Scientific Report for the CNR STM 2016

Prof. HUANG Lingfeng

Department of Ecological Science and Engineering
College of the Environment and Ecology
Xiamen University
Xiang'an South Road,
Xiamen, 361102, Fujian, P.R. China
Tel. & Fax: +86-592-2188455
E-mail: huanglf@xmu.cdu.cn

<u>Title of the program:</u>

Effect of eutrophication on the isotopic variation ($\delta^{13}C$, $\delta^{15}N$) of the first trophic levels in the Yundang Lagoon (Xiamen, China)

Period: 8-22 August 2016 at Consiglio Nazionale delle Ricerche – Istituto per l'Ambiente Marino Costiero (CNR-IAMC), Località Sa Mardini, Torregrande, 09170 Oristano, Italy

Summary of activities

The activities conducted between 8 and 22 August 2016 at the Institute for Coastal Marine Environment of Oristano of CNR in collaboration with CNR host, Dr. Paolo Magni, included:

1) Presentation of the first results of the isotopic analysis conducted on samples collected in the Yundang lagoon.
2) Discussion of the results and preparation of the whole dataset available for different primary producers and consumers for further analysis.
3) Univariate and multivariate analysis of the data set.
4) Discussion of the new results and preparation of graphs and tables.
5) Presentation of results and discussion for the preparation of a manuscript to be submitted to an international journal.
6) Planning of new joint research activities and project proposals.
7) Preparation of the manuscript and the report.

Through a series of activities, we have finished a draft manuscript titled as "*Effect of eutrophication on the isotopic variation ($\delta^{13}C$, $\delta^{15}N$) of the first trophic levels in the Yundang Lagoon (Xiamen, China)*", and the both sides have reached a strong will for further cooperative research and made a preliminary plan.

In short, my visit at the Oristano section of the Institute for Coastal Marine Environment, National Research Council of Italy (CNR-IAMC) under the Short Term Mobility Program (Year 2016) was fruitful. I acknowledge with thanks the funding support from CNR-Italy and all the activities hosted by Dr. Paolo Magni in the Oristano section of CNR-IAMC.

Signature: Huang Lingfeng

August 22, 2016

ANNEX 1. DRAFT MANUSCRIPT PREPARED FOR SUBMISSION TO AN INTERNATIONAL PEER-REVIEWED JOURNAL

Effect of eutrophication on the isotopic variation ($\delta^{13}C$, $\delta^{15}N$) of the first trophic levels in the Yundang Lagoon (Xiamen, China)

Xinqing Zheng[1], Paolo Magni[2], Serena Como[2], Lingfeng Huang[3]

1. Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, China.

2. National Research Council of Italy, Institute for Coastal Marine Environment (CNR-IAMC), 09170 Oristano, Italy.

3. College of the Environment and Ecology, Xiamen University, Xiamen 361102, PR China

1 Introduction

Aquatic eutrophication is a significant problem globally, primarily due to the discharge of large amounts of wastewater derived from agricultural, industrial and municipal activities. Micro- and macro-algal blooms are commonly increasingly in coastal waters subjected to excessive anthropogenic inputs of nutrients (Worm and Lotze, 2006; Kraufvelin et al., 2006; Balducci et al., 2001). Coastal lagoons are semi-closed coastal waters surrounded by sand spits or sandbanks, and they are some of the most productive ecosystems worldwide, offering optimal niches for numerous aquatic species that utilize these areas as refuges or breeding grounds (Nixon, 1981). Different types of primary producers can be found in coastal lagoons, such as phytoplankton, micro-phytobenthos (MPB), mangroves, salt marsh plants, and macroalgae (or seagrasses), as well as the epiphytes of these species(Carlier et al., 2008; Lin et al., 2007; Maksymowska et al., 2000; Vizzini and Mazzola, 2008). The diverse potential carbon sources add to the complexity of a coastal lagoon ecosystem and also increase the difficulty in describing the trophic relationship. Given that both photosynthetic producers and debris from vascular plants and algae are present in the coastal lagoons, the assessment of which organic carbon sources are mainly assimilated by consumers is challenging. To date, no paradigm has stated the role of the main carbon sources in the trophic dynamics of the coastal lagoon food web, which seems to depend on the composition of primary producers (Carlier et al. 2007; Magni et al. 2008; Rodríguez-Graña et al., 2008; Vizzini and Mazzola, 2008).

The stable isotope technique can trace the end carbon source of consumers, and it is a useful tool for determining food web structure. The IsoSource model by Phillips and Greg (2003) facilitates the identification of the food source for consumers and has been widely applied to ecological studies on estuaries, salt marshes, and coastal lagoons (Choy et al., 2009; Quan et al., 2007; Svensson et al., 2007;

Schaal et al., 2008). However, this model cannot quantify which components support the smooth operation of the food web based only on stable isotope analysis. Junger and Planas (1994) considered the biomass of consumers to be a relevant variable for accurate quantification of the relative contribution of organic carbon sources in the aquatic food web. Their study proved to be an excellent trial and was considered to be feasible (Choy et al., 2009), because the biomass of consumers represents the mass of living consumers in a given area and directly reflects the amount of assimilated carbon sources. Primary consumers are the key conduit that transfers organic matter or primary producers to higher trophic levels. Hence, the contributions of organic carbon sources to primary consumers can indirectly reflect their relative importance to the overall aquatic food web as the trophic base fueling the web.

Yundang Lagoon is a coastal eutrophic shallow (average depth of approximately 2.5 m), brackish (annual mean salinity of 25 to 26) and small (the total area of the lagoon is 1.5 km^2) lagoon located at the western part of Xiamen Island, southeast of China. In the past three decades, Yundang Lagoon was highly polluted due to the discharge of municipal sewage from many outlets around the lagoon and the weak or even stagnant hydrodynamics in this enclosed environment. Microalgae (typically diatoms and dinoflagellates) and macroalgae (dominated by *Ulva lactuca*) blooms occurred alternately year round, in which *Ulva* blooms are typically in cool seasons and microalgal blooms occurs in warm seasons. Understanding the dynamics the first level producers (here, including the primary producers and the particulate organic maters derived from primary producers) with the variations of the ambient environments is important for the management of the lagoon ecosystem, as well as looking for feasible approaches to solve the eutrophication problems.

The aim of this study is to reveal the eutrophication effects on the variations of first level producers in Yundang Lagoon by the use of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) technique and common analysis approaches. To this end, the environmental variables (Chl *a*, TOC, Cat/Nat and TOC/Chl a) and stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of the first level producers were investigated and analyzed among the main sectors of the lagoon (i.e., diversion canal, main canal, inner lagoon and outer lagoon) in March and September, representing the cool and warm season, respectively. In addition, the isotopic composition of the first level producers including phytoplanktons (PHY), macroalgae (ULV), microphytobenthos (MPB), decaying leaves of mangrove plants (LMP) and sedimentary organic matter (SOM).

2 Materials and methods

2.1. Study sites

The study was conducted in March and September 2010 in the Yundang Lagoon, which connects coastal waters through a small canal controlled by a sluice. The laggon is separated into several parts, including a diversion canal (DC), main canal (MC), inner lagoon (IL), and outer lagoon (OL) (Fig. 1). Water exchange between the lagoon and inshore water is limited, and the retention time of lagoon water is approximately 3 d. Given the massive domestic sewage discharge in the area, eutrophication has become an extremely serious threat in the lagoon water, with the concentration of dissolved inorganic nitrogen reaching 0.35 mmol/L (Li et al., 2007). Macroalgal blooms, which are typically dominated by *Ulva lactuca*, occur annually during winter and spring. Biomass of fresh *U. lactuca* varied from around 1000 g /m^2 to 4200 g/m^2 during this period (Zheng and Huang, 2011).

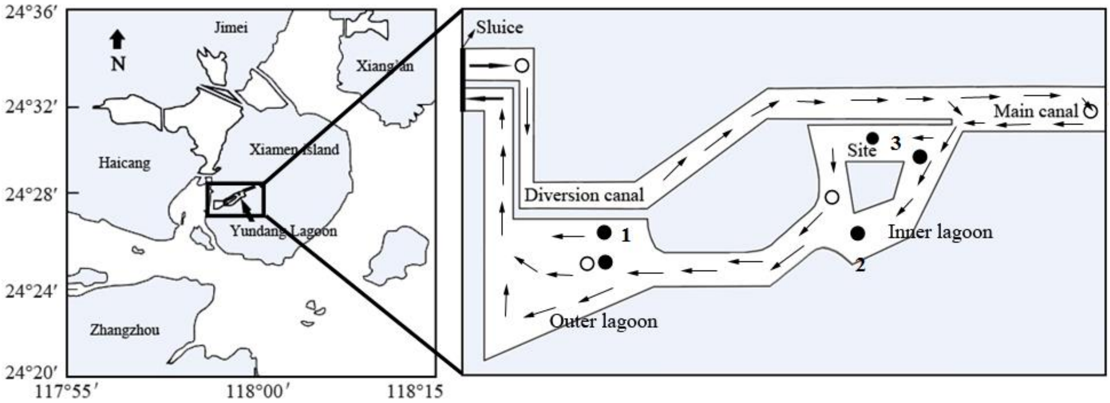

Fig.1 The study site. The white circles represent the sites for POM, while the black circles represent the sites for SOM and *Ulva lactuca*. The stations near the shore (Stn.1 and Stn.3) were chosen to sample the decaying leaves of mangrove and microphytobenthos. The arrow shows the direction of the water flow.

2.2 Sampling collection and processing

At each date, water samples for the determination of POM were collected at four stations randomly chosen in the deeper areas (water depth approximately 4 to 5 m) of each sector of the lagoon. At each station, 2 L of surface seawater was collected for both the chemical and stable isotopic composition of POM and passed through a 170 µm mesh. The filtrate was further filtered using pre-combusted (450°C, 2 h) Waterman GF/F filters to obtain the POM.

The macroalgae *U. lactuca* and decaying leaves of mangrove plants were hand-picked from the inner and outer lagoon, respectively, and transported back to the laboratory. The samples were cleaned carefully with 0.22-µm filtered seawater. Corers were used to collect the sediments. Five to ten sediment samples were collected from stations 1 to 3. The collected surface sediments (2 cm) of all samples from one station were pooled as one combined sample.

Microphytobenthos (MPB) were collected based on the principle that benthic algae, such as Pennatae daitom, can move up and down the surface of the sediments. Briefly, sediments near the shore (depths less than 0.5m) were sampled using a box corer (20 cm×20 cm). The top 2 to 3cm of sediment layers were collected in the field using a scoop and placed in a 25 cm×35 cm clean container. The sediments were first covered by a 0.2to 0.5cm layer of pre-burned sands (550°C, particle size: 0.5mm to 1 mm). A 63-µm mesh was placed on top of the sand to prevent the animals living in the sediments from moving into the sand layers. The sand was then covered with a second layer of sand (0.5 mm). The filtered seawater was sprayed until the sands were wet. After 6 h, the second layer of sand was collected and mixed well in 0.22-μm filtered seawater. After 5 min of sedimentation with the sand, the seawater was siphoned away and then filtered through a 63-μm mesh to remove the suspended fine sands. The filtrate was filtered onto pre-combusted GF/F filters to obtain MPB.

Pure phytoplankton was obtained by the phytoplankton cultivated in filtered seawater collected from Yundang Lagoon in illumination incubators at temperatures relevant to the field environment (~15°C in March and ~28.5°C in September) for 8 days. After that, the samples were filtered using pre-combusted (450°C, 2 h) Waterman GF/F filters.

The samples were dried in an oven at 60°C for 48 h. The POM and SOM samples were acidified before analysis because numerous organic materials contain inorganic carbonates that are more enriched in $\delta^{13}C$ than other components. For POM, the samples were acidified for 48 h in a clean dryer using HCl vapor. The samples were then dried in an oven for 48 h. The sediments were acidified using a 1mol/L HCl solution and stirred every 1 h until no bubbles were observed. The sediments were then washed five times using Milli-Q water to remove the residual HCl. The acidified sediments were dried in an oven for 48 h. Except POM, all other samples were ground into powder that was sieved using a 100-μm mesh and stored in sample vials before further analysis.

2.3 Chemical analysis

2.3.1 Atomic *C*/*N* ratio of organic matter sources

We used a Varie El III elemental analyzer to measure the total organic carbon (TOC) and total nitrogen (TN) of organic matter (OM) sources mentioned above. The atomic carbon-to-nitrogen ratio (C_{at}/N_a) was calculated with the formula (TOC×14)/(TN×12).

2.3.2 Chlorophyll *a* of POM

POM samples from 4 stations were extracted with 5ml Dimethyl Formamide for 6 h. Chlorophyll *a* (Chl *a*) in the extract was measured with a Turner Designs 10 AU digital fluorescence spectrometer. The practical concentration of POM was conversed according to the volume of the filtered seawater.

2.3.3 Stable isotopic composition of organic matter sources

The $\delta^{13}C$ and $\delta^{15}N$ signals of the samples were measured using an isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., USA) attached to a Flash EA1112 HT elemental analyzer. The sample was burned into CO_2 and N_2 under high temperature. The ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were detected by IRMS and then compared to international standards (Pee Dee Belnite and atmospheric N_2) after which the $\delta^{13}C$ and $\delta^{15}N$ were calculated using the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = (R_{sample} - R_{standard})/R_{standard}\times 1000$$

The detection limits were 0.1‰ and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The corresponding R represents the $^{13}C/^{12}C$ and $^{15}N/^{14}N$.

According to the proportion of phytoplankton in the POM, the $\delta^{13}C$ and $\delta^{15}N$ of non-living POM (NPOM) in Yundang Lagoon was calculated as follows:

$$\delta^{13}C_{NPOM} \text{ or } \delta^{15}N_{NPOM} = (\delta X_{POM} - R\delta X_{Phy})/(1-R)$$

where δX_{POM} and δX_{Phy} represent the $\delta^{13}C$ or $\delta^{15}N$ of POM and phytoplankton, respectively. R represents the proportion of phytoplankton in POM calculated as follows:

$$R = OC_{Phy}/TOC = (40\times \text{Chl } a)/TOC$$

where OC_{Phy} and TOC represent the organic carbon of the phytoplankton and POM in terms of $mg\cdot L^{-1}$, respectively.

2.4 Data analysis and statistics

To test for differences in the spatial and temporal distribution of POM, we used a 2-way mixed model analyses of variance (ANOVA). The analyses took into consideration the sectors of the lagoon (4 levels: DC, MC, IL and OL; fixed), the dates (two levels: March and September; fixed and orthogonal to distance) as factors and the stations as replication units. With regard to dates, we considered two different dates (March and September) as fixed factor in the analysis. Whether time should be considered as fixed or random factor in the ANOVA model is often unclear (Bennington and Thayne, 1994). In our study, we considered dates as fixed factor because sampling dates were not chosen randomly from a pool of possible dates within a year, but were selected using the criterion that they should represent different periods of the year (i.e. the cool seasons characterized by macro-algal blooms and the warm seasons with red tide). This analysis was used to test for changes in the following variables: (1) variables concerning the chemical composition of POM, Chl *a*, TOC, C:N and TOC:Chl *a* ratios, (2) $\delta^{13}C$ and $\delta^{15}N$ of POM and (3) $\delta^{13}C$ and $\delta^{15}N$ of phytoplankton in the POM. Homogeneity of variances was checked using Cochran's C-test (Winer et al.,1991), and when necessary, data were log (x þ 1) transformed to remove the heterogeneous variances. In the analyses of variance, when significant differences were found among Sectors or in the interaction between Sectors and Dates, a posteriori multiple comparisons were done using SNK (Student Newman Keuls) tests (Underwood, 1997).

The difference in C_{at}/N_a and $\delta^{13}C$ and $\delta^{15}N$ for OM sources was tested by Tukey's HSD test among stations and independent-sample *t* test between months, respectively.

Based on the $\delta^{13}C$ and $\delta^{15}N$ signatures, the feasible contributions of several potential OM sources to POM in the inner lagoon were determined using IsoSource model, which is the mixing model developed by Phillips and Gregg (Phillips and Gregg, 2003).

Data were analyzed using SPSS 16.0.

3 Results

3.1 Chemical composition of POM

Table 1. Results of ANOVAs to test for the differences among sites of Yundang lagoon (i.e. diversion canal, DC, main canal, MC, inner lagoon, IL, and outer lagoon, OL) and between dates (i.e. March and September 2010) and SNK test on mean values of Chl *a*, TOC, CNratio, TOC/Chl a ratio, $\delta^{13}C$ and $\delta^{15}N$ of POM and phytoplankton (living component of POM).

		Chl *a*			TOC			C:N ratio			TOC:Chl *a* ratio		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Sites=S	3	3.67	45.89	0.00	2.25	29.88	0.00	75.96	28.88	0.00	0.18	16.11	0.00
Dates=D	1	11.06	138.27	0.00	1.48	19.62	0.00	9.88	3.76	0.06	0.06	5.89	0.02
S × D	3	3.64	45.51	0.00	1.33	17.67	0.00	12.49	4.75	0.01	0.06	5.90	0.00
Residual	24	0.08			0.08			2.63			0.01		
Total	31												
transformation		Ln(×+1)			none			none			none		
Cochran C test		0.33			0.35			0.71(P<0.01)			0.67(P<0.01)		
		March:MC>DC=OL>IL			March:DC=MC>IL=OL						March: nah		
		Sept:DC>all			Sept:MC>all						Sept: DC>all		
		DC: March>September			DC: March>September						DC: March<September		
		MC:March<September			MC:March<September						MC:NS		
		IL: March<September			IL: March<September						IL: March>September		
		OL: March<September			OL: March<September						OL: NS		
		$\delta^{13}C$ POM			$\delta^{15}N$ POM			$\delta^{13}C$ PHY			$\delta^{15}N$ PHY		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Sites=S	3	24.38	379.54	0.00	68.47	657.62	0.00	4.86	83.77	0.00	68.47	657.62	0.00
Dates=D	1	24.97	388.87	0.00	0.14	1.36	0.25	17.76	306.22	0.00	0.14	1.36	0.25
S × D	3	35.10	546.55	0.00	0.45	4.35	0.01	2.87	49.55	0.00	0.45	4.35	0.01
Residual	24	0.06			0.10			0.06			0.10		
Total	31												
transformation		none			none			none			none		
Cochran C test		0.44			0.32			0.38			0.32		
		March: DC>all			March: DC>OL>IL>MC			March:DC<OL<IL<MC			March:DC<IL<MC<OL		
		Sept: DC<MC<IL<OL			Sept: DC<IL<MC<OL			Sept: nah			Sept: DC<IL<MC<OL		
		DC: March>September			DC: March>September			DC: March>September			DC: March<September		
		MC:March<September			MC:NS			MC:March>September			MC:March>September		
		IL: March<September			IL: NS			IL: March>September			IL: NS		
		OL: March<September			OL: NS			OL: NS			OL: NS		

The analysis detected a significant sectors × dates interaction for Chl *a*, TOC and TOC:Chl *a* ratio (i.e. Chl *a*, TOC, *C*:*N* and TOC:Chl *a* ratios; Table 1). The SNK test within sectors × dates interaction (P = 0.05) indicated that the mean values of Chl *a* were significantly lower in March than in September in all sectors except DC where an opposite pattern was found (March > September; SNK test; Fig. 2). As a consequence, the values of Chl *a* were lower in DC than in all other sectors, which did not differ from each other in September (September: DC < MC = IL = OL). In march, the values of Chl *a* were the highest in MC, intermediate in DC and IL and the lowest in OL (March: MC > DC = OL > IL).

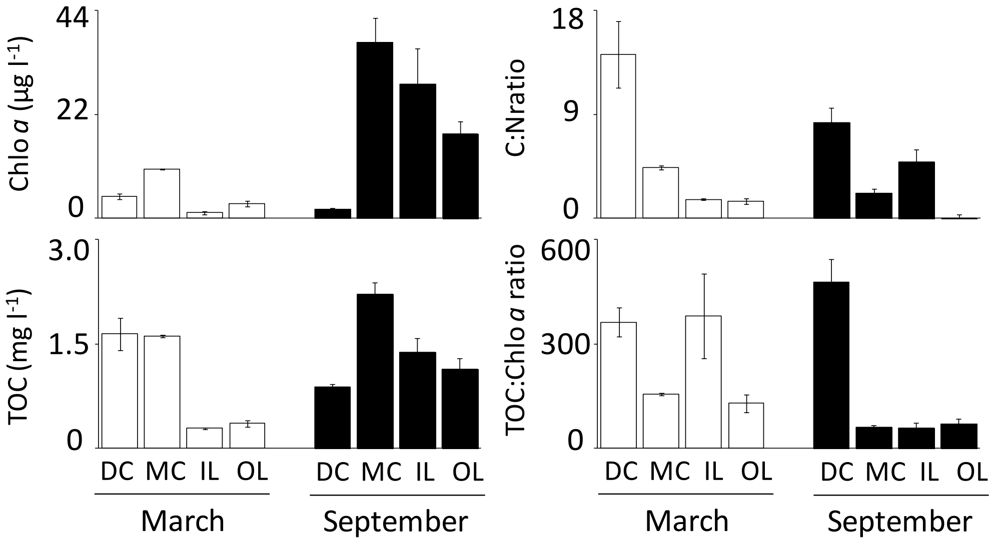

Fig.2. Mean (n= 4, ±SE) values of Chl *a*, TOC, C:N and TOC:Chl *a* of POM at the study sites (i.e. DC, MC, IL and OL) of Yundang Lagoon in March and September 2010.

Similar to what was observed in Chl *a*, the mean values of TOC were significantly lower in March than in September in all sectors except DC where an opposite pattern was found (March > September; SNK test; Fig. 2). As a consequence, the TOC values tended to be the lowest in DC, intermediate in IL and OL and the highest in MC in September but the SNK test did not discriminate alternative hypotheses (Fig. 2). In march, the values of TOC were the higher in DC and MC than in IL and OL (March: MC = DC > IL = OL; Fig. 2).

The analyses did not revealed clear cut differences among sectors in TOC:Chl *a* ratio in March whereas it peaked in DC in September. This peak was possibly due to the fact that Chl *a* decreased more than TOC, indicating major contribution of non living OM in POM.

Finally, the analyses revealed a significant effect of sectors, but no a significant effect of dates or distance × dates interaction in the CN ratio of POM. The values tended to be the highest in DC than in MC, IL and OL which did not differ from each other but the SNK test did not discriminate alternative hypotheses, possibly to the peak in IL in March (Fig. 2). Overall, this result indicates a more refractory material in DC, consistently over the study period.

3.2 Stable isotopic composition of POM

A significant sectors × dates interaction was found for $\delta^{13}C$ and $\delta^{15}N$ values of POM (Table 1). The analyses revealed that POM were more ^{13}C-enriched in September than in March in all sectors except DC where the most depleted $\delta^{13}C$ values were found in September (Fig. 3). As a consequence, while DC had more ^{13}C-enriched values than all other sectors, which did not differ from each other in March (March: DC > MC = IL = OL), DC had the lowest $\delta^{13}C$ values found within the lagoon in September (September: DC < MC = IL = OL).

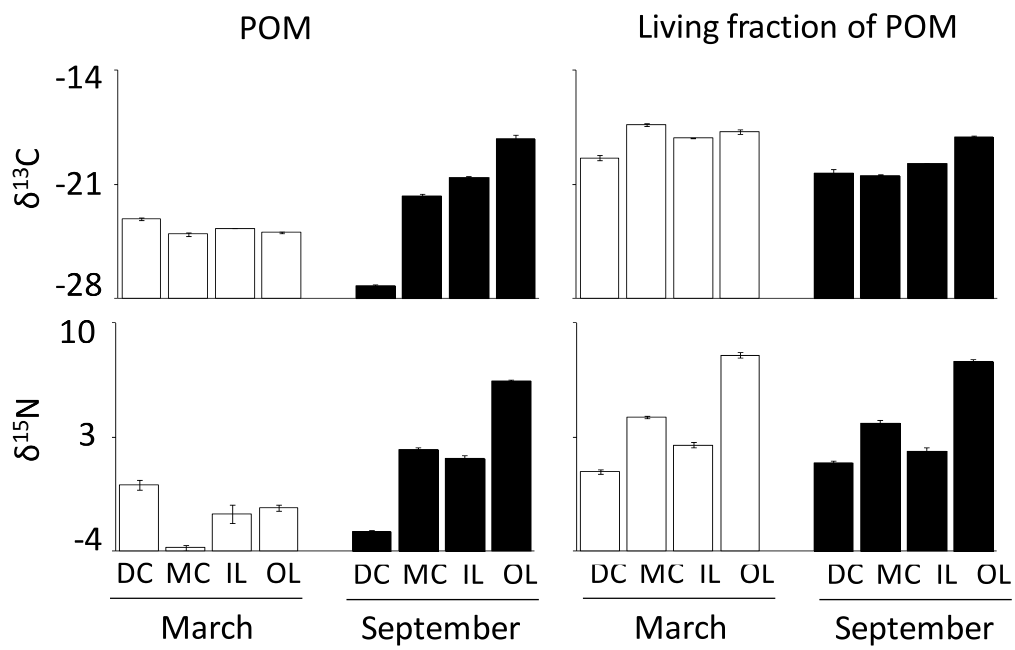

Fig.3. Mean (n= 4, ±SE) values of stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of POM and its living fraction at the study sites (i.e. DC, MC, IL and OL) of Yundang Lagoon in March and September 2010.

As for $\delta^{13}C$, $\delta^{15}N$ values of POM were more variable in DC, being higher in March than September. No differences between dates were instead found in the other sectors of the lagoon (i.e. MC, IL and OL). In March, the highest $\delta^{15}N$ values were in DC, intermediate in OL and IL and the lowest in MC, following the order DC > OL > IL > MC. In September, $\delta^{15}N$ values followed an opposite pattern being the lowest in DC, intermediate in IL and MC and the highest in OL (September: DC < IL < MC < OL).

A significant sectors × dates interaction was also found for $\delta^{13}C$ and $\delta^{15}N$ values of phytoplankton (Table 1). Phytoplankton was found to be more ^{13}C-enriched in March than in September in DC and MC while no difference between dates were detected in IL and OL. In March, the lowest $\delta^{13}C$ values were in DC, the intermediate values in IL and OL and the highest $\delta^{13}C$ values in MC, following an opposite spatial pattern to what was observed in POM (where DC had the highest $\delta^{13}C$ values). This may be indicative of a major contribution of non living ^{13}C-enriched fraction of POM. Conversely, a pattern similar to what was observed in POM was found in September, being the $\delta^{13}C$ values the highest in OL, indicating that POM reflect the C signature of phytoplankton. The SNK test however did not discriminate alternative hypotheses. The $\delta^{15}N$ values were the lowest in DC and the highest in OL in both dates (DC < IL < MC < OL).

Table 2 Stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of various organic matter sources in Yundang Lagoon.

Sources	C_{at}/N_{at}				$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
	Inner lagoon		Outer lagoon		Inner lagoon		Outer lagoon		Inner lagoon		Outer lagoon	
	March	September	March	September	March	September	March	September	March	September	March	September
SOM	13.57±1.48 a/A (n=9)	18.01±4.96 a/B (n=6)	16.60±2.99 a/A (n=9)	16.91±3.36 a/A (n=4)	-19.96±2.02 a/A (n=9)	-21.63±0.13 a/A (n=6)	-21.70±0.55 a/A (n=9)	-21.55±0.19 a/A (n=6)	4.45±0.39 a/A (n=9)	4.86±0.57 a/A (n=4)	3.72±0.47 a/A (n=9)	4.87±0.61 a/B (n=4)
ULV	7.24±0.26 b (n=3)	-	6.97±0.13 b (n=3)	-	-11.79±0.23 b (n=3)	-	-12.16±0.28 b (n=3)	-	3.12±0.27 b (n=3)	-	4.67±0.25 b (n=3)	-
MPB	6.91±0.17 b/A (n=4)	6.45±0.08 b/B (n=4)	7.02±0.12 b/A (n=4)	6.84±0.22 b/B (n=4)	-19.10±0.14 a/A (n=4)	-15.87±0.61 b/B (n=4)	-21.36±0.09 a/A (n=4)	-16.99±0.35 b/B (n=4)	4.49±0.52 a/A (n=4)	5.52±1.22 a/A (n=4)	6.35±0.55 c/A (n=4)	5.88±0.17 b/A (n=4)
LMP	17.41±2.37 c/A (n=3)	16.41±2.41 a/A (n=4)	15.97±2.16 a/A (n=3)	17.47±1.67 a/A (n=4)	-27.02±0.02 c/A (n=3)	-26.45±0.22 c/B (n=4)	-25.76±0.16 c/A (n=3)	-26.19±0.06 c/A (n=4)	1.83±0.03 c/A (n=3)	2.64±0.12 b/B (n=4)	3.66±0.03 a/A (n=3)	3.17±0.08 c/B (n=4)

SOM: sedimentary organic matter; ULV: *Ulva lactuca*; MPM: microphytobenthos; LMP: leaves of mangrove plants.

"-" represents no samples used for the measurement in September because *U. lactuca* does not exist in September in Yundang Lagoon due to high temperature (>25°C).

The letters behind the data represents the results of statistical tests. The different lowercase in each row (a, b and c) showed the significance between different type of OM sources in March or September. The significance of OM between March and September was tested by independent samples *t* test, and the difference is shown by the capital (A and B). The different level is $p<0.05$.

3.3 C_{at}/N_{at} and stable isotopic composition of OM sources and their contribution to POM

The C_{at}/N_{at} and stable isotopic composition of OM sources are shown in Table 3. The LMP and SOM had the highest C_{at}/N_{at} ratios with values ranging from 13.57 to 18.01 for SOM and from 15.97 to 17.47 for LMP. The *U. lactuca* and MPB was around 7 in C_{at}/N_{at}, and the gap between months was very minor in MPB though statistical differences were detected (Table 2).

Carbon stable isotopes were the most enriched (-12.16~-11.79‰) in *U. lactuca* and the most depleted (-27.02~-25.76‰) in LMP in March. There was no difference between SOM and MPB either in IL or in OL. In September, the *U. lactuca* disappeared due to high temperature. The MPB was the most enriched in $\delta^{13}C$ (-16.99~-15.87‰) during this period—there was significant more enrichment than in March.

There were significant differences in the $\delta^{15}N$ values among the OM sources. In IL, SOM and MPB samples collected in March and September were the most enriched in $\delta^{15}N$ (4.45~5.52‰), and LMP was the most depleted (1.83~2.64‰). In the OL, the MPB showed the most enrichment in $\delta^{15}N$ (5.88~6.35‰); LMP was the most depleted in $\delta^{15}N$ (3.17~3.66‰).

4Discussion

4.1 Isotopic characterization of OM sources

Ulva lactuca collected from Yundang Lagoon in March was most enriched for $\delta^{13}C$ (around -12‰) with values close to those reported in other coastal systems, i.e. Tapong Bay, Taiwan (Lin et al., 2007), Ría de Arosa, Spain (Page and Lastra, 2003) and Sage Lot Pond in Waquoit Bay, USA (Martinetto et al., 2006). Pure phytoplankton cultivated in filtered seawater from the lagoon was -20.47‰ to -17.35‰ for $\delta^{13}C$ and 0.85‰ to 8.03‰ for $\delta^{15}N$, which concurs with previously published literature (Table 3). The $\delta^{13}C$ of the MPB varied seasonally, which was more depleted in samples collected in March than those in many previous studies by Moncreiff and Sullivan (2001), Kang et al. (2003) and Choy et al. (2009), but similar the 20‰ in the Yangtze River estuary in China and an eelgrass *Zostera marina* bed in the western Baltic Sea (Jaschinski et al., 2008; Quan et al., 2007). More enriched $\delta^{13}C$ of MPB in September (Table 2) may be related to high radiance levels. High radiance would lead to high carbon requirements as well as low metabolism of ^{13}C for primary producers and enrichment of ^{13}C (Durako and Hall, 1992; Grice et al., 1996).

Detritus from mangrove plants mainly comprising *Kandelia candel* showed the most depleted $\delta^{13}C$ in Yundang Lagoon with values within the range typical for terrestrial organic matter (Table 3, Fig. 3). This was similar to previous studies in Deep Bay in the eastern Pearl River estuary (Lee, 2000) in theTamsui River, Taiwan (Kao and Chang, 1998), but more enriched than the Dapong Bay, Taiwan (Lin et al., 2007).

Table 3 Atomic ratio of organic carbon to total nitrogen (Cat/Nat) and stable isotope signatures (δ13C and δ15N, respectively) of different organic matter in the published literature. The number in superscript showed the data sources.

Sources	C_{at}/N_{at}	$\delta^{13}C$	$\delta^{15}N$
Ulva lactuca	7.08[1]	-11.2~-10.8‰[2-4]	3.2~11.0‰[2-4]
Kandelia candel	15.97~17.47	-28.0~-24.0‰[2, 5-6]	3.98~12.56‰[2, 5]
Microphytobenthos	7.5[7]	-20.2~-13.7‰[8-12]	6.0~10.8‰[8-12]
Terrigenous organic detritus	>12[13-14]	-28.0‰~ -23‰[14-16]	0.2‰~6.1‰[14-16]
Sewage	6.9[14]	-26.5‰~-22.4‰[14, 16-18]	-4.5~2.3‰[14, 16-18]
Phytoplankton	≈6.7[19]	-22.0~-18.0‰[8-9, 15, 18]	3.3~11.7‰[8-9, 15, 18]

1. Zheng et al., 2013; 2. Lin et al., 2007; 3. Martinetto et al., 2006; 4. Page and Lastra, 2003; 5. Kao and Chang, 1998; 6. Lee, 2000; 7. Kraufvelin et al., 2006; 8. Baeta et al., 2009; 9. Choy et al., 2009; 10. Kanaya and Kikuchi, 2008; 11. Shang et al., 2008; 12. Yokoyama et al., 2009; 13. Teixeira and Perkey, 2003; 14. Maksymowska et al., 2000; 15. Middelburg and Nieuwenhuize, 1998; 16. Thornton and McManus, 1994; 17. Sampaio et al., 2010; 18. Gaston and Suthers, 2004; 19. Redfield et al., 1963.

4.2 Isotopic variation (δ13C, δ15N) of the first trophic levels

In Yundang Lagoon, we measured high values of the C_{at}/N_{at} ratio (11.2~17.2) and TOC/Chl *a* values exceeding 400 in POM_{dc} (Fig. 2). This indicates that the seawater entering Yundang Lagoon contained massive terrigenous organic detritus and a low proportion of fresh phytoplankton in POM_{dc}. This conclusion was further confirmed by the stable isotopic composition of POM_{dc}. Depleted $\delta^{13}C$ and $\delta^{15}N$ were detected in our case, -27 to -23‰ for $\delta^{13}C$ and -2.82 to 0.05‰ for $\delta^{15}N$, which is close to the values in terrestrial organic detritus reported by Middelburg and Nieuwenhuize (1998), and Thornton and McManus (1994) (Table 3). The higher levels of $\delta^{13}C$ and $\delta^{15}N$ depletion in POM_{dc} from September may be due to a higher proportion of terrestrial organic detritus in the lagoon perhaps because of increased rainfall during this period.

The POM_{mc} was extremely high in Chl *a* and C_{at}/N_{at} ratio was also close to phytoplankton in spite of the temporal differences (Fig. 2). However, the origin of OM varied significantly temporally. In March, the TOC/Chl *a* value exceeded 150 and suggested that POM_{mc} largely originated from the terrigenous OM or other sources such as sewage. The results concur with stable isotope findings in which pure phytoplankton grown in filtered seawater from the main canal were far more enriched with $\delta^{13}C$ than POM_{mc} (Table 2). Considering that the sewage enters near the main canal and Lake Songbai which is located in the upstream of the lagoon, we suspect that depletion in $\delta^{13}C$ of POM_{mc} is closely associated with the input of massive wastewater. According to the relative contribution of pure phytoplankton in POM_{mc}, the $\delta^{13}C$ and $\delta^{15}N$ values of non-living POM (NPOM) in March was determined to be -26.59‰ for $\delta^{13}C$ and -6.63‰ for $\delta^{15}N$, respectively (Table 2). This is close to the values (-26.5‰ for $\delta^{13}C$ and -4.5‰ for $\delta^{15}N$) of sewage previously reported by Maksymowska (Maksymowska et al., 2000). In September, the composition of POM_{mc} changed sharply. About 50 TOC/Chl *a* values and stable isotope ratios near the pure fresh phytoplankton showed a big contribution from lagoon-borne phytoplankton.

POM in the inner and outer lagoon has many potential OM sources. These include the input from the diversion and main canal, but also the phytoplankton, re-suspended sedimentary organic matter, seaweed, and vascular plants including the mangrove plant *Kandelia candel* around the lagoon. Our results showed a clear temporal variation in the origin of POM in these areas. In March, although the C_{at}/N_{at} values of POM_{il} and POM_{ol} (5.8-9.2) were close to values typical for fresh phytoplankton (Redfield et al., 1963), low concentrations of Chl *a*, high TOC/Chl *a* values (>100) (Fig. 2) and depleted $\delta^{13}C$ away from that of phytoplankton (Fig. 3) indicate that the POM in the inner and outer lagoons were not composed of phytoplankton.

Table 4 Feasible contribution of potential OM to POM in the Yundang Lagoon. Data in the table are mean and feasible contributions indicating 1st (left) to 99th (right) percentiles, respectively.

Sources	Inner lagoon		Outer lagoon	
	March	September	March	September
POM_{dc}	13.4(0~53)	8.7(7~11)	10.5(0~42)	/
POM_{mc}	61.9(45~72)	5.2(0~22)	63.5(46~73)	/
POM_{dc}+POM_{mc}	75.3(64~99)	13.9(7~29)	74.0(67~88)	/
Phytoplankton	3.9(0~17)	82.9(71~89)	1.7(0~8)	/
Ulva lactuca	2.1(0~9)	*	1(0~5)	*
Sedimentary organic matter	4.3(0~18)	1.4(0~6)	4.6(0~18)	/
Mangroves	14.3(1~27)	1.7(0~7)	18.7(11~28)	/

"*" represent the absence of the carbon sources during the time. "/" represent no result from the calculation of IsoSource model.

To further quantify the contribution that various OM sources have on the POM, the potential OM sources to POM_{il} were determined including POM_{dc}, POM_{mc}, lagoon-borne phytoplankton, *U. lactuca* and the detritus from mangrove plants (DMP). We hypothesized that $\delta^{13}C$ and $\delta^{15}N$ of POM_{dc} and POM_{mc} did not vary significantly when they was transported to the inner lagoon because of the short distance (<1km) from the diversion canal and main canal to the inner lagoon. Their contribution to POM_{il} was 61.9% (45-72%) for POM_{mc}, 13.4% (0-53%) for POM_{dc}, 3.9% (0-17%) for phytoplankton, 2.1% (0-9%) for *U. lactuca* and 14.3% (0-39%) for DMP, respectively in March (Table 4). According to posteriori aggregation, the combined contribution of exogenous OM (POM_{dc} and POM_{mc}) to POM_{il} reached 75.3% （64-99%）(Table 4). Thus, we concluded that the POM in the inner and outer lagoon in March mostly originates from the exogenous OM especially from sewage in the main canal.

There is a high dependence of the POM on exogenous OM in March. This may be related to the short water retention time. Although Yundang Lagoon is a choked lagoon (Fig. 1), water retention time is short (<3 d) due to daily tidal drainage. There is much seawater input into Yundang Lagoon along the diversion canal. This accelerates water circulation and water exchange within the lagoon. This is also attributed to low temperature and *U. lactuca* bloom in March, which suppressed the development of phytoplankton perhaps because of the competition for inorganic nutrients and allelopathic effects caused by the secondary metabolites from *U. lactuca* (Nan et al., 2008; Tang and Gobler, 2011).

POM in September largely originates from lagoon-borne phytoplankton, evidenced by TOC/Chl *a* values below 50 (Fig. 2) and stable isotope signatures close to the phytoplankton (Table 2). Phytoplankton blooms during this period because rich nutrients stimulate their explosive growth with gradually increasing temperatures since June.

In conclusion, in Yundang Lagoon, ultra-eutrophication caused by the fast urbanlization of Xiamen City has shown a great effects on the seasonal variation of the first level producers in the lagoon system, which may be realed by the isotopic changes in the organisms and the associated particulate organic matters. For better understanding of the eutrophic effects in the lagoon ecosystems, further studies on the consumers and hence the entire food web of the lagoon are expected.

Acknowledgments

This paper was prepared under a Short Term Mobility Programme (STM2016) of the National Research Council of Italy granted to LH. We are grateful to all the staff in the Management Center of the Yundang Lagoon, especially Xunyi Fu and Xiangwei Xu for their assistance in collecting the samples. This work was funded by the Natural Science Foundation of Fujian (Grant No. 2014J01127), and the National Natural Science Foundation of China (Grant No. 41506123).

References

Baeta, A., Pinto, R., Valiela, I., Richard, P., Niquil, N., Marques, J.C., 2009. δ^{15}N and δ^{13}C in the Mondego estuary food web: Seasonal variation in producers and consumers. Mar. Environ. Res. 67, 109-116.

Balducci, C., Sfriso, A., Pavoni, B., 2001. Macrofauna impact on Ulva rigida C. Ag. production and relationship with environmental variables in the lagoon of Venice. Mar. Environm. Res. 52, 27–49.

Barnes, R.S.K., 1980. Coastal lagoons. Cambridge University Press.

Carlier, A., Riera, P., Amouroux, J.M., Bodiou, J.Y., Desmalades, M., 2008. Food web structure of two Mediterranean lagoons under varying degree of eutrophication. J. Sea Res. 60, 264–275.

Carlier, A., Riera, P., Amouroux, J.M., Bodiou, J.Y., Escoubeyrou, K., Desmalades, M., Caparros, J., Grmare, A., 2007. A seasonal survey of the food web in the Lapalme Lagoon (northwestern Mediterranean) assessed by carbon and nitrogen stable isotope analysis. Estuar. Coast. Shelf Sci. 73, 299–315.

Choy, E.J., Richard, P., Kim, K.-R., Kang, C.-K., 2009. Quantifying the trophic base for benthic secondary production in the Nakdong River estuary of Korea using stable C and N isotopes. J. Exp. Mar. Bio. Ecol. 382, 18-26.

Cifuentes, L.A., Sharp, J.H., Fogel, M.L., 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnol. Oceanogr. 33, 1102-1115.

Costa, T.L., Araújo, M.P., Knoppers, B.A., Carreira, R.S., 2011. Sources and distribution of particulate organic matter of a tropical estuarine-lagoon system from NE Brazil as indicated by lipid biomarkers. Aquat. Geochem. 17, 1-19.

Durako, M., Hall, M., 1992. Effects of light on the stable carbon isotope composition of the seagrass *Thalassia testudinum*. Mar. Ecol. Prog. Ser. 86, 99-101.

Gaston, T.F., Suthers, I.M., 2004. Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ of liver, muscle and bone in a rocky reef planktivorous fish: the relative contribution of sewage. J. Exp. Mar. Bio. Ecol. 304, 17-33.

Grice, A., Loneragan, N., Dennison, W., 1996. Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. J. Exp. Mar. Bio. Ecol. 195, 91-110.

Jaschinski, S., Brepohl, D.C., Sommer, U., 2008. Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. Mar. Ecol. Prog. Ser. 358, 103-114.

Kanaya, G., Kikuchi, E., 2008. Spatial changes in a macrozoobenthic community along environmental gradients in a shallow brackish lagoon facing Sendai Bay, Japan. Estuar. Coast. Shelf S. 78, 674-684.

Kang, C.K., Kim, J.B., Lee, K.S., Lee, P.Y., Hong, J.S., 2003. Trophic importance of benthic microalgae to macrozoobenthos in coastal bay systems in Korea: dual stable C and N isotope analyses. Mar. Ecol. Prog. Ser. 259, 79-92.

Kao, W.-Y., Chang, K.-W., 1998. Stable carbon isotope ratio and nutrient contents of the *Kandelia candel* mangrove populations of different growth forms. Botanical Bulletin of Academia Sinica 39, 39-45.

Kraufvelin, P., Salovius, S., Christie, H., Moy, F.E., Karez, R., Pedersen, M.F., 2006. Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the marine amphipod *Gammarus locusta*. Aquat. Bot. 84, 199-209.

Lee, S., 2000. Carbon dynamics of Deep Bay, eastern Pearl River estuary, China. II: Trophic relationship based on carbon-and nitrogen-stable isotopes. Mar. Ecol. Prog. Ser. 205, 1-10.

Li, J., Huang, L.F., Guo, F., Cai, A.Y., Ying, Z., 2007. Absorption of N, P nutrients by *Gracilaria tenuistipitata* and its resistant effect on the outbreak of red tide. Journal of Xiamen University (in Chinese) 46, 221-225.

Lin, H.J., Kao, W.Y., Wang, Y.T., 2007. Analyses of stomach contents and stable isotopes reveal food sources of estuarine detritivorous fish in tropical/subtropical Taiwan. Estuarine, Estuar. Coast. Shelf Sci. 73, 527-537.

Liu, M., Hou, L.J., Xu, S.Y., Ou, D.N., Yang, Y., Yu, J., Wang, Q., 2006. Organic carbon and nitrogen stable isotopes in the intertidal sediments from the Yangtze Estuary, China. Mar. Pollut. Bull. 52, 1625-1633.

Magni, P., Rajagopal, S., van der Velde, G., Fenzi, G., Kassenberg, J., Vizzini, S. Mazzola, A., Giordani, G., 2008. Sediment features, macrozoobenthic assemblages and trophic relationships (δ13C and δ15N analysis) following a dys-trophic event with anoxia and sulphide development in the Santa Giusta la-goon (western Sardinia, Italy). Mar. Pollut. Bull. 57, 125–136.

Maksymowska, D., Richard, P., Piekarek-Jankowska, H., Riera, P., 2000. Chemical and isotopic composition of the organic matter sources in the Gulf of Gdansk (Southern Baltic Sea). Estuar Coast. Shelf S. 51, 585-598.

Martinetto, P., Teichberg, M., Valiela, I., 2006. Coupling of estuarine benthic and pelagic food webs to land-derived nitrogen sources in Waquoit Bay, Massachusetts, USA. Mar. Ecol. Prog. Ser. 307, 37-48.

Middelburg, J.J., Nieuwenhuize, J., 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. Mar. Chem. 60, 217-225.

Miltner, A., Emeis, K.-C., 2000. Origin and transport of terrestrial organic matter from the Oder lagoon to the Arkona Basin, Southern Baltic Sea. Org. Geochem. 31, 57-66.

Moncreiff, C.A., Sullivan, M.J., 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 215, 93-106.

Nan, C., Zhang, H., Lin, S., Zhao, G., Liu, X., 2008. Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures. Aquat. Bot. 89, 9-15.

Nixon, S.W., 1981. Nutrient dynamics, primary production and fisheries yields of lagoons. In: Proceedings of International Symposium on Coastal Lagoons. Bor-deaux, France. Oceanologica Acta SP, pp. 357–371.

Page, H., Lastra, M., 2003. Diet of intertidal bivalves in the Ria de Arosa (NW Spain): evidence from stable C and N isotope analysis. Mar. Biol. 143, 519-532.

Papadimitriou, S., Kennedy, H., Kennedy, D.P., Duarte, C.M., Marb, N., 2005. Sources of organic matter in seagrass-colonized sediments: A stable isotope study of the silt and clay fraction from *Posidonia oceanica* meadows in the western Mediterranean. Org. Geochem. 36, 949-961.

Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261-269.

Phillips, D.L., Newsome, S.D., Gregg, J.W., 2005. Combining sources in stable isotope mixing models: alternative methods. Oecologia 144, 520-527.

Quan, W., Fu, C., Jin, B., Luo, Y., Li, B., Chen, J., Wu, J., 2007. Tidal marshes as energy sources for commercially important nektonic organisms: stable isotope analysis. Mar. Ecol. Prog. Ser. 352, 89-99.

Redfield, A., Ketchum, B., Richards, F., 1963. The influence of organisms on the composition of seawater. Comparative and Descriptive Oceanography, 26-27.

Rodríguez-Graña, L., Calliari, D., Conde, D., Sellanes, J., Urrutia, R., 2008. Food web of a SW Atlantic shallow coastal lagoon: spatial environmental variability does not impose substantial changes in the trophic structure. Marine Ecology Series. 362, 69–83.

Ruttenberg, K.C., 1997. Phosphorus distribution, C: N: P ratios, and $\delta^{13}C_{oc}$ in arctic, temperate, and tropical coastal sediments: tools for characterizing bulk sedimentary organic matter. Mar. Geol. 139, 123-145.

Sampaio, L., Freitas, R., Máguas, C., Rodrigues, A., Quintino, V., 2010. Coastal sediments under the influence of multiple organic enrichment sources: An evaluation using carbon and nitrogen stable isotopes. Mar. Pollut. Bull. 60, 272-282.

Shang, X., Zhang, G.S., Zhang, J., 2008. Relative importance of vascular plants and algal production in the food web of a Spartina-invaded salt marsh in the Yangtze River estuary. Mar. Ecol. Prog. Ser. 367, 93-107.

Tang, Y.Z., Gobler, C.J., 2011. The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. Harmful Algae 10, 480-488.

Teixeira, M.J., Perkey, D.W., 2003. Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). Estuar Coast. Shelf S. 57, 1023-1048.

Thornton, S.F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. Estuar Coast. Shelf S. 38, 219-233.

Vizzini, S., Mazzola, A., 2008. The fate of organic matter sources in coastal environments: a comparison of three Mediterranean lagoons. Hydrobiologia, 611, 67–79.

Volkman, J.K., Revill, A.T., Holdsworth, D.G., Fredericks, D., 2008. Organic matter sources in an enclosed coastal inlet assessed using lipid biomarkers and stable isotopes. Organic Geochemistry 39, 689-710.

Worm B, Lotze H K. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. Limnol. Oceanogr. 51, 569–579.

Yamamuro, M., 2000. Chemical tracers of sediment organic matter origins in two coastal lagoons. J. Marine Syst. 26, 127-134.

Yokoyama, H., Sakami, T., Ishihi, Y., 2009. Food sources of benthic animals on intertidal and subtidal bottoms in inner Ariake Sound, southern Japan, determined by stable isotopes. Estuar Coast. Shelf S. 82, 243-253.

You, B.S., Wang, T.C., Fan, C.X., Zhong, J.C., Yin, H.B., Li, B., 2008. Effects of sediment resuspension on aqueous nutrient loading in grass type zone of Lake Taihu. Environmental Science (in Chinese) 29, 26-31.

Zeitzschel, B., 1970. The quantity, composition and distribution of suspended particulate matter in the Gulf of California. Mar. Biol. 7, 305-318.

Zheng, X.Q., Huang, L.F., Li, Y.C., Huang, B.Q., 2013. The feeding selectivity of an herbivorous amphipod *Ampithoe valida* on three dominant macroalgal species of Yundang Lagoon. Acta Ecologica Sinica (in Chinese) 33, 7166-7172.

Zhou, Q., Xie, P., Xu, J., Ke, Z.X., Guo, L.G., Cao, T., 2009. Seasonal variations in stable isotope ratios of two biomanipulation fishes and seston in a large pen culture in hypereutrophic Meiliang Bay, Lake Taihu. Ecol. Eng. 35, 1603-1609.