microorganisms of deep sea will be one of spot for exploitation.
The pollutant of aldehydes widely exists in the environment, for example methanol, acetaldehyde, acraldehyde (Zhou et al., 2005; Gesser and Fu, 1990; Xi et al., 1999). Previous studies have reported that the superfluous acetaldehyde in furniture materials have been noticed by people, and that is harmful to humans (Homann et al., 2000). While acetaldehyde dangers have not been noticed yet by people. Acetaldehyde is toxic when applied externally for prolonged periods, an irritant, and a probable carcinogen. At present, enzymatic degradation by the acetaldehyde dehydrogenase is the main efficient way to degrade acetaldehyde (Lubin and Westerfield, 1945; Svegliati-Baroni et al., 2001). Unfortunately, the extraction of acetaldehyde dehydrogenase is costly and difficult. Furthermore, as the complex chemical constituents of acetaldehyde industrial wasted water, gas and residue, such as high heavy metal, strong acid and alkali, so the degradation of acetaldehyde by the acetaldehyde dehydrogenase is infeasible (van der Ploeg et al., 1994; Lachenmeier and Sohnius, 2008; Kurkiviuru et al., 2007). To address this issue, recent studies have more focused on microbial catalyzing, especially the bacteria in deep sea.

Although technique of microbial pure culture which obtains microbial pure culture by isolation, purification, culture of microorganism has encountered un-overstepped obstacle for studying diversity of microorganism, it is irreplaceable for study of cellular structure, physiology, genetics and application of microorganism. To isolate microorganisms as far as possible, the technique of microbial pure culture was improved. New technique without high concentration of nutrients and manual preparation could provide primitive ecology for microorganisms, and the enrichment of microorganisms in situ is one of frequently used techniques. Kaebberlein et al. (2002) have obtained many more stains used this technique than traditional separation methods. Furthermore, molecular ecological techniques have recently been used to study bacterial diversity in situ (Kirk et al., 2004). This stems from the fact that many microorganisms are not easily obtainable as cultured strains in laboratory, which would allow for identification and characterization.

In this study, with the aim to know about the acetaldehyde-degrading bacterial phylogenetic diversity and to search strains with the potential application of acetaldehyde degradation in the deep sea water of the West Pacific Ocean, enrichment in situ and in the laboratory respectively, the uncultured and cultured bacterial phylogenetic diversity and the ability of acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean were investigated. Besides, the potential application of acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean was suggested.

2 Materials and methods

2.1 Sampling

Enrichment in laboratory: Deep sea water was sampled at the depth of 2 000 m in the West Pacific Ocean (21°03′30″N, 118°23′17″E) in September 2012. A total of 30 mL deep sea water was loaded in 50 mL reagent bottle with 5 g absorbing 40% acetaldehyde of active carbon, and the reagent bottle was stored in a cold storage (8°C) for 6 months.

Enrichment in situ: regent bottles were placed at the depth of 2 000 m in the West Pacific Ocean (21°03′30″N, 118°23′17″E) in September 2012. There were active carbon granules which absorbed 40% acetaldehyde in these regent bottles, and some pores were on these regent bottles for internal and external material exchange, and bacteria were enriched in situ for six months.

2.2 Total genomic DNA extraction, PCR amplification of 16S rRNA gene, and clone library structure

Total genomic DNA was extracted from two kinds of samples, respectively, using PowerSoil DNA Isolation Kit (MOBIO). The quality and concentration of the soil DNA were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). The overall size of the total DNA was checked by running an aliquot of DNA on a 1.0% agarose gel, and then was stored in -20°C until further processing.

The resulting genomic DNA was used as templates to amplify the bacterial 16S rRNA gene fragment using common primers 27F and 1492R. The PCR mixture (50 μL) contained 2 μL DNA template, 5 μL 10×Taq buffer (Mg²⁺), 4 μL dNTP mixture (2.5 mmol/L), 1 μL of each primer (10 μmol/L), 0.5 μL EXTaq DNA polymerase (5 U/μL). The PCR program was carried out with 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 90 s, annealing at 55°C for 90 s and elongation at 72°C for 90 s, and final elongation at 72°C for 10 min. PCR products were purified using Gel Extraction Kit (Omega). PCR products were ligated into pT7-2 Vector and transformed into E. coli DH5α. PCR was performed to detect whether the picked clones were positive recombinants. Two different clone libraries were structured. A total of 200 positive clones were selected from each clone library and sequenced using primers T3 and T7 by Shanghai Majorbio Company.

2.3 16S rRNA genes phylogenetic diversity and bacterial diversity analysis

After removing the sequences of plasmid vector, 1 400–1 500 bp 16S rRNA genes were obtained in this study, and were checked chimera on the DECIPHER-Find Chimeras. These sequences were aligned on the RdP Pipeline Tools to identify sequences similarity. Sequences with similarity not less than 97% were classified as one OTUs, while similarity less than 97% were classified different OTUs. The representative sequences were selected, and then phylogenetic trees based on gene sequences of 16S rRNA were constructed using neighbor-joining method in MEGA 5.0 combined with bootstrap analysis setting with 1 000 replications, respectively. Coverage estimator based on Coverage C (Chao and Lee, 1992) and species richness was assessed with Chao1 (Chao, 1984), while evenness and diversity of species were estimated by Evenness index (E') and Shannon diversity index (H') (Chao and Shen, 2003).

2.4 Isolation and identification of cultivable acetaldehyde-degrading bacteria

To further screen cultivable acetaldehyde-degrading bacteria, acetaldehyde was used as sole carbon source for the growth of acetaldehyde-degrading bacteria. Two different samples (5 g) were vortex shocked with 5 min, and then serial 10-fold dilutions (each sample in sterile sea water) were plated on the corresponding solid modified 2216E (0.1% yeast extract, 3% agar powder, 720 mg/L acetaldehyde and sea water) and incubated for 48 h at 37°C. Subsequently, individual bacteria were removed from agar plates, placed on new medium and checked for purity and stored in 20% glycerol at ~80°C.

The genomic DNA of acetaldehyde-degrading bacteria was extracted using TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0. The resulting genomic DNA was used as templates to amplify the bacterial 16S rRNA gene fragment using common primers 27F and 1492R. PCR products were sequenced in Shanghai Majorbio Company after analysis by electrophoresis on 1.5% agarose gel. A total of 1 400–1 500 bp of Bacterial 16S
rRNA genes sequences were obtained in this study and aligned on the NCBI database. The most appropriate relative sequences were selected and it can be sure genus or family of bacteria until the similarity not less than 99%.

2.5 Detection of acetaldehyde-degrading ability of cultivable acetaldehyde-degrading bacteria

To detect the tolerance of cultivable acetaldehyde-degrading bacteria toward acetaldehyde, bacterial cells were grown in 5 mL 2216E with different concentrations of acetaldehyde (500 mg/L, 800 mg/L, 100 mg/L, 1 500 mg/L, and 2 000 mg/L) at 37°C, 200 r/min. The OD_{560} was detected after 48 h to determine whether these bacteria could tolerate the acetaldehyde.

To further investigate the abilities of acetaldehyde-degradation of these bacteria, acetaldehyde was used as sole carbon for the growth of these bacteria (1 g/L NH\textsubscript{4}NO\textsubscript{3}, 500 mg/L acetaldehyde and sea water). Acetaldehyde-degrading bacteria cells were cultured at 37°C, 200 r/min for 24 h in 2216E medium. The cultures were centrifuged at 10 000 g for 10 min, and the bacterial pellets were washed twice with sterile medium (1 g/L NH\textsubscript{4}NO\textsubscript{3}, and sea water), and then resuspended in medium (1 g/L NH\textsubscript{4}NO\textsubscript{3} and sea water). Subsequently, acetaldehyde-degrading bacteria (2% inoculation amount) were cultured in medium (1 g/L NH\textsubscript{4}NO\textsubscript{3}, 500 mg/L acetaldehyde and sea water) at 37°C, 200 r/min for 24 h and the medium without acetaldehyde-degrading bacteria were used as the blank control. Then, the cultures were centrifuged at 10 000 g for 10 min, and the supernatants were collected to analysis acetaldehyde using HPLC. Previous reports have described the method of detecting acetaldehyde, 200 μL 1 000 mg/L of 2, 4-dinitrophenylhydrazine (DNPH) and 20 μL acetic acid was added to each of 200 μL samples supernatants and incubated for 15 min at 37°C for derivatization. Each of samples was mixed with 1 200 μL extraction agent (ethyl acetate:n-hexane=1:5, v/v) for vortex shocked 5 min, and then sat for 30 min, supernatants were collected and then were vacuum-dried for 6 h using a speed vacuum concentrator (Labconco, Kansas City, MO, USA). The derivative samples were re-dissolved in solution (acetonitrile:water=85:15, v/v) and then analyzed by Agilent E2695 (Agilent Technologies, Wilmington, DE, USA) equipped with Acclaim 120 C18 (4.6 mm×250 mm) and 2998 PDA detector. Samples were analyzed at 40°C using ethyl acetate:n-hexane=1:5 (v/v) as a mobile phase at a flow rate of 1 mL/min. The concentration of the acetaldehyde-DNPH (ACH-DNPH) was determined by the calibration curves of peak areas vs. the standard curve of acetaldehyde-DNPH, which were obtained from HPLC analysis. In addition, the concentration of acetaldehyde in the blank control was detected to calculate the volatilization of acetaldehyde during cultivation.

2.6 Nucleotide sequence accession number

Bacterial 16S rRNA sequences obtained in this study were deposited in GenBank under accession numbers KM873053-KM873119 and KM873133-KM873144.

3 Results

3.1 Uncultivated acetaldehyde-degrading bacterial community composition of the West Pacific Ocean’s deep sea in laboratory and in situ

To analyze acetaldehyde-degrading bacterial community in deep sea water of the West Pacific Ocean, two sample treatments including enrichment in laboratory and in situ were used. To further measure the bacterial diversity presented within two different samples, a series of alpha diversity indices were used (Table 1). Coverages were 91.5% and 92.5% for enrichment in laboratory and in situ, respectively, which showed these data were authentic. Shannon, Simpson, and Chao1 diversity indices account for evenness and richness as well as the total number of species obtained. The overall diversity of bacterial populations was showed with Shannon diversity index of 2.29 and 2.07, Evenness index of 0.654 9 and 0.597 2, Chao1 richness estimator of 101 and 40.75 in sample of enrichment in laboratory and in situ, receptively.

Table 1. Analysis of the acetaldehyde-degrading bacterial diversity in deep-sea water from the South China Sea

|                          | In laboratory | In situ |
|--------------------------|---------------|---------|
| Numbers of sequenced clones | 200           | 200     |
| Numbers of OTUs          | 33            | 32      |
| Coverages/\%             | 91.5          | 92.5    |
| Shannon diversity index (H’) | 2.29         | 2.07    |
| Evenness index (E’)       | 0.654 9       | 0.597 2 |
| Chao1                     | 101           | 40.75   |

As showed in Table 2, a total of 33 and 32 OTUs were obtained from 200 clones in sample of enrichment in laboratory and in situ, receptively. To identify these OTUs, BLASTn database was used for 16S rRNA gene identification, and then MEGA 5.0 was employed for phylogenetic analysis. In laboratory, three OTUs belonging to Vibrio genus were obtained: OTU ACH-14L-309 and ACH-14L-70 showed 99% and 97% 16S rRNA gene similarities to Vibrio harveyi ATCC BAA-1116, respectively. OTU ACH-14L-307 showed 98% 16S rRNA gene similarity to Vibrio crosstacist strain LGP 7, OTU ACH-14L-308 showed 99% 16S rRNA gene similarity to Streptococcus pseudopneumoniae IS7493, and OTU ACH-14L-306 showed 99% 16S rRNA gene similarity to Halomonas meridiana strain DSM 5425, OTU ACH-14L-67 showed 94% 16S rRNA gene similarity to Thalassobacter stenotrophicus strain 5SM22, OTU ACH-14L-86 exhibited 99% 16S rRNA gene similarity with Staphylococcus caprae strain ATCC 35338, OTU ACH-14L-287 showed 99% 16S rRNA gene similarity with Marinobacter vinificrum strain FBB, and two OTUs which belonged to Desulfituromusa genus were obtained, namely OTU ACH-14L-77 and ACH-14L-60 which have showed 97% and 96% 16S rRNA gene similarities to Desulfuromusa fertireducens strain 102 and Desulforhopalus singaporensec strain S’pore T1, respectively. In situ, two OTUs belonging to Vibrio genus were also obtained, namely OTU ACH-14S-98 and OTU ACH-14S-76 which have 99% 16S rRNA gene similarities to Vibrio harveyi ATCC BAA-1116 and Vibrio chagosii strain R-3712, respectively. OTU ACH-14S-95 showed 99% 16S rRNA gene similarity with Desulfuromusa ferrireductans strain 5SM22, OTU ACH-14S-85 exhibited 95% 16S rRNA gene similarity with Pelagibacillus litoralis strain CL-ES2. Four OTUs belonging to marinobacter genus were obtained: OTU ACH-14S-211, ACH-14S-74, ACH-14S-63 and ACH-14S-68. All of OTUs’ information in laboratory or in situ was shown in Table 2.

3.2 Comparing of uncultivated acetaldehyde-degrading bacterial diversity of the West Pacific Ocean’s deep sea in laboratory and in situ

Previous studies have showed that different ways of sample treatments could enrich different bacteria (Rochelle et al., 1994; Smalla et al., 2007). Eight bacterial phyla were detected, while two OTUs were unclassified. As showed in Fig. 1, the most predominant phyla was γ-proteobacteria (63.5%), followed by Firmicutes (14%), α-proteobacteria (6.5%), β-proteobacteria (5%), δ-
Table 2. Summary of the 16S rDNA sequences identified in the deep sea water of the South China Sea by enriching in situ or in laboratory.

| Accession No. | Nearest relative | Identity/% |
|---------------|-----------------|------------|
| KM873087      | Vibrio harveyi ATCC BAA-1116 (NR_102976.1) | 99%         |
| KM873094      | Vibrio chagassii strain R-3712 (NR_025480.1) | 99%         |
| KM873102      | Escherichia fergusonii ATCC 35469 (NR_074902.1) | 99%         |
| KM873115      | Haemophilus parainfluenzae ATCC 33392 (NR_042878.1) | 99%         |
| KM873108      | Sheuannella japonica strain KMM 3299 (NR_025012.1) | 99%         |
| KM873091      | Pseudoalteromonas donghaensis strain HS1 (NR_104537.1) | 97%         |
| KM873091      | Pseudoalteromonas phenolica strain O-BC30 (NR_028809.1) | 98%         |
| KM873114      | Alteromonas hispanica strain F-32 (NR_043274.1) | 97%         |
| KM873109      | Alteromonas macleodi strain 'Balearic Sea AD45 (NR_074797.1) | 99%         |
| KM873088      | Idiomarina solitaria OS145 (NR_027560.1) | 99%         |
| KM873105      | Colwellia psychrerythraea strain ATCC 27364 (NR_037047.1) | 98%         |
| KM873117      | Marinobacter adhaerens HPI5 (NR_074765.1) | 99%         |
| KM873095      | Marinobacter vinifirmus strain FB1 (NR_043666.1) | 99%         |
| KM873100      | Marinobacter algicola DG893 (NR_042807.1) | 98%         |
| KM873098      | Marinobacter algicola DG893 (NR_042807.1) | 99%         |
| KM873095      | Halomonas meridiana strain DSM 5425 (NR_042066.1) | 99%         |
| KM873093      | Jannaschia sp. CCS1 (NR_074163.1) | 91%         |
| KM873092      | Pelagibacter litoralis strain CL-ES2 (NR_044158.1) | 95%         |
| KM873111      | Sulfuromonas lithotrophicum strain 42BKT (NR_024802.1) | 95%         |
| KM873112      | Sulfurimonas autotrophica DSM 16294 (NR_074551.1) | 92%         |
| KM873097      | Arcobacter nitrofigilis DSM 7299 (NR_102873.1) | 94%         |
| KM873116      | Arcobacter nitrofigilis DSM 7299 (NR_102873.1) | 92%         |
| KM873090      | Desulfuribacillus spongaporensis strain S'pore T1 (NR_028742.1) | 96%         |
| KM873096      | Micrococcus luteus NCTC 2665 (NR_075062.1) | 99%         |
| KM873106      | Corynebacterium tuberculosis strain Medalle X (NR_028975.1) | 99%         |
| KM873101      | Propionibacterium acnes (NR_040847.1) | 98%         |
| KM873103      | Streptococcus mitis strain NS51 (NR_028664.1) | 99%         |
| KM873104      | Dolosigranulum pigrum strain B91/1468 (NR_026998.1) | 99%         |
| KM873119      | Jeotgalibacillus marinus strain 581 (NR_025351.1) | 96%         |
| KM873110      | Bacillus subterraneus strain COO3B (NR_104749.1) | 99%         |
| KM873118      | Bacillus niabensis strain 4T19 (NR_043334.1) | 98%         |
| KM873113      | Trichodesmium erythraeum IMS101 (NR_074275.1) | 87%         |
| KM873107      | Uncultured bacterium gene for 16S ribosomal RNA (AB250585.1) | 90%         |
| KM873054      | Vibrio harveyi ATCC BAA-1116 (NR_102976.1) | 99%         |
| KM873071      | Vibrio harveyi ATCC BAA-1116 (NR_102976.1) | 97%         |
| KM873056      | Vibrio crassostreae strain LGP 7 (NR_044078.1) | 98%         |
| KM873076      | Escherichia coli strain K-12 (NR_102804.1) | 99%         |
| KM873077      | Haemophilus parainfluenzae ATCC 33392 (NR_042878.1) | 99%         |
| KM873060      | Sheuannella japonica strain KMM 3299 (NR_025012.1) | 99%         |
| KM873064      | Pseudoalteromonas phenolica strain O-BC30 (NR_028809.1) | 97%         |
| KM873063      | Alteromonas macleodi strain 'Balearic Sea AD45 (NR_074797.1) | 99%         |
| KM873062      | Idiomarina baltica OS145 (NR_027560.1) | 99%         |
| KM873069      | Colwellia psychrerythraea strain ATCC 27364 (NR_037047.1) | 98%         |
| KM873061      | Marinobacter vinifirmus strain FB1 (NR_043666.1) | 99%         |
| KM873057      | Halomonas meridiana strain DSM 5425 (NR_042066.1) | 99%         |
| KM873081      | Neisseria meningitidis MC58 strain MC58 (NR_103915.1) | 98%         |
| KM873075      | Aureimonas alabamensis strain S21B (NR_043764.1) | 99%         |
| KM873072      | Thalassobacter stenothericus strain 55M22 (NR_027051.1) | 90%         |
| KM873084      | Marinella mobilis strain E6 (NR_044447.1) | 99%         |
| KM873070      | Pseudovibrio denitrificans strain DN34 (NR_029112.1) | 91%         |
| KM873058      | Sulfovirum sp. NBC37-1 (NR_074503.1) | 95%         |
| KM873085      | Arcobacter nitrofigilis DSM 7299 (NR_102873.1) | 99%         |

to be continued
Continued from Table 2

| OTUs      | Number of clones | Accession No. | Nearest relative                                      | Identity/% |
|-----------|-----------------|---------------|-------------------------------------------------------|------------|
| ACH-14L-207 | 1              | KM873086     | *Aerococcus nitrofigilis* DSM 7299 (NR_102873.1)       | 94%        |
| ACH-14L-77  | 1              | KM873068     | *Desulfuromusa ferrireducens* strain 102 (NR_043214.1) | 97%        |
| ACH-14L-60  | 7              | KM873074     | *Desulforhopalus stagnoporensis* strain S’pore T1 (NR_028742.1) | 96%        |
| ACH-14L-310 | 1              | KM873053     | *Kocuria hiflizophila* DC2201 (NR_074786.1)            | 99%        |
| ACH-14L-245 | 1              | KM873083     | *Rotia muclugirosa* DY-18 (NR_074693.1)                | 96%        |
| ACH-14L-12  | 1              | KM873080     | *Veillonella cretii* strain ATCC 17747 (NR_025046.1)    | 96%        |
| ACH-14L-308 | 12             | KM873055     | *Streptococcus pseudopneumoniae* IS7493 (NR_074987.1) | 99%        |
| ACH-14L-261 | 2              | KM873062     | *Gemella haemolysans* ATCC 10379 (NR_025903.1)         | 99%        |
| ACH-14L-86  | 10             | KM873066     | *Staphylococcus caprae* strain ATCC 35538 (NR_024655.1) | 99%        |
| ACH-14L-88  | 1              | KM873065     | *Bacillus arsenicus* strain Con a/3 (NR_042217.1)      | 98%        |
| ACH-14L-78  | 2              | KM873067     | *Bacillus subterraneus* strain COO13B (NR_104749.1)    | 99%        |
| ACH-14L-298 | 6              | KM873059     | *Trichodesmium erythraeum* IMS101 (NR_074275.1)        | 86%        |
| ACH-14L-30  | 1              | KM873079     | Uncultured *Compositomonas* sp. clone 1_145 (KF75866.1) | 98%        |

3.3 Isolation of acetaldehyde-degrading bacteria of the west Pacific Ocean’s deep sea in laboratory and in situ

To further screen and investigate the acetaldehyde-degrading bacteria of the West Pacific Ocean’s deep sea, we have isolated cultivable bacteria from deep sea water which were enriched in laboratory and in situ. In laboratory, a total of four acetaldehyde-degrading strains were obtained (Table 3). Two strains belonging to *vibrio* genus were obtained, namely, strain ACH-L-1 and strain ACH-L-2 which exhibited 99% 16S rRNA gene similarities with *vibrio harveyi* ATCC BAA-1116 and *vibrio rotiferianus* CAIM 577, respectively. One strain ACH-L-5 showed 99% 16S rDNA gene similarity with *halomonas axilaxis* strain Althf1 and another strain ACH-L-8 exhibited 99% 16S rDNA gene similarity with *halomonas meridiana* strain DSM 5425. While in situ, a total of eight acetaldehyde-degrading strains were isolated. Strain ACH-S-1 showed 99% 16S rDNA gene similarity to *vibrio rotiferianus* CAIM 577 and it was also obtained in laboratory. Another strain, namely, ACH-S-12 has shown 99% 16S rDNA gene similarity to *vibrio parahaemolyticus* RIMD 2210633. Strain ACH-S-6 which belonged to *halomonas* genus exhibited 16S rDNA gene similarity to *halomonas axilaxis* strain Althf1. Furthermore, two strains as well as ACH-S-7 and ACH-S-10 have showed 97% and 99% 16S rDNA gene similarity to *pseudoalteromonas piscicida* strain IAM 12932 and *pseudoalteromonas xanthomarina* strain KMM 1447, respectively. Strains ACH-S-8 and ACH-S-9 which showed 99% 16S rDNA gene similarity to *bacillus aquimaris* strain TF-12 were obtained. Another strain ACH-S-11 exhibited 99% 16S rDNA gene similarity to *pseudoalteromonas xanthomarina* strain KMM 1447. These results showed that bacteria belonging to *vibrio* and *halomonas* were the main bacterial population for acetaldehyde-degrading bacterial community in deep sea. Furthermore, these results also showed that bacteria of *vibrio* and *halomonas* genus are more suitable for cultivation in laboratory’s conditions than other bacteria.

3.4 Ability of acetaldehyde-degrading for cultivable acetaldehyde-degrading bacteria

To further investigate the ability of acetaldehyde-degrading of these strains, we have detected the tolerance of cultivable acetaldehyde-degrading bacteria suffering acetaldehyde and the degradation of acetaldehyde by using HPLC analysis. In Table 3, our results have showed that all of strains can grow in medium which contain high concentration of acetaldehyde (0.8–1.5 mg/mL). There were three strains, namely, ACH-L-5, ACH-L-8 and ACH-S-12 can tolerate 1.5 g/L acetaldehyde. Follow eight strains, ACH-L-2, ACH-S-1, ACH-S-6, ACH-S-7, ACH-S-10, ACH-S-11, ACH-S-8 and ACH-S-9 can suffer 1 g/L acetaldehyde. Strain ACH-L-1 can tolerate 0.8 g/L acetaldehyde. Then we have detected the tolerance of cultivable acetaldehyde-degrading bacteria suffering acetaldehyde and the degradation of acetaldehyde by using HPLC analysis. In Table 3, our results have showed that all of strains can grow in medium which contain high concentration of acetaldehyde (0.8–1.5 mg/mL). There were three strains, namely, ACH-L-5, ACH-L-8 and ACH-S-12 can tolerate 1.5 g/L acetaldehyde. Follow eight strains, ACH-L-2, ACH-S-1, ACH-S-6, ACH-S-7, ACH-S-10, ACH-S-11, ACH-S-8 and ACH-S-9 can suffer 1 g/L acetaldehyde. Strain ACH-L-1 can tolerate 0.8 g/L acetaldehyde. Then we have detected the ability of acetaldehyde-degrading of these strains by using acetaldehyde as sole carbon for growth. We have found that
Fig. 1. Phylogenetic analysis of uncultured bacteria which enrichment in laboratory based on 16S rRNA genes sequences. All of bacterial 16S rRNA genes sequences are identified by accession and their ID number or their genus on the tree. The tree was constructed by neighbor-joining method. Bootstrap values (expressed as percentages of 1 000 replications) are shown at branching points.
Fig. 2. Phylogenetic analysis of uncultured bacteria which enrichment in situ based on 16S rRNA genes sequences. All of bacterial 16S rRNA genes sequences are identified by accession and their ID number or their genus on the tree. The tree was constructed by neighbor-joining method. Bootstrap values (expressed as percentages of 1 000 replications) are shown at branching points.
most of strains can degrade acetaldehyde. In particular, there were four strains, namely ACH-L-5, ACH-L-8, ACH-S-12 and ACH-S-6, could degrade all of acetaldehyde in medium (500 mg/L) after 24 h. Followed by strain ACH-S-11 which could degrade 82.94% acetaldehyde, strain ACH-S-9 was 73.92%, strain ACH-S-8 have degraded 55.44%, and strains ACH-L-2, ACH-L-1, ACH-S-7, ACH-S-1, ACH-S-10 have degraded 53.90%, 50.40%, 39.50%, 27.00% and 17.10% acetaldehyde, respectively. As shown in Table 4, these results showed that bacteria belonging to the genus *Halomonas* have better ability of acetaldehyde degradation than other genus’ bacteria.

### 4 Discussion

Although, a large number of marine microorganism species have been reported, the vast majority was still unknown (Ding and Valentine, 2015; Arrigo, 2005). Meanwhile, marine microorganisms were more and more used in many fields, including biomedicine, industrial production, environmental management, and in situ. The bacterial names are presented. Red indicates high abundance and green low abundance.

![Fig. 3. Heatmap analysis of uncultured bacterial community structure at genus level (except genus *Vibrio*) in enrichment in laboratory and in situ. The bacterial names are presented. Red indicates high abundance and green low abundance.](image)

![Table 3. Analysis of the acetaldehyde-degrading ability of cultured bacterial strains from the deep sea water of the South China Sea by enriching in situ or in laboratory.](table)

| Strain   | Accession No. | Nearest relative                  | Identity/% | MCTA | DA |
|----------|---------------|-----------------------------------|------------|------|----|
| In laboratory |               |                                    |            |      |    |
| ACH-L-1  | KM873133      | *Vibrio harveyi* ATCC BAA-1116 (NR_102976.1) | 99         | 0.8  | 50.40 |
| ACH-L-2  | KM873134      | *Vibrio rotiferianus* CAIM 577 (NR_042081.1) | 99         | 1    | 53.90 |
| ACH-L-5  | KM873135      | *Halomonas axialensis* strain Althf1 (NR_027219.1) | 99         | 1.5  | 100  |
| ACH-L-8  | KM873140      | *Halomonas meridiana* strain DSM 5425 (NR_042066.1) | 99         | 1.5  | 100  |
| In situ |               |                                    |            |      |    |
| ACH-S-1  | KM873138      | *Vibrio rotiferianus* CAIM 577 (NR_042081.1) | 99         | 1    | 27.00 |
| ACH-S-12 | KM873144      | *Vibrio parahaemolyticus* RIMD 2210633 (NR_074196.1) | 99         | 1.5  | 100  |
| ACH-S-6  | KM873138      | *Halomonas axialensis* strain Althf1 (NR_027219.1) | 99         | 1    | 100  |
| ACH-S-7  | KM873139      | *Pseudoalteromonas donghuaensis* strain H51 (NR_104537.1) | 97         | 1    | 39.50 |
| ACH-S-10 | KM873142      | *Pseudoalteromonas piscicida* strain IAM 12932 (NR_040946.1) | 99         | 1    | 17.10 |
| ACH-S-11 | KM873143      | *Pseudoalteromonas santiharmonia* strain KMM 1447 (NR_041044.1) | 99         | 1    | 82.94 |
| ACH-S-8  | KM873140      | *Bacillus aquimarina* strain TF-12 (NR_025241.1) | 99         | 1    | 55.44 |
| ACH-S-9  | KM873141      | *Bacillus aquimarina* strain TF-12 (NR_025241.1) | 99         | 1    | 73.92 |

Note: MCTA represents the max tolerable concentration of acetaldehyde (g/L) and DA the degradation rate of acetaldehyde (%).
and the study of marine microorganisms was always one of the hot spots over the last decades (Gao et al., 2017; Bernan et al., 1997; Telecom, 2002). Deep sea water could also harbor many microorganisms, especially some typical microorganisms with bioactive functions, such as acetaldehyde-degrading bacteria and polysaccharides-degrading bacteria (Horikoshi, 1998; Koyama and Yoshida, 2016). In previous studies, bacterial diversity studies were analyzed by using streak plate method, sequencing of 16S rRNA (Caporaso et al., 2011). While most of these studies were performed in laboratory. To further investigate the diversity of acetaldehyde-degrading bacteria in the West Pacific Ocean’s deep sea. We have analyzed the diversity of cultured and uncultured acetaldehyde-degrading bacteria of deep sea water which were enriched in laboratory and in situ by using streak plate method and sequencing of 16S rRNA. Meanwhile, we also have detected the ability of acetaldehyde-degradation of these cultured bacteria. Our studies have showed that α, γ, δ, ε-Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria dominated the bacterial community by sequencing of 16S rRNA in deep sea water with enrichments in laboratory or in situ. But there were only some strains which belonged to γ-Proteobacteria and Firmicutes were obtained by using streak plate method. Meanwhile, these results indicated that most of acetaldehyde-degrading bacteria in deep sea water were not suitable for growth in laboratory’s conditions. On the other hand, as shown in Table 1, the uncultured bacterial diversity richness which enrichment in laboratory is better than enrichment in situ. Although the bacterial diversity richness in laboratory is better than in situ, but the main bacterial community structures are similar. Thus, our study has showed that bacteria of Vibrio and Halomonas composed the dominant population. Conversely, we have obtained more acetaldehyde-degrading bacterial strains in situ than in laboratory. It seems that more uncultured bacteria were enriched in laboratory conditions than in situ conditions, while bacterial strains were suitable grown in situ environments. We have also demonstrated that these bacteria belonging to geni Vibrio and Halomonas played a key role in degradation of aldehydes material of marine.

In phylum level, previous studies have demonstrated that bacteria of Proteobacteria dominated bacterial community in environments, contain α, γ, δ, and ε-Proteobacteria (O’Sullivan et al., 2002; Franzmann, 1996; Borneman and Triplett, 1997). Previous studies of marine bacterial diversity have showed that bacteria belonging to α-Proteobacteria were the main uncultured bacterial species, while the main cultured bacterial species were the γ-Proteobacteria (Borneman and Triplett, 1997). Interestingly, in this study, γ-Proteobacteria were the dominating bacteria whether cultured or uncultured bacteria. In genus level, Vibrio and Halomonas were the dominating genus of uncultured bacteria, and we have obtained some strains belonging to Vibrio and Halomonas, especially only strains of Vibrio and Halomonas were found from enrichment in laboratory. The results of detecting ability of acetaldehyde-degrading have showed these strains belonging to Vibrio and Halomonas were better than the other strains for the ability of acetaldehyde-degrading. It seems that bacteria of Vibrio and Halomonas play a key role in the degradation of acetaldehyde in deep sea water. Similarly, previous studies have reported that bacteria of Vibrio and Halomonas could grow in the high aldehydes concentration (Vedadi and Meighen, 1997; Sripo et al., 2002), but further and systemic investigation should be carried out. In our study, we investigated the diversity of bacteria of acetaldehyde-degrading in deep sea water of the West Pacific Ocean, and compared the different diversity of acetaldehyde-degrading bacteria between with two samples which enrichment in situ and in laboratory. Some acetaldehyde-degrading bacterial strains were obtained, especially four strains belonging to Vibrio and three strains of Halomonas, which have potentials in degradation of aldehydes material.

Previous studies have reported a strain, namely Acetobacter pasteurianus belonging to Rhodobacterales, α-Proteobacteria, which have utilized acetaldehyde or ethanol in medium as carbon source for growth (Kanchanarat et al., 2010; Takekuma et al., 1993). In this study, 16S rRNA genes of Rhodobacterales and Rhizobiales were also found, it indicates that these bacteria belonging to α-Proteobacteria play key roles in carbon or nitrogen cycles in marine environments. Unfortunately, bacterial strains of α-Proteobacteria were not isolated because of unsuitable culture condition. Previous reports have investigated typical marine bacterial diversity and found that these bacteria belonging to β-Proteobacteria mainly survive in estuary or nearshore area and almost not distribute in deep sea (Sekiguchi et al., 2002; Wu et al., 2004). Similarly, our studies do not find bacteria belonging to β-Proteobacteria, except one 16S rRNA gene from enrichment in situ. The bacteria of δ, ε-Proteobacteria which have ability of sulfate reducing or sulfur-oxidizing were frequently found in hydrothermal area and cold seep of deep sea. Meanwhile, in this study, we also have obtained 16S rRNA genes of δ, ε-Proteobacteria from the deep sea water whether enrichment in situ or in laboratory. These results may suggest that these bacteria may play important roles in marine carbon cycling and nitrogen cycling.

Bacteria of Firmicutes were frequently isolated from offshore and sediment in shallow sea, and there were debatable about it was marine aborigines or originate from mainland (Ravenschlag et al., 1999). Meanwhile, the bacteria belonging to Enterococcus,

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**Table 4. The ability of acetaldehyde-degrading in bacterial strains**

| Strain | Degradation of acetaldehyde every 24 h/mg g⁻¹ | Reference |
|--------|------------------------------------------|------------|
| Metschnikowia pulcherrima C6 | 662.4 | Li and de Orduña (2011) |
| Zygossaccharomyces bailii C23 | 547.2 | Li and de Orduña (2011) |
| Candida stellata C35 | 720.0 | Li and de Orduña (2011) |
| Hansenula anomala C4 | 345.6 | Li and de Orduña (2011) |
| Candida vini C2 | 360.0 | Li and de Orduña (2011) |
| Hanseniaspora uvarum C1 | 604.8 | Li and de Orduña (2011) |
| Schizo-saccharomyces pombe C7 | 57.6 | Li and de Orduña (2011) |
| ACH-L-5 | 833.3 | this study |
| ACH-L-8 | 684.9 | this study |
| ACH-S-12 | 609.8 | this study |
| ACH-S-6 | 675.7 | this study |
Planomicrobium, Bacillus, have shown that the ability of degrading acetaldehyde. In this study, not only 16S rRNA genes of Firmicutes were detected, but also cultured bacteria of Enterococcus, Planomicrobium, Bacillus (belong to Firmicutes) were isolated. As far as we know, this was the first report that the cultured bacteria of Firmicutes from deep sea could degrade acetaldehyde.

As we all know that there are a larger number of microorganism species in ocean, and many of marine microorganisms have excellent bioactivities for application (Kobayashi and Ishibashi, 1993; Okami et al., 1976; Manivasagan et al., 2016). Unfortunately, most of marine microorganisms are still unknown. In our study, we have screened 16S rRNA genes of uncultured bacteria and isolated cultured bacteria from the deep sea water of the West Pacific Ocean by using enrichment in situ and in the laboratory, and then detected the ability of acetaldehyde-degradation of cultured bacteria, respectively. Our study showed that the γ-Proteobacteria (occupied 80% clones) was dominant in the deep sea water of the West Pacific Ocean, especially these bacteria belonging to Vibrio and Halomonas were widely distributed in deep sea environments and composed the main acetaldehyde-degrading bacterial community in deep sea water whether enrichment in situ or laboratory. As far as we known, this is the first systematic report of acetaldehyde-degrading bacteria in the deep sea water of the West Pacific Ocean. Our study will provide a reference for investigation of acetaldehyde-degrading bacteria in the West Pacific Ocean. Based on above investigations, the potential applications of acetaldehyde-degrading bacterial stains from the deep sea water of the West Pacific Ocean was suggested.

References
Arrigo K R. 2005. Marine microorganisms and global nutrient cycles. Nature, 437(7057): 349–355
Bernan V S, Greenstein M, Maiese W M. 1997. Marine microorganisms as a source of new natural products. Advances in Applied Microbiology, 43: 57–90
Boresman J, Triplett F W. 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology, 63(7): 2647–2653
Caporaso J G, Lauber C L, Walters W A, et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America, 108(5): 4516–4522
Chao Anne. 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics, 11(4): 265–270
Chao Anne, Lee S M. 1992. Estimating the number of classes via sample coverage. Journal of the American Statistical Association, 87(417): 210–219
Chao Anne, Shen T J. 2003. Nonparametric estimation of Shannon’s index of diversity when there are unseen species in sample. Environmental and Ecological Statistics, 10(4): 429–443
Ding Haibing, Valentine D L. 2008. Methanotrophic bacteria occupy benthic microbial mats in shallow marine hydrocarbon seeps, Coal Oil Point, California. Journal of Geophysical Research: Biogeosciences, 113(G1): G01015
Ding H, Valentine D L. 2012. Methanotrophic bacteria occupy benthic microbial mats in shallow marine hydrocarbon seeps, Coal Oil Point, California. Journal of Geophysical Research Biogeosciences, 113(G1): 222–256
Franzmann P D. 1996. Examination of Antarctic prokaryotic diversity through molecular comparisons. Biodiversity and Conserva- tion, 5(11): 1295–1299
Gao Boliang, Jin Min, Li Li, et al. 2017. Genome sequencing reveals the complex polysaccharide-degrading ability of novel deep-sea bacterium Flammeovirga pacifica WPAGA1. Front Microbiol, 8: 600
Gesser H D, Fu Shali. 1990. Removal of aldehydes and acidic pollutants from indoor air. Environmental Science & Technology, 24(4): 495–497
Homann N, Tellonen J, Meurman J H, et al. 2000. Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. Carcinogenesis, 21(4): 663–668
Horikoshi K. 1998. Barophiles: deep-sea microorganisms adapted to an extreme environment. Current Opinion in Microbiology, 1(3): 291–295
Ista L K, Callow M E, Finlay J A, et al. 2004. Effect of substrate surface chemistry and surface energy on attachment of marine bacteria and algal spores. Applied and Environmental Microbiology, 70(7): 4151–4157
Kaeberlein T, Lewis K, Epstein S S. 2002. Isolating “uncultivable” microorganisms in pure culture in a simulated natural environment. Science, 296(5570): 1127–1129
Kunchanarach W, Theeragold G, Yakushi T, et al. 2010. Characterization of thermotolerant Acetobacter pasteurianus strains and their quinoprotein alcohol dehydrogenases. Applied Microbiology and Biotechnology, 85(3): 741–751
Kelecom A. 2002. Secondary metabolites from marine microorganisms. Anais da Academia Brasileira de Ciências, 74(1): 151–170
Kirk J L, Beaudette L A, Hart M, et al. 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods, 58(2): 169–188
Kobayashi J, Ishibashi M. 1993. Bioactive metabolites of symbiotic marine microorganisms. Chemical Reviews, 93(5): 1753–1769
Koyama S, Yoshida T. 2016. Electrical collection of membrane-intact and dehydrogenase-positive symbiotic bacteria from the deep-sea bivalve Calyptogena Okutanii. Electrochemistry, 84(5): 358–360
Kurkiviouri J, Salaspuvo V, Kaliohaavaa P, et al. 2007. Acetaldehyde production from ethanol by oral streptococci. Oral Oncology, 43(2): 181–186
Lachenmeier D W, Sohnis E M. 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. Food and Chemical Toxicology, 46(6): 2903–2911
Li Erhu, de Orduña R M. 2011. Evaluation of the acetaldehyde production and degradation potential of 26 enological Saccharomyces and non-Saccharomyces yeast strains in a resting cell model system. Journal of Industrial Microbiology & Biotechnology, 38(9): 1391–1398
Lubin M, Westerfield W W. 1945. The metabolism of acetaldehyde. Journal of Biological Chemistry, 161: 503–512
Manivasagan P, Nam S Y, Oh J. 2016. Marine microorganisms as potential biofactories for synthesis of metallic nanoparticles. Critical Reviews in Microbiology, 42(6): 1007–1019
Okami Y, Okazaki T, Kitahara T, et al. 1976. Studies on marine microorganisms. V. A new antibiotic, aplasmomycin, produced by a Streptomyces isolated from shallow sea mud. Journal of Antibiotics, 29(10): 1019–1025
Okazaki T, Kitahara T, Okami Y. 1975. Studies on marine microorganisms: IV. A new antibiotic SS-228 Y produced by Chainia isolata. Chemical Toxicology, 46(8): 2903–2911
O’Sullivan L A, Weightman A J, Fry J C. 2002. New degenerate Cyto- phaga-Flexibacter-Bacteroides-Specific 16S ribosomal DNA-targeted oligonucleotide probes reveal high bacterial diversity in river estuaries. Applied and Environmental Microbiology, 68(1): 201–210
Ravenschlag K, Sahm K, Pernthaler J, et al. 1999. High bacterial diversity in permanently cold marine sediments. Applied and Environmental Microbiology, 65(9): 3982–3989
Rochelle P A, Cragg B A, Fry J C, et al. 1994. Effect of sample handling on estimation of bacterial diversity in marine sediments by 16S rRNA gene sequence analysis. FEMS Microbiology Ecology, 15(1–2): 215–225
segikuchi H, Koshikawa H, Hiroki M, et al. 2002. Bacterial distribu-
tion and phylogenetic diversity in the Changjiang estuary before the construction of the Three Gorges Dam. Microbial Ecology, 43(1): 82–91

Smalla K, Oros-Sichler M, Milling A, et al. 2007. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: do the different methods provide similar results?. Journal of Microbiological Methods, 69(3): 470–479

Sripo T, Phongdara A, Wanapu C, et al. 2002. Screening and characterization of aldehyde dehydrogenase gene from Halomonas salina strain AS11. Journal of Biotechnology, 95(2): 171–179

Svegliati-Baroni G, Ridolfi F, Di Sario A, et al. 2001. Intracellular signaling pathways involved in acetaldehyde-induced collagen and fibronectin gene expression in human hepatic stellate cells. Hepatology, 33(5): 1130–1140

Takemura H, Kondo K, Horinouchi S, et al. 1993. Induction by ethanol of alcohol dehydrogenase activity in Acetobacter pasteurianus. Journal of Bacteriology, 175(21): 6857–6866

van der Ploeg J, Smidt M P, Landa A S, et al. 1994. Identification of chloroacetaldehyde dehydrogenase involved in 1,2-dichloroethane degradation. Applied and Environmental Microbiology, 60(5): 1599–1605

Vedadi M, Meighen E. 1997. Critical glutamic acid residues affecting the mechanism and nucleotide specificity of Vibrio harveyi aldehyde dehydrogenase. European Journal of Biochemistry, 246(3): 698–704

Wu M, Song Liansheng, Ren Jianping, et al. 2004. Assessment of microbial dynamics in the Pearl River Estuary by 16S rRNA terminal restriction fragment analysis. Continental Shelf Research, 24(16): 1925–1934

Xi Zhuge, Dai Shugui, Zhang Lin, et al. 1999. Studies on the damage of calf thymus DNA induced by 3 kinds of aldehyde pollutants using ultraviolet and fluorescence spectrum. Environmental Chemistry (in Chinese), 18(5): 453–457

Zhou Zhijun, Liu Yingxi, Zeng Junning, et al. 2005. Determination of 13 kinds of aldehyde and ketone pollutants of indoor air by HPLC at one time. Journal of Environment and Health (in Chinese), 22(4): 297–299
Annual pattern of zooplankton communities and their environmental response in a subtropical maritime channel system in the northern Bay of Bengal, Bangladesh

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Received 21 April 2017; accepted 18 September 2017

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Abstract

Zooplankton plays an important role in aquatic food webs by fluxing of energy from primary producer to subsequent trophic levels in the food chain. The annual pattern of zooplankton communities and potential environmental drivers were studied in the Kohelia channel, Bangladesh from summer 2014 to spring 2015. Samples were collected using net at a depth of 1 m. A total of 32 species belonged to 18 orders, 27 families and 15 taxonomic groups were identified. Of these species, 22 distributed in all four seasons of which 8 were dominant and highly contributing to the total communities. Species number peaked in summer next to winter and fall in spring while maximum abundance was in summer and minimum in spring. Multivariate analyses showed that there was a clear annual pattern in the zooplankton communities. Species diversity and evenness peaked in spring but fall in autumn while the high value of species richness was found in winter. Biological-environmental best matching (BIO-ENV) analyses confirmed that community pattern of zooplankton was mainly driven by transparency, salinity, and temperature individually or combined with water nutrients. These results demonstrate that annual pattern of the zooplankton community shaped by channel environmental factors in subtropical channel ecosystems, thus might be used for community-based subtropical coastal water bioassessment.

Key words: zooplankton, trophic levels, subtropical channel, community structure, multivariate approach

Citation: Abdullah Al Mamun, Alam Md. Didarul, Akhtar Aysha, Xu Henglong, Islam Md. Shafiquil, Mustafa Kamal Abu Hena, Uddin MMuslem, Alam Md. Wahidul. 2018. Annual pattern of zooplankton communities and their environmental response in a subtropical maritime channel system in the northern Bay of Bengal, Bangladesh. Acta Oceanologica Sinica, 37(8): 65–73, doi: 10.1007/s13131-018-1184-6

1 Introduction

Zooplankton are heterotrophic non motile/weak swimming aquatic animals living in all types of water bodies like fresh, coastal or marine ecosystems (Ferdous and Muktadir, 2009). They are key components in the aquatic ecosystem having effective role in the tropical food web and behave as a linker between the lower and higher levels (Liu et al., 2013; Sahu et al., 2013; Arashekevich et al., 2002). Most significantly they treat as intermediate nexus for fluxes of energy from primary producers like as phytoplankton and microbes to consumer levels of food chain (Iqbal et al., 2014; Madin et al., 2001), and also provide essential indication about the trophic condition of secondary production in the aquatic ecosystems (Abdullah Al et al., 2018). Moreover, the fertility of ecosystems depends on availability of zooplankton while eventually it provides information of fisheries potentiality and sustainability (Abu Hena et al., 2016; Srichandan et al., 2015). Furthermore, it is acquainted that the world richest fisheries directly or indirectly depend on zooplankton availability (Srichandan et al., 2013) because they lead the displacement of the organisms that depended on them (Savari et al., 2013; Prabhahar et al., 2011). Studies have reported that variation in distribution, abundances and composition of zooplankton greatly influenced the existing aquatic habitats on depending communities like fishes, e.g., pelagic fishes feed on zooplankton for their entire life stages or early life stages (Abu Hena et al., 2016; Iqbal et al., 2014). Therefore, the relationship between zooplanktons and physiochemical parameters directly/indirectly related to monsoonal activity of the bay water especially during monsoon period, e.g., May to July when effective rainfall and nutrients input from upland influence the community patterns like distribution, abundances and composition (Srichandan et al., 2014; Fernandes and Ramaiah, 2009). Moreover, it has been widely used as potential bioindicators for ecological succession of coastal waters, also discriminating environmental status due to short living and quick response to certain environmental changes (Ferdous and Muktadir, 2009; Bianchi et al., 2003; Ramaiah and Nair, 1997). Up to date, several studies have been carried out about zooplankton and related environmental paramet-
ers in the coastal waters of the Bay of Bengal but very limited in the Bangladesh coastal waters especially northern part. Previous studies reported that the community patterns of zooplankton are governed by hydrological parameters of those ecosystems where transparency, salinity, temperature and nutrients are the significant influencing factors for variation of the community patterns (Abu Hena et al., 2016; Khan et al., 2015; Iqbal et al., 2014).

Bangladesh has the greatest significant of available resources both fauna and flora due to unbroken 710 km coast line and stable continental shelf also place in Genjatic delta region (Gonga-Brahamaputtra and Meghna) (Abu Hena and Khan, 2009), which carried out by several rivers, estuaries and open maritime channels systems, i.e., Moheshkhali, Kutubdia and Kohelia channels (Abu Hena et al., 2005). Their valuable economic contribution in the fisheries sectors and coastal carbon sequestration having massive growth of natural aquatic vegetation (e.g., mangroves, salt marshes and seagrasses) are noticeable. For example, Moheshkhali channel is habitat of around 35 finfish species and 10 shrimp species (Rashed-Un-Nabi et al., 2011) also seven species of cephalopods (Abu Hena et al., 2005). These fisheries species are fond of zooplanktons as their foodstuffs during foraging stages in the channels. In contrast, from the economical and geographical point of view, Kohelia channel has great contribution having massive growth of aquatic vegetation which provides feeding, spawning and nursery ground, also supply abundant food and relatively safe ecological niche for many valuable species like shrimp (e.g., Penaeus monodon) and other commercial fishes. Being as important wetland and aquatic ecosystem, the research on zooplankton along with limnology are still scarce in this area. The research on hydrological and biological properties are important of this channel system prior to take necessary steps on ecological health management and resource conservation.

In the present study, a one-year baseline survey was carried out in the channel from summer 2014 to spring 2015. Our objectives of this study were: (1) to document the species composition and abundances of zooplankton; (2) to reveal the annual variation in zooplankton communities; and (3) to summarize the annual environmental response of zooplankton communities in such a subtropical channel system.

2 Materials and methods

2.1 Study area, data collection and sample processing

Three sampling stations were selected in the Kohelia channel: Sta. 1 was located at the mouth of the channel in the southeast part of the Dalghat (Monirtek); Sta. 2 was in the middle part of the channel near Zapua; and Sta. 3 was the joint of two channels of Kohelia and Moheshkhali near Materbari (Fig. 1). Samples were collected in summer (June 2014), autumn (September 2014), winter (December 2014) and spring (March 2015), respectively.

Zooplankton samples were collected using a conical shaped plankton net with a mesh size of 325 μm. A digital flow meter was set up at the mouth of the net to record the amount of water filtered through the net during sampling. Samples were collected at three stations from the surface water at 1 m depth for 10–15 min.

After collection, all samples were preserved in 5% formalin solution (Goswami, 2004). For effective sorting, the samples were stained with rose bangle (coloring reagent) and left for 24 h in the laboratory. All zooplankton species attained pink color, which helped to sort out easily and identification was carried out using fine brush, needles, forceps and microscope.

Enumeration was done according to Goswami (2004) zooplankton abundance was expressed as individuals per liter (ind./L).

The sorted zooplanktons were preserved in 70% ethanol solution. Preserved samples were identified morphologically under the microscope according to the previous studies (Conway, 2012; Al-Yamani et al., 2011; Mulyadi, 2004; Conway et al., 2003; Buckland-Nicks et al., 2002; Goddard, 2001).

Environmental parameters such as water temperature (°C), salinity, pH, and water transparency (cm) were estimated in situ.
using centigrade thermometer, refractometer (TANAKA New S-100, Japan), digital pen pH meter (HANNA instruments, model HI 98107) and secchi disk, respectively. Sub-subsurface (~1 m) water samples were collected for measuring dissolved oxygen (DO), total dissolved solid (TDS), total suspended solid (TSS), nitrite-nitrogen (NO₂⁻N) and soluble reactive phosphate-phosphorus (PO₄-P), following the standard method of APHA (1989).

2.2 Data analysis

Species diversity (H′) (Shannon and Weaver, 1949), species richness (d) (Margalef, 1968) and species evenness (J′) (Pielou, 1966) were used to summarize the biodiversity pattern of zooplankton. These three indices were calculated according to the following formula:

\[ H' = -\sum_{i=1}^{S} P_i \ln P_i, \]

\[ J' = H' / \ln S, \]

\[ d = (S - 1) / \ln N, \]

where \( H' \) is observed diversity index, \( P_i \) is proportion of the total count arising from the \( i \)th species, \( S \) is total number of species, and \( N \) is total number of individuals.

Multivariate analysis of annual variations in the zooplankton communities were analyzed using PRIMER v7.0.11 with the routine PREMANOVA (Abdullah Al et al., 2018; Clark and Gorley, 2015; Anderson et al., 2008). Bray-Curtis similarity and Euclidean distance matrices were computed on square root root transformed species abundance data and on log-transformed/normalized environmental data, respectively. The species contribution as top rank contributors in each sampling period was conducted by SIMPER analysis. The species distribution among four sampling period was analyzed by the sub-module of CLUSTER on Bray-Curtis similarities from the standardized species abundance data. The annual variation in community patterns were coordinated using the sub-module of dbrDA (distance based redundancy analysis) of PREMANOVA in PRIMER (Anderson et al., 2008), while the annual pattern of environmental variables was coordinated using the routine PCA (principle component analysis) (Abdullah Al et al., 2018; Clark and Gorley, 2015).

Univariate analysis of Pearson correlation matrix was conducted using the IBM SPSS v.22 (Landau and Everitt, 2004) to identify any existences of variance between the environmental parameters and zooplankton communities.

3 Results

3.1 Environmental variables

The average values of nine environmental variables were: water temperature showed a clear annual variation, ranging from 23.67°C to 30.67°C from summer to spring; salinity ranged from 12.33 to 34.33, with minimum (12.33) in summer and maximum (34.33) in winter; transparency varied from 17.17 cm (summer) to 75.67 cm (spring); the average pH ranged from 7.24 to 7.54; DO fluctuated from 4.76 mg/L (summer) to 6.35 mg/L (autumn); TDS varied from 30.93 μg/L to 32.67 μg/L, maintaining relatively stable in all seasons; TSS varied from 0.58 μg/L to 1.00 μg/L with a peak in the winter; NO₂⁻N varied from 0.34 mg/L (summer) to 0.53 mg/L (peaked in autumn); and PO₄-P varied from 0.33 mg/L to 0.55 mg/L (peaked in the spring).

3.2 Taxonomic composition and species distribution

The species composition, average abundances and the ranks of the top 8 contributors in each sampling period were given in Table 1. A total of 32 zooplankton species, belonging to 18 order, 27 families and 15 taxa were identified during the study period. Of these, 22 species occurred in all seasons and were defined as “common” species, while eight species (Amphipoda, Acetes, Heterocope, Temora longicornis, Cyclops, Mesopodopsis orientalis and Scylla serrata) of the top 10 ranked contributors at each season defined as “dominant” species (Table 1). It was noteworthy that the other 10 species were occurred in specific seasons, and defined as endemic species with seasons. For example, Ammothera longipes was found only in summer; Macrobrachium rosenbergii and Linuche unguiculata were noted in winter and spring, respectively.

A dendrogram for species distribution in all the four seasons showed that 32 species formed seven groups at 60% Bray-Curtis similarity level based on their occurrences and abundances. Groups 1 and 2 consisted of 25 common/dominant species with higher composition and abundances in the total zooplankton communities. Of these 32 species, 26 occurred in summer, 30 in autumn and 28 species in winter and spring, respectively, which indicated a clear succession in the zooplankton communities from summer to spring (Fig. 2).

3.3 Seasonal variation of zooplankton communities

In terms of average values, species number peaked in winter, followed by winter and fall in spring while the maximum abundance occurred in the summer and gradually dropped in the spring (Fig. 3). The zooplankton communities represented clear annual variations in terms of both relative abundances and species composition (Fig. 4). It was noted that Mesopodopsis orientalis, Acetes erythraeus, Americamysis bahia, Ampelisca bocki, Cyclops bicuspidatus, Peneaus indicus, Scylla serrata and Temora longicornis were the primary contributors for the total composition of the communities in all the four seasons, e.g., Americamysis bahia, Peneaus indicus, Scylla serrata and Ampelisca bocki were contributors for summer; Americamysis bahia, Mesopodopsis orientalis and Cyclops bicuspidatus were for autumn; Americamysis bahia, Mesopodopsis orientalis, Cyclops bicuspidatus, Temora longicornis, Ampelisca bocki and Peneaus indicus; and Mesopodopsis orientalis, Acetes erythraeus, Cyclops bicuspidatus, Temora longicornis, Ampelisca bocki and Peneaus indicus for winter and spring, respectively.

Based on 12 data points, the distance-based redundancy analysis (dbrDA) demonstrated a clear annual pattern of the zooplankton communities (Fig. 5). For example, the first axis (dbrDA1, 60.1% of total variation) separated the zooplankton samples in spring and winter (on the left) from those in autumn and summer (on the right), while the second axis (dbrDA2, 23.7% of total variation) discriminated the samples in winter and autumn (upper) from those at the other two samples (lower) (Fig. 5a).

Vector overlay of Pearson correlations of the eight dominant species with the dbrDA axis was shown in Fig. 5b. Although these species were the top 8 ranked contributors in each samples, vector for 4 zooplankton species (Americamysis bahia, Cyclops bicuspidatus, Peneaus monodon and Leucothoe spinicarpa) pointed toward the sample cloud in autumn (upper in right), one species (Mesopodopsis orientalis) toward that in winter (upper left),
two species (Acetes erythraeus and Cheiriphotis megacheles) toward those in spring (lower left) and three (Amphipola bocchi, Penaeus indicus and Scylla serrata) toward those in summer (lower right).

The seasonal variation in species richness, evenness and diversity indices during study period was shown in Fig. 6. The species richness peaked in the winter but dropped in the autumn (Fig. 6a), while the species evenness and diversity represented the highest value in spring and lowest in autumn (Figs 6b and c).

### 3.4 Relationship between zooplankton and environmental parameters

The seasonal changes in environmental variables were summarized by principle component analysis (PCA) in Fig. 5. Vector overlay of these environmental variables with the PCA axis was shown in Figs 5c and d and coefficient of correlation (RELSTE) analysis showed that annual variations in zooplankton communities were significantly correlated with the changes in environmental variables ($p=0.552, P<0.05$).

Biological-environmental best matching analyses (BIOENV) demonstrated that the seasonal shift in community structure of zooplankton was mainly driven by salinity, transparency, temperature and nutrients ($p=0.773, P<0.05$) individually or combined with other environmental parameters (Table 2).

Univariate statistical analysis of correlations between environmental parameters and abundances of eight dominant species of zooplankton was summarized in Table 3. Among the eight species, four (Mesopodopsis orientalis, Amphipola bocchi, Cyclops bicuspidatus and Penaeus indicus) were significantly correlated with TSS, pH and DO ($p<0.05$). For example, two species Mesopodopsis orientalis and Cyclops bicuspidatus were positively significant correlated with TSS but Penaeus indicus were significantly negative correlated with water pH (Table 3).

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**Table 1.** Species list with average seasonal abundance (N, ind./L), and the top 10 ranks (Rk) by contribution of each species to the average Bray-Curtis similarity during the study period in the Kohelia channel, northern Bay of Bengal, Bangladesh

| Species               | Summer N | Summer Rk | Autumn N | Autumn Rk | Winter N | Winter Rk | Spring N | Spring Rk |
|-----------------------|-----------|-----------|----------|-----------|----------|-----------|----------|-----------|
| *Acetes erythraeus*   | ++        | 10        | +        | 9         | ++       | 4         | +++      | 1         |
| *Acetes indicus*      | ++        | 8         | +        | ...       | ++       | ...       | 10       | +         |
| *Ameconymy baha*      | +++       | 1         | +++      | 1         | +++      | 1         | +        | 5         |
| *Ammonia longipes*    | +         | ...       | +        | ...       | +        | 10        | +        | 7         |
| *Amphipola bocchi*    | +++       | 3         | +        | 8         | +        | 8         | +        | 6         |
| *Canthocalanus*       | ++        | 6         | +        | ...       | +        | ...       | +        | 10        |
| *Canthocalanus pauper*| +         | ...       | +        | ...       | +        | ...       | +        | 5         |
| *Cheiriphotis megacheles* | +     | ...       | +        | ...       | +        | ...       | +        | 5         |
| *Cyclops bicuspidatus*| ++        | 7         | +++      | 2         | ++       | 2         | +        | ...       |
| *Eudene tergestina*   | +         | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Gammarus roesel*     | +         | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Leucotrochus spinicarpa* | -      | ...       | -        | ...       | +        | ...       | +        | ...       |
| *Linuche unguiculata* | -         | ...       | -        | ...       | +        | 9         | +        | ...       |
| *Lucifer faxoni*      | +         | ...       | +        | ...       | +        | 7         | +        | 8         |
| *Lucifer hanseni*     | +         | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Macra chinsensis*    | +         | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Mechrobactrum rassenbergii* | -     | ...       | -        | ...       | +        | ...       | +        | ...       |
| *Mesopodopsis orientalis* | +     | ...       | ++       | 3         | ++       | 2         | +        | ...       |
| *Microptus oligobranchia* | +      | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Neptuna pelagica*    | +         | ...       | +        | ...       | -        | ...       | -        | ...       |
| *Nerocolia phaioleuca*| +         | ...       | -        | ...       | +        | ...       | +        | ...       |
| *Oniscus astellus*    | +         | ...       | +        | ...       | +        | ...       | -        | ...       |
| *Penaeus indicus*     | ++        | 4         | +        | 6         | +        | ...       | ++       | 2         |
| *Penaeus merguiensis* | +         | ...       | ++       | 4         | ++       | 3         | +        | 4         |
| *Penaeus monodon*     | ++        | 5         | -        | ...       | -        | ...       | +        | ...       |
| *Sagitta bedoti*      | +         | ...       | +        | ...       | +        | 5         | +        | ...       |
| *Scylla serrata*      | +++       | 2         | +        | 10        | -        | ...       | -        | ...       |
| *Solen brevis*        | +         | ...       | +        | 7         | -        | ...       | +        | ...       |
| *Spadella cephaloptera* | +       | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Tanaisus lillobargi* | +         | ...       | +        | ...       | +        | ...       | +        | 9         |
| *Temora longicornis*  | ++        | 9         | +        | 5         | +        | 6         | ++       | 3         |
| *Trachycardium asiaticum* | +      | ...       | +        | ...       | +        | ...       | +        | ...       |
| *Umbonium vestiariun* | +         | ...       | +        | ...       | +        | ...       | +        | ...       |
In the present study, a total of 32 zooplankton species, belonging to 15 taxonomic groups, were identified during the all four seasons from the Kohelia channel, Bay of Bengal. This value is comparable with the reported values by Abu Hena et al. (2016), who reported 11 taxonomic groups including 33 species from Bakkhal sub-tropical estuary, Cox’s Bazar, Bangladesh. The maximum species number and abundance was recorded in post monsoon and minimum was in monsoon period reported by Abu Hena et al. (2016), while Sharif et al. (2017) reported 37 major zooplankton groups with the maximum occurrences in monsoon from the Meghna River Estuary, Bangladesh. In our previous in-

**Fig. 2.** Dendrogram of the species distribution during four seasons using group average clustering based on Bray-Curtis similarities from square root transformed species abundance data of each species of zooplankton of Kohelia Channel. + represents presence and – absence.

**4 Discussion**

In the present study, a total of 32 zooplankton species, belonging to 15 taxonomic groups, were identified during the all four seasons from the Kohelia channel, Bay of Bengal. This value is comparable with the reported values by Abu Hena et al. (2016), who reported 11 taxonomic groups including 33 species from Bakkhal sub-tropical estuary, Cox's Bazar, Bangladesh. The maximum species number and abundance was recorded in post monsoon and minimum was in monsoon period reported by Abu Hena et al. (2016), while Sharif et al. (2017) reported 37 major zooplankton groups with the maximum occurrences in monsoon from the Meghna River Estuary, Bangladesh. In our previous in-

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**Table 1.** Bray-Curtis similarity/%

| Species               | Summer | Autumn | Spring | Winter |
|-----------------------|--------|--------|--------|--------|
| Tanaissus lilljeborgi | +      | +      | +      | +      |
| Cheiriphotis megacheles | -      | +      | +      | +      |
| Umbonium vestiarium   | +      | +      | +      | +      |
| Spadella cephaloptera  | -      | +      | +      | +      |
| Mactra chinensis       | +      | +      | +      | +      |
| Sagitta bedoti         | +      | +      | +      | +      |
| Lucifer hanseni        | +      | +      | +      | +      |
| Gammarus roeseli       | +      | +      | +      | +      |
| Penaeus indicus        | +      | +      | +      | +      |
| Ampelisca bocki        | +      | +      | +      | +      |
| Temora longicornis     | +      | +      | +      | +      |
| Acetes erythraeus      | +      | +      | +      | +      |
| Penaeus merguiensis    | +      | +      | +      | +      |
| Mesopodopsis orientalis| +      | +      | +      | +      |
| Cyclops bicuspifidatus | +      | +      | +      | +      |
| Lucifer faxoni         | +      | +      | +      | +      |
| Acetes indicus         | +      | +      | +      | +      |
| Canthocalanus pauper   | +      | +      | +      | +      |
| Leucothoe spinicarpa   | +      | +      | +      | +      |
| Penaeus monodon        | +      | +      | +      | +      |
| Americamysis bahia     | +      | +      | +      | +      |
| Trachycardium asiaticum| +      | +      | +      | +      |
| Micronephys oligobranchi| +     | +      | +      | +      |
| Oniscus asellus        | +      | +      | +      | +      |
| Evadne tergestina      | +      | +      | +      | +      |
| Scylla serrata         | +      | +      | +      | +      |
| Neptunus pelagicus     | +      | +      | +      | +      |
| Linuche unguiculata    | +      | +      | +      | +      |
| Macrobrachium rosenbergii| +    | +      | +      | +      |
| Nerocila phaioleura    | +      | +      | +      | +      |
| Solen brevis           | +      | +      | +      | +      |
| Ammothella longipes    | +      | +      | +      | +      |
investigation, we found 32 species from 15 taxonomic groups with maximum abundance in winter and minimum in summer, in same place with another channel system Kutubdia in Bangladesh (Abdullah Al et al., 2018), while Iqbal et al. (2014) reported 12 taxa with maximum abundance in winter and minimum in pre-monsoon in southeastern coastal waters in Bangladesh. Furthermore, Khan et al. (2015) reported eight groups at Sitakunda coast in Bangladesh in the Bay of Bengal, while Savari et al. (2013) reported 15 groups of zooplankton in the Oman Sea with peak abundance which is observed in northeast monsoon period, who explained that water hydrographic condition could be leaded to variation of species composition and distribution. In contrast, present study was a little bit different from previous reports which might be due to the geographical location and ecological condition of the present study area. For example, most of the previous studies were conducted in estuarine environment, while the present study was in the open maritime channel which could probably had different ecological parameters, e.g., food availability, transparency, salinity variation and nutrients of water, which directly or indirectly influenced the abundance and composition of zooplankton.

Based on the present data, 22 species were present in all the four seasons and others 10 species occurred in specific seasons. This implies that ecological conditions such as water parameters, food availability of the study area were different from one season to another throughout the year. Abdullah Al et al. (2018) and Sharif et al. (2017) stated that majority of the zooplankton groups in the northern Bay of Bengal driven coastal waters are shifted due to environmental condition, e.g., water temperature fluctuation, salinity variation, transparency and dissolved oxygen degradation, which was reflected in our present findings. Of these 32 species, Americamysis bahia was the most dominant species for three seasons while Ampelisca bocki, Cyclops bicuspidatus, Mesopodopsis orientalis, Acetes erythraeus and Peneaus indicus were dominant species in all seasons, although Scylla serrata was dominant only in summer. In terms of abundances of the dominant species, it should be noted that eight species belonging to groups of mysids, copepods, Acetes, shrimp larvae, crab larvae and amphipods were most dominated in this study area probably due to ecological parameters, e.g., salinity, transparency and pH of this channel system. In addition, the sustainability of mangroves and salt marshes habitats by providing shelter zone and adequate food supply for zooplankton were reported elsewhere (Abdullah Al et al., 2018; Abu Hena et al., 2016).

The species richness peaked in winter but dropped in autumn, while the species evenness and diversity showed similar patterns of changing whereas the highest in spring and the lowest in autumn. It is well recognized that estuary has dynamic environmental characteristics, which govern unique habitat for living organisms that is why abundance and composition of zooplankton was higher than other places (Abdullah Al et al., 2018; Abu Hena et al., 2016). Bearing these similarities, densely vegetated mangroves and salt marsh habitats, and estuarine habitats in monsoon period of the Kohelia channel also provided favorable ecological niches for zooplankton communities. In the coastal waters, copepod density has reported to be highly related with salinity level during rainy season when freshwater added in water it turned to be declined (Abdullah Al et al., 2018; Abu Hena et al., 2016; Cook et al., 2007; Rakhes et al., 2006; Hirst et al., 1999).

Present study findings also reflected these arguments, and indic-
ating that copepods showed positive correlation with water temperature, salinity and transparency. Thus, these imply that with increasing salinity, temperature and transparency directly/indirectly influenced the composition and abundance of copepods than other zooplankton communities.

It has been increasingly recognized that multivariate analysis is more effective than univariate analysis for detecting the changing of community structure (Abdullah Al et al., 2018; Clark and Gorley, 2015). In this study, clustering analysis based on Bray-Curtis similarity, dbRDA and PCA of 32 species revealed a significant relationship between community variation in the zooplankton communities and the changes in the environmental parameters. Best matching analyses showed that temperature, salinity and nutrients probably the main drivers to the annual patterns of the zooplankton communities in such a subtropical channel system. Seasonal cycle and situation of zooplankton species depend on water salinity, nutrients and transparency (Abdullah Al et al., 2018; Hwang et al., 2010; Mohanty et al., 2010; Osore et al., 2004). Based on present study, it has revealed that zooplankton communities of Kohelia channel in the northern Bay of Bengal governed by different hydrological factors of the channel environment where monsoonal activities of the Bay support very suitable ecological niche for mysids as well as other dominant zooplankton groups most prominently for copepods, amphipods and shrimps.

In conclusion, this is one of the pioneer research focusing on

![Distance-based redundancy analysis (dbRDA) for the zooplankton communities with correlation of 10 dominant species with dbRDA axis (a, b) and principle component analysis (PCA) for the environmental parameters with correlations of zooplankton communities in PC axis (c, d) with required data transformed from four seasons.](image)

![Species richness (a), species evenness (b) and species diversity (c) of zooplankton communities of Kohelia channel in the northern Bay of Bengal, Bangladesh.](image)
Table 2. Summary of results from biota-environment (BIOENV) analysis showing the ten best matches of environmental variables with spatial variations in the zooplankton abundances during the study period

| Rank | Environmental variables                  | $\rho$ value | $P$ value |
|------|------------------------------------------|--------------|-----------|
| 1    | Trans, NO$_2$-N                          | 0.773        | <0.05     |
| 2    | T, Sal, Trans, DTS, NO$_2$-N             | 0.770        | <0.05     |
| 3    | T, Sal, Trans, PO$_2$-P, NO$_2$-N        | 0.767        | <0.05     |
| 4    | T, Sal, Trans, NO$_2$-N                 | 0.762        | <0.05     |
| 5    | Sal, Trans, PO$_2$-P, NO$_2$-N          | 0.762        | <0.05     |
| 6    | T, Sal, Trans, NO$_2$-N                 | 0.761        | <0.05     |
| 7    | Sal, Trans, NO$_2$-N                    | 0.761        | <0.05     |
| 8    | Sal, Trans, DTS, PO$_2$-P, NO$_2$-N     | 0.759        | <0.05     |
| 9    | T, Trans, NO$_2$-N                      | 0.756        | <0.05     |
| 10   | Sal, Trans, NO$_2$-N                    | 0.753        | <0.05     |

Note: $\rho$ value is Spearman correlation coefficient and $P$ value statistical significance level. T represents water temperature, Sal salinity, Trans transparency, DTS total dissolved solid, PO$_2$-P water soluble reactive phosphate-phosphorus, and NO$_2$-N water nitrite nitrogen.

Table 3. Pearson correlation between average values of the eight dominants zooplankton species with average environmental variables during the study period

| Species            | T     | Trans | $pH$ | Sal | TSS | TDS | DO  | NO$_2$-N | PO$_2$-P |
|--------------------|-------|-------|------|-----|-----|-----|-----|----------|----------|
| Acetes erythraeus  | 0.355 | 0.881 | -0.842 | 0.786 | 0.210 | -0.676 | 0.325 | 0.702 | 0.470 |
| Americanysis baha  | -0.471 | -0.860 | 0.906 | -0.716 | -0.122 | 0.846 | -0.086 | -0.677 | -0.460 |
| Annelolis bocki    | 0.774 | -0.427 | -0.340 | -0.636 | -0.824 | -0.489 | -0.974 | -0.564 | 0.438 |
| Cyclops bicuspidatus | -0.694 | 0.747 | 0.116 | 0.884 | 0.973 | 0.057 | 0.774 | 0.889 | -0.553 |
| Mesopodopsis orientalis | -0.614 | 0.801 | 0.036 | 0.896 | 0.956 | -0.124 | 0.603 | 0.942 | -0.583 |
| Peneaus indicus    | 0.678 | 0.656 | -0.973 | 0.495 | -0.175 | -0.763 | 0.039 | 0.400 | 0.749 |
| Scylla serrata     | 0.512 | -0.714 | 0.049 | -0.851 | -0.782 | -0.136 | -0.943 | -0.759 | 0.189 |
| Temora longicornis | 0.307 | 0.854 | -0.797 | 0.783 | 0.223 | -0.572 | 0.428 | 0.676 | 0.487 |

Note: *Correlation is significant at the $P<0.05$ level. DO represents dissolved oxygen, TSS total suspended solid, and pH water hydrogen ion concentration. Significant values were in bold text. See Table 2 for other abbreviations.

annual variation in community patterns of zooplankton with response to environmental changes in the subtropical channel of Kohelia in the northern Bay of Bengal, southeastern coastal zone of Bangladesh. Of these 32 species, 22 distributed in all season of which eight species were found dominant with the highest abundances and composition in the zooplankton communities. Due to strategic location of the Kohelia channel, this place is highly suitable for most of the aquatic animals especially molluscs and shrimps. Besides, the existence of aquatic vegetation like mangroves and salt marshes could also provide the suitable habitats for the presence of pelagic mollusc and shrimp in this channel system. The changing of the salinity, transparency, temperature and nutrients drove the annual pattern in zooplankton community structure. Thus, this finding revealed that the zooplankton community represented structural variation shaped by the environmental drivers in tropical channel system, which might be used as bioassessment of monitoring program for assessing coastal ecosystems. We recommended that further more studies are needed to justify this statement.

Acknowledgements
A special thank goes to Md. Khusrhid Alam Bhuiyan for illustrating the study area map, Dev Mallick for helping sps data analysis, and Xu Guanqian for helping PRIMER data analysis. The authors are pleased to Zheng Wang, and Gao Yangyang for reviewing the earliest version of the manuscript and creative comments which helped to improve the manuscript significantly, and financial support provided by Bangladesh Nature (BNF) is highly acknowledged.

References
Abdullah Al M, Akhtar A, Abu Hena M K, et al. 2018. Seasonal pattern of zooplankton communities and their environmental response in subtropical maritime channels systems in the Bay of Bengal, Bangladesh. Acta Ecologica Sinica, https://doi.org/10.1016/j.chnaes.2017.11.001
Abu Hena M K, Japar Sidik B, Idris M H, et al. 2016. Seasonal distribution of zooplankton composition and abundance in a sub-tropical mangrove and salt marsh estuary. Malaysian Journal of Science, 35(2): 257–270
Abu Hena M K, Sharifuzzaman H, Affabuddin M S, et al. 2005. Coastal resources utilization and conservation issues in Cox’s Bazar, Bangladesh. STREAM Journal, 4: 7–10
Abu Hena M K, Khan M A A. 2009. Coastal and estuarine resources of Bangladesh: management and conservation issues. Maejo International Journal of Science and Technology, 3(2): 313–342
Al-Yamani F Y, Skryabin V, Gubanova A, et al. 2011. Marine Zooplankton Practical Guide for the Northwestern Arabian Gulf, Vol. 1. Kuwait: Kuwait Institute for Scientific Research
Anderson M J, Gorley R N, Clarke K R. 2008. PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. Plymouth, UK: PRIMER-E Ltd
APHA (American Public Health Association), 1989. Standard Methods for the Examination of Water and Waste Water. 17th ed. Washington, DC: American Public Health Association
Arashkevich E, Wassmann P, Pasternak A, et al. 2002. Seasonal and spatial changes in biomass, structure, and development progress of the zooplankton community in the Barents Sea. Journal of Marine Systems, 38(1–2): 125–145
Bianchi F, Acri F, Aubry F B, et al. 2003. Can plankton communities be considered as bio-indicators of water quality in the Lagoon of Venice?. Marine Pollution Bulletin, 46(8): 964–971
Buckland-Nicks J, Gibson G, Koss R, 2002. Phylum mollusca: gastropoda. In: Young C M, Sewell M A, Rice M E, eds. Atlas of Marine Invertebrate Larvae. San Diego: Academic Press
Clarke K R, Gorley R N. 2015. PRIMER v7: User Manual/Tutorial. Plymouth, USA: PRIMER-E Ltd
Conway D V P. 2012. Marine zooplankton of southern Britain: Part 1.
Plankton respiration in the northern South China Sea during summer and winter

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Received 21 January 2017; accepted 18 July 2017

Abstract

Plankton respiration is an important part of the carbon cycle and significantly affects the balance of autotrophic assimilation and heterotrophic production in oceanic ecosystems. In the present study, respiration rates of the euphotic zone plankton community (CR eu) size fractionated chlorophyll a concentration (Chl a), bacterial abundance (BAC), and dissolved oxygen concentration (DO) were investigated during winter and summer in the northern South China Sea (nSCS). The results show that there were obvious spatial and temporal variations in CR eu in the nSCS (ranging from 0.03 to 1.10 μmol/(L·h)), CR eu in winter ([0.53±0.27] μmol/(L·h)) was two times higher than that in summer ([0.26±0.20] μmol/(L·h)), and decreased gradually from the coastal zone to the open sea. The distribution of CR eu was affected by coupled physical-chemical-biological processes, driven by monsoon events. The results also show that CR eu was positively correlated with Chl a, BAC, and DO, and that BAC contributed the highest CR eu variability. Furthermore, the results of the stepwise multiple linear regression suggest that bacteria and phytoplankton were the dominant factors in determining CR eu (R² = 0.82, p<0.05) in the nSCS. Based on this relationship, we estimated the integrated water column respiration rate (CR int) within 100 m of the investigated area, and found that the relationship between the biomass of the plankton community and respiration may be nonlinear in the water column.

Keywords: northern South China Sea, plankton community, respiration, phytoplankton, bacteria

Citation: Zeng Xiangxi, Hao Qiang, Zhou Guangdong, Le Fengfeng, Liu Chenggang, Zhou Wenli. 2018. Plankton respiration in the northern South China Sea during summer and winter. Acta Oceanologica Sinica, 37(8): 74–83, doi: 10.1007/s13131-018-1213-5

1 Introduction

Plankton respiration, the ability of planktonic organisms to consume oxygen and release CO₂, is the key link to organic-inorganic transformation in oceanic ecosystems, as well as one of the most important aspect of studies of the carbon cycle. The total respiration of the marine ecosystem consumes up to 186 Gt/a of organic carbon (17.2 Pmol/a O₂), which is approximately equal to the oceanic gross primary production, and most of the organic carbon consumption is associated with plankton respiration (Williams and del Giorgio, 2005). Although plankton respiration exists throughout the water column, the respiration rate is usually higher in the upper layer where photosynthesis occurs and biological events are concentrated. It was estimated that the planktonic community respiration in the euphotic zone alone may be responsible for approximately 50% of the total organic carbon consumption in the open ocean (del Giorgio and Duarte, 2002).

The respiration of plankton communities is usually expressed as the consumption rate of dissolved oxygen. Plankton respiration rates are mainly dependent on the biomass of plankton communities and their metabolic activities, which are controlled by environmental factors such as temperature, nutrients, and dissolved organic carbon (Williams and del Giorgio, 2005). Temperature is a critical factor for controlling the metabolic rate of plankton communities, and therefore, on a global scale, the respiration of plankton communities increases gradually from the polar regions to the tropical areas with increasing temperatures (Rivkin and Legendre, 2001; Garcia-Corrál et al., 2017). The respiration of the plankton community in the Antarctic Ocean has been shown to be 14.1–52.8 mmol/(m²·d), while the mean value in a tropical area was 32.3–131.3 mmol/(m²·d) (Zhang, 1999). The variation in the respiration rate is significantly affected by the biomass dominant species in the plankton community, and therefore, heterotrophic bacteria, phytoplankton, or zooplankton probably control plankton respiration in the different ecosystems. In some productive coastal zones, community respiration rate has been shown to be positively correlated with phytoplankton biomass, where more than 60% of the change in...
respiration in the upper layer could be explained by changes in
gross primary productivity (Pringault et al., 2009). However, in
the oligotrophic open sea area, the oxygen consumption of het-
erotrophic bacteria usually contributed more than 50% of com-
munity respiration (Schwaerter et al., 1988; Rivkin and Legendre,
2001). Moreover, it was reported that zooplankton grazing was
enhanced in a late spring bloom, and that plankton community
respiration was also related to zooplankton biomass to a certain de-
gree (Ahrrens and Peters, 1991; Chen et al., 2003). Ocean circula-
tion and river input can carry a large quantity of nutrients and
dissolved organic carbon that affect horizontal transportation or
vertical movement, where violent fluctuations in nutrient concen-
tration can significantly affect the biomass of plankton com-
munities, thereby influencing community respiration rates.
Therefore, a certain degree of coupling exists between plankton
respiration and physical processes.

In comparison with photosynthesis, there are relatively few
investigations of plankton community respiration in China’s
seas. Previous researchers have found that plankton community
respiration at the Changjiang (Yangtze River) Estuary exhibited
extremely wide distributions in characteristics that gradually de-
creased from the estuary to the open sea, and that plankton com-
munity respiration was positively correlated with bacterial
abundance (Ning and Coudé, 1991). Li et al. (2002) combined ap-
parent oxygen utilization and the range and thickness of an anox-
ic area to calculate the oxygen consumption of an anoxic area
near the Changjiang Estuary, which was estimated to be approx-
imately 1.59×10^6 t. Subsequent investigations in the East China
Sea determined that the factors governing plankton community
respiration in different trophic systems were distinct, where bac-
teria were found to be the main contributors of plankton com-
munity respiration in the mesotrophic system, protozoans were
the dominant contributors of plankton community respiration in
the oligotrophic system, and the influence of temperature on
plankton community respiration was small (Chen et al., 2003).
Research in the Taiwan Strait also verified that the correlation
between plankton community respiration and chlorophyll a con-
centration in winter and summer was not significant, thereby in-
dicating that phytoplankton was not the main contributor to
community respiration rate (Huang et al., 2005). Wang et al.
(2014) compared plankton community respiration and primary
production in the Taiwan Strait and in the nSCS during summer,
and reported the distribution patterns of autotrophic and hetero-
trophic regions. In the South China Sea, several studies have in-
dicated that primary production exhibits significant seasonal
variation and is coupled with monsoon-driven patterns of ocean
circulation (Liu et al., 2002; Ning et al., 2004). However, research
on seasonal variations in plankton community respiration in the
South China Sea is lacking, and even less research has focused on
the relationship between plankton community respiration and
ecological factors here.

In the present study, we described distributions in the rates of
plankton community respiration in the euphotic zone (CReu)
during different monsoon seasons in the nSCS. We conducted a
comprehensive observation of ecological factors, and in situ in-
cubation measurements of CReu during two seasonal cruises
(winter and summer). The relationships between CReu and eco-
logical factors were also analyzed. This work aimed to provide
basic data for studying the micro-ecology and carbon cycle of the
nSCS, and help to understand the coupling between plank-
tron communities and the environmental forces at the regional
scale.

2 Materials and methods

2.1 Study area and sampling

Samples were collected during winter and summer of 2009 in the
nSCS (18°30′–23°00′N, 111°00′–117°00′E). The winter cruise
was from 11 to 23 February 2009 and comprised 22 stations (two
transects perpendicular to the coast line and two transects paral-
lel to the coast line, Fig. 1). The summer cruise was from 12 to 23
August 2009, and also comprised 22 stations (same configuration
as the winter cruise, Fig. 1). To understand the spatial variation of
the different variables, the nSCS was categorized into the coastal
zone (<50 m), continental shelf region (50–200 m), and open sea
(>200 m) according to depth.

Samples of seawater were collected for the determination of
temperature (T), dissolved oxygen concentration (DO), chloro-
phyll a concentration (Chl a), and bacterial abundance (BAC)
using 5 dm³ Niskin bottles at the following depths: surface layer
(0 m), 10 m, 25 m, 50 m, 100 m, 150 m, and 200 m. There were
seven and five sample stations for measuring CReu in winter and
summer, respectively. Sampling depths corresponded to light
levels of 100%, 10%, and 1% of the surface irradiance. Water
samples in each layer were pre-screened through 200 μm mesh
to remove larger zooplankton, and were then incubated in parallel.

Fig. 1. Sampling stations (●) in the northern South China Sea
(nSCS) during winter and summer of 2009.

2.2 Methods of measurement

2.2.1 Plankton community respiration

Plankton community respiration rates are usually represen-
ted by the difference in dissolved oxygen between initial and dark
treatments (Robinson et al., 2002; Chen et al., 2003). In this study,
plankton community respiration was determined by measuring
the change in the rate of DO during a dark incubation period.
Owing to limits imposed by the plan of the cruise, and by logistic-
al support, only a very few stations were reached between mid-
night and dawn, and thus water samples for CReu were collected
in the morning of each sampling day (6:00–12:00). First, each pre-
screened water sample was aliquoted into two 250 cm³ acid-
washed black glass bottles (black bottles were pre-sterilized after
being immersed in diluted hydrochloric acid for 8 h and re-
peatedly washed (Pringault et al., 2009) until they overflowed.
The bottles were then carefully sealed using a silica gel plug with
electrodes, paying careful attention to eliminating air bubbles in the bottles during this process. Next, the bottles were placed in a large incubation tank on deck to maintain the in situ temperature by pumping surface seawater into the tank (Ganf, 1974; Markager et al., 1992; Xu et al., 2006). As the study area included estuary and upwelling regions with higher activities of heterotrophic organisms, a 6 h incubation time was chosen to avoid the influence of the growth of microzooplankton and bacterioplankton. DO was determined in culture bottles using a six-channel oxygen meter with high-sensitivity SI1302 microcathode oxygen electrodes (Strathkelvin Instruments, Ltd, North Lanarkshire, Scotland), and probes were calibrated by iodometry before each incubation experiment according to the National Standards of the PRC (2008b). The dissolved oxygen meter recorded DO values once per second, and plankton community respiration rates were calculated by the differences in DO during the incubation period.

2.2.2 Measurements of environmental parameters

Sampling and procedures to determine T, salinity, and DO were conducted according to the method of China National Specifications Oceanographic Survey Standards (National Standards of the PRC, 2008a, b).

Bacterial abundance was analyzed by FCM (flow cytometry; FACS Calibur, Becton Dickinson Co., USA) according to Marie et al. (1999). A 5 cm³ subsample of water was fixed with paraformaldehyde (1%), quick-frozen in liquid nitrogen, and stored at –80°C in the laboratory. After thawing at room temperature, an aliquot of the PRC, 2008a, b). After analyzing the content of each filter, the bacteria, 2008, Vol. 37, No. 8, P. 74–83

Table 1. Integrated averages of the rates of plankton community respiration (CR_{eu}) and related environmental parameters in the northern South China Sea (nSCS) during the winter and summer of 2009.

| Parameter     | Winter               | Summer               |
|---------------|----------------------|----------------------|
|               | Coastal zone         | Continental shelf    | Open sea             | Coastal zone         | Continental shelf    | Open sea             |
| T/°C          | 20.2±1.2             | 21.7±0.8             | 21.3±0.9             | 26.6±2.1             | 25.1±1.0             | 20.6±0.5             |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| DO/μmol dm⁻³  | 551.12±56.54         | 499.78±21.62         | 422.87±31.97         | 394.95±74.65         | 434.59±16.32         | 378.25±13.30         |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| Chla/ mg m⁻³  | 3.05±3.05            | 0.39±0.09            | 0.18±0.04            | 1.86±1.69            | 0.26±0.05            | 0.09±0.03            |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| Net           | 1.66±2.20            | 0.05±0.04            | 0.01±0.00            | 1.04±1.04            | 0.03±0.02            | 0.01±0.00            |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| Nano          | 0.86±0.72            | 0.10±0.05            | 0.03±0.01            | 0.58±0.74            | 0.06±0.03            | 0.02±0.00            |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| Pico          | 0.53±0.36            | 0.24±0.07            | 0.14±0.04            | 0.25±0.17            | 0.18±0.04            | 0.07±0.02            |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| BAC/10⁶ cell cm⁻³ | 1.3±0.4              | 0.9±0.1              | 0.6±0.1              | 1.3±0.3              | 0.8±0.2              | 0.4±0.0              |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |
| CR_{eu}/μmol L⁻¹ h⁻¹ | 0.82                | 0.63±0.15            | 0.36±0.15            | 0.67                 | 0.35±0.01            | 0.09±0.04            |
| n             | 8                    | 6                    | 8                    | 8                    | 6                    | 8                    |

Note: T represents temperature, DO dissolved oxygen, RAC bacterial abundance, and Chl α chlorophyll a concentration; and these parameters were calculated in water column. CR_{eu} represents the rates of plankton community respiration, and was calculated in euphotic zone. Values are mean±standard deviation and n number of sample stations.
differences and spatial variation. In the winter, \(T\), DO, Chl \(a\) and BAC were \((21.8±2.1)^\circ\text{C}, (484.55±64.52)\ \mu\text{mol/dm}^3, (0.73±1.58)\ \text{mg/m}^3\), and \((0.9±0.4)\times10^6\ \text{cell/cm}^3\), respectively. In terms of horizontal distribution, \(T\) increased with increasing distance from the coastline, while DO, Chl \(a\) and BAC gradually decreased from the coastal zone to the open sea. The contributions of net-, nano- and pico-fractions to Chl \(a\) were 49%, 27% and 24%, respectively. In terms of vertical distribution, \(T\) decreased with increasing water depth. Coastal water was uniform owing to vertical mixing, and thermocline intensity was weak in the nSCS (Fig. 2).

In comparison with winter, the entire study area was characterized by high \(T\)((24.8±4.8)^\circ\text{C}), low DO ((419.46±77.71) \mu\text{mol/dm}^3), and low biomass (including Chl \(a\) ((0.50±1.23) mg/m\(^3\)) and BAC ((0.8±0.4)\times10^6 cell/cm\(^3\)) in summer. In terms of horizontal distribution, \(T\), Chl \(a\) and BAC gradually decreased from the coastal zone to the open sea; however, the highest value of DO was encountered in the continental shelf area. Net-, nano and pico-fractions contributed 50%, 29% and 21% to Chl \(a\), respectively, which was similar in comparison with that in winter. In terms of vertical distribution, the intensity of the thermocline in the summer was relatively strong. The thermocline in the coastal zone appeared at a depth of 20–30 m, while the thermocline of the open sea appeared at 50–75 m layer (Fig. 2). Both Chl \(a\) and BAC presented the phenomenon of subsurface maximum, significantly (Le et al., 2015; Zeng et al., 2017).

### 3.2 CR\(\text{eu}\) in the northern South China Sea

#### 3.2.1 Distribution of CR\(\text{eu}\)

The distribution of CR\(\text{eu}\) at depths with 100%, 10% and 1% of surface incident irradiance in winter and summer is shown in Fig. 3. The CR\(\text{eu}\) in winter was significantly higher than that in summer. The CR\(\text{eu}\) ranged between 0.10–1.10 \mu\text{mol/(L·h)} and 0.03–0.67 \mu\text{mol/(L·h)} in winter and summer, respectively. The mean value of CR\(\text{eu}\) in winter was (0.53±0.27) \mu\text{mol/(L·h)}, which was approximately two times the summer value ((0.26±0.20) \mu\text{mol/(L·h)}). The horizontal distribution of CR\(\text{eu}\) at each layer was similar in winter and summer, both decreasing from the coastal zone to the open sea (Fig. 3 and Table 1). CR\(\text{eu}\) in both the coastal zone and the continental shelf in winter remained high (0.82 and (0.63±0.15) \mu\text{mol/(L·h)}, respectively), while in the summer, the highest CR\(\text{eu}\) (0.67 \mu\text{mol/(L·h)}) was encountered in the coastal zone.

In terms of vertical distribution, the CR\(\text{eu}\) maximum mainly appeared at the subsurface layer in both seasons, with the exception of the coastal zones. From the surface to the bottom of the euphotic zone, the CR\(\text{eu}\) values at each sample depth were (0.56±0.26), (0.56±0.24) and (0.47±0.33) \mu\text{mol/(L·h)}, respectively, in winter. The CR\(\text{eu}\) maximum in the coastal zone generally appeared at the surface, while in the continental shelf and open sea, it generally appeared at depths with light attenuated to 10% of the surface irradiance. In summer, CR\(\text{eu}\) values at depths with 100%, 10% and 1% of surface incident irradiance were (0.19±0.10), (0.22±0.15) and (0.26±0.25) \mu\text{mol/(L·h)}, respectively. The CR\(\text{eu}\) maximum appeared at depths with light attenuated to 1% in the summer. It is worth noting that, Sta. A2 was not taken into consideration for this calculation, as only data for surface CR\(\text{eu}\) was obtained during the experiment.

#### 3.2.2 Relationships between CR\(\text{eu}\) and ecological factors

By comparing CR\(\text{eu}\) and ecological factors in the different regions of the nSCS (Table 1), it could be seen that CR\(\text{eu}\) and ecological factors exhibited distinct differences between the different regions of the nSCS. In the winter, \(T\) was lower than in the summer, where the temperature difference between winter and summer was 1–6\(^\circ\text{C}\). However, for CR\(\text{eu}\), the values for DO, Chl \(a\) and BAC were higher in winter than in summer, and their distributions were relatively consistent with each other. In terms of horizontal distribution, the values for CR\(\text{eu}\), Chl \(a\) and BAC in the coastal zone were significantly higher than those in the contin-

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**Fig. 2.** Vertical distribution of temperature (\(T\)) in the northern South China Sea (nSCS) during the winter and summer of 2009. a and c. Stations sampled in winter, b and d. stations sampled in summer, a and b. Transect S1, and c and d. Transect S2.
ental shelf and open sea. DO presented a progressively decreasing trend from the coastal zone and continental shelf to the open sea. Because thermal conductivity was higher in the continental shelf than in the ocean, \( T \) increased with increasing distance to the coastline in the winter, but gradually decreased with increasing distance to the coastline in the summer. From the aforementioned results, it can be seen that there is a high consistency between \( CR_{eu} \) and ecological factors in the different areas of the nSCS.

To understand the influence of the aforementioned ecological factors on \( CR_{eu} \), linear regression was used to analyze relationships between \( CR_{eu} \) and \( T \), DO, BAC, and Chl a in the euphotic zone (Fig. 4). The results show that biological factors (Chl a and BAC) were significantly positively correlated with \( CR_{eu} \). The most significant correlation was observed between BAC and \( CR_{eu} \), where BAC could explain 55\% of the variability in \( CR_{eu} \) and Chl a could explain 36\%. Of the abiotic factors, DO was significantly positively correlated with \( CR_{eu} \) \((R^2=0.45, p<0.05)\), while \( T \) displayed a relatively weaker negative correlation with \( CR_{eu} \) \((R^2=0.20, p<0.05)\).

4 Discussion

4.1 Variation in \( CR_{eu} \) and environmental control

In the South China Sea, in general, there is a significant concomitant relationship between physical, chemical and biological processes at the large- and mesoscales (Ning et al., 2004), such as biological processes in the upper layers were greatly affected by monsoon-driven oceanic movements. In summer, estuary and coastal upwellings are induced by the prevailing southwest monsoon, but the upper water of the whole sea area generally presents as oligotrophic, with a lower plankton community biomass.

Fig. 3. Distribution of the rates of plankton community respiration (\( CR_{eu} \)) in the northern South China Sea (nSCS) during the winter and summer. a, c, e. \( CR_{eu} \) in winter and b, d, f. \( CR_{eu} \) in summer.
owing to the influence of strong anti-cyclonic circulation on the basin scale (Liu et al., 2002). However, in the winter when the northeast monsoon prevails, the temperature decreases and the vertical mixing effect becomes strong. Coastal waters, driven by the northeast monsoon, move towards the southwest, and bring abundant nutrients into the South China Sea. Meanwhile, a large and strong cyclonic western boundary current forms in the western South China Sea, thereby causing an upwelling of lower layers, and thus supplements nutrients in the euphotic zone (Behrenfeld and Falkowski, 1997). Hence, the primary productivity in winter increases, which supports the higher standing stock of phytoplankton during this time. In addition, the dissolved organic matter, either released from the phytoplankton or from the process of zooplankta consuming phytoplankta, can be used by bacteria for growth and propagation to enhance bacterial abundance (Liu et al., 2007). High levels of nutrients would also lead to higher BAC, for bacteria can only effectively use dissolved organic matter when sufficient quantities of inorganic nutrients are present (Tupas and Koike, 1990; Cotner et al., 1997; Donachie et al., 2001). This study also demonstrates that the intensity of the thermocline was relatively weak in the winter (Fig. 2), and the biomass of the plankton community (both Chl a ((0.73±1.58) mg/m³) and BAC ((0.9±0.4)×10^6 cell/cm³)) in the water column in winter was significantly higher than that in the summer ((0.50±1.23) mg/m³ and (0.8±0.4)×10^6 cell/cm³, respectively). In addition, owing to the dual influences of the infusion of eutrophic rivers and offshore upwelling, high levels of nutrients and a high plankton community biomass are commonly reported in the coastal zone as opposed to in the open sea (Smith, 2006; Liu et al., 2007; Legrand et al., 2015). Similarly, high values of both Chl a and BAC encountered in the coastal zone were observed in the present study, along with a relatively high nutrient concentration, while the lowest values appeared in the open sea (Table 1).

The distribution of CR_eu, which also exhibited high temporal and spatial variability in the nSCS, was consistent with data for Chl a and BAC. Seasonally, CR_eu was substantially higher in the winter ((0.53±0.27) μmol/(L·h)) than in summer ((0.26±0.20) μmol/(L·h)). In terms of horizontal distribution, the CR_eu observed in the coastal zone and continental shelf ((0.82±0.20) and (0.50±0.25) μmol/(L·h), respectively) were quite higher than that observed in the open sea ((0.25±0.17) μmol/(L·h)). The highest CR_eu was observed in the continental shelf during winter, while it was observed in the coastal zone during summer. In terms of vertical distribution, the highest CR_eu occurred in the layers where the Chl a and BAC maxima appeared. Previous studies indicate that plankton respiration is positively correlated with the biomass of the plankton community (Robinson et al., 2002; Chen et al., 2003). However, as the composition and biomass of the
plankton community change concomitantly with the nutritional conditions and physicochemical environment, there is also a change in the respiration rate. In the East China Sea, bacteria were shown to be the main contributor of plankton respiration when nitrate concentrations were greater than 0.3 mmol/m³; however, when nitrate concentration was not greater than 0.3 mmol/m³, protozoans governed the respiration of the plankton community (Chen et al., 2003). In addition, a study on plankton respiration in the eastern Atlantic found that Bac and Chl α could explain the variability in the respiration of the plankton community by 70% and 61%, respectively (Robinson et al., 2002). In comparison with the aforementioned ocean regions, nutrients in the nSCS were relatively abundant, and the nitrate concentration at most stations was greater than 0.3 mmol/m³. Thus, it is unlikely that phytoplankton and bacterial biomass decreased, and that protozoans became the major component of plankton respiration as a result of nutrient limitation. The results of the present study indicate that $CR_{\text{pou}}$ in the nSCS was significantly and positively correlated with Bac and Chl a, where Chl a could explain 36% of the variability in $CR_{\text{pou}}$ and Bac could explain 55% of the variability in $CR_{\text{pou}}$, suggesting that heterotrophic bacteria are the main contributors to plankton respiration in the nSCS, and that phytoplankton and heterotrophic bacteria affect the majority of $CR_{\text{pou}}$ in a cooperative manner.

Our results also indicate that the abiotic factors $T$ and DO were weakly correlated with $CR_{\text{pou}}$, with relatively lower $R^2$, when compared with the correlations between $CR_{\text{pou}}$ and biotic factors. In the nSCS, $CR_{\text{pou}}$ was negatively correlated with $T$ ($R^2=0.20$, $p<0.05$), which was inconsistent with the reports of a previous study conducted in the Chesapeake Bay, where the seasonal variation in $CR_{\text{pou}}$ was positively correlated with temperature (Sam- pou and Kemp, 1994). Generally, plankton respiration demonstrates a significant sensitivity to fluctuations in temperature, where increasing temperature would accelerate the respiration rate (Ganf, 1974). In general, $Q_{10}$—the temperature sensitivity coefficient—of plankton respiration has been shown to be approximately 2.4 (Jankowski et al., 2014). In other words, with every 10°C increase in temperature, plankton respiration would also increase 2.4 times the original value. The temperature-dependent nature of plankton respiration has also been demonstrated in the North Atlantic Ocean Gyre (García-Corrall et al., 2014). However, the respiration rate of the plankton community in this case was also controlled by the biomass of the plankton community (García-Corrall et al., 2014), where this was mainly dependent on nutrient level and the concentration of dissolved organic carbon. In the nSCS, the increasing temperature would enhance the thermocline, thereby preventing the high-nutrient water under the thermocline to be transported into the euphotic zone, which restrains the growth of phytoplankton. Therefore, increased temperature indirectly reduces primary production in the water, and consequently lowers the biomass of phytoplankton and bacteria (Liu et al., 2007; Lo et al., 2015; Hao et al., 2016), thereby decreasing the respiration of the plankton community. According to the results of the present study, we believe that in the nSCS, the effect of biomass on $CR_{\text{pou}}$ is more pronounced than temperature. This could be the main reason for the negative correlation between $CR_{\text{pou}}$ and $T$ observed in the nSCS. In addition, the $CR_{\text{pou}}$ in the nSCS was positively correlated with DO ($R^2=0.45$, $p<0.05$). The results of a previously published study indicated that in waters with relatively abundant levels of nutrients, low levels of DO (31.25–62.50 $\mu$mol/dm³) would limit the respiration of the plankton community, and thus the influence of DO above the limited value on respiration was not very significant (Sam- pou and Kemp, 1994). In the present study, the minimum values of DO in winter and summer in the nSCS were 286.07 $\mu$mol/dm³ and 177.55 $\mu$mol/dm³, respectively, which were both substantially higher than the DO concentration that is considered to be limiting in plankton community respiration. Thus, the DO in the study area would not impose a restriction on $CR_{\text{pou}}$. The positive correlation observed between DO and $CR_{\text{pou}}$ could be a result of the following scenarios: (1) DO increased with increasing primary productivity, where approximately 10% of the primary productivity was released in the form of soluble inorganic carbon, thereby accelerating the growth of bacteria; (2) DO was generally positively correlated with phytoplankton biomass, which was also the main contributor of respiration under conditions of darkness (Markager et al., 1992; Zheng et al., 1992). Therefore, taken together, the aforementioned information indicates that abiotic factors do not directly influence the variability of $CR_{\text{pou}}$ in a significant manner, but they mainly exert indirect effects on the variability of $CR_{\text{pou}}$ via their effects on biotic factors.

4.2. Comparisons with previous studies

In comparison with the published findings regarding respiration in subtropical ocean waters, the distribution of $CR_{\text{pou}}$ in the nSCS presented consistency in a general trend, and also preserved the peculiarity of the investigated area (Table 2). On one hand, the $CR_{\text{pou}}$ in the study area decreased sharply from the coastal zone to the open sea, which was consistent with trends of plankton respiration gradually decreasing from the inner to the outer southeastern continental shelf in the USA (Pomeroy et al., 2000) and the continental shelf regions of the Mediterranean and the East China Sea (Le Ferla et al., 2006; Chen et al., 2003). Affected by factors such as the input of terrestrial organic matter and near shore upwelling, nutrients in the coastal zone and the continental shelf are relatively rich, and effectively support the high plankton community biomass observed in these regions, which causes the relatively higher respiration of plankton communities in the coastal zone and inner continental shelf (Iriarte et al., 1991; Chen et al., 2003). On the other hand, plankton community respiration in the winter in the nSCS was significantly higher than that in the summer, in contrast to the findings of a higher-latitude continental shelf near Georgia, USA (Jiang et al., 2010). Bearing in mind the $Q_{10}$ value of plankton (2.4) (Jankowski et al., 2014), the temperature difference between winter and summer in the continental shelf of Georgia was between 10–20°C (which exerted a significant influence on $CR_{\text{pou}}$), while the temperature difference between winter and summer in the present study was approximately 1–6°C, and appears to have had little influence on the respiration of the plankton community.

A comparison between the $CR_{\text{pou}}$ in the nSCS and that of open sea regions of similar latitudes can be seen in Table 2. This comparison shows that the $CR_{\text{pou}}$ in the nSCS in summer was higher than the values observed in oligotrophic regions (e.g., Central North Pacific Ocean Gyre, North Atlantic Subtropical Gyre, Eastern Tropical Atlantic Gyre) (Williams and Purdie, 1991; Gonzláez et al., 2001; Robinson et al., 2002), and was slightly lower than that in the open sea areas of upwelling with relatively abundant nutrients (North West African Upwelling) (Robinson et al., 2002).

It is also important to note that variations in sampling and incubation time could lead to slight differences in the resulting measurements of plankton respiration. As aforementioned, it is ideal to collect water samples before dawn to ensure consistency between metabolic level and the circadian rhythm of plankton communities (Gasol et al., 1998). However, some studies have indicated that the activities of bacteria and the abundance of het-
erlotrophic flagellates were the highest at midnight or during early morning (Kuipers et al., 2000). Therefore, collecting water samples before dawn could result in the estimated respiration rate of plankton communities to seem high, while collecting water samples during the daytime could result in an underestimation. Thus, in the present study, the later collection times may have resulted in an underestimation of the respiration rate. Likewise, differences in incubation may also result in differences in respiration rate estimation. In general, the incubation time is 24 h. Under the assumption that the respiration rate increases linearly with time, a 24 h incubation is ideal to record the complete diurnal variation of plankton community respiration. However, Bender et al. (1999) points out that the accelerated growth, grazing, and death of microzooplankton in the absence of large grazers during the incubation period in bottles may result in underestimations of plankton community respiration rates, and that using short incubation times can, at least partially, alleviate this problem. In the present study, the incubation time was 6 h, which could have caused a slight overestimation of CR eu, even though it weakened the bottle effect. Despite the sampling and incubation times in the present study, the differences in CR eu between this study and other similar studies are not substantial, and trends in the large-scale distribution of CR eu tendency are comparable. Therefore, we suggest that the error from methodological variation did not exert a significant influence on large-scale seasonal variations in respiration and spatial tendencies. 4.3 Possibility of estimating respiration rate by environmental factors The correlations between plankton community respiration and environmental parameters have been previously used to estimate plankton community respiration, either partially or entirely (Jensen et al., 1990; Chen et al., 2003). In addition, it has been shown that the correlation between plankton community respiration and a single parameter was significantly lower than the correlation between plankton community respiration and multiple parameters (Chen et al., 2003). In the present study, the biomass, physical environment, and community structure were considered in the multiple linear regression (since DO in the investigated area was much higher than the DO concentration limiting plankton respiration, it was not considered here). As indicated in Table 3, in the regression group with two variables, the combination of BAC and Chl a was the closest predictor of CR eu, and could explain 82% of the variation in CR eu, indicating that bacteria and chlorophyll were the main factors influencing plankton respiration in the nSCS. After adding the variable of temperature, and using the size-fractioned chlorophyll concentration instead of Chl a, R² did not significantly increased, indicating that temperature and community structure have little predictive effect on CR eu. Thus, the combination of BAC and Chl a was not suitable for estimating respiration rate by environmental factors.

### Table 2. Comparison of rate of plankton community respiration (CR eu) in the eutrophic zones of subtropical oceans

| Region | Season | T/°C | CR eu (calculated by O₂)/mmol·m⁻³·d⁻¹ | Reference |
|--------|--------|------|------------------------------------|-----------|
| South-eastern USA continental shelf (28.0°–34.0°N, 78.0°–83.0°W) | Inner shelf | winter | 26.4 | ND | Pomeroy et al. (2000) |
| | Middle shelf | winter | 16.8 | ND | Pomeroy et al. (2000) |
| | At the edge of the Gulf Stream | winter | 7.2 | ND | Pomeroy et al. (2000) |
| | Continental shelf off Georgia, USA (30.8°–32.2°N, 79.5°–81.5°W) | winter | 10.0–30.0 | 0.3–3.3 | ND | Jiang et al. (2010) |
| | nSCS (18.5°–22.0°N, 111.0°–117.0°E) | winter | 20.2–25.5 | 4.6–19.6 | 548 | this study |
| South-eastern USA continental shelf (28.0°–34.0°N, 78.0°–83.0°W) | Inner shelf | summer | 22.8 | ND | Pomeroy et al. (2000) |
| | Middle shelf | summer | 31.2 | ND | Pomeroy et al. (2000) |
| | At the edge of the Gulf Stream | summer | 24 | ND | Pomeroy et al. (2000) |
| | CNPO (Central North Pacific Ocean Gyre; 28.0°–29.0°N, 154.0°–155.0°W) | summer | 24.9–25.3 | 0.1–1.6 | 53 | Williams and Purdie (1991) |
| | NAS (North Atlantic Subtropical Gyre; 28.0°–38.0°N, 38.0°–18.0°W) | summer | 20 | ND | 142 | González et al. (2001) |
| | ETRA (Eastern Tropical Atlantic Gyre; 15.5°S–14.2°N) | summer | 22.2–28.9 | ND | 159 | Robinson et al. (2002) |
| | NWAF (North West African Upwelling; 14.9°–21.2°N) | summer | 22.6 | ND | 211 | Robinson et al. (2002) |
| | nSCS (18.5°–22.0°N, 111.0°–117.0°E) | summer | 25.5–30.3 | 1.7–16.0 | 208 | this study |

Note: Data are represented as means and ranges. ND means no data.

### Table 3. Results of multiple linear regression between CR eu and related factors in the northern South China Sea (nSCS)

| Factor | Function | R² |
|--------|----------|----|
| T Chl a | CR eu = –0.0159 × T + 0.30318 × Chl a + 0.695 98 | 0.38 |
| T BAC | CR eu = –0.032913 × T + 0.70614 × BAC + 0.503 764 | 0.67 |
| BAC Chl a | CR eu = 0.69414 × BAC + 0.311 92 × Chl a − 0.391 58 | 0.82 |
| T BAC Chl a | CR eu = –0.010008 × T + 0.686117 × BAC + 0.276 569 × Chl a − 0.123 779 | 0.83 |
| T BAC Net | CR eu = –0.018753 × T + 0.707 924 × BAC + 1.144 718 × Net + 0.106 085 | 0.86 |
| T BAC Net Nano | CR eu = –0.01399 × T + 0.803 224 × BAC − 0.331 087 × Net + 1.043 628 × Nano − 0.140 226 | 0.87 |
| T BAC Net Nano Pico | CR eu = –0.021476 × T + 0.823 222 × BAC − 0.411 904 × Net + 1.342 398 × Nano − 0.291 136 × Pico + 0.095 266 | 0.89 |

Note: Net, Nano and Pico represent the concentration of net-Chl, nano-Chl and pico-Chl, respectively. The units of CR eu, T, BAC, Chl a, Net, Nano, and Pico are μmol/(L·h), °C, 10⁶ cell/mL³, mg/m³, mg/m³, mg/m³, and mg/m³, respectively.
could be the most effective predictor of the distribution of $CR_{eu}$ in the nSCS.

Based on the multiple linear regression equation of $CR_{eu}$, BAC and Chl $a$, and the measurements of Chl $a$ and BAC in the water column at each station, plankton respiration in the water column ($CR_{int}$) within 100 m of the study area was estimated. The average $CR_{int}$ in winter and summer in the nSCS was $(17.32±20.27)$ mmol/(m$^3$·d) and $(11.81±12.18)$ mmol/(m$^3$·d), respectively, which is consistent with the distribution of $CR_{eu}$. However, $CR_{int}$ below the euphotic zone of the open ocean—estimated from the multiple linear regression equation of $CR_{eu}$, BAC and Chl $a$—contained negative values. The reason might be the inconsistencies in plankton biomass and respiration inside and outside the euphotic zone. The changes in bacterial metabolism, which was caused by the sharply decreasing temperature from the upper euphotic zone to deeper layers, might have resulted in the above-mentioned inconsistency. It has been found in controlled experiments on respiration that when the controlled-environmental temperature is 5°C lower than the in situ temperature, plankton respiration dropped by about 20%–50%, and bacterial respiration also presented significant temperature dependency (Wang, 2014). Our observations indicated that the average temperature in the 100–200 m deep waters was 7.6°C lower than the average temperature in the waters above 100 m. The rapid drop in temperature possibly caused the decrease in bacterial metabolism below the euphotic zone, thereby causing a greater decrease in plankton respiration. Moreover, there were differences in the quality and quantity of organic substances available for consumption by the microbial communities in the upper and deeper layers of the ocean (Jiao et al., 2014). The dissolved organic matter brought from the outside and the dissolved organic carbon generated by primary productivity mainly exist in the euphotic zone, and nutrient concentration in the euphotic zone is relatively high and easily utilized by bacteria; below the euphotic zone, the growth of bacteria is slow because of the inefficient degradation of dissolved organic matter and the low availability of nutrient substances. Hence, owing to the changes in conditions such as temperature and nutrient availability in the water column, there may have been a nonlinear relationship between plankton biomass and respiration outside the euphotic zone. Using the empirical relationship obtained from the parameters in the euphotic zone may lead to an overestimation of plankton respiration in the water column.

5 Conclusions

In this study, we conclude that plankton respiration in the euphotic zone of the nSCS is higher in winter than in summer, and respiration in the coastal zone is higher than that in the open sea, thereby exhibiting significant seasonal and spatial variations. Plankton respiration was positively correlated with plankton community biomass, indicating a close coupling effect between plankton respiration and primary productivity, which is also driven by monsoon-circulation processes. Similar to in other oligotrophic ocean systems, heterotrophic bacteria are the main contributor to respiration. Because respiration is controlled by the biomass of plankton communities, temperature is not a dominant factor in the upper layer of the nSCS, and the influence of temperature on seasonal variations in plankton respiration is lower than that of nutrient availability. In addition, we have demonstrated that there is a significant error in estimating plankton respiration throughout the water column when using the empirical relationship between plankton respiration and ecological factors in the euphotic zone. This indicates that the relationship between plankton respiration and ecological factors may change as the depth changes. In future, the respiration of plankton communities throughout the water column and possible substitute factors should be considered when making estimations. More importantly, it is necessary to integrate observations of primary production and plankton respiration to describe variations in net community production to further elucidate the details of the organic carbon budget in the South China Sea.

Acknowledgements

The authors thank the Environmental Monitoring Center of South China Sea Branch, State Oceanic Administration for providing temperature, DO, and nutrient data. The authors are also grateful to the officers and crew of the “China Marine Surveillance 81” cruise for their assistance.

References

Ahrens M A, Peters R H. 1991. Plankton community respiration: relationships with size distributions and lake trophy. Hydrobiologia, 224(2): 77–87

Behrenfeld M J, Falkowski P G. 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnology and Oceanography, 42(1): 1–20

Bender M, Orschao J, Dickson M L, et al. 1999. In vitro $O_2$ fluxes compared with $^{14}$C production and other rate terms during the JGOFS Equatorial Pacific experiment. Deep Sea Research Part I: Oceanographic Research Papers, 46(4): 637–654

Booth B C. 1988. Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific Ocean in May and August, 1984. Marine Biology, 97(2): 275–286

Chen C, Shiah F K, Gong G C, et al. 2003. Planktonic community respiration in the East China Sea: importance of microbial consumption of organic carbon. Deep Sea Research Part II: 82

Cotner J B, Ammerman J W, Peele E R, et al. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology, 13(2): 141–149

del Giorgio P A, Duarte C M. 2002. Respiration in the open ocean. Nature, 420(6914): 379–384

Donachie S P, Christian J R, Karl D M. 2001. Nutrient regulation of bacterial production and eucaryote activities in the subtropical North Pacific Ocean. Deep Sea Research Part II: Topical Studies in Oceanography, 48(8–9): 1719–1732

Ganf G G. 1974. Rates of oxygen uptake by the planktonic community of a shallow equatorial lake (Lake George, Uganda). Oecologia, 15(1): 17–32

García-Coral L S, Barber E, Regaudie-de-Gioux A, et al. 2014. Temperature dependence of planktonic metabolism in the subtropical North Atlantic Ocean. Biogeosciences, 11(2): 4529–4540

García-Coral L S, Holding J M, Carillo-de-Albornoz P, et al. 2017. Temperature dependence of plankton community metabolism in the subtropical and tropical oceans. Global Biogeochemical Cycles, 31(7): 1141–1154

Gasol J M, Doval M D, Pinhassi J, et al. 1998. Diel variations in bacterial heterotrophic activity and growth in the northwestern Mediterranean Sea. Marine Ecology Progress Series, 164: 107–124

González N, Anadón R, Mouríño B, et al. 2001. The metabolic balance of the planktonic community in the North Atlantic Subtropical Gyre: the role of mesoscale instabilities. Limnology and Oceanography, 46(4): 946–952

Hao Qiang, Liu Chenggang, Le Fengfeng, et al. 2016. Phytoplankton photosynthesis-irradiance parameters during summer and winter in the northern South China Sea. Haiyang Xuebao (in Chinese), 38(4): 22–30

Holm-Hansen O, Lorenzen C J, Holmes R W, et al. 1965. Fluorometric determination of chlorophyll. ICES Journal of Marine Science, 30(1): 3–15

Huang Bangqin, Hong Huaosheng, Xu Xiangzhong, et al. 2005. Study
on respiratory electron transport system (ETS) of phytoplankton in Taiwan Strait and Xiamen Harbour. Chinese Journal of Oceanology and Limnology, 23(2): 176–182
Iriarte A, Daneri G, Garcia V M T, et al. 1991. Plankton community respiration and its relationship to chlorophyll a concentration in marine coastal waters. Oceanologica Acta, 14(4): 379–388
Jankowski K, Schindler D E, Lisi P J. 2014. Temperature sensitivity of community respiration rates in streams is associated with watershed geomorphic features. Ecology, 95(10): 2707–2714
Jensen I L, Sand-Jensen K, Marcher S, et al. 1990. Plankton community respiration along a nutrient gradient in a shallow Danish estuary. Marine Ecology Progress Series, 61(1–2): 75–85
Jiang Liqing, Cai Weijun, Wang Yongchen, et al. 2010. Pelagic community respiration on the continental shelf off Georgia, USA. Biogeochemistry, 98(1–3): 101–113
Jiao N, Robinson C, Azam F, et al. 2014. Mechanisms of microbial carbon sequestration in the ocean-future research directions. Biogeoosciences, 11(9): 5285–5306
Kuipers B, Van Noort G J, Vosjan J, et al. 2000. Diel periodicity of bacterioplankton in the euphotic zone of the subtropical Atlantic Ocean. Marine Ecology Progress Series, 201: 13–25
La Ferla R, Azzaro M, Maimone G. 2006. Microbial respiration and trophic regimes in the Northern Adriatic Sea (Mediterranean Sea). Estuarine, Coastal and Shelf Science, 69(1–2): 196–204
Le Fengfeng, Cai Yuming, Sun Jun, et al. 2015. Seasonal variation of picoplankton abundance and biomass in the northern South China Sea in summer and winter of 2009. Haiyang Xuebao (in Chinese), 29(2): 41–51
Legrand C, Fridolfsson E, Bertos-Fortis M, et al. 2015. Interannual variability of phyto-bacterioplankton biomass and production in coastal and offshore waters of the Mediterranean Sea. Ambio, 44(S3): 427–438
Li Daoji, Zhang Jing, Huang Daji, et al. 2002. Oxygen depletion off the Changjiang (Yangtze River) Estuary. Science in China Series D: Earth Sciences, 45(12): 1137–1146
Liu K K, Chao S Y, Shaw P T, et al. 2002. Monsoon-forced chlorophyll a and primary productivity in the Taiwan Strait and the northern South China Sea in summer 2012. Journal of Tropical Oceanography (in Chinese), 33(4): 61–68
Li Fengfeng, Cai Yuming, Sun Jun, et al. 2015. Seasonal variation of picoplankton abundance and biomass in the northern South China Sea in summer and winter of 2009. Haiyang Xuebao (in Chinese), 29(2): 41–51
Lin Wei, Chen Bingzhang, et al. 2014. Metabolic states of benthic community respiration rates in streams is associated with water temperature. Journal of Tropical Oceanography (in Chinese), 33(4): 61–68
Liu Chenggang, Ning Xiuren, Cai Yuming, et al. 2007. Bacterioplankton in the euphotic zone of the subtropical Atlantic Ocean. Marine Ecology Progress Series, 291: 13–25
Maire D, Brussaard C P D, Thyrhaug R, et al. 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied and Environmental Microbiology, 65(1): 45–52
Markager S, Jespersen A M, Madsen T V, et al. 1992. Diel changes in dark respiration in a plankton community. Hydrobiologia, 238(1): 119–130
National Standards of the PRC. 2008a. GB/T 12763. 2–2007 Specifications for Oceanographic Survey-Part 2: Marine Hydrographic Observation (in Chinese). Beijing: China Standards Press, 5–8
National Standards of the PRC. 2008b. GB/T 12763. 4–2007 Specifications for oceanographic survey-Part 4: Survey of chemical parameters in sea water (in Chinese). Beijing: China Standards Press, 7–10
Ning X, Chai F, Xue H, et al. 2004. Physical-biological oceanographic coupling influencing phytoplankton and primary production in the South China Sea. Journal of Geophysical Research: Oceans, 109(C10): C10005
Ning Xiuren, Coudé C. 1991. Interrelationships among chlorophyll a, bacteria, ATP, POC and microbial respiration rate in the Changjiang estuary and plume area. Haiyang Xuebao (in Chinese), 13(6): 831–838
Pomroy E R, Sheldon J E, Sheldon W M, et al. 2000. Seasonal changes in microbial processes in estuarine and continental shelf waters of the south-eastern U. S. A. Estuarine, Coastal and Shelf Science, 51(4): 415–428
Pringault O, Tesson S, Rochelle-Newall E. 2009. Respiration in the light and bacterio-phytoplankton coupling in a coastal environment. Microbial Ecology, 57(2): 321–334
Rivkin R B, Legendre L. 2001. Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science, 291(5512): 2398–2400
Robinson C, Serret P, Tilstone G, et al. 2002. Plankton respiration in the Eastern Atlantic Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 49(5): 787–813
Sampou P, Kemp W M. 1994. Factors regulating plankton community respiration in Chesapeake Bay. Marine Ecology Progress Series, 110(2–3): 249–258
Schwaerter S, Sondergaard M, Riemann B, et al. 1988. Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. Journal of Plankton Research, 10(3): 515–531
Smith V H. 2006. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnology and Oceanography, 51(5): 161–172
Tupas L, Koike I. 1990. Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. Limnology and Oceanography, 35(5): 1145–1155
Wang Na. 2014. Community and bacterial respiration in the South China Sea and Fujian coastal waters [dissertation] (in Chinese). Xiamen: Xiamen University
Wang Na, Lin Wei, Chen Bingshang, et al. 2014. Metabolic states of the Taiwan Strait and the northern South China Sea in summer 2012. Journal of Tropical Oceanography (in Chinese), 33(4): 61–68
Williams P J L B, del Giorgio P A. 2005. Respiration in Aquatic Ecosystems: History and Background. Solid State Ionics, 25(1): 100–122
Xu Shenbo, Wang Wenqi, Zhang Yuna, et al. 2006. Manufacture of the device for breath measuring and its use in water environmental monitoring. Journal of Laiyang Agricultural College (in Chinese), 20(10): 109(C10): C10005
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Abstract

Dissolved organic matter (DOM) represents a significant source of nutrients that supports the microbial-based food web in seagrass ecosystems. However, there is little information on how the various fractions of DOM from seagrass leaves contributed to the coastal biogeochemical cycles. To address this gap, we carried out a 30-day laboratory chamber experiment on tropical seagrasses *Thalassia hemprichii* and *Enhalus acoroides*. After 30 days of incubation, on average 22% carbon (C), 70% nitrogen (N) and 38% phosphorus (P) of these two species of seagrass leaf litter was released. The average leached dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) of these two species of seagrass leaf litter accounted for 55%, 95% and 65% of the total amount of C, N and P lost, respectively. In the absence of microbes, about 75% of the total amount of DOC, monosaccharides (MCHO), DON and DOP were quickly released via leaching from both seagrass species in the first 9 days. Subsequently, little DOM was released during the remainder of the experiment. The leaching rates of DOC, DON and DOP were approximately 110, 40 and 0.70 μmol/(g·d). Leaching rates of DOM were attributed to the nonstructural carbohydrates and other labile organic matter within the seagrass leaf. *Thalassia hemprichii* leached more DOC, DON and MCHO than *E. acoroides*. In contrast, *E. acoroides* leached higher concentrations of DON than *T. hemprichii*, with the overall leachate also having a higher DON: DOP ratio. These results indicate that there is an overall higher amount of DOM leachate from *T. hemprichii* than that of *E. acoroides* that is available to the seagrass ecosystem. According to the logarithmic model for DOM release and the *in situ* leaf litter production (the Xincun Bay, South China Sea), the seagrass leaf litter of these two seagrass species could release approximately 4×10^3 mol/d DOC, 1.4×10^3 mol/d DON and 25 mol/d DOP into the seawater. In addition to providing readily available nutrients for the microbial food web, the remaining particulate organic matter (POM) from the litter would also enter microbial remineralization processes. What is not remineralized from either DOM or POM fractions has potential to contribute to the permanent carbon stocks.

Key words: dissolved organic matter, *Thalassia hemprichii*, *Enhalus acoroides*, leaf litter, leaching

Citation: Liu Songlin, Jiang Zhijian, Zhou Chenyuan, Wu Yunchao, Arbi Iman, Zhang Jingping, Trevathan-Tackett Stacey M. 2018. Leaching of dissolved organic matter from seagrass leaf litter and its biogeochemical implications. Acta Oceanologica Sinica, 37(8): 1–7, doi: 10.1007/s13131-018-1233-1

1 Introduction

Seagrass beds rank among the most productive autotrophic ecosystems on the planet, despite only covering 0.15% of global sea surface area (Duarte and Chiscano, 1999; Hemmings and Duarte, 2000). A large fraction of seagrass production (up to 50%) is allocated to the growth of aboveground biomass (Duarte et al., 1996), however the relatively high C:N:P ratios and low palatability of seagrass leaves (Vizzini et al., 2002; Duarte et al., 2010) leads to the generally low use of seagrass production by herbivores (Cebrián et al., 1996; Cebrian and Duarte, 2001). Instead, most seagrass leaf production senesces and contributes to the detrital pool (Cebrián et al., 1996; Chiù et al., 2013). Decomposition within the seagrass ecosystems is common for senesced seagrass leaves (Mateo et al., 2006), although it has been recently found that seagrass production can contribute significant biomass to other habitats via export (Duarte and Krause-Jensen, 2017). According to global data summarized, Duarte and Krause-Jensen (2017) estimated that 50% of seagrass biomass produced is decomposed, with export and herbivory accounting for 24% and 19%, respectively.

Leaf litter that enters the decomposition process provides a source of C and nutrient recycling within seagrass meadows and neighboring ecosystems (Ziegler and Benner, 1999; Holmer and Olsen, 2002; Yarbro and Carlson, 2008; Jiménez et al., 2017). The
decomposition of seagrass leaves begins with a rapid initial leaching or autolytic production of dissolved organic matter (DOM), which typically represents most of the labile organic matter content of seagrass leaf (Peduzzi and Herndl, 1991; Lavery et al., 2013). Subsequently, microbial breakdown of more recalcitrant organic matter (e.g., lignin and cellulose) could last for months to years (Godshalk and Wetzel, 1978; Peduzzi and Herndl, 1991). Most of DOM released from seagrass leaf litter occurs during the first few weeks (Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014), but can continue for many months as progressively more cell walls are penetrated by microbes (Harrison, 1989). For example, Lavery et al. (2013) found the Posidonia sinuosa released approximately 50% DOM in the first 14 days and estimated it would take about 3 years to release the next 50% DOM. The DOM that is released from seagrass leaf litter provides an important ecosystems service as it supports microbial production and thus microbial-based food webs (Robertson et al., 1982; Våhätalo and Søndergaard, 2002; Lavery et al., 2013). Therefore, seagrass leaf senescence, abscission and subsequent decomposition together represent an ample and constant source of DOM to the ecosystems (Kirkman and Reid, 1979; Mateo and Romero, 1996; Ziegler and Benner, 1999; Apostolaki et al., 2009).

The leaching process can be easily predicted and quantified using a single-component exponential decay model (Maie et al., 2006; Lavery et al., 2013). However, most studies describing these DOM leaching dynamics focus on DOC and chromophoric DOM (CDOM) (Våhätalo and Søndergaard, 2002; Vichkovitten and Holmer, 2004; Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014). As a result, there is little information on the contributions of seagrass dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) to seagrass meadow nutrient cycling. In order to fill in these research gaps, we used a laboratory incubation technique to reveal the release dynamics of DOM from the two tropical seagrass species: Thalassia hemprichii and Enhalus acoroides. Our aim was to investigate the species-specific leaching patterns of the different DOM fractions: DOC, monosaccharide (MCHO), DON and DOP. The results will help to illuminate the DOM availability of leachate from seagrass leaf litter in a tropical meadow to strengthen our understanding of the effects of seagrass leaf detritus on the biogeochemistry in seagrass ecosystems.

2 Materials and methods

2.1 Study site and sample collection

A mixed tropical seagrass meadow lies in the southern shallow waters of the Xincun Bay (18°24′34″~18°24′42″N, 109°57′42″~109°57′58″E), South China Sea, dominated by Thalassia hemprichii (Ehrenb. ex Solms) Asch. and Enhalus acoroides (L.L.) Royle (Huang et al., 2006). The T. hemprichii and E. acoroides meadows occupy about 50% and 40% of southern shallow water region in the Xincun Bay (unpublished data), respectively, and are an important source for the Xincun Bay DOM (Liu et al., 2016b). A vast amount of leaf litter of these two tropical seagrass species accumulate in the southern shallow waters of the Xincun Bay. The oldest leaf blades still attached to the shoots were collected from seagrass meadows during low tide. After collection, the seagrass leaves were transported to the laboratory, scraped free of epiphytes using a razor blade, washed with ambient seawater, then cut into pieces for the leaching experiment.

2.2 Experimental setup

Before the experiment, a separate subset of seagrass leaves was dried to a constant weight in order to calculate the wet weight: dry weight conversion of the two seagrass species. All glass bottles used in the sample collection and experimental process were acid-cleaned (7 days in 10% HCl), rinsed with Milli-Q water and then pre-combusted (500°C, 5 h) for removing organic carbon. For the leaching experiment, 20 g wet weight (approximately 2 g dry weight) of senescent seagrass leaves were placed into acid-washed 1.000 mL glass serum bottles (n=3) (Maie et al., 2006). Next, 500 mL of sterile, artificial seawater (using the in situ salinity of 31) and 2.0 mL saturated HgCl₂ solution was added to each bottle to ensure no microbes were active for the experiment, i.e., a “microbe-independent” leaching experiment (Wang et al., 2014). All bottles were left open to the atmosphere, covered slackly with clean aluminum foil and incubated at room temperature (25°C) in the dark for 30 days. At selected times (Days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30), the incubating seawater was collected for DOC and nutrient measurements. The fresh artificial seawater was analyzed for the initial background contents of DOC and nutrients. In between sampling times, water level was monitored during the incubation, and analyzed concentrations were adjusted for water evaporation losses (Maie et al., 2006). After the 30-day incubation, the seagrass leaves were dried to constant weight and the dry mass measured.

2.3 Sample analysis

Water samples were filtered through pre-combusted (450°C for 4 h) GF/F filters. For DOC analysis, samples were stored in acid-washed brown apragaz bottles at -20°C before analysis on a TOC analyzer (TOC-VcpH, Shimadzu, Japan). MCHO was determined by the TPTZ (2, 4, 6-tripyridyl-s-triazine) method (Myklestad et al., 1997). Briefly, samples were measured by oxidizing the free reduced sugar with Fe³⁺ in alkaline conditions, followed by spectrophotometric analysis (UV-2600, Shimadzu, Japan) of a colored product of reduced Fe²⁺ and TPTZ. DON was calculated as the difference between total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN=NO₃⁻+NO₂⁻+NH₄⁺). DOP was determined as the difference between total dissolved phosphorus (TDP) and dissolved inorganic phosphorus (DIP=PO₄³⁻) (Bronk et al., 2000; Barrón and Duarte, 2009). TDN and TDP were measured with the persulfate oxidation method (Valderrama, 1981; Bronk et al., 2000; Pierzynski, 2000). DIN and DIP were measured spectrophotometrically according to standard colorimetric techniques following the methods developed by Grasshoff et al. (2009) using a spectrophotometer (UV-2600, Shimadzu, Japan).

At the beginning of the experiment, the leaf nonstructural carbohydrate content was measured. After drying of the leaf litter, the leaf samples were ground to a size of 0.18 mm (~80-mesh sieve). Approximately, 0.1 g was extracted twice in hot 80% ethanol. The soluble sugar content was determined by the anthrone-sulfuric acid method (Yemm and Willis, 1954). Subsequently, the starch content of the remaining materials was quantified by gelatinization at 100°C for 15 min and solubilization in 7% perchloric acid (Allen, 1989). Nonstructural carbohydrate content was estimated by multiplying soluble sugar and starch concentrations (nonstructural carbohydrate=soluble sugar+starch) (Orth and Moore, 1986; Burke et al., 1996). In addition, the dry weight (DW) and elemental contents of the leaf litter before and after the incubation were measured as well. Leaf samples were oven-dried at 60°C to a constant weight for determining the leaf DW. elemental C and N using the dried seagrass were measured by Elemental CHNS analyzer model Vario EL cube (Vario EL, Elemental Analyser system GmbH, Germany). The elemental P
content was analyzed by a colorimetric method (Fourquean et al., 1992). Each sample of above parameters was measured two or three times with a coefficient of variation of ≤2%.

2.4 Data analysis

Leaf characteristics, releasing rates, cumulative releasing concentrations of DOC, MCHO, DON and DOP and their stoichiometry ratios during the 30-day incubation were compared between the two seagrass species using student’s t-test. Data were log transformed if the assumption of homogeneity of variance was violated. The seagrass species effect with α<0.05 was considered statistically significant. Meanwhile, the effect sizes of releasing rates, cumulative releasing concentrations of DOC, MCHO, DON and DOP, and their stoichiometry ratios between the two seagrass species were calculated by using Cohen’s d test. Napierian logarithm functions were carried out for fitting the accumulation concentrations of DOM from seagrass leaf litter (Maie et al., 2006; Lavery et al., 2013). All above mentioned statistical analysis was performed with Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA), SigmaPlot 12.0 (Systat Software Inc., Chicago, IL) and IBM SPSS Statistics 19.0 software (IBM SPSS Statistics 19, IBM Corporation, Somers, NY).

3 Results

3.1 Characteristics of seagrass leaves

Characteristics of the seagrass leaves in the Xincun Bay used for the experiment were summarized in Table 1. There were no significant differences in leaf soluble sugar, starch, C and N content between the two seagrass species. However, the soluble sugar in the leaves of T. hemprichii was slightly lower than that of E. acoroides, while the opposite was shown for species differences in starch and nonstructural carbohydrates. Elemental contents were similar between T. hemprichii and E. acoroides. Additionally, after the 30-day incubation, the remaining weights of both seagrass species were ~70% of the initial DW. There was no significant difference in the elemental content between the two seagrass species after 30 days of decomposition. In addition, there was approximately 22% C, 70% N and 38% P released from the seagrass during the 30 days leaching period. In other words, the C, N and P released from the seagrass leaf litter were about 6,000, 1,200 and 30 μmol/g (DW), respectively.

3.2 Characteristics of leaching dissolved organic matter

More than 75% of the total DOC, MCHO, DON and DOP was released from the two species of seagrass leaf litter in the first 9 days (Fig. 1). The accumulated leachate concentrations of DOC, DON and DOP (over the 30 days) were observed not to be significantly different between the two seagrass species (Figs 1a, c, d; p>0.05). However, the comparison (T. hemprichii vs. E. acoroides) of effect sizes of DOC, DON and DOP using Cohen’s d were 0.87, -1.87 and 8.48, respectively (small effect=0.2, medium effect=0.5, large effect=0.8). Additionally, the MCHO released from T. hemprichii leaf litter was 1.6-fold higher than E. acoroides after the 30-day incubation (Fig. 1b, p<0.05, Cohen’s d=6.28). There were no significant differences of the leaching rates of DOM between the two seagrass species (Figs 2a, c, d; p>0.05), with the exception of MCHO (Fig. 2b, p<0.05). Meanwhile, the Cohen’s d test of leaching rates (T. hemprichii vs. E. acoroides) of DOC, MCHO, DON and DOP were 0.87, 4.45, -0.75 and 3.01, respectively. Furthermore, the released amount of DOC, MCHO, DON and DOP leached from leaf litter were lower than the C, N and P content lost from the leaf litter, respectively. The DOC, DON and DOP accounted for 55%, 95% and 65% of the total C, N and P amounts lost of seagrass leaf litter, respectively.

Over the 30-day incubation, the DOC:DON:DOP ratio of the released DOM from T. hemprichii and E. acoroides were 161:50:1 and 169:89:1, respectively. In addition, the DON:DOM ratio of the total released DOM from T. hemprichii was significantly lower than that of E. acoroides (T. hemprichii vs. E. acoroides, p<0.05, Cohen’s d=−0.82). In contrast, the DOC:DON (T. hemprichii vs. E. acoroides, p>0.05, Cohen’s d=0.54) and DOC:DOP (T. hemprichii vs. E. acoroides, p<0.05, Cohen’s d=−0.15) were similar between the two seagrass species (Table 3).

4 Discussion

Nonstructural carbohydrates and amino acids reflect much of the labile organic carbon in seagrass leaves and are typically released during the initial leaching phase of decomposition (Vichkovitten and Holmer, 2004; Lavery et al., 2013). The variation of nonstructural carbohydrate, soluble sugar and starch content of T. hemprichii and E. acoroides was much higher than previous reports for Cymodocea spp., Halodule spp., Halophila stipulosa, Syringodium isoetifolium and Zostera muelleri, but lower than Amphibolis griffithii, Syringodium filiforme, Zostera marina and Zostera noltii (Table 4). The DOC releasing rates of our study (119.2 μmol/(g·d)) was most similar to that of Thalassia testudinum (127.8 μmol/(g·d)) (Maie et al., 2006), but less than other morphologically smaller (sub)tropical taxa, e.g., S. filiforme (288 μmol/(g·d)) (Wang et al., 2014).

On account of the nutrients released from seagrass leaf litter contained not only the DOC, DON and DOP but also the inorgan-

Table 1. The chemical characteristics (soluble sugar, starch, nonstructural carbohydrates, elemental contents and the total elemental weights for experiment) of seagrass leaves before and after the decomposition incubation experiments (mean±SEM)

| Parameters                              | Before leaching | After leaching |
|-----------------------------------------|-----------------|----------------|
|                                         | Thalassia hemprichii | Enhalus acoroides | Thalassia hemprichii | Enhalus acoroides |
| Soluble sugar/mg·g⁻¹ (DW)               | 32.0±8.3        | 35.5±6.5        | -              | -                |
| Starch/mg·g⁻¹ (DW)                      | 47.3±8.7        | 40.2±11.4       | -              | -                |
| Nonstructural carbohydrates/mg·g⁻¹ (DW) | 79.2±17.1       | 75.7±17.9       | -              | -                |
| C/% (DW)                                | 33.9±1.2        | 32.1±1.6        | 36.8±1.8       | 36.5±1.0         |
| N/% (DW)                                | 2.30±0.39       | 2.52±0.15       | 1.04±0.13      | 1.02±0.20        |
| P/% (DW)                                | 0.22±0.03       | 0.28±0.03       | 0.19±0.04      | 0.25±0.05        |
| Total C weight/mg (DW)                  | 707±51.8        | 683±68.5        | 548±76.9       | 533±74.5         |
| Total N weight/mg (DW)                  | 48.0±7.8        | 53.7±6.7        | 15.5±3.8       | 14.9±1.1         |
| Total P weight/mg (DW)                  | 4.59±0.16       | 5.86±0.57       | 2.83±0.14      | 3.66±0.23        |

Note: There were no significant differences for between-species comparisons (p>0.05). DW represents dry weight.
Fig. 1. The cumulative leaching of DOC (a), MCHO (b), DON (c) and DOP (d) from *T. hemprichii* and *E. acoroides* leaf litter during the 30-day incubation in the absence of microbes. Values represent means±SEM. No significant differences between the seagrass species for the DOC, DON and DOP cumulative concentrations were found. Asterisks represents significantly different of MCHO cumulative concentrations between the seagrass species after the 30-day incubation. The molar percentages of the DOM that was leached from *T. hemprichii* and *E. acoroides* (Days 3, 9, 15, 21, and 27) were shown in above or below the error bars.

Fig. 2. The leaching rates of DOC (a), MCHO (b), DON (c) and DOP (d) from *T. hemprichii* and *E. acoroides* leaf litter in the absence of microbes after the 30-day incubation. Different superscript letters indicate that the differences in ratios between the two seagrass species were significant (*p*<0.05). Values are represented as means±SEM.
ic components and other elemental organic matter (Lavery et al., 2013; Jiménez et al., 2017). Thus the DOM content was lower than the total mass lost for each seagrass species. However, we can roughly estimate that the leached DOM accounted for 30% of the lost materials of each seagrass. The average DOC:DON of the two seagrasses in this study was far below the refractory DOM stoichiometry ratios (16:1) for the open ocean (Aminot and Kérouel, 2004), but was close to the in situ DOC:DON ratio of DOM of the Xincun Bay (6:1) (Liu et al., 2016b). The DOC:DON:DOP ratios from seagrass leachate in this study supports the previous hypothesis that there are high contributions of seawater DOM originating from seagrass sources in winter, while the other contributions to seawater DOM is mainly from river runoff (Liu et al., 2016b). This suggests that the seagrass DOM could be providing substantial nutrients to in situ microbial communities (Peduzzi and Herndl, 1991; Vihátaalo and Sondergaard, 2002; Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014). For example, Vihátaalo and Sondergaard (2002) found 28% of leached DOM was taken up by microorganisms during 30 days. Additionally, the relatively low DOC:DON ratio is likely linked to the high historical nutrient loading that occurs in the Xincun Bay (Liu et al., 2016a), and thus can alter the N content of the leaf standing stock (Duarte, 1990). Nevertheless, we cannot calculate the exact percentage of the seagrass leaf litter contribution to the DOM of in situ water column only according to the DOM contents and the ratios. As a matter for future research, we recommend the δ13C analyses of DOC to evaluate the exact percentage of the seagrass leaf litter contribution to the water column DOM.

We would expect that after DOM release that DOP would be preferentially remineralized in the seawater due to P limitations in many tropical seagrass ecosystems, followed by N and C (Ogawa and Tanoue, 2003; Aminot and Kérouel, 2004; Ziegler et al., 2004). The DON:DOP of the DOM leachate from T. hemprichii was lower than what was released from E. acoroides, suggesting that the DOM from T. hemprichii may contribute more dissolved nutrients to the ecosystems than E. acoroides. Furthermore, MCHO, one of the highest bioavailability substances among different carbohydrates, can directly and quickly be assimilated by microbes (Peduzzi and Herndl, 1991; Vichkovitten and Holmer, 2004). Previous studies suggest the leached DOM of leaf litter from different seagrass species have distinct bioavailability linked to nonstructural carbohydrate, protein-associated DOM (Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014).

### Table 2. Single logarithm function fitted to the accumulation of DOM fractions over 30 days of leaching

| DOM fraction | Thalassia hemprichii | Enhalus acoroides |
|--------------|----------------------|------------------|
| DOC          | y = 1.253 + 0.326lnx, R² = 0.999 7 | y = 1.770.1 + 0.31.1lnx, R² = 0.995 1 |
| MCHO         | y = 0.859 + 0.380lnx, R² = 0.999 3 | y = 0.6595 + 0.380lnx, R² = 0.977 0 |
| DON          | y = 3.844 + 0.251.4lnx, R² = 0.993 1 | y = 3.8944 + 0.257.1lnx, R² = 0.994 5 |
| DOP          | y = 9.523 + 0.396lnx, R² = 0.924 6 | y = 8.613 + 0.331lnx, R² = 0.933 7 |

Note: x is time in days and y accumulation concentration (μmol/g DW).

### Table 3. Ratios of the composition of DOM released from T. hemprichii and E. acoroides leaf litter

| Seagrass species | DOC:DON | DOC:DOP | DON:DOP | DOC:DON:DOP |
|------------------|---------|---------|---------|-------------|
| *Thalassia hemprichii* | 3.22±1.23<sup>a</sup> | 161±36.8<sup>b</sup> | 50.0±11.6<sup>b</sup> | 161:50:1<sup>b</sup> |
| *Enhalus acoroides* | 2.46±0.67<sup>a</sup> | 169±40.6<sup>b</sup> | 68.8±19.8<sup>ab</sup> | 169:69:1<sup>b</sup> |

Note: Different superscript letters indicate the differences in ratios between the two seagrass species were significant (p<0.05). Values are represented as means±SEM.

### Table 4. Review of literature that reports nonstructural carbohydrate, soluble sugar and starch content for seagrass leaf (nonstructural carbohydrate=soluble sugar+starch)

| Species | Nonstructural carbohydrate | Soluble sugar | Starch | References |
|---------|-----------------------------|---------------|--------|------------|
| *Amphibolis griffithii* | 188 | 38 | 150 | Mackey et al. (2007) |
| *Cymodocea rotundata* | 9 | 1 | 8 | Lawler et al. (2006) |
| *Cymodocea serrulata* | 3 | 1 | 2 | Lawler et al. (2006) |
| *Halodule uninervis* | 32 | 22.1 | 9.9 | Sheppard et al. (2007) |
| *Halodule spinulosa* | 15 | 1 | 14 | Lawler et al. (2006) |
| *Halodule minor* | 9 | 1 | 8 | Lawler et al. (2006) |
| *Halophila engelmannii* | 52–124 | | | Davies et al. (1987) |
| *Halophila ovalis* | 102 | 30 | 72 | Longstaff et al. (1999) |
| *Halophila spinulosa* | 19.2 | 12.9 | 6.3 | Sheppard et al. (2007) |
| *Posidonia oceanica* | 40–140 | | | Invers et al. (2004) |
| *Posidonia sinuosa* | 78 | 28 | 50 | Collier et al. (2009) |
| *Syringodium filiforme* | 170–220 | | | Siegal-Willott et al. (2010) |
| *Syringodium isoetifolium* | 35 | 19 | 16 | Lawler et al. (2006) |
| *Thalassia testudinum* | 46–70 | | | Lee and Dunton (1996) |
| *Zostera muelleri* | 34.3 | 24.3 | 10 | Sheppard et al. (2007) |
| *Zostera marina* | 111.13 | 78.53 | 32.6 | Burke et al. (1996) |
| *Zostera noltii* | 95.2 | 90.4 | 4.8 | Olivé et al. (2007) |
| *Thalassia hemprichii* | 79.2 | 32.0 | 47.3 | this study |
| *Enhalus acoroides* | 75.7 | 35.5 | 40.2 | this study |

Note: All data are reported as mg/g (DW).
the nonstructural carbohydrates were similar between the *T. hemprichii* and *E. acoroides*, the higher releasing rate and amount of MCHO also suggested the higher bioavailability of DOM leaching from *T. hemprichii* than *E. acoroides*.

More than 75% of the total DOM was released during first 9 days, which is similar to those found in previous studies (Maie et al., 2006; Chiu et al., 2013). The releasing rates of DOM, DON and DOP declined rapidly after the first 9 days and remained steady for the remainder of the experiment. The average aboveground biomass of *T. hemprichii* and *E. acoroides* in the Xincun Bay has been reported to be about 43 g/m² and 64.24 g/m², respectively (Liu et al., 2016a). According to the seagrass distribution area, the total biomass of *T. hemprichii* and *E. acoroides* in the Xincun Bay has been estimated to be 4.33×10⁷ g DW and 5.14×10⁷ g DW, respectively. Chiu et al. (2013) calculated that about 80% leaf production of *T. hemprichii* can become leaf detritus in southern tip of Taiwan. In consideration of approximate geographical conditions of the Xincun Bay with Chiu et al. (2013) study area, we assume the *T. hemprichii* and *E. acoroides* leaf production is similar. In addition, the *T. hemprichii* and *E. acoroides* leaf turnover time is approximately 45 days and 105 days, respectively (Hemminga et al., 1999). Therefore, the total leaf litter production of *T. hemprichii* and *E. acoroides* is proximately 1.16×10⁸ g/d in the Xincun Bay. Assuming the leaching period only last 30 days, and using the DOM leaching rates and the estimated leaf litter production, we estimate that the DOM production in the Xincun Bay is about 4 035 mol/d DOC, 1 377 mol/d DON and 25 mol/d DOP. Although we only performed a microbe-independent leaching experiment, the microbes do not contribute much to the direct loss of organic matter from detrital leaves of seagrass during initial leaching period (Harrison and Mann, 1975; Vähätalo and Sondergaard, 2002). Rather, the leaf-associated microbial communities play a more active role later on in the decomposition process via enzymatic remineralization of the detritus (Godshalk and Wetzel, 1978). Thus the estimated amount of DOM leached from seagrass was reasonable without the presence of microbes. These sources of DOM provide important pathways for transferring vascular plant production to the microbial food webs and higher trophic levels in seagrass and adjacent coastal waters in the Xincun Bay. In addition, the average DOC:DON:DOP ratios (162:55:1) leached by the two seagrasses was much lower than terrestrial DOC:DON:DOP (1 368:232:1) in the Xincun Bay (Liu et al., 2016b), indicating higher bioavailability of DOM leaching from seagrass than the terrestrial DOM. Furthermore, seagrass leaves contain recalcitrant organic matter and calcium carbonate that likely survives the leaching phase and may contribute to sediment carbon sequestration (Trevathan-Tackett et al., 2017) and sand for the beach or and dune system (Jiménez et al., 2017), respectively.

5 Conclusions

After 30 days of leaching, about 22%, 70% and 38% of seagrass C, N and P were released, respectively, for near-sesescence seagrass leaves. The estimates above indicate that significant quantities of seagrass-sourced DOM is released to the water column independent of microbial processes. Both species contributed similar quantity of DOM, with *T. hemprichii* possibly being of greater bioavailability to the food webs in seagrass meadows. Meadow-wide, the seagrass leaf litter can provide more than 4 000 mol/d DOC, 1 350 mol/d DON and 25 mol/d DOP to the Xincun Bay. However, previous reviews suggest that this rate of DOM release could last several months longer than the timeframe of this study due to further breakdown of the cell walls by microbes (Harrison, 1989; Peduzzi and Herndl, 1991; Mateo and Romero, 1996; Chiu et al., 2013). Therefore, the microbial-dependent release of DOM as well as the remaining POM represent two different pools of C, N and P to the ecosystem. We recommend the use of microbe-centric analyses (e.g., gene expression) in combination of DOM and POM chemical analyses to better understand micro-level exchanges between microbial communities and the nutrients seagrasses provide to create a complete the OM cycle and stoichiometry budget for the Xincun Bay.

References

Allen S E. 1989. Chemical Analysis of Ecological Materials. Oxford: Blackwell Scientific Publications

Aminot A, Kérouel R. 2004. Dissolved organic carbon, nitrogen and phosphorus in the N–E Atlantic and the N–W Mediterranean with particular reference to non-refractory fractions and degradation. Deep Sea Res Part I Oceanogr Res Pap, 51(12): 1975–1999

Apostolaki E T, Marbà N, Holmer M, et al. 2009. Fish farming enhances biomass and nutrient loss in *Posidonia oceanica* (L.) Delile. Estuar Coast Shelf Sci, 81(3): 390–400

Barros C, Duarte C M. 2009. Dissolved organic matter release in *Posidonia oceanica* meadow. Mar Ecol Prog Ser, 374: 75–84

Brönk D A, Lomas M W, Gilbert P M, et al. 2000. Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. Mar Chem, 69(1–2): 163–178

Burke M K, Dennison W C, Moore K A. 1996. Non-structural carbohydrate reserves of eelgrass *Zostera marina*. Mar Ecol Prog Ser, 137: 195–201

Cebrian J, Duarte C M. 2001. Detrital stocks and dynamics of the seagrass *Posidonia oceanica* (L.) Delile in the Spanish Mediterranean. Aquat Bot, 70(4): 295–309

Cebrián J, Duarte C M, Marbà N, et al. 1996. Herbivory on *Posidonia oceanica*: magnitude and variability in the Spanish Mediterranean. Mar Ecol Prog Ser, 130: 147–155

Chiu S H, Huang Y H, Lin H J. 2013. Carbon budget of leaves of the tropical intertidal seagrass *Thalassia hemprichii*. Estuar Coast Shelf Sci, 125: 27–35

Collier C J, Lavery P S, Ralph P J, et al. 2009. Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. J Exp Mar Biol Ecol, 370(1–2): 89–103

Dawes C, Chan M, Chinn R, et al. 1987. Proximate composition, photosynthetic and respiratory responses of the seagrass *Halophila englemannii* from Florida. Aquat Bot, 27(2): 195–201

Duarte C M. 1990. Seagrass nutrient content. Mar Ecol Prog Ser, 67: 201–207

Duarte C M, Chiscano C L. 1999. Seagrass biomass and production: a reassessment. Aquat Bot, 65(1–4): 159–174

Duarte C M, Krause-Jensen D. 2017. Export from seagrass meadows contributes to marine carbon sequestration. Front Mar Sci, 4: 46

Duarte C M, Marbà N, García E, et al. 2010. Seagrass community metabolism: assessing the carbon sink capacity of seagrass meadows. Globa Biogeochem Cycles, 24(4): GB4032

Duarte C M, Merino M, Agawin N S R, et al. 1998. Root production and belowground seagrass biomass. Mar Ecol Prog Ser, 171: 97–108

Foursqrean W J, Ziemann J C, Powell G V N. 1992. Phosphorus limitation of primary production in Florida Bay: evidence from C:NP ratios of the dominant seagrass *Thalassia testudinum*. Limnol Oceanogr, 37(1): 162–171

Godshalk G L, Wetzel R G. 1978. Decomposition of aquatic angiosperms. III. *Zostera marina* L. and a conceptual model of decomposition. Aquat Bot, 5: 329–354

Grasshoff K, Kremling K, Ehrhardt M. 2009. Methods of Seawater Analysis. 3rd ed. New York: John Wiley & Sons

Harrison P G. 1989. Detrital processing in seagrass systems: a review of factors affecting decay rates, remineralization and detritivory. Aquat Bot, 35(3–4): 263–288
Harrison P G, Mann K H. 1975. Detritus formation from eelgrass (Zostera marina L.): the relative effects of fragmentation, leaching, and decay. Limnol Oceanogr, 20(6): 924–934

Hemminga M A, Duarte C M. 2000. Seagrass Ecology. Cambridge: Cambridge University Press

Hemminga M A, Marbá N, Stapel J. 1999. Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems. Aquat Bot, 65(1–4): 141–158

Holmer M, Olsen A B. 2002. Role of decomposition of mangrove and seagrass detritus in sediment carbon and nitrogen cycling in a tropical mangrove forest. Mar Ecol Prog Ser, 230: 87–101

Huang Xiaoping, Huang Liangmin, Li Yinghong, et al. 2006. Main seagrass beds and threats to their habitats in the coastal sea of South China. Chin Sci Bull, 51(S2): 136–142

Invers O, Krammer G P, Pérez M, et al. 2004. Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass Posidonia oceanica. J Exp Mar Biol Ecol, 303(1): 97–114

Jiménez M A, Beltran R, Traverset A, et al. 2017. Aeolian transport of seagrass (Posidonia oceanica) beach-cast to terrestrial systems. Estuar Coast Shelf Sci, 196: 31–44

Kirkman H, Reid D D. 1979. A study of the role of the seagrass Posidonia australis in the carbon budget of an estuary. Aquat Bot, 7: 173–183

Lavery P S, McMahon K, Weyers J, et al. 2006. Near-infrared reflectance spectroscopy is a rapid, cost-effective predictor of seagrass nutrients. J Chem Ecol, 32(6): 1353–1365

Lee K S, Dunton K H. 1996. Production and carbon reserve dynamics of the seagrass Thalassia testudinum in Corpus Christi Bay, Texas, USA, Mar Ecol Prog Ser, 143: 201–210

Liu Songlin, Ji Zhijian, Zhang Jingjing, et al. 2016a. Effect of nutrient enrichment on the source and composition of sediment organic carbon in tropical seagrass beds in the South China Sea. Mar Pollut Bull, 110(1): 274–280

Liu Songlin, Jiang Chunjian, Zhou Chenyuan, et al. 2016b. Temporal and spatial variation of dissolved organic matter and its controlling factors in seagrass bed ecosystem of Xincun Bay, Hainan Island. Chinese Journal of Ecology (in Chinese), 35(5): 2144–2151

Longstaff B J, Loneragan N R, O’Donohue M J, et al. 1999. Effects of light deprivation on the survival and recovery of the seagrass Halophila ovalis (R.Br.) Hook. J Exp Mar Biol Ecol, 234(1): 1–27

Mackey P, Collier C J, Lavery P S. 2007. Effects of experimental reduction of light availability on the seagrass Amphibolis griffithii. Mar Ecol Prog Ser, 342: 117–126

Maie N, Jaffé R, Miyoshi T, et al. 2006. Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland. Biogeochemistry, 78(3): 285–314

Mateo M A, Romero J. 1996. Evaluating seagrass leaf litter decomposition: an experimental comparison between litter-bag and oxygen-suction methods. J Exp Mar Biol Ecol, 202(2): 97–106

Mateo M A, Cebrián J, Dunton K, et al. 2006. Carbon flux in seagrass ecosystems. In: Larkum A W D, Orth R J, Duarte C M, eds. Seagrasses: Biology, Ecology and Conservation. Dordrecht: Springer, 159–192

Myklestad S M, Skånøy E, Hestmann S. 1997. A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater. Mar Chem, 56(3–4): 279–286

Ogawa H, Tanoue E. 2003. Dissolved organic matter in oceanic waters. J Oceanogr, 59(2): 129–147

Olivé I, Brun F G, Vergara J J, et al. 2007. Effects of light and biomass partitioning on growth, photosynthesis and carbohydrate content of the seagrass Zostera noltii Hornem. J Exp Mar Biol Ecol, 345(2): 90–100

Orth R J, Moore K A. 1986. Seasonal and year-to-year variations in the growth of Zostera marina L. (eelgrass) in the lower Chesapeake Bay. Aquat Bot, 24(4): 335–341

Peduzzi P, Herrndl G J. 1991. Decomposition and significance of seagrass leaf litter (Cymodocea nodosa) for the microbial food web in coastal waters (Gulf of Trieste, northern Adriatic Sea). Mar Ecol Prog Ser, 71(2): 163–174

Pierzynski G M. 2000. Methods of phosphorus analysis for soils, sediments, residuals, and waters. Raleigh: North Carolina State University

Robertson M L, Mills A L, Ziemann J C. 1982. Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. Mar Ecol Prog Ser, 7: 279–285

Sheppard J K, Lawler I R, Marsh H. 2007. Seagrass as pasture for seacows: landscape-level dunging habitat evaluation. Estuar Coast Shelf Sci, 71(1–2): 117–132

Siegal-Willott J L, Harr K, Hayek L A C, et al. 2010. Proximate nutrient analyses of four species of submerged aquatic vegetation consumed by Florida manatee (Trichechus manatus latirostris) compared to romaine lettuce (Lactuca sativa var. longifolia). J Zoo Wildl Med, 41(4): 594–602

Trevathan-Tackett S M, Macreadie P I, Sanderman J, et al. 2017. A global assessment of the chemical recalcitrance of seagrass tissues: implications for long-term carbon sequestration. Front Plant Sci, 8: 925

Viháatalo A V, Søndergaard M. 2002. Carbon transfer from detrital leaves of eelgrass (Zostera marina) to bacteria. Aquat Bot, 73(3): 265–273

Valderrama J C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar Chem, 10(2): 109–122

Vichkovtten T, Holmer M. 2004. Contribution of plant carbohydrates to sedimentary carbon mineralization. Org Geochem, 35(9): 1035–1066

Vizzini S, Sarà G, Michener R H, et al. 2002. The role and contribution of the seagrass Posidonia oceanica (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecol, 23(4): 277–285

Wang Xuchen, Chen R F, Cable J E, et al. 2014. Leaching and microbiological degradation of dissolved organic matter from salt marsh plants and seagrasses. Aquat Sci, 76(4): 595–609

Yarbro L A, Carlson Jr P R. 2008. Community oxygen and nutrient fluxes in seagrass beds of Florida Bay, USA. Estuar Coasts, 31(5): 877–897

Yemm E W, Willis A J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem J, 57(3): 508–514

Ziegler S, Benner R. 1999. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Mar Ecol Prog Ser, 180: 149–160

Ziegler S, Kaiser E, Benner R. 2004. Dynamics of dissolved organic carbon, nitrogen and phosphorus in a seagrass meadow of Laguna Madre, Texas. Bull Mar Sci, 75(3): 391–407
Assessment of the consecutive harmful dinoflagellate blooms during 2015 in the Izmit Bay (the Marmara Sea)

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Received 8 August 2017; accepted 11 December 2017

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Abstract

A series of red tides were observed during 2015 in the Izmit Bay (the Marmara Sea) which is located in the most industrialized and populated region of Turkey. Six samplings were carried out in this area following the red tides. Nitrite-N, nitrate-N, ammonia, silica and orthophosphate concentrations were analyzed spectrophotometrically. Physicochemical conditions were measured by CTD probe. Plankton quantification was performed using counting chambers under microscopes. *Prorocentrum micans* was the most abundant species, except on May 14, 2015, when *Noctiluca scintillans* was dominant. The abundance of *P. micans* reached average 18×10^6 ind./L on May 3, 2015 in the Karamürsel station, simultaneously with elevated levels of NH_3 and o-PO_4^{3-}. The sample was also abundant in dead amphipods ((72±12) ind./L) that had been covered by mucilage aggregates produced by *P. micans*. The highest biomass (calculated by carbon) was recorded as (268±26.0) mg/L on May 14 in the Hereke station. Beside the anthropogenic wastewater discharges, unknown sources and resuspensions caused increases in nutrient levels. After long term northeaster gusts (35 km/h for 5 d) an upwelling occurred on November 6, 2015 after wind-induced sediment resuspension. Although nutrient discharges remarkably decreased over 30 years through established wastewater treatment plants, harmful phytoplankton blooms still occur. Comparing the present results with other studies in nearby Mediterranean seas reveals that the most intense harmful dinoflagellate bloom in recent years occurred in the Izmit Bay. Therefore, additional protection measures are necessary for a cleaner Izmit Bay. These incidents also demonstrate that contaminants, accumulated in sediment, may have long-lasting effects on enclosed marine ecosystems.

Key words: red tide, harmful algal bloom, phytoplankton, dinoflagellate, Izmit Bay, Marmara Sea, mucilage

Citation: Ergül Halim Aytekin, Aksan Serdar, İşiroğlu Merve. 2018. Assessment of the consecutive harmful dinoflagellate blooms during 2015 in the Izmit Bay (the Marmara Sea). Acta Oceanologica Sinica, 37(8): 91–101, doi: 10.1007/s13131-018-1191-7

1 Introduction

Marine phytoplankton generates approximately half of the global primary production and affects the fate of sea life, with respect to fishery yields and sustainable marine ecosystems (Boyce et al., 2010). Domestic and industrial discharges cause nutrient enrichment and consequently, alterations of phytoplankton composition in terms of biodiversity and abundance in many coastal areas (Smith et al., 1999). Annual nitrogen and phosphorus input to ecosystems has more than doubled globally during the last fifty years (Falkowski et al., 2000; Smil, 2000). Since the availability of nutrients, necessary for growth and reproduction of phytoplankton, is often lower than the biological demand, additional nutrient supply is necessary for phytoplankton growth (Corbett, 2010). Therefore, in addition to common sources (e.g., terrestrial inflows, airborne particulates) anthropogenic discharges (e.g., industrial and domestic effluents, agricultural runoffs) and sediment resuspensions affect nutrient enrichment, leading to an increase in phytoplankton abundance. Under favorable oceanographic conditions (e.g., temperature and salinity), sufficient nutrient levels may cause excessive phytoplankton reproduction and harmful algal blooms may occur (Heisler et al., 2008). These enrichments mostly happen in coasts near urbanized and industrialized settlements (Ferreira et al., 2011; Heisler et al., 2008). However, biogeochemical structure and environmental pressures may vary for a coastal marine ecosystem and exact mechanisms of algal bloom occurrences remain unknown, as in the Izmit Bay.

The Izmit Bay, one of the most polluted marine ecosystems of Turkey, contains a wide range of contaminants including organic and inorganic compounds such as heavy metals, radionuclides, POPs, and nutrients (Balkis et al., 2007; Ergül et al., 2013a, b; Karademir et al., 2013; Tolun et al., 2006). The bay is located to the northeast of the Marmara Sea (Turkey) and a permanent pycnocline and a two-layered stratification are observed throughout year. Upper layer salinity is generally less than 24.0, and this reaches 36.0 below the halocline layer (Ergül, 2016). There are around 2 million inhabitants living in Kocaeli Province surrounding the Izmit Bay. Population and industrial activities have been increasing rapidly since the 1980s, and the bay receives a considerable amount of domestic and industrial discharge from its drainage basin. Since the 1960s, more than 400 large industrial plants have been built around the bay, including the largest shipyards, metallurgy, paper mill, fertilizer, and petrochemical facilities of Turkey. In addition to 70 shipyards, there are 40 ports located along the coast of the bay. In 2015, the bay received approximately 400 and 80 tonnes of total nitrogen (TN) and phosphorus (TP) discharges from wastewater treatment plants, respectively. Moreover, almost all effluents were dis-
charged without treatment before 2000, and 41.0% of domestic and industrial discharge has remained untreated until 2010 (ECR, 2013; ISU, 2016). At the current time, there are no available data for untreated sewage discharges, agricultural runoffs, etc.) in the Izmit Bay. Eutrophication is also a serious problem (Morkoc et al., 2001; Okay et al., 2001) and phytoplankton blooms were recently reported in the bay (Ergul et al., 2014, 2010).

Phytoplankton blooms, causing brownish-red surface water with bad odor and mucilage formation, drew public attention to domestic and industrial discharges as potential triggering elements. Moreover, meteorological factors likely have an effect on algal bloom occurrence. Therefore, investigation into mechanisms of algal blooms and their harmful effects on the ecosystem will be crucial for evaluating effective environmental factors and solving future problems in an enclosed marine ecosystem, close to a populated and industrialized city, such as the Izmit Bay. This study evaluated (1) serial phytoplankton blooms with mucilage formation, (2) accompanied physicochemical and meteorological conditions in 2015, (3) evaluation of possible reasons for harmful algal blooms in the Izmit Bay, and (4) a comparison between the present results and remarkable diatom and dinoflagellate blooms around nearby Mediterranean seas.

2 Materials and methods

2.1 Study area

The Izmit Bay is a semi-enclosed coastal ecosystem located in the most industrialized area of the Marmara region (Turkey). The 49 km long bay with an area of 300 km² has three basins connected by shallow and narrow passages. The eastern basin, the smallest component of the system, is approximately 5.0 km wide, 13 km long, 47.0 km² in surface area and relatively shallow with a maximum depth of 40.0 m. The central basin, which is the largest component of the system, is approximately 9.5 km wide, 22 km long, 165 km² in surface area and the depth increases up to 208 m in the southern section (Kuscu et al., 2002). A narrow and shallow area (i.e., 55 m depth) at Dil Creek shore separates the central and western basins which are approximately 8.5 km wide, 14 km long and 47.0 km² in surface area. The bottom topography of the western basin slopes downward toward the Marmara Sea and increases to a depth of approximately 100 m near its opening (Fig. 1). Stratification in the water column occurs throughout the year because of salinity differences. Saltier water originates from the Mediterranean Sea in the lower layer, whereas less saline water originates from the Black Sea in the upper layer (Morkoc et al., 1996). Vertical mixing between the two layers is restricted over the Marmara Basin and plays a key role in the principal physicochemical characteristics of the bay (Algan et al., 1999).

2.2 Sampling and methodology

Six surveys were carried out when red tides occurred to sample surface water for quantitative analysis of nitrite-N (NO2−-N), nitrate-N (NO3−-N), ammonia (NH4+), silica (SiO2) and orthophosphate (o-Po4−3) and phytoplankton identification in the Izmit Bay. These were carried out on April 12, April 30, May 3, May 14, July 11, and November 6, 2015 at Uşaklı, Sekapark, Karamürsel, Hereke, Marina and Marina stations, respectively in the Izmit Bay (Fig. 1). Temperature, salinity, dissolved oxygen (DO), turbidity, pH and chlorophyll a (Chl a) levels were measured in the water for each samplings. Although phytoplankton blooms were observed throughout the bay, marine water samples were taken at the locations densest in phytoplankton abundance during bloom times.

Composite surface water samples were taken with a Nansen bottle (3 L) twice hanging from the edge of a landing dock approximately 50 m away from the coast at all sites, these were put into amber glass bottles and kept cool for immediate transportation to the laboratory. The first Nansen bottle samples was filtered with a 0.45 μm, 47 mm Whatman filter paper and used for NO2−-N, NO3−-N, NH4+, o-Po4−3 and SiO2 analysis via spectrophotometric methods (Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Murphy and Riley, 1962), while the other Nansen bottle sample (3 L) was used for phytoplankton identification and quantification. A 10 mL tubular plankton chamber was used for pre-identification under an inverted microscope while plankters were alive. Then samples were fixed using 4.00% formaldehyde solution for better identification and quantifying. Before the counting procedure, the samples were kept in a dark room in tubular glass for precipitation. Then phytoplankters were identified and quantified via a Nageotte counting chamber under a light microscope. Counting procedures were repeated at least three times. Results were given as individual per liter.

To estimate the biomass of phytoplankton, dimensions of each taxa were measured under a light microscope equipped with a camera (Olympus BX51). Measured dimensions were used to calculate cell volumes according to calculated values for each species’ similar dimensions in a previous study by Olenina et al. (2006) in the Baltic Sea. To calculate cell biomass in terms of organic carbon content as pg C/cell, equations by Menden-Deuer and Lessard (2000), given below, were used:

\[
\text{cell biomass} = 0.288 \times \text{biovolume}^{0.811} \\
\text{for Bacillariophyceae (diatoms)} \\
\text{cell biomass} = 0.216 \times \text{biovolume}^{0.339} \\
\text{for taxaons other than diatoms.}
\]

The carbon content for each species was multiplied by individual number to calculate the organic carbon mass for each species group. Results were given as mg/L (calculated by carbon) in the text.

A data sonde (Hydrolab DS-5) was used to measure temperature, salinity, dissolved oxygen (DO), turbidity, pH and Chl a, in the water (Fig. 1). The device was calibrated before each deployment, waiting at least two minutes for warm up and then it was submerged at a constant speed (i.e., 0.2 m/s) where the water column had enough depth. At least thirty results of measurements were used to calculate average value. The Chl a and DO data were tested using a classical acetone extraction and Winkler methods, respectively. Differences between concentrations in classical methods and probe measurements were less than 5.00%. Standard deviations were calculated for statistical sufficiency. Meteorological records were obtained from Turkish State Meteorological Service stations located in Izmit.

Trophiic index (TRIX) values were calculated in order to quantify eutrophic state and water quality. To calculate TRIX, concentrations of dissolved inorganic nitrogen (c(DIN), i.e., NO2−-N + NO3−-N + NH4+-N), o-Po4−3 (c(P), μg/L), DO deviation from saturation (d(DO), %) and Chl a concentration (c(Chl a), mg/L) were used in the equation below (Vollenweider et al., 1998):

\[
\text{TRIX} = \frac{\log_{10}(c(\text{Chl} \ a) \times d(\text{DO}) \times c(\text{DIN}) \times c(\ P)) + 1.5}{1.2}
\]
Pearson correlation coefficients (two-tailed) were calculated among variables to better explain the relationship between parameters and these are used in the text where necessary.

3 Results and discussion

On March 19, 2015, the Izmit Bay which is located at the eastern end of the Marmara Sea, was covered by phytoplankton and a light red appearance was observed over almost all the bay’s surface (Fig. 2a). This appearance was disappeared after approximately two days. On April 12, phytoplankton abundance, which was dominated by *Prorocentrum micans* Ehrenberg, 1834, increased, surface water partly reached a brownish-red appearance, and mucilage formation was observed (Fig. 2b).

Six different phytoplankton species (*Achnantes brevipes* C. Agardh, 1824, *Cerataulina pelagica* (Cleve) Hendey, 1937, *Dictyocha speculum* Ehrenberg, 1834, *Melosira* sp., *Navicula* sp., and *Prorocentrum micans*), which belong to three different groups (four Bacillariophyceae, one Dictyochophyceae, and one Dinophyceae) were observed intermittently in the samples on April 12, April 30 and May 3, 2015 with average surface water temperatures of (14±1.0)°C, and salinity of 21±3.0. Regarding abundance, dinoflagellates were dominant with rates of over 99.0% and *P. micans*, which is known as a potential high biomass-forming dinoflagellate (Jeong et al., 2005) was the most abundant species in those three blooms (average 1.10×10^6, 5.40×10^6 and 17.9×10^6 ind./L, respectively; Table 1, Fig. 3a). Mucilage formations occurred in these blooms and a milky light-brown flocculated layer was observed on the sea surface. After the blooms, red appearances and mucilage layers disappeared within a few days from surface waters. In the microscopy slides, mucus aggregates were also observed amongst *P. micans* clusters (Fig. 2c). Therefore, formation of mucilage should be excreted by *P. micans* after their excessive proliferation (Figs 2c, d). On May 3, the mucilage layer was very intense, and dead amphipods, which were coated with amorphous mucus pellets, were observed in the samples in Karamürsel ((72±12) ind./L, Fig. 2d) with the highest surface water turbidity ((287±46.0) NTU) and Chl *a* concentrations ((180±15.0) μg/L, Fig. 4b). Although several phytoplankton blooms have been reported from the Izmit Bay since 1986 (Taş et al., 2016), to our knowledge, this incident was the most intense.

![Map of Izmit Bay and sampling sites. P represents industrial ports, S shipyards, and W wastewater treatment plants.](image1)

**Fig. 1.** Map of Izmit Bay and sampling sites. P represents industrial ports, S shipyards, and W wastewater treatment plants.

![Pictures of aerial view of the phytoplankton blooms without (a) and with (b) mucilage formations on March 19 and April 12, 2015, respectively; light microscope slides of *P. micans* with mucilage formation, in May 3, 2015 (c); and a dead amphipod individual covered with mucilage layer (d). Arrows indicate pervaded *P. micans* and mucilage clusters on the amphipod body.](image2)

**Fig. 2.** Pictures of aerial view of the phytoplankton blooms without (a) and with (b) mucilage formations on March 19 and April 12, 2015, respectively; light microscope slides of *P. micans* with mucilage formation, in May 3, 2015 (c); and a dead amphipod individual covered with mucilage layer (d). Arrows indicate pervaded *P. micans* and mucilage clusters on the amphipod body.
Moreover, the presence of dead amphipods after a phytoplankton bloom was reported for the first time in the present study. Mortis causa of the amphipods was not clear, and there are no (open) scientific literature regarding marine mucilage-dependent amphipod mortality. However, Bruno et al. (1989) reported that farmed and wild fish may also be killed by the smothering of gills due to phytoplankton mucus production. Therefore, given its appearance under stereomicroscope, it is reasonable that mucilage aggregates probably blocked amphipod gills and thus respiration was prevented, leading to their death (Fig. 2d). Also, oxygen concentration decrease may be considered as another reason of amphipod mortality. However we have no simultaneous dissolved oxygen data to prove this claim. Amphipod and copepods are known predators of dinoflagellates including bloom causative species (i.e., P. micans), therefore high amphipod abundance in the samples are most likely connected to their feeding behavior (Yi et al., 2017). It should be noted that phytoplankton abundance as well as nutrient concentrations were approximately three fold higher near the shore because of conglomeration after drifting via waves and those values were excluded from further assessment in the present study.

In the bloom conditions on May 3, 2015, nutrient levels remarkably increased, and raised inorganic nitrogen, and excessive o-PO$_4^{3-}$ concentrations were measured (Figs 3c, d). On that day, most intense dinoflagellate bloom occurred concurrently with the highest concentrations of DIN and o-PO$_4^{3-}$ ever recorded during the study. Therefore, the existence of elevated levels of nutrients should have triggered excessive dinoflagellate (i.e., P. micans) proliferation and caused excretion (i.e., mucilage formation) from P. micans cells, under the favorable meteorological conditions.
conditions. It should be noted that, very calm weather and light winds were recorded for two days immediately before the bloom on May 3. Therefore, increased nutrients, which are most likely derived from undesirable or illegal discharges, could not be dispersed by winds, currents, precipitations, etc. from the surface water and finally were consumed by phytoplankton. In fact, the Izmit Bay’s renewal capacity is known to be insufficient because of a relatively long residence time of the water masses (Morkoç et al., 2001) and in this incident, light winds, and accordingly weak currents, allowed nutrients accumulation in surface waters. Thus, appropriate conditions occurred for excessive *P. micans* proliferation.

Following phytoplankton increases, which occurred on May 14, July 11 and November 6, 2015 and unlike other blooms, biodiversity reached 10, 14, and 10 species, respectively, during these incidents in the Izmit Bay. During the study, 17 taxa which have not bloom formation were also recorded in different classes (Barclayophyceae, Dinophyceae, Euglenophyceae, Oligotrichaceae, and Coscinodiscaceae) in addition to six bloom taxa (Table 1, Fig. 3a).

On May 14, both DIN and o-PO$_4^{3-}$ concentration decreased compared to May 3 and in this event biodiversity reached ten species (Figs 3a, c, d). The dominant species at this time was *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 (Figs 3a, d) with surface water temperatures of 15.0°C and salinity of 25.9 in the Hereke station, which was the highest salinity level recorded during the study (Fig. 4). Corresponding to individual number, Barclayophyceae (diatom) was dominant in the second order at a rate of 16.6%, following Dinophyceae (diatomflagellate) (75.5%). Euglenophyceae and Oligotrichaceae species were also observed in this red tide with rates of 7.70% and <1.00%, respectively. On that day, although the lowest phytoplankton abundance was found (54.0×10$^3$ ind./L), the highest phytoplankton biomass (258±26.0 mg/L) was recorded, because of the presence of *N. scintillans* (34.0×10$^3$ ind./L), which is known for its large cell volume (Figs 3a, b).

During the red tide, the *N. scintillans* bloom was also reported from the Marmara Sea on May 17 (NASA, 2015). This organism is not known as toxin producer, but it is, however, able to accumulate toxic levels of ammonia and can thus cause massive mortality to marine organisms because of ammonia excretion into the ambient waters (Faust and Gulledge, 2002; Okaichi and Nishio, 1976). In fact, the highest NH$_4^+$ concentration in second order was measured on May 14; however, its concentration was 12 fold higher than the sum of NO$_3^-$ and NO$_2^-$ concentrations in second order. During the study, the NH$_3$ concentration decreased again (average 480×10$^3$ ind./L) and a light red appearance was observed on the sea surface without mucilage forma-

### Table 1. Phytoplankton diversity in the surface water of the Izmit Bay in the bloom conditions during 2015

| Species | Classis | Sampling date |
|---------|---------|---------------|
| *Achnanthes brevipes* C. Agardh, 1824 | B | 12 Apr. 2015 |
| *Cerataulina pelagica* (Cleve) Hendey, 1937 | + | 30 Apr. 2015 |
| *Chaetoceros decipiens* Cleve, 1873 | - | 3 May 2015 |
| *Cocinodiscus radiatus* Ehrenberg, 1840 | - | 14 May 2015 |
| *Cocinodiscus granii* Gough, 1905 | - | 11 Jul. 2015 |
| *Melosira* sp. | + | 6 Nov. 2015 |
| *Nitzschia longissima* (Brébisson) Ralfs, 1861 | - | 12 Apr. 2015 |
| *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928 | - | 30 Apr. 2015 |
| *Skeletonema costatum* (Greville) Cleve, 1873 | - | 3 May 2015 |
| *Dictyocha speculum* Ehrenberg, 1839 | Dc | 14 May 2015 |
| *Dinophysis acuminata* Claparède & Lachmann, 1859 | Dn | 11 Jul. 2015 |
| *Neoceratium furca* (Ehrenberg) F. Gomez, D. Moreira & P. Lopez-Garcia, 2010 | - | 6 Nov. 2015 |
| *Neoceratium fusus* (Ehrenberg) F. Gomez, D. Moreira & P. Lopez-Garcia, 2010 | - | 6 Nov. 2015 |
| *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 | - | 12 Apr. 2015 |
| *Oxytoxum longiceps* Schiller | - | 30 Apr. 2015 |
| *Proorocentrum micans* Ehrenberg, 1834 | + | 3 May 2015 |
| *Proorocentrum scutellum* Schröder, 1900 | - | 14 May 2015 |
| *Protothecopsis diabolus longipes* Cleve, 1900 | - | 11 Jul. 2015 |
| *Balech, 1974* | - | 6 Nov. 2015 |
| *P. Lopez-Garcia* | - | 6 Nov. 2015 |
| *Protoperotheidium diversum* (Ehrenberg, 1840) Balech, 1974 | - | 12 Apr. 2015 |
| *Eutreptiella* sp. | E | 30 Apr. 2015 |
| *Favella campanula* (Schmidt, 1902) Jörgensen, 1924 | O | 3 May 2015 |
| *Tintinnopsis campanula* Ehrenberg, 1840 | - | 14 May 2015 |

**Note:** B represents Bacillariophyceae, Dc Dictyochophyceae, Dn Dinophyceae, E Euglenophyceae, and O Oligotrichaceae.
tion, while surface water temperatures of 25.0°C and salinity of 21.4 were recorded at Marina station in the Izmit Bay. With respect to individual count, Dinophyceae was the dominant group, with rates of 86.8%. Bacillariophyceae and Oligotricha species were also observed with rates of 12.9% and <1.00%, respectively. In this red tide, the highest phytoplankton diversity, with 14 species (eight Dinophyceae, four Bacillariophyceae and two Oligotricha) were observed simultaneously with the second highest DIN:P ratio as 0.5 (Fig. 3a). Thus, different phytoplankton species found an opportunity to proliferate. Although the highest DIN:P ratio was determined on May 14, as was discussed above, this rate was related to the elevated level of ammonia, which possibly derived from *N. scintillans* excretion. Regarding the increased diatom abundance, the lowest silica concentration (0.22±0.01 mg/L) was determined on July 11, because of the silica usage in the cell wall of diatoms. On that day, one of the most common diatom species, *Skletonema costatum* reached 60.0×10^3 ind./L which was the highest abundance determined among diatom species throughout the study.

After July 2015, a significant phytoplankton increase was not observed until late autumn in the Izmit Bay. On November 6, a new bloom which was dominated by *P. micans* was observed again, without mucilage formation. Although the incident remained limited and almost no red appearance was observed in the central and western basins, phytoplankton abundance reached 1.10×10^6 ind./L and its diversity reached ten species (eight Dinophyceae and two Bacillariophyceae) in the Marina station located in the eastern basin of the bay. Interestingly, this bloom occurred a few days after a relatively long term strong wind blew. During that time, northeastern gusts, with an average speed of 35 km/h (ranged from 33 to 41 km/h) blew around the Izmit Bay for 5 d between 28 October and 2 November (Fig. 4a-right panel). Over the following days, the wind speed gradually decreased, and on November 6, the last phytoplankton bloom was observed in 2015, when average wind speed was 12 km/h, surface water temperatures were 15°C, and salinity was 22.0 (Fig. 4a).

It is well known that the Izmit Bay has been exposed to treated and/or untreated wastewater discharges since the 1960s. Hence, an elevated level of organic and inorganic contaminants including nutrients accumulated in the surface sediment of the bay, is...
present (Aktan et al., 2005; Ergül et al., 2013a; Karademir et al., 2013; Morkoç et al., 2001). Beside the unknown amount of land based effluents from several sources (e.g., agricultural runoffs, untreated anthropogenic wastewater, underground water), ~400 t total nitrogen and ~100 t total phosphorus were routinely discharged from wastewater treatment plants located around the Izmit Bay in 2015 (Fig. 5). The northern and southern sides of the bay are surrounded by hills, while the eastern and western edges are open from either side. Due to this geographical structure, a corridor occurs in the east-west direction. Therefore, following the ~35 km/h northeastern gusts for 5 d, wind induced sediment resuspensions occurred in the east, the shallowest basin of the Izmit Bay, and ambient water was contaminated with nutrients that were used by phytoplankton for their reproduction. Unlike other cases, NO$_3^-$-N concentration (0.040±0.003) mg/L was slightly higher than the NH$_4^+$ level (0.030±0.005) mg/L in this bloom. This high rate of nitrate may be related to oxidation of resuspended ammonia after nitrification processes in the surface sediment (Fig. 3c).

In terms of numbers of individuals, dinoflagellates were dominant with rates of over 99.0%, as in the first three blooms. Therefore, on November 6, 2015, silica content was remained relatively high (0.76±0.02) mg/L due to low usage by diatoms (Fig. 3d).

Since DIN:Si ratios ranged between 0.08 and 0.56 (i.e., <1), and DIN:P ratios ranged between 0.03 to 3.11 (i.e., below 16:1) in all samplings, nitrogen was the limiting factor during bloom conditions. It is known that N broadly limits phytoplankton growth in coastal marine ecosystems (Nixon, 1986) and N limitations have been reported from the Izmit Bay during bloom conditions (Aktan and Dede, 2008; Aktan et al., 2005), and other parts of the Marmara Sea (Turkoglu, 2013, 2016; Balkis et al., 2010) as in the present study.

According to previous studies (Morkoç et al., 2001) and Kocaeli Metropolitan Municipality records (ISU, 2016), discharges of total suspended matter (TSM), biological oxygen demand (BOD), total nitrogen (TN) and total phosphorus (TP) into the Izmit Bay gradually decreased 40, 40, 14 and 20 fold, respectively, since 1984, owing to established wastewater treatment plants (Fig. 5). Currently, it is estimated that more than 80.0% of discharge from the wastewater treatment plants has domestic origin, whereas the remaining discharge is from industrial sources. Nevertheless, the present results show that, despite precautions in place to decrease nutrient inputs via these plants, algal blooms still occur, with harmful effects including mucilage presence and invertebrate deaths.

The trophic index (TRIX), which was developed by Vollenweider et al. (1998) to determine the trophic state and quality of coastal marine ecosystems, ranged from 6.1 to 10 (6.9, 10, 6.3, 6.1, and 6.8 for April 30, May 3, May 14, July 11, and November 6, 2015, respectively). Therefore, based on TRIX values, the eutrophication status of the Izmit Bay was classified as degraded and there was a very high trophic level during the bloom conditions. It should be noted that the eutrophic state of the surface waters of the Izmit Bay fluctuated during the study and in some periods reached good quality and low trophic levels. On the other hand, because of the low current regime and the relatively long residence time of the water masses in the Izmit Bay (Morkoç et al., 2001), nutrient accumulation over certain periods of the year caused deterioration of the trophic state. Also, consecutive high TRIX values may be an important sign for future environmental problems for the bay. In fact, significant correlations between TRIX and total phytoplankton abundance (TPPA) (r=0.98, p<0.01, n=5) indicate that contents of the water encouraged the bloom conditions and following that the water quality of the bay was highly affected by the blooms. Positive significant correlations between o-PO$_4^{3-}$ and TPPA (r=0.96, p<0.01, n=6) and between NO$_3^-$-N and TPPA (r=0.86, p<0.05, n=6) revealed that variations of total phytoplankton density were linked to the nutrient concentrations. Also, a positive significant correlation between total phytoplankton biomass and the DIN:P ratio (r=0.98, p<0.01, n=6) indicates nitrogen limitation. Consequently, despite the improvement work over the last few decades by the municipality of the city around the Izmit Bay, based on adopted legislation in the frame of European Union directives, harmful algal blooms still occur with the abovementioned harmful effects.

In fact, the marine eutrophication issue and phytoplankton blooms, encountered in coastal marine ecosystems of Mediterranean seas, has been addressed in a number of studies since the 1960s (Karydis and Kitsiou, 2012). Through regulation and prevention, based on the Barcelona convention and the directives regarding the European Union policy on eutrophication the Water Framework Directive 2000/60/EC (EC, 2000), Mediterranean countries have modeled their legislation to reduce nutrient inputs to the marine ecosystems (Karydis and Kitsiou, 2012; Saliba, 1995). Nevertheless, despite the precautions, numerous intense phytoplankton blooms have been observed in Mediterranean coastal waters. Hence, we have listed recent intense dinoflagellate and diatom blooms (i.e., >10$^6$ ind./L) with temperature, salinity and nutrient data in the adjacent seas (i.e., the Marmara and Black Seas) and near Mediterranean coastal waters (i.e., the Aegean, Ionian and Adriatic Seas), in order to evaluate recent cases since 2000 and to compare them with the present results from the Izmit Bay (Table 2).

Temporal distribution of intense diatom and dinoflagellate blooms spread predominantly over three seasons: spring, summer and winter, in the Mediterranean coastal surface waters since 2000. In terms of frequency, the bloom conditions occurred mostly in spring (five diatom and five dinoflagellate blooms) followed by summer (four diatom and two dinoflagellate blooms) and winter (two diatom and three dinoflagellate blooms), whereas intense autumn blooms were recorded less frequently (two diatom and one dinoflagellate blooms). Typically, dinoflagellate blooms occurred in spring and early summer, while diatom blooms occurred in winter and early spring.

![Fig. 5. Daily average domestic and industrial biological oxygen demand (BOD), total suspended matter (TSM), total nitrogen (TN) and total phosphorus (TP) loads (as tonnes) into Izmit Bay between 1984 and 2015.](image-url)
### Table 2.  List of the most recent dinoflagellate and diatom blooms around Mediterranean coastal surface waters with temperature, salinity and nutrient data, including input sources

| Area                  | Causative species                      | Period    | $T/\degree C$ | $S$ | $\text{NO}_2-N \text{+ NO}_3-N/\text{mg.L}^{-1}$ | $\text{NH}_4/\text{mg.L}^{-1}$ | $\text{PO}_4^{3-}$ or TP$^+$/mg.L$^{-1}$ | $\text{SiO}_2$ or SiO$_{4}^{2-}$/mg.L$^{-1}$ | Input |
|-----------------------|----------------------------------------|-----------|---------------|-----|-----------------------------------------------|-------------------------------|--------------------------------------------|---------------------------------------------|-------|
| Marmara Sea           |                                        |           |               |     |                                               |                               |                                            |                                             |       |
| İstanbul Shore$^{(3)}$ | *Nitzschia longissima* (Brébisson) Ralfs, 1861 | winter 2000 | 14           | 25  | -                                             | -                             | -                                          | -                                           | I, D  |
| Golden Horn Estuary   | *Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975 TP | summer 2001 | 23           | 17  | 0.03                                          | 0.34                          | -                                          | -                                           | I, D  |
| (İstanbul) Strait$^{(3)}$ | *Skeletonema marinoi* Sarno & Zingone, 2005 | spring 2010 | 11           | 17  | 0.11                                          | 0.07                          | 0.11                                       | 0.72                                        |       |
| Çanakkale Strait$^{(6, 5)}$ | *Pseudo-nitzschia pungens* (Grunow ex Cleve) G. R. Hasle, 1993 | summer 2001 | 23           | 27  | 0.01                                          | -                             | 0.03                                       | 0.33$^*$                                    | D     |
|                       | *P. pungens*                           | winter 2002 | 8            | 30   | 0.04                                          | -                             | 0.04                                       | 0.15$^*$                                    |       |
|                       | *Prorocentrum micans* Ehrenberg, 1834 Ceratium spp. | E summer 2003 | 21           | 22  | <0.01                                         | -                             | 0.01                                       | 0.12$^*$                                    |       |
| İzmit Bay$^a$         |                                        |           |               |     |                                               |                               |                                            |                                             |       |
|                       | *P. micans*                            | spring 2015 | 15           | 13  | 0.02                                          | 0.01                          | 0.15                                       | 0.84                                        | I, D  |
|                       | *P. micans*                            | spring 2015 | 15           | 24  | 0.11                                          | 0.28                          | 42.0                                       | 0.82                                        |       |
|                       | *P. micans*                            | autumn 2015 | 15           | 22  | 0.05                                          | 0.03                          | 0.35                                       | 0.76                                        |       |
| Black Sea             |                                        |           |               |     |                                               |                               |                                            |                                             |       |
| Trabzon Shore$^{(5)}$ | *Scyphosia trochoidea* (Stein) Loeblich III, 1976 | winter 2000 | 9            | 19  | 0.40                                          | -                             | 0.09                                       | D                                           |       |
| Constanta Shore$^{(7)}$ | *Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902 | summer 2010 | 24           | 11  | -                                             | -                             | -                                          | -                                           | U, R, A |
| Rize Shore$^{(6)}$    |                                        |           |               |     |                                               |                               |                                            |                                             |       |
|                       | *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 | spring 2011 | 11           | 16  | 0.18                                          | -                             | 0.01                                       | 0.51                                        | D     |
| Aegean and Ionian Sea |                                        |           |               |     |                                               |                               |                                            |                                             |       |
| Thermoikos Bay$^{(9, 10)}$ | *Skeletonema costatum* (Greville) Cleve, 1873 | E spring 2000 | 11           | 36  | 0.04                                          | 0.02                          | 0.13                                       | 0.16$^*$                                    | D, A  |
|                       | *Leptocylindrus minimus* Gran, 1915 |           |               |     |                                               |                               |                                            |                                             |       |
|                       | *Prorocentrum obtusidens* Schiller, 1928 | >1, 2 winter 2001 | -         | -    | -                                             | -                             | -                                          | -                                           | D, A  |
|                       | *Prorocentrum redfieldii* Bursa, 1959 | >6, 0 winter 2001 | -          | -    | -                                             | -                             | -                                          | -                                           | D, A  |
|                       | *P. pungens*                           | >1, 8 spring 2002 | -        | -    | -                                             | -                             | -                                          | -                                           | D, A  |
|                       | *N. scintillans*                       | >5.4 spring 2004 | -          | -    | -                                             | -                             | -                                          | -                                           | D, A  |
| Amvrakikos Bay$^{(9)}$ | *Alexandrium insuetum* Ballech, 1985 | >2.6 spring 2003 | -          | -    | -                                             | -                             | -                                          | -                                           | D, R  |
| Kalloni Bay$^{(11)}$  | *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle, 2003 | spring 2005 | 12           | 36  | 0.07                                          | 0.01                          | 0.01                                       | 0.72                                        | D, R, A |
| Adriatic Sea          |                                        |           |               |     |                                               |                               |                                            |                                             |       |
| Trieste Bay$^{(2)}$   | *S. marinoi*                           | E spring 2000 | 2.6          | -    | -                                             | -                             | -                                          | T, I, R                                     |       |
|                       | *Chaetoceros spp.*                     | autumn 2000 | 4.6          | -    | -                                             | -                             | -                                          | T, R, A                                    |       |
|                       | *Chaetoceros spp.*                     | E spring 2009 | 2.3          | -    | -                                             | -                             | -                                          | T, R, A                                    |       |
| Pesaro-Foglia Coast$^{(3)}$ | Diatom species                      | autumn 2000 | 16           | 17   | 0.56                                          | 0.01                          | 0.28$^*$                                   | -                                           | R, A, I, D |
| Kastela Bay$^{(4)}$   | *L. minimus*                          | E summer 2005 | 6.0          | 27   | 0.01                                          | 0.02                          | 0.01                                       | 0.09$^*$                                    | I, D, R |
| Rovinj Coast$^{(5)}$  | *Chaetoceros ricovisibilis* Schiller in Hustredt, 1930 | Summer 2009 | 1.4          | 26   | 0.47                                          | 0.55                          | 0.03                                       | 3.42$^*$                                    | U, T, R, A |

Note: $A_i$ represents total abundance, $T$ temperature, $S$ salinity, $I$ industrial, $R$ solar irridance, $D$ domestic, $T$ terrigenic, $U$ upwelling, $A$ agriculture, and $E$ early. 1) Deniz and Taş (2009), 2) Taş and Okus (2011), 3) Taş and Yılmaz (2015), 4) Turkoglu (2010), 5) Turkoglu (2008), 6) Feyzioglu and Seyhan (2007), 7) Mihailov et al. (2013), 8) Kopuz et al. (2014), 9) Nikolaidis et al. (2005), 10) Koukaras and Nikolaidis (2004), 11) Spatharis et al. (2007), 12) Cabrini et al. (2012), 13) Penna et al. (2004), 14) Bužančić et al. (2016), 15) Bosak et al. (2016), * the present study, $\text{SiO}_2$ and “total phosphorus.”
However, in some cases, dinoflagellate blooms were reported in winter (Scissipedia trochoidea (Stein) Loeblich III, 1976, and Prorocentrum redfieldii Bursa, 1959 from Trabzon Shore and Tharmaniaos Bay, respectively) whereas diatom blooms were reported in summer seasons (Pseudo-nitzschia Pungens (Grunow ex Cleve) G. R. Hasle, 1993, Thalassiosira nitzschioides Schiller in Hustedt, 1930, Leptocylindrus minimus Gran, 1915 and Chaetoceros erixissibilis Schiller in Hustedt, 1930 from the Çanakkale Strait, Constanța Bay, Kastela Bay and Rovinj coasts, respectively; Table 2).

Phytoplankton blooms may occur in a wide range of temperatures and salinity (8.00–27.0°C and 11.0–36.0, respectively) and species-specific characters seem to be determined in bloom formations. Nutrient values also had diversified levels (<0.01–0.56, 0.01–0.55, 0.01–42.0 and 0.12–3.42 for NO$_3^-$-N + NO$_2^-$-N, NH$_3$, PO$_4^{3-}$ and SiO$_4$, respectively) and in almost all blooms, the ratios of nitrogen to phosphorus were remarkably different from the classical Redfield ratio (i.e., 16:1; Table 2). Consequently, these data suggest that a combination of nutrients is more important than its levels for diatom and dinoflagellate bloom formations, and termination of proliferation is determined by limiting nutrients. In addition, the blooms are predominantly influenced by temperature and salinity while sufficient light intensity and favorable amounts of nutrients exist in the water.

The spatial distribution of these blooms revealed that dense incidents mainly arose in the coastal waters of the northern parts of the Mediterranean. Although bloom cases have been reported, the abundance of diatoms and dinoflagellates did not exceed 10$^6$ ind./L in the Levantine Sea. Extreme proliferations of both groups were reported from the Black, Marmara, Aegean and Ionian Seas. However, despite the remarkable dinoflagellate abundance, incidents that exceeded 10$^6$ ind./L were reported merely for diatoms from the Adriatic Sea since 2000 (Table 2). Because the northern parts of the Mediterranean are richer in nutrients than the southern parts (Ignatiades, 2005), denser phytoplankton blooms are expected in the northern regions. In fact, the densest dinoflagellate bloom (70×10$^6$ ind./L) was reported from the Golden Horn Estuary, adjacent to the Istanbul Strait as well as the Black and Marmara Seas, in summer 2001 (Taş and Okuş, 2011). Besides being a semi-enclosed basin, the Golden Horn Estuary was known for its polluted water with foul odor because of untreated industrial and domestic discharges. However, the estuary was rehabilitated in the mid-2000s and bloom conditions have not been reported since 2010. Another dense bloom (40×10$^6$ ind./L) due to proliferation of diatoms was recorded in Pesaro coastal in the north Adriatic in autumn 2000. Beside local land-based sources, the Po River was considered as the responsible nutrient carrier for this bloom (Penna et al., 2004). Like above-mentioned blooms, most cases occurred under the land based anthropogenic inputs around the northern Mediterranean. However, legislations, based on EU directives, were set up to prevent further deterioration, and the decreasing bloom densities can be considered possible improvements in many Mediterranean coasts.

A comparison with blooms from nearby coastal surface waters of the Mediterranean seas showed that dinoflagellate blooms in the Izmit Bay were the densest consecutive blooms ever reported in recent years (Table 2). Like other incidents, domestic and industrial inputs were effective in those blooms. Additionally, unlike other incidents, wind-induced sediment resuspension caused a dinoflagellate bloom in the Izmit Bay, and this case indicates that accumulated contaminants in the surface sediment can be a potential nutrient source. This represents long term effects of untreated discharges on an elongated coastal marine ecosystem.

4 Conclusions

Beside low-speed water mass movement and routinely discharged nutrients via wastewater treatment plants from a populated city, various reasons might have contributed to the occurrence of the algal bloom conditions in the Izmit Bay, such as the inadequacy of advanced treatment technology, untreated and/or illegal domestic and industrial discharges, agricultural run-offs, and wind-induced resuspensions. Therefore, the results of the present study suggest that there is a need for increased action to prevent harmful algal blooms, in accordance with the environmental deterioration in the Izmit Bay. This can be achieved by improvements to wastewater treatment technology, including nutrient removal capability, prevention of untreated discharges, illegal bilge bailing and controlling agricultural fertilizer usage. Finally, the removal of the upper layer of the eastern basin’s surface sediment should be considered to prevent undesirable effects of wind induced resuspensions.

References

Aktan Y, Dede A, Ciftçi P S. 2008. Muclage event associated with diatoms and dinoflagellates in Sea of Marmara, Turkey. Harmful Algae News, 36
Aktan Y, Tüfekcii V, Tüfecki H, et al. 2005. Distribution patterns, biomass estimates and diversity of phytoplankton in Izmit Bay (Turkey). Estuarine, Coastal and Shelf Science, 64(2–3): 372–384
Algan O, Altok H, Yülç H. 1999. Seasonal variation of suspended particulate matter in two-layered Izmit Bay, Turkey. Estuarine, Coastal and Shelf Science, 49(2): 235–250
Artüz I, Baykut F. 1987. Hydrography of the Marmara Sea and Scientific Examination Regarding to Water Pollution (in Turkish). İstanbul: İstanbul Üniversitesi Çevre Sorunları Araştırma Merkezi
Balkis N, Atabay H, Türe’Ten I, et al. 2010. Role of single-celled organisms in mucilage formation on the shores of Buyukada Island (the Marmara Sea). Journal of the Marine Biological Association of the United Kingdom, 91(4): 771–781
Balkis N, Şenel E, Aksu A. 2007. Trace metal distributions in water column and surface sediments of Izmit Bay (Turkey) after Marmara (Izmit) earthquake. Fresenius Environmental Bulletin, 16(8): 910–916
Bendschneider K, Robinson R J. 1952. A New Spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research, 11: 87–96
Bosak S, Godrijan J, Šilović T. 2016. Dynamics of the marine planktonic diatom family Chaetoceraceae in a Mediterranean coastal zone. Estuarine, Coastal and Shelf Science, 180: 69–81
Boyce D G, Lewis M R, Worm B. 2010. Global phytoplankton decline over the past century. Nature, 466(7306): 591–596
Bruno D W, Dear G, Seaton D D. 1989. Mortality associated with phytoplankton blooms in Sea of Marmara, Turkey. Estuarine, Coastal and Shelf Science, 3289–3300
Bužančić M, Glada Ž, N, Marasović I, et al. 2016. Eutrophication influence on phytoplankton community composition in three bays on the eastern Adriatic coast. Oceanologia, 58(4): 302–316
Cabrini M, Fornasaro D, Cossarini G, et al. 2012. Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. Estuarine, Coastal and Shelf Science, 115: 113–124
Corbett D R. 2010. Resuspension and estuarine nutrient cycling: insights from the Neuse River Estuary. Biogeoosciences, 7(10): 3289–3300
Deniz N, Taş S. 2009. Seasonal variations in the phytoplankton community in the north-eastern Sea of Marmara and a species list. Journal of the Marine Biological Association of the United Kingdom, 89(2): 269–276
EC. 2000. Directive of the European Parliament and of the Council 2000/60/EC Establishing a Framework for Community Action in the Field of Water Policy. Brussels: Official Journal of the European Communities, European Commission, 1–72

ECR. 2013. 2012 Environmental Case Report of Kocaeli Province (in Turkish). Environmental Case Report. Ankara, Turkey: Ministry of Environment and Urban Planning, 195

Ergül H A. 2016. Evaluation of seasonal physicochemical conditions and chlorophyll-a concentrations in Izmit Bay, Marmara Sea. Journal of Black Sea/Mediterranean Environment, 22(3): 201–217

Ergül H A, Aksan S, Ipsiroglu M, et al. 2014. Assessment of consecutive plankton blooms on march and April 2014 in Izmit Bay (the Marmara Sea). In: 1st International Congress of Applied Ichthyology & Aquatic Environment. Volos, Greece: University of Thessaly

Ergül H A, Belvermig M, Kilic O, et al. 2013a. Natural and artificial radiouclide activity concentrations in surface sediments of Izmit Bay, Turkey. Journal of Environmental Radioactivity, 126: 125–132

Ergül H A, Küçük A, Terzi M. 2010. A study on the red tide of Izmit Bay—June 2010. In: Ozturk B, ed. Marmara Denizi 2010. Istanbul: TUDAV Press, 464–468

Ergül H A, Varol T, Ay Ü. 2013b. Investigation of heavy metal pollutants at various depths in the Gulf of Izmit. Marine Pollution Bulletin, 73(1): 389–393

Falkowski P, Scholes R, Boyle E, et al. 2000. The global carbon cycle: a test of our knowledge of earth as a system. Science, 290(5490): 291–296

Faust M A, Gulledge R A. 2002. Identifying Harmful Marine Dinoflagellates. Contributions from the United States National Herbarium. Washington DC: Smithsonian Institution, National Museum of Natural History

Feriz J, Gilbert P M, Burkholder J M, et al. 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae, 8(1): 3–13

Ignatiades L. 2005. Scaling the trophic status of the Aegean Sea, eastern Mediterranean. Journal of Sea Research, 54(1): 51–57

ISU. 2016. Average annual discharges to Izmit Bay from wastewater treatment plants (in Turkish). In: Facilities D O T, ed. Kocaeli Metropolitan Municipality. Kocaeli: Municipality Press

Jeong H J, Park J Y, Nho J H, et al. 2005. Feeding by red-tide dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, 45(3): 569–579

Mihailov M E S, Buga L, Malcu V, et al. 2013. Characteristics of upwelling algal bloom and hypoxia events in the western black sea in 2010. Fresenius Environmental Bulletin, 22(10): 2981–2990

Morkoc E, Okay O, Geveci A. 1996. Towards a clean Izmit bay (in Turkish). Project No: 15.1.005. Gebze Kocaeli, Turkey: Tubitak Press

Morkoc E, Okay O S, Tolun L, et al. 2001. Towards a clean Izmit Bay. Environment International, 26(3): 157–161

Mullin J B, Riley J P. 1955. The colorimetric determination of silicate with special reference to sea and natural waters. Analytica Chimica Acta, 12: 162–176

Murphy J, Riley J P. 1962. A modified single solution method for the determination of phosphate in natural-waters. Analytica Chimica Acta, 27: 31–36

NASA. 2015. Blooms in the Sea of Marmara. https://visibleearth.nasa.gov/view.php?id=85947 [2015-05-28/2015-06-08]

Nikolaidis G, Koukaras K, Aligiaziki K, et al. 2005. Harmful microalgal episodes in Greek coastal waters. Journal of Biological Research, 3: 77–85

Nixon S W. 1986. Nutrient dynamics and the productivity of marine coastal waters. In: Halwagy R, Clayton D, Bebebehani M, eds. Coastal Eutrophication. Oxford, UK: The Alden Press, 97–115

Okaichi T, Nishio S. 1976. Identification of ammonia as the toxic principle of red tide of Noctiluca scintillans. Bull Plankton Soc Jpn, 23: 75–80

Okay O S, Tolun L, Telli-Karakoc F, et al. 2001. Izmit Bay (Turkey) ecosystem after Marmara earthquake and subsequent refinery fire: the long-term data. Marine Pollution Bulletin, 42(5): 361–369

Olenina I, Hajdu S, Edler L, et al. 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. Baltic Sea Environment Proceedings No. 106. Helsinki: Helsinki Commission, Baltic Marine Environment Protection Commission, 144

Penna N, Capellacci S, Ricci F. 2004. The influence of the Po River discharge on phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea. Marine Pollution Bulletin, 48(3–4): 321–326

Saliba L. J. 1995. Development of regional coastal water quality standards in the Mediterranean. Water Science and Technology, 32(9–10): 17–24

Sml M. V. 2000. Phosphorus in the environment: natural flows and human interferences. Annual Review of Energy and the Environment, 25: 53–88

Smith V H, Tilman G D, Nekola J C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution, 100(1–3): 179–196

Spaethis S, Danielidis D B, Tsitsis G. 2007. Recurrent Pseudo-nitzschia calliantha (Bacillariophyceae) and Alexandrium insuetum (Dinophyceae) winter blooms induced by agricultural runoff. Harmful Algae, 6(6): 811–822

Taş S, Ergül H A, Balkis N. 2016. Harmful algal blooms (HABs) and mucilage formations in the Sea of Marmara. In: Özsoy E, Çağatay M N, Balkis N, et al., eds. The Sea of Marmara, Marine Biodiversity, Fisheries Conservation and Governance, Istanbul, Turkey: Turkish Marine Research Foundation (TUDAV), 768–786

Taş S, Okuş E. 2011. A review on the bloom dynamics of a harmful dinoflagellate prorocentrum minimum in the Golden Horn Estuary. Turkish Journal of Fisheries and Aquatic Sciences, 11(4): 673–681

Tas S, Yılmaz N. 2015. Potentially harmful microalgae and algal blooms in a eutrophic estuary in the Sea of Marmara (Turkey). Mediterranean Marine Science, 16(2): 432–443

Tolun L, Martens D, Okay O S, et al. 2006. Polycyclic aromatic hydrocarbon contamination in coastal sediments of the Izmit Bay (Marmara Sea): case studies before and after the Izmit Earthquake. Environment International, 32(6): 758–765

Turl V, Hagström Å, Kovač N, et al. 2010. Composition and function of mucilage macroaggregates in the northern Adriatic. Aquatic Microbial Ecology, 61(3): 279–289

Turkoglu M. 2013. Red tides of the dinoflagellate Noctiluca scintillans
associated with eutrophication in the Sea of Marmara (the Dardanelles, Turkey). Oceanologia, 55(3): 709–732
Turkoglu M. 2016. First harmful algal bloom record of tycoplanktonic dinoflagellate *Prorocentrum lima* (Ehrenberg) F. Stein, 1878 in the Dardanelles (Turkish Straits System, Turkey). Journal of Coastal Life Medicine, 4(10): 765–774
Türkoğlu M. 2008. Synchronous blooms of the coccolithophore *Emiliania huxleyi* and three dinoflagellates in the Dardanelles (Turkish Straits System). Journal of the Marine Biological Association of the UK, 88(3): 433–441
Türkoğlu M. 2010. Temporal variations of surface phytoplankton, nutrients and chlorophyll a in the Dardanelles (Turkish Straits System): a coastal station sample in weekly time intervals. Turkish Journal of Biology, 34(3): 319–333
Vollenweider R, Giovanardi F, Montanari G, et al. 1998. Characterization of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: proposal for a trophic scale, turbidity and generalized water quality index. Environmetrics, 9(3): 329–357
Yi Xiaoyan, Huang Yousong, Zhuang Yunyun, et al. 2017. *In situ* diet of the copepod *Calanus sinicus* in coastal waters of the South Yellow Sea and the Bohai Sea. Acta Oceanologica Sinica, 36(6): 68–79
Distribution and controlling factors of phytoplankton assemblages associated with mariculture in an eutrophic enclosed bay in the East China Sea

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Received 17 October 2017; accepted 11 December 2017

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Abstract

The distribution of phytoplankton and its correlation with environmental factors were studied monthly during August 2012 to July 2013 in the Yantian Bay. A total of 147 taxa of phytoplankton were identified, and the average abundance was in the range of 0.57×10^4 to 7.73×10^4 cell/L. A total of 19 species dominated the phytoplankton assemblages, and several species that are widely reported to be responsible for microalgae blooms were the absolutely dominant species, such as Skeletonema costatum, Navicula sp., Thalassionema nitzschiioides, Pleurosigma sp., and Licmophora abbreviata. The monthly variabilities in phytoplankton abundance could be explained by water temperature, dissolved oxygen, salinity, dissolved inorganic nitrogen (DIN), and suspended solids. The results of a redundancy analysis showed that pH and nutrients, including DIN and silicate (SiO_4^{4-}), were the most important environmental factors controlling phytoplankton assemblages in specific months. It was found that nutrients and pH levels that were mainly influenced by mariculture played a vital role in influencing the variation of phytoplankton assemblages in the Yantian Bay. Thus, a reduction of mariculture activities would be an effective way to control microalgae blooms in an enclosed and intensively eutrophic bay.

Key words: Sansha Bay, phytoplankton, eutrophication, microalgae blooms, bioremediation, East China Sea

Citation: Huo Yuanzi, Wei Zhangliang, Liu Qiao, Yang Fangfang, Long Lijuan, Zhang Qi, Bi Hongsheng, He Qing, He Peimin. 2018. Distribution and controlling factors of phytoplankton assemblages associated with mariculture in an eutrophic enclosed bay in the East China Sea. Acta Oceanologica Sinica, 37(8): 102–112, doi: 10.1007/s13131-018-1238-9

1 Introduction

Many coastal regions are at risk from eutrophication, which is a process resulting from an increase in anthropogenic nutrient inputs and other biogenic elements in estuarine waters, intensive mariculture activities, and other serious disasters (Picart et al., 2015). Mariculture has expanded in recent years, with an increased output to meet the growing global demand for aquatic products (Halwart et al., 2007; Willart et al., 2013; Chen and Qiu, 2014; Ferreira et al., 2014). However, the intensive mariculture of fish, shrimp, shellfish, and other economically important aquatic animals can lead to dissolved inorganic nutrients being released directly into seawater, and can also result in feces and pseudo feces depositing into sediments, which would subsequently increase the nutrient concentration in sea water due to decomposition. Intensive mariculture could lead to changes in the structure of dissolved inorganic nutrients and the sedimentary environment in coastal waters, especially in semi-enclosed and enclosed marine areas (de Jonge et al., 2002; Neori et al., 2004). The high nutritional status can change the characteristics of the ecosystem and cause a series of adverse ecological events, including phytoplankton blooms, macroalgae blooms, and other serious disasters (Nagasoe et al., 2010; Gilbert and Burkholder, 2011; Huo et al., 2012; Schumacher et al., 2014).

Phytoplankton blooms cause mass mortalities of wild and farmed animals worldwide, with catastrophic impact on aquaculture and local economies (Richlen et al., 2010). Harmful phytoplankton blooms also contaminate shellfish with toxins, making them unsafe for human consumption and can cause a variety of health problems (Flewelling et al., 2005; Peng et al., 2012). The distribution of phytoplankton assemblages is primarily influenced by environmental factors, such as water temperature and nutrient levels (Byun et al., 2007; Paeli et al., 2011). Several previous
ous studies have described the relationships between the phytoplankton distribution and environmental factors in coastal waters. Silicate (SiO$_4^{4-}$) and soluble reactive phosphorus (SRP), which are both influenced by hydrodynamic conditions, were the most important environmental factors influencing the phytoplankton distribution in the semi-enclosed environment of the Bohai Bay (Peng et al., 2012). Dissolved nitrate and pH have been shown to control the seasonal fluctuations of phytoplankton along the eastern coast of the Gulf of Suez in Egypt (Nassar et al., 2015). Shen et al. (2011) reported that temperature and turbidity had significant effects on the pattern of phytoplankton assemblages during wet and dry seasons in the Zhujiang (Pearl River) Estuary in China. Therefore, understanding the relationship between phytoplankton and environmental factors is of fundamental importance in studies of the mechanisms that distribute phytoplankton assemblages and the establishment of control measures (Peng et al., 2012).

The Sansha Bay is a typical enclosed bay along the coast of the East China Sea and is one of the most important aquaculture bases in southeastern China. It is seriously impacted by both poor hydrological exchange and anthropogenic activities (Wu et al., 2015). There are 220,000 fish cages in the Sansha Bay, with Pseudosciaena crocea and Crassostrea gigas and Apostichopus japonicus were also cultivated on a large scale in recent decades. The excess nutrients from mariculture activities, which enter the environment as dissolved ammonia, feces, and uneaten feed can stimulate rapid phytoplankton growth (Li et al., 2010). Yu et al. (2014) demonstrated that anthropogenic disturbance is the major controlling factor of habitat degradation in the Sansha Bay.

This study was conducted in the Yantian Bay, which has an area of approximately 405.86 km$^2$ (Wu et al., 2015). In recent years, the Yantian Bay has experienced serious eutrophication mainly due to intensive mariculture (Hu et al., 2014; Yu et al., 2014), which is a weak source of CO$_2$, resulting in low pH values (Wei et al., 2016). The rate of habitat degradation in the Yantian Bay, one of the six sub-bays of the Sansha Bay, is more severe (33.50%) than in the other sub-bays mainly due to mariculture activities. To the best of our knowledge, little information is available regarding the relationship between phytoplankton assemblages and environmental factors in enclosed and intensively eutrophic mariculture sea areas.

In this study, the composition and distribution of phytoplankton assemblages and the relationships between the environmental factors and phytoplankton were evaluated based on data obtained from 12 continuous cruises conducted during the period from August 2012 to July 2013 in the Yantian Bay. The aims of this study are to investigate phytoplankton assemblages and their relationship with environmental properties in intensively eutrophic mariculture areas and to identify the most important variables that determine the distribution of phytoplankton in these areas.

2 Materials and methods

2.1 Study area and survey methods

The Yantian Bay (26.72°–26.84°N, 119.76°–119.83°E), which is an enclosed sub-bay inside the Sansha Bay at the coast of the

Fig. 1. The locations of the Sansha Bay on the coast of the East China Sea (a), the Yantian Bay inside the Sansha Bay (b) and ten sampling sites in the Yantian Bay during the period of August 2012 to July 2013 (c).
East China Sea (Fig. 1). During our investigations, *P. crocea*, *C. gigas* and *A. japonicus* were cultured all year round. In this area, the macroalgae, *Gracilaria lemaneiformis*, is most commonly cultivated during September and December, and *Laminaria japonica* typically cultivated during January and May. There are no macroalgae cultivated during June and August in the Yantian Bay. Ten sampling sites were selected in the Yantian Bay, and samples were taken monthly during the period of August 2012 to July 2013 (Fig. 1). Sampling Site 1 was located in a non-mariculture area; sampling Site 3 was located in an area where *C. gigas* was cultured; sampling Site 4 was located in an area where *P. crocea* was cultured; while the other sampling sites were distributed in seaweed cultivation areas. A total of 12 cruises were conducted in this study. Sampling activities were conducted onboard a local fishing boat. Water samples and phytoplankton samples were collected using Niskin bottles controlled by a hand-operated winch (Widco, Fort Lauderdale, FL, USA) in the middle of every month.

### 2.2 Physico-chemical properties

During each survey, the surface temperature, salinity, suspended solids (SS), pH and dissolved oxygen (DO) were measured in the field using a multi-parameter kit (MSS, HACH, Loveland, CO, USA). Seawater samples were taken at each sampling site during the slack tide period at a depth of 15–20 cm below the surface, and were analyzed for the determination of ammonium (NH₄-N), nitrite (NO₂-N), nitrate nitrogen (NO₃-N), soluble reactive phosphorus (PO₄-P), silicate (SiO₄²⁻), chemical oxygen demand (COD) and chlorophyll a (Chl a). Seawater samples for the measurement of dissolved inorganic nutrients were filtered through cellulose membranes (0.45 μm), which were pre-immersed in 10% HCl for at least 10 h and rinsed with distilled water many times before use, and one to two drops of mercury (II) chloride was also added. Water samples used for Chl a determination were filtered onto GF/F glass-fiber filters onboard and wrapped in tinfoil. All seawater samples were preserved in a freezer at −30°C onboard and then transported to the laboratory under cold conditions and preserved in a freezer at −30°C in the laboratory. NH₄-N, NO₂-N, NO₃-N, PO₄-P and SiO₄²⁻ concentrations were measured according to the GB/T 12763.4 (2007) protocol. COD was measured directly according to the GB/T17378.4 (2007) protocol. Chl a was extracted from samples using 90% aqueous acetone, and the concentration of Chl a was determined using a fluorometer (Turner Designs, San Jose, CA, USA) (Parsons et al., 1984).

### 2.3 Phytoplankton community structure

At each sampling site, 1 000 mL seawater was sampled at a depth of 15–20 cm below the surface using Niskin bottles. These samples were immediately preserved with neutralized formaldehyde at a final concentration of 4% for the determination of phytoplankton. The samples were transported back to the laboratory under cool conditions, and 250 mL seawater samples were then placed in an Utermöhl counting chamber. Phytoplankton cells of greater than 5 μm diameter were identified and counted using an inverted microscope (Eclipse 100, Nikon, Tokyo, Japan) at 200× and 400× magnification. The entire chamber was examined and each cell was counted as a unit. At least 400 individuals of the more abundant species were counted from each sample with a 10% error. Diatoms were identified to species level if possible. The density of phytoplankton was recorded as cell/L, and was calculated based on the volume of seawater examined. The dominance index (Y) of the phytoplankton species, species diversity index (H'), richness index (J), Margalef index (d), and evenness index (J) were calculated according to Wang et al. (2005), Shannon and Wiener (1963), Margalef (1968), and Pielou (1975). A species with Y≥0.02 was considered to be a dominant phytoplankton species.

### 2.4 Statistical analysis

Based on the results of a test of normality, Pearson correlation analyses were used to determine correlations between environmental factors and total phytoplankton abundance, total diatom abundance and total dinoflagellate abundance in the Yantian Bay throughout the whole period investigated. Significant and highly significant correlations were defined at P<0.05 and P<0.01, respectively. Statistical analyses were conducted using SPSS 19.0. Multivariate ordination techniques were used to determine the relationship of environmental factors with the phytoplankton community in each month investigated using CANOCO for Windows 4.5 (Lepš and Šmilauer, 2003). Selected environmental parameters (sea surface temperature, pH, salinity, DO, Chl a, COD, DIN, dissolved inorganic phosphorus (DIP), SiO₄²⁻, DIN/SRP ratio and DIN/SiO₄²⁻ ratio) were adopted as the explanatory variables. Three forms of nitrogen were integrated as dissolved inorganic nitrogen (DIN), whereas PO₄-P was regarded as DIP in this study. All environmental parameters were transformed (log₁₀(x)) before analysis except for pH. In the data matrix of phytoplankton species abundance, only those species with an abundance greater than 5% in at least one sample were used in the analysis (Peng et al., 2012). The phytoplankton species data were transformed (log₁₀(x+1)) before analysis to obtain consecutive distributions. A detrended correspondence analysis (DCA) was applied to the phytoplankton species data to determine whether linear or unimodal ordination methods should be applied. The DCA revealed that the maximum gradient length of the four axes was less than three; therefore, linear methods were considered to be suitable ordination techniques in this study and a redundancy analysis (RDA) was applied to assess the relationships between phytoplankton and environmental parameters (Lepš and Šmilauer, 2003). A Monte Carlo simulation was used to test the significance of the environmental parameters when explaining the phytoplankton data by the RDA using CANOCO for Windows 4.5 (Lepš and Šmilauer, 2003).

### 3 Results

#### 3.1 Physico-chemical properties

During the study period, the spatio-temporal distribution of the environmental factors varied among the investigation months. The results observed for these environmental factors were reported by Wu et al. (2015).

#### 3.2 Phytoplankton composition

A total of 147 taxa of phytoplankton belonging to six taxonomic groups, were identified in the Yantian Bay during the investigation period. Diatoms and dinoflagellates accounted for 78.23% and 14.97% of the phytoplankton taxa, respectively, and there were 10 phytoplankton taxa belonging to Cyanophyta, Chlorophyta, Myzozoa and Ochrophyta (Table 1). The number and abundance of phytoplankton taxa varied in the different investigation months. Only *Skeletonema costatum*...
dominated the phytoplankton community in May 2013, while nine species dominated the phytoplankton community during September and November in 2012. *Skeletonema costatum* dominated the phytoplankton community in all investigation months, and accounted for 6.75%–93.98% of the total phytoplankton abundance. *Navicula* sp. and *Pleurosigma* sp. were the dominant species in eight investigation months, and accounted for 2.58%–27.30% and 2.11%–14.67% of the phytoplankton abundance, respectively. *Pseudo-nitzschia delicatissima* accounted for 29.39%, 14.41% and 46.91% of the phytoplankton abundance during August, September and October in 2012, respectively. *Detonula pumila*, *Thalassionema nitzschioides*, *Cerataulina pelagica* and *Licmophora abbreviata* accounted for 37.67%, 36.94%, 25.12% and 15.12% of the phytoplankton abundance during July, April, July and April in 2013, respectively. The other ten dominant diatoms accounted for less than 8% of the phytoplankton abundance in the investigation months. *Prorocentrum gracile*, the only dominant dinoflagellate, accounted for 2.30% and 4.25% of the phytoplankton abundance in September 2012 and March 2013, respectively.

### 3.3 Phytoplankton diversity

The average Shannon–Wiener index ($H'$) value varied from 0.89 to 3.54 among the different investigation months throughout the study period (Table 3). The average $H'$ was as low as 0.89 in January 2013, increased to 3.00 in March 2013, and decreased gradually to 1.52 in June 2013. The average $H'$ was maintained at a relatively high value of 2.66–3.54 from September to November in 2012. The Pielou index ($J$) was in the range of 0.21–0.75 during the study period, and the $J$ values varied in the same way as the

### Table 1. The number and average abundance ($\times 10^4$ cell/L) of phytoplankton taxa in specific taxonomic groups across ten sampling sites

| Taxonomic group | Item          | 2012          | 2013          |
|-----------------|---------------|---------------|---------------|
|                 | Aug. | Sep. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. |
| Diatom          | n    | 54   | 53   | 48   | 48   | 59   | 50   | 46   | 35   | 41   | 38   | 42   | 47   |
|                 | N    | 7.71 | 1.40 | 2.58 | 0.76 | 2.1  | 6.47 | 0.63 | 0.53 | 0.85 | 5.18 | 2.92 | 4.76 |
| Dinoflagellate  | n    | 9    | 5    | 3    | 4    | 3    | 2    | 4    | 3    | 5    | 9    | 9    | 4    |
|                 | N    | 0.029| 0.039| 0.027| 0.017| 0.0096| 0.047| 0.0078| 0.026| 0.0067| 0.069| 0.072| 0.0078|
| Cyanophyta      | n    | 1    | 1    | 1    | 1    | 1    | 0.0004| 0.0004| 0.018|
|                 | N    | 0.0074| 0.0030| 0.00022| 0.0074| 0.0048| 0.0056| 0.0015| 0.054|
| Chlorophyta     | n    | 2    | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 2    | 2    | 2    | 2    |
|                 | N    | 0.0074| 0.0030| 0.00022| 0.0074| 0.0048| 0.0056| 0.0015| 0.054|
| Myzooza         | n    | 1    | 1    | 1    | 1    | 1    | 0.0026| 0.0015| 0.0056| 0.0002| 0.0002| 0.0004|
|                 | N    | 0.0074| 0.0030| 0.00022| 0.0074| 0.0048| 0.0056| 0.0015| 0.054|
| Ochrophyta      | n    | 1    | 1    | 1    | 1    | 1    | 0.0004| 0.0004| 0.0007| 0.0004|
|                 | N    | 0.0074| 0.0030| 0.00022| 0.0074| 0.0048| 0.0056| 0.0015| 0.054|
| Total           | n    | 63   | 60   | 51   | 54   | 62   | 53   | 52   | 42   | 51   | 48   | 54   | 54   |
|                 | N    | 7.73 | 1.45 | 2.60 | 0.78 | 2.10 | 6.52 | 0.64 | 0.57 | 0.87 | 5.26 | 3.90 | 4.84 |

Note: $n$ indicates the number of phytoplankton species in specific taxonomic groups and $N$ the average abundance of phytoplankton in specific taxonomic groups across ten sampling sites.

### Table 2. Monthly dominant phytoplankton species and the average abundance ($\times 10^4$ cell/L) of each dominant species across ten sampling sites

| Species                        | 2012          | 2013          |
|--------------------------------|---------------|---------------|
|                                | Aug. | Sep. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. |
| Skeletonema costatum           | 3.51 | 0.41 | 0.60 | 0.24 | 1.39 | 5.62 | 0.24 | 0.035| 0.13 | 4.94| 2.41 | 0.52 |
| Navicula sp.                   | 0.054| 0.08 | 0.29 | 0.39 | 0.15 | 0.15 | 0.12 | 0.075|
| Pleurosigma sp.                | 0.086| 0.074| 0.11 | 0.063| 0.067| 0.054| 0.063| 0.13 |
| Cylindrotheca closterum        | 0.021| 0.035| 0.043| 0.13 |
| Licmophora abbreviata          | 0.064| 0.018| 0.13 |
| Detonula pumila                | 0.11 | 0.038| 1.82 |
| Bacillaria paxillifera         | 0.45 | 0.12 | 0.18 |
| Pseudo-nitzschia delicatissima| 2.45 | 0.20 | 1.19 |
| Thalassionema nitzschioides    | 0.021| 0.018| 0.32 |
| Ditylum brightwellii           | 0.029| 0.15 |
| Rhizosolenia setigera          | 0.031| 0.033| 0.22 |
| Paralia sulcata                | 0.030| 0.086|
| Climacospheria monilgera       | 0.030| 0.046|
| Cerataulina pelagica           | 1.19 |
| Chaetoceros abnormis           | 0.18 |
| Thalassiosira rotula           | 0.22 |
| Nitzchia sigma                 | 0.058|
| Grammatophora undulata         | 0.21 |
| Prorocentrum gracile           | 0.033| 0.024|
Table 3. Three indexes of the phytoplankton community in the different investigation months in the Yantian Bay

| Month | Shannon-Wiener index (H') | Pielou index (J') | Margalef index (d) |
|-------|---------------------------|------------------|-------------------|
|       | Min. | Max. | Average | Min. | Max. | Average | Min. | Max. | Average |
| 2012  |      |      |         |      |      |         |      |      |         |
| Aug.  | 1.41 | 3.07 | 2.14    | 0.32 | 0.60 | 0.44    | 1.83 | 3.15 | 2.35    |
| Sep.  | 2.68 | 4.24 | 3.54    | 0.59 | 0.86 | 0.75    | 2.27 | 3.25 | 2.62    |
| Oct.  | 2.09 | 3.77 | 2.66    | 0.46 | 0.80 | 0.57    | 1.84 | 3.11 | 2.34    |
| Nov.  | 2.58 | 3.84 | 3.25    | 0.61 | 0.84 | 0.74    | 1.73 | 2.94 | 2.27    |
| Dec.  | 1.11 | 2.45 | 1.77    | 0.25 | 0.57 | 0.40    | 1.60 | 2.96 | 2.16    |
| 2013  |      |      |         |      |      |         |      |      |         |
| Jan.  | 0.58 | 1.17 | 0.89    | 0.14 | 0.27 | 0.21    | 1.39 | 1.86 | 1.71    |
| Feb.  | 1.72 | 3.73 | 2.74    | 0.41 | 0.84 | 0.70    | 1.20 | 2.58 | 1.74    |
| Mar.  | 2.67 | 3.46 | 3.00    | 0.65 | 0.80 | 0.73    | 1.59 | 2.52 | 1.91    |
| Apr.  | 1.97 | 3.54 | 2.67    | 0.54 | 0.74 | 0.67    | 0.83 | 3.03 | 1.79    |
| May   | 0.31 | 3.26 | 1.49    | 0.07 | 0.81 | 0.37    | 1.25 | 1.90 | 1.75    |
| Jun.  | 1.19 | 2.09 | 1.52    | 0.25 | 0.51 | 0.33    | 1.59 | 2.56 | 2.26    |
| Jul.  | 2.35 | 3.28 | 2.81    | 0.51 | 0.71 | 0.62    | 1.75 | 2.60 | 2.14    |

$H'$ values among the different investigation months (Table 3). The lowest value of the Margalef index (d) of 1.71 was recorded in January 2013, and it was maintained at 1.74–1.91 from February to May in 2013. The d values were in the range of 2.14–2.62 during the period of August to December in 2012 and June to July in 2013.

3.4 Phytoplankton distribution

The average phytoplankton density was 3.00×10^4 cell/L throughout the whole year. The highest average density of 7.73×10^3 cell/L was recorded in August 2012, and the lowest average density of 0.57×10^3 cell/L was recorded in March 2013. The average phytoplankton density across all sampling sites was in the range of 0.78×10^4 cell/L to 6.52×10^4 cell/L in the other ten investigation months.

The geographical distribution of phytoplankton during the study period is shown in Fig. 2. In the spring months of March and April, the phytoplankton were distributed relatively uniformly throughout the Yantian Bay, but in May there was an increase from sampling Site 1 with 0.27×10^3 cell/L inside the bay to sampling Site 10 with 18.76×10^3 cell/L at the mouth of the bay. During summer, the phytoplankton were distributed relatively uniformly from different sampling sites in June, and the phytoplankton abundance decreased from inside the bay to the mouth of the bay in July and August, with the highest density of 16.23×10^4 cell/L at sampling Site 7 in August 2012. During autumn, the phytoplankton were also distributed relatively uniformly in September and November, but the density was as high as 5.10×10^4 cell/L at sampling Site 2 and continuously decreased to 1.04×10^4 cell/L at sampling Site 10 in October 2012. During winter, the phytoplankton abundance was relatively high at sampling sites inside the bay and at the mouth of the bay compared with the abundance at sampling sites in the middle of the bay in December 2012. The density increased to 4.22×10^5–9.75×10^4 cell/L, with no obvious pattern in the spatial distribution in January 2013. It then decreased to 0.16×10^4–2.00×10^4 cell/L, with the lowest phytoplankton abundance occurring at the mouth of Yantian Bay in February 2013.

3.5 Relationships between phytoplankton abundance and environmental factors

The results of the Pearson correlation analysis across the whole study period showed that total phytoplankton abundance and total diatoms were significantly correlated with water temperature, DIN, and DIN/SiO_4^2− (P<0.01, Table 4) and DO (P=0.016 and 0.017, Table 4). The total dinoflagellate abundance was significantly correlated with salinity and suspended solids (P=0.002 and <0.001, Table 4).

The relationship between environmental factors and phytoplankton abundance in each month investigated was analyzed by RDA (Table 5 and Fig. 3). The results of Monte Carlo tests showed that only the first canonical axis was significant during these investigation months, which indicated that these environmental factors may be important for explaining the phytoplankton community compositions. The first axis explained 20.1% to 43.4% of the phytoplankton variations in the investigation months. There were different significant environmental factors that explained the variability of the phytoplankton composition in the investigation months according to the RDA with forward selection. Silicate levels were found to statistically explain the variation in the composition of phytoplankton in November 2012 and July 2013, while pH was the significant environmental factor in August 2012, September 2012, February 2013, and April 2013. In December 2012, March 2013 and May 2013, the significant environmental factors were DO, DIN and salinity, respectively. During the study period in October 2012, January 2013 and June 2013, no environmental factor was significantly correlated with phytoplankton abundance in the Yantian Bay.

4 Discussion

The composition and abundance of phytoplankton in the Sansha Bay in May, August and November in 1990, and in February 1991, sampled using a conical phytoplankton net, was reported by Lin (1993). It is difficult to compare the phytoplankton abundance in this study with the results reported by Lin (1993) because of the different sampling methods, but the difference in the dominant phytoplankton species over the 20 years can be evaluated. *Skeleto-nea costatum*, *Ditylum brightwellii* and *Nitzschia sigma*, which were the dominant species in the early 1990s also dominated the phytoplankton community in our study, while *Biddulphia sinensis*, *Biddulphia regia*, *Coscinodiscus centra-lis*, *Coscinodiscus oculusiridis*, *Chaetoceros decipiens*, *Bacillaria paradoxoa*, *Thalassiothrix frauenfeldii*, *Ceratium fusus* and *Noctiluca scintillans* were dominant species in the early 1990s, but not in the early 2010s. *Noctiluca scintillans* was the dominant dinoflagellate in the early 1990s in the Sansha Bay (Lin, 1993),
but *P. gracile* was the only dominant dinoflagellate in September and March in the 2010s in the Yantian Bay. The $H'$ values in the current study were much smaller than in the corresponding months in the 1990s (Lin, 1993), which indicated that the phyto-
plankton composition and abundance had changed substantially over the 20 years.

The phytoplankton abundance was significantly different among the different months investigated. The monthly variations of the phytoplankton community and their abundance in the Yantian Bay were greatly influenced by environmental factors (Table 4). The most important environmental factor influencing the monthly variations of phytoplankton abundance was water temperature. Water temperature can control the seasonal dynamics of phytoplankton successions (Dupuis and Hann, 2009). Some researchers have reported that the abundance of diatoms is inversely related to temperature, with a high abundance of diatoms at temperatures below 18°C (da Silva et al., 2005; Turner et al., 2009). In the Yantian Bay, the highest total diatom abundance probably occurs during periods with a relatively high water temperature, which differs from the situation in the Bohai Bay (Peng et al., 2012). The abundance of small-celled diatoms, such as S. castatum, Navicula sp. and Pleurosigma sp., increases during warm water periods, because warm water is well known to be less viscous than cold water, favoring a species with a small cell size (Tunin-Ley et al., 2007).

Nutrient availability is one of key factors influencing the monthly variations of the phytoplankton community (Llope et al., 2009; Ward et al., 2011; Peng et al., 2012). Under normal conditions, phytoplankton takes up N and P at the Redfield ratio (16:1). In the Yantian Bay, the observed molar ratio of N/P was higher than the Redfield ratio, indicating either a N surplus or a P-limited system (Wu et al., 2015). There was no significant correlation between phytoplankton abundance and the PO$_4$-P concentration, which was mostly higher than the eutrophication threshold value of 0.045 mg/L (Wu et al., 2015). These results indicated that phytoplankton abundance was not limited by P availability in the Yantian Bay during the study period. Total phytoplankton and total diatom abundance were significantly negatively correlated with the DIN concentration in this study. This may be explained by the increasing phytoplankton abundance, which led to nitrogen being rapidly depleted. These results differed from those results reported by Lin (1993), who reported a negative correlation between phytoplankton abundance and PO$_4$-P in the 1990s in the Sansha Bay.

Salinity is another important environmental factor that can influence the seasonal variations of phytoplankton (McQuoid, 2005). In this study, total dinoflagellate abundance was significantly negatively correlated with salinity (Table 4). A relatively low salinity is favorable for the explosive growth of dinoflagellates, which increases the possibility of red tide outbreaks (Yuan et al., 2014). There was a significant correlation between total dinoflagellate abundance and the concentration of SS in this study. Dinoflagellates are unable to survive in very turbulent water (Margalet, 1978). Wang and Huang (2003) reported that low turbidity may also favor the growth of Karenia mikimotoi and Prorocentrum dentatum. The fine particles remain in suspension, resulting in high level so flight scattering and considerably reducing light penetration into the water column (Oliver et al., 2010), which would limit the growth and photosynthesis of phytoplankton (Shen et al., 2011).

The phytoplankton composition and abundance varied between different sampling sites, which were due to different

| Month | Environmental factor | Eigenvalues | Variation explains solely/ | F | p |
|-------|----------------------|-------------|---------------------------|---|---|
| Aug.  | pH                   | 0.434       | 43.4                      | 6.129 | 0.002 |
| Sep.  | pH                   | 0.295       | 29.5                      | 3.351 | 0.002 |
| Nov.  | SiO$_4$              | 0.201       | 20.1                      | 2.015 | 0.042 |
| Dec.  | DO                   | 0.227       | 22.7                      | 2.355 | 0.030 |
| 2013  |                      |             |                           |     |    |
| Feb.  | pH                   | 0.264       | 26.4                      | 2.872 | 0.002 |
| Mar.  | DIN                  | 0.271       | 27.1                      | 2.978 | 0.022 |
| Apr.  | pH                   | 0.311       | 31.1                      | 3.613 | 0.004 |
| May   | salinity             | 0.357       | 35.7                      | 4.448 | 0.014 |
| Jul.  | SiO$_4$              | 0.256       | 25.6                      | 2.756 | 0.020 |

Table 4. Pearson correlation between total phytoplankton abundance, total diatom abundance, total dinoflagellate abundance and environmental factors across the whole study period in the Yantian Bay

| Environmental factor | Total       | Diatom      | Dinoflagellate | n  |
|----------------------|-------------|-------------|----------------|----|
| Temperature          | 0.315 **    | 0.313 **    | 0.106          | 120|
| Salinity             | 0.164       | 0.167       | -0.277 **      | 120|
| pH                   | -0.058      | -0.059      | -0.019         | 120|
| DO                   | -0.221 *    | -0.219 *    | -0.046         | 120|
| SS/mg·L$^{-1}$       | -0.157      | -0.154      | -0.336 **      | 120|
| COD                  | -0.079      | -0.083      | 0.088          | 120|
| DIN                  | -0.399 **   | -0.397 **   | -0.176         | 120|
| PO$_4$–P             | -0.150      | -0.147      | -0.079         | 120|
| SiO$_4$              | -0.055      | -0.055      | -0.079         | 120|
| DIN/PO$_4$–P         | -0.176      | -0.177      | -0.012         | 120|
| DIN/SiO$_4$          | -0.402 **   | -0.399 **   | -0.155         | 120|

Note: ** Highly significant correlation at the 0.01 level (2-tailed); * significant correlation at the 0.05 level (2-tailed).

Table 5. Apartitioning analysis of environmental factors influencing phytoplankton assemblages in the Yantian Bay during the period of August 2012 to July 2013
Fig. 3. Correlation plots of the redundancy analysis (RDA) for the relationship between the environmental variables and phytoplankton taxa. The numbers with letters represent the following species: n1 represents Cerataulina pelagica, n2 Chaetoceros abnormis, n3 Chaetoceros lauderi, n4 Chaetoceros sp., n5 Coscinodiscus sp., n6 Cylindrotheca closterium, n7 Detonula pumila, n8 Ditylum brightwellii, n9 Melosira nummuloides, n10 Skeletonema costatum, n11 Thalassiosira rotula, n12 Bacillaria paxillifera, n13 Chaetoceros curvisetus, n14 Pseudo-nitzschia delicatissima, n15 Chaetoceros debilis, n16 Melosira granulata, n17 Navicula sp., n18 Nitzschia sigma, n19 Pleurosigma sp., n20 Rhizosolenia setigera, n21 Thalassionema nitzschioides, n22 Thalassiosira sp., n23 Prorocentrum gracile, n24 Licmophora abbreviata, n25 Grammatophora undulata, n26 Melosira granulata, n27 Nitzschia lorenziana, n28 Nitzschia sp., n29 Pseudo-nitzschia pungens, n30 Asteroplanus karianus, n31 Climasphenia moniliger, n32 Paralia sulcata, n33 Planktonia blanda, n34 Scenedesmus quadricauda, n35 Coscinodiscus jonesianus, n36 Triceratium alternans, and n37 Thalassiosira nordenskiöldii.
phytoplankton species adapting to changes in the physico-chemical environment in the specific investigation months. Although water samples were collected in different functional areas, all sampling sites were impacted by mariculture activities, as shown by the geographical distribution of dissolved inorganic nutrients (Wu et al., 2015).

During the whole investigation period, the phytoplankton community was mainly composed of nereid diatom species, such as the diatom *S. costatum*, *Navicula* sp., *P. delicatissima* and the dinoflagellate *P. gracile*. The concentration of SiO$_4^-$ could influence the structure of phytoplankton assemblages, with diatoms becoming the dominant species when the SiO$_4^-$ concentration was higher than 2 μmol/L and the levels of all other nutrients were sufficient (Egge, 1998). *Skeletomena costatum* dominated the phytoplankton community during the whole year. According to the results of the RDA analysis, its abundance was significantly correlated to high SiO$_4^-$ and DIN concentrations, suggesting that *S. costatum* prefers water with high levels of nutrients. The results of this study are consistent with other studies that have found that *S. costatum* prefers nutrient-rich seawater (Patil and Anil, 2011; Peng et al., 2012). *Navicula* sp., *Pleurosigma* sp., *C. closterium* and *P. sulcata* also have a preference for seawater containing high nutrient concentrations, and their abundance has been shown to be significantly correlated with levels of nutrients and/or SiO$_4^-$ (Du et al., 2016; El-Kassas and Gharib, 2016; Zhang et al., 2016). These diatom species could be considered as bioindicators for assessing environmental quality in specific ecosystems. *Detonula pumila* was one of the dominant species in the phytoplankton assemblage in November 2012 and July 2013 (Table 2 and Fig. 3), and its abundance was significantly correlated to the SiO$_4^-$ concentration in the RDA. The results of this study were consistent with those of Yuan et al. (2014) who found that the abundance of *D. pumila* was strongly related to silicate levels in the Sanggou Bay, Ailian Bay, and Lidao Bay at the coast of the Yellow Sea. *Pseudo-nitzschia delicatissima* was one of the dominant species in the summer period in the Yantian Bay, and is considered to be an estuarine species that is typically observed in the Yellow Sea. *Pseudo-nitzschia delicatissima* was one of the dominant species in the summer period in the Yantian Bay, and is considered to be an estuarine species that is typically observed in the Yellow Sea. *Pseudo-nitzschia delicatissima* was one of the dominant species in the summer period in the Yantian Bay, and is considered to be an estuarine species that is typically observed in the Yellow Sea.
ton Res, 31(5): 489–502

Edger J K. 1998. Are diatoms poor competitors at low phosphate concentrations? J Mar Syst, 16(3–4): 191–198

El-Kassas H Y, Gharib S M. 2016. Phytoplankton abundance and structure as indicator of water quality in the drainage system of the Burullus Lagoon, southern Mediterranean coast, Egypt. Environ Monit Assess, 189(9): 530

Engström-Öst J, Koski M, Schmidt K, et al. 2002. Effects of toxic cyanobacteria on a plankton assemblage: community development during decay of Nodularia spumigena. Mar Ecol Prog Ser, 232: 1–14

Ferreira J G, Saurel C, Lencart e Silva J D, et al. 2014. Modelling of interactions between coastal and offshore aquaculture. Aquaculture, 426–427: 154–164

Flewelling L J, Naar J P, Abbott J P, et al. 2005. Brevetoxicosis: red tides and marine mammal mortalities. Nature, 435(7043): 755–756

Gao Kunshan, Helbling E W, Häder D P, et al. 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Mar Ecol Prog Ser, 470: 167–189

Gibert P M, Burkleholder J M. 2011. Harmful algal blooms and eutrophication: “strategies” for nutrient uptake and growth outside the Redfield comfort zone. Chin J Oceanol Limnol, 29(4): 724–738

Halwart M, Soto D, Arthur J R. 2007. Cage Aquaculture: Regional Relevance and Global Overview. FAO Fisheries Technical Paper. No. 498. Rome: FAO, 241

Hu Ming, Wei Zhangliang, Han Hongbin, et al. 2014. The survey and assessment of water environmental quality in the mariculture area in the enclosed Sansha Bay. Journal of Shanghai Ocean University (in Chinese), 23(4): 582–587

Huo Yuanzi, Wu Hailong, Chai Zhaoyang, et al. 2012. Bioremediation efficacy of Gracilaria verrucosa for an integrated multi-trophic aquatic system with Pseudosclerma crocused in Xiangshan harbor, China. Aquaculture, 326-329: 99–105

Lepš J, Smilauer P. 2003. Multivariate Analysis of Ecological Data Using CANOCO. New York: Cambridge University Press, 1–122

Li Chaolun, Zhang Yongshao, Song Sun, et al. 2010. Species composition, density and seasonal variation of phytoplankton in Sanggou Bay, China. Progress in Fishery Sciences (in Chinese), 31(4): 1–8

Lin Jinmei. 1993. Distribution of phytoplankton in Sansha Bay, Fujian. Journal of Oceanography in Taiwan Strait (in Chinese), 14(7): 1065–1069

Liu Dongyan, Sun Jun, Zou Jinhong, et al. 2005. Phytoplankton succession during a red tide of Skeletonema costatum in Jiaozhou Bay of China. Mar Pollut Bull, 50(1): 91–94

Llope M, Chan K S, Ciannelli L, et al. 2009. Effects of environmental conditions on the seasonal distribution of phytoplankton biomass in the North Sea. Limnol Oceanogr, 54(2): 512–524

Mahmood T, Fang Jiaxiang, Jiang Zengjie, et al. 2016. Carbon and nitrogen flow, and trophic relationships, among the cultured species in an integrated multi-trophic aquaculture (IMTA) bay. Aquacult Environ Interact, 8: 207–219

Margalef R. 1968. Perspectives in Ecological Theory. Chicago: University of Chicago Press, 1–111

Margalef R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol Acta, 1(4): 493–509

McQuoid M R. 2005. Influence of salinity on seasonal germination of rosettes in the Arabian gulf region, with special reference to the identification and phylogeny of the fish-killing dinoflagellate Cochlodinium polykrikoides. Harmful Algae, 9(2): 279–293

Picart S S, Allen J I, Butenschön M, et al. 2015. What can ecosystem models tell us about the risk of eutrophication in the North Sea?. Clim Change, 132(1): 111–125

Pielou C E. 1975. Ecological Diversity. New York: John Wiley, 1–117

Shen Pingping, Li Yang, Liangmin, et al. 2011. Spatio-temporal variability of phytoplankton assemblages in the Pearl River estuary, with special reference to the influence of turbidity and temperature. Cont Shelf Res, 31(16): 1672–1681

Shannon C E, Wiener W. 1963. The Mathematical Theory of Communication. Urbana: University of Illinois Press, 1–117

Su Yuezhong. 2009. Investigation and prevention-control counter-measures on Cryptocorynium irivians Brown in the main culture zones of Fujian province. Fish Sci Technol Inf (in Chinese), 36: 29–35

Tunin-Ley A, Labat J P, Gasparini S, et al. 2007. Annual cycle and diversity of species and infraspecific taxa of Ceratium (Dinophyceae) in the Ligurian sea, northwest Mediterranean. Jourunal of Phycolory, 43: 1149–1163

Velle L J, Naar J P, Abbott J P, et al. 2005. Brevetoxicosis: red tides and marine mammal mortalities. Nature, 435(7043): 755–756

Wang Junhui, Huang Xiqing, 2003. Ecological characteristics of Procorntum dentatum and the cause of harmful algal bloom formation in China Sea. Chinese Journal of Applied Ecology (in Chinese), 14(7): 1065–1069

Wang Junlong, Yuan Qi, Shen Xinyou. 2005. Ecological characteristics of the phytoplankton of the Yangtze River estuary and adjacent waters. Journal of Fishery Sciences of China (in Chinese), 12(3): 300–306

Ward B B, Rees A P, Somerfield P J, et al. 2011. Linking phytoplankton community composition to seasonal changes in f-ratio. ISME J, 5(11): 1759–1770

Wei Zhaoyan, Jiang Zengjie, Hu Ming, et al. 2016. Seasonal variation of sea-air CO2 flux in mariculture area in Yantian Harbor, Sansha Bay. Journal of Shanghai Ocean University (in Chinese), 25(1): 106–115

Wang Junhui, Huang Xiqing, 2003. Ecological characteristics of Procorntum dentatum and the cause of harmful algal bloom formation in China Sea. Chinese Journal of Applied Ecology (in Chinese), 14(7): 1065–1069

Wang Junlong, Yuan Qi, Shen Xinyou. 2005. Ecological characteristics of the phytoplankton of the Yangtze River estuary and adjacent waters. Journal of Fishery Sciences of China (in Chinese), 12(3): 300–306

Ward B B, Rees A P, Somerfield P J, et al. 2011. Linking phytoplankton community composition to seasonal changes in f-ratio. ISME J, 5(11): 1759–1770

Wei Zhaoyan, Jiang Zengjie, Hu Ming, et al. 2016. Seasonal variation of sea-air CO2 flux in mariculture area in Yantian Harbor, Sansha Bay. Journal of Shanghai Ocean University (in Chinese), 25(1): 106–115
Wilfart A, Prudhomme J, Blancheton J P, et al. 2013. LCA and emergy accounting of aquaculture systems: towards ecological intensification. J Environ Manage, 121: 96–109

Wu Hailong, Hu Yuanzi, Hu Ming, et al. 2015. Eutrophication assessment and bioremediation strategy using seaweeds co-cultured with aquatic animals in an enclosed bay in China. Mar Pollut Bull, 95(1): 342–349

Yuan Mingli, Zhang Cuixia, Jiang Zengjie, et al. 2014. Seasonal variations in phytoplankton community structure in the Sanggou, Ailian, and Lidao Bays. J Ocean Univ China, 13(6): 1012–1024

Yu Ge, Sun Peng, Liu Guangxing, et al. 2014. Diagnostic model construction and example analysis of habitat degradation in enclosed bay: II. spatiotemporal variations in habitat degradation in Sansha Bay. J Ocean Univ China, 32(3): 636–644

Zhang Xia, Zhang Jingping, Huang Xiaoping, et al. 2014. Phytoplankton assemblage structure shaped by key environmental variables in the Pearl River Estuary, South China. J Ocean Univ China, 13(1): 73–82

Zhang Shan, Leng Xiaoyun, Feng Yuanyuan, et al. 2016. Ecological provinces of spring phytoplankton in the Yellow Sea: species composition. Acta Oceanol Sin, 35(8): 114–125