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Carbon and nitrogen flow, and trophic relationships, among the cultured species in an integrated multi-trophic aquaculture (IMTA) bay

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ABSTRACT: Stable isotopic signatures of organic carbon ($\delta^{13}C$) and total nitrogen ($\delta^{15}N$) were measured on suspended particulates and sediments in order to understand the sources of organic matter (OM), water quality and flow of organic carbon and nitrogen among integrated multi-trophic aquaculture (IMTA) species, as well as to evaluate the role of IMTA practice in accumulation and assimilation of OM during wet and dry seasons. OM distribution and composition were studied during 2011 in Sanggou Bay (SGB) of northern China, a system that receives terrestrial and oceanic inputs, and which is used for IMTA ventures. Results showed that higher terrestrial input of OM occurs during the wet compared to the dry season in the SGB. OM in suspended particulates (POM) showed marine- and terrestrial-derived signatures during the wet season, as revealed from their ranges in $\delta^{13}C$ (−27.4 to −20.7‰) and $\delta^{15}N$ (4.7 to 9.4‰). Sedimentary organic matter (SOM) showed signatures of marine-derived OM during both seasons, with ranges in $\delta^{13}C$ and $\delta^{15}N$ of −22.4 to −21.4‰ and 1.7 to 6.4‰, respectively. Shellfish and combined (shellfish, seaweed) cultures in SGB have the potential to reduce OM received from the fish cages as well as from the seasonal inputs from rivers. Mixing with Yellow Sea water, combined with prevailing circulation, favours the dispersal, dilution and transformation of OM and maintains and improves water quality. Based on our results, and compared with previous studies, the water quality of the SGB is likely to be sustained by IMTA activities.

KEY WORDS: IMTA · POM · SOM · Carbon isotope · Nitrogen isotope · Trophic levels · Sanggou Bay

INTRODUCTION

Excess amounts of carbon and nitrogen produced either from land-based or offshore aquaculture activities are considered to be one of the main sources of pollution in coastal environments. Increasing coastal area development as well as aquaculture activities have been of particular concern to the health of coastal ecosystems. Land-based aquaculture waste is often discharged directly into shallow coastal areas, causing excessive organic and nutrient loads (Alabaster 1982). Offshore cage culture is considered to be a direct source of organic matter (OM) to the surrounding waters in the form of suspended detritus (Karakassis et al. 2000, Mazzola & Sarà 2001), which mainly consists of uneaten feed and excretion products from the cultured fish (Holby & Hall 1991, Hall et al. 1992). Furthermore, anthropogenic input provides

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additional nutrient and OM enrichment in the coastal marine system (Evgenidou & Valiela 2002). This waste affects not only the area in close proximity to the sources but can alter a wider coastal zone at various ecosystem levels; reducing the biomass, density and diversity of the benthos, plankton and nekton, and modifying natural food webs and stimulating eutrophication (Gowen et al. 1991, Pillay 1991, Vollenweider 1992, Duarte 1995). However, the offshore cultivation of shellfish together with seaweed could reduce the impact of OM waste and nutrients on the environment, as substantiated by land-based integrated aquaculture practice (Shpigel et al. 1991, Shpigel & Neori 1996). The aquaculture-derived nutrients can be removed by seaweed biofilters (Buschmann et al. 2008). Such a combined species cultivation method, so-called integrated multi-trophic aquaculture (IMTA), is practiced in Chinese coastal zones. Besides the feasible ventures in mariculture schemes, the combination of trophic levels among cultured species in IMTA systems is also important in improving water quality. The IMTA of shellfish, seaweed and fish is common on the coast of northern China and has been in practice over 3 decades (Fang et al. 1996a,b, 2009).

Sanggou Bay (SGB) receives OM from both natural and anthropogenic sources, which subsequently impact the water quality of the bay. SGB is surrounded by a population of ca. 0.6 million in Rongcheng City of Shandong Peninsula. River runoff from Rongcheng City is considered the main source of nutrients into SGB and is composed on average of 65% crop land waste and 35% urban waste (Project SPEAR; Ferreira et al. 2007). Stable isotope analysis has been used successfully in determining sources of nutrition for consumers, evaluation of trophic relationship among organisms, understanding different sources of OM (terrestrial and marine) and environmental impact assessment (Wada et al. 1987, Risk & Erdmann 2000, Costanzo et al. 2001). Stable isotope ratios of organic carbon ($\delta^{13}C$) and total nitrogen ($\delta^{15}N$) have also been used to determine the impact of aquaculture waste on the environment (Ye et al. 1991, Vizzini & Mazzola 2004, Yokoyama et al. 2006, Jiang et al. 2012). Aquaculture waste enters the food web and alters the natural isotopic composition of OM sources at both the base and upper trophic levels. Nitrogen-rich fish waste mainly affects $\delta^{15}N$ values without or little alteration of $\delta^{13}C$ (Vizzini & Mazzola 2004). Aquaculture and human waste can affect at different levels of the ecosystem — reducing the biomass, density and diversity of the benthos, plankton and nekton — and modify natural food webs in coastal areas (Gowen et al. 1991, Pillay 1991).

In the present study, our first goal was to investigate the carbon and nitrogen flow from (1) phytoplankton, particulate OM (POM), sediment OM (SOM) or seaweed to filter feeders and (2) trash fish (feed provided to fish in fish cages) or plankton to omnivorous fish in an IMTA system in SGB using dual isotopic technique. A second objective was to study the isotopic profile ($\delta^{13}C$ and $\delta^{15}N$) of SOM and POM to understand the sources of carbon and nitrogen in SGB. Our study focused on understanding the role of lower trophic levels in the reduction of OM and clarifying whether aquaculture- and land-derived OM impact the water quality of the bay.

MATERIALS AND METHODS

Study area

The SGB (37° 01′ to 37° 09′ N and 122° 24′ to 122° 35′ E) is located in Rongcheng Town, in Weihai City, on the Shandong Peninsula in northeastern China (Fig. 1). The bay is semi-enclosed and opens into the Yellow Sea (YS) in the east, covering an area of 144 km^2. Freshwater inputs to the bay are mainly from one large river (the Gu River) and some small rivers (Ba, Sanggan, Yetao and Xiaolou Rivers). The bay experiences seasonal terrigenous inputs, with freshwater inflow being maximum in summer and with an average discharge of 1.7×10^8 m^3 to 2.3×10^8 m^3 (Rongcheng River Report 2012, www.rcsl.gov.cn). Water in the bay is well mixed and depth varies between 7.5 and 21 m (Zhao et al. 1996). IMTA is an important commercial activity in SGB. On the basis of culturing activities, the bay is divided into 4 culture areas. The southwest is used for shellfish and fish culture (hereafter, SF+F), the central part is dominated by polyculture of shellfish and seaweed (SF+SW), and the outer bay is cultivated with seaweed (SW) monoculture along the eastern boundary that opens into the YS (Fig. 1). Fish is cultured between May and October, while bivalve culture lasts between 1 and 2 yr. Red seaweed and kelp are cultivated from June–October and November–April, respectively (Zhao et al. 1996, SPEAR 2007). Shellfish and seaweed are cultivated in long lines around fish cages. Bivalve production includes the Chinese scallop *Chlamys farreri* (~60×10^3 t yr^{-1}) and the Pacific oyster *Crassostrea gigas* (~15×10^3 t yr^{-1}). Seaweed production includes kelp *Saccharina japonica* (~84×10^3 t yr^{-1}) and red alga *Gracilaria lemaneiformis*

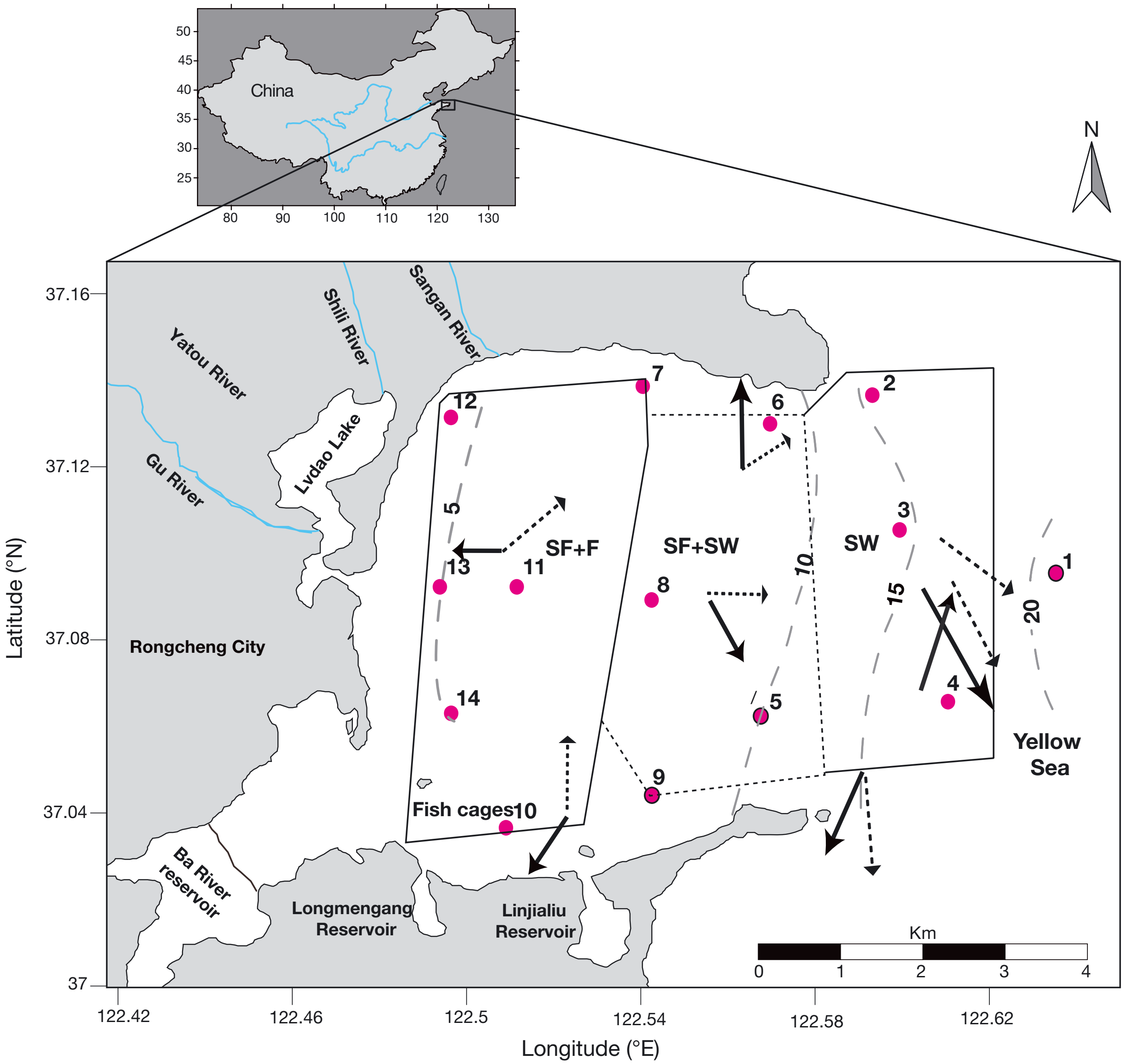

Fig. 1. Map of Sanggou Bay, showing culture areas (polygons with solid and dotted lines) and 14 stations (red dots) of cruises in August 2011 (wet season, summer) and January 2012 (dry season, winter). Culture areas include combined culture of shellfish and fish (SF+F), shellfish and seaweed (SF+SW) and monoculture of seaweed (SW). Solid arrows denote surface water current and dashed arrows bottom flow (source: Ferreira et al. 2007). Grey dashed lines denote isobaths (m)

(~25 × 10^3 t yr^{-1}). The production of Japanese flounder *Paralichthys olivaceus* is ~24 × 10^3 t yr^{-1} (Rongcheng Fisheries Technology Extension Station 2012 statistics [www.rchy.gov.cn], summarized in Table 1).

Sampling and analysis

Samples for hydrographic parameters, POM, SOM, phytoplankton, zooplankton, shellfish (oyster and scallop), seaweed, cultured fish and trash fish were collected in August 2011 (wet season, i.e. summer) and January 2012 (dry season, i.e. winter). Surface water samples were collected using a Niskin water sampler at 14 stations covering all 3 culture areas in SGB (Fig. 1). The water samples were immediately screened through a 200 µm mesh net to remove larger zooplankton and debris. They were filtered under vacuum onto prewashed, pre-combusted (450°C, 4h) and pre-weighed Whatman GF/F filter papers (0.7 µm pore size). The samples were subsequently stored at −40 °C in a freezer until laboratory analysis.

Table 1. Summary of aquaculture in Sanggou Bay, where species are cultured in combination (SF+F, SF+SW) and monoculture (SW) in integrated multi-trophic aquaculture (IMTA). Additional details on the cultured area, annual production, and stocking, harvesting and culture periods for the different groups are also given (data from Zhao et al. 1996, Ferreira et al. 2007, Rongcheng Fisheries Technology Extension Station 2012 statistics [www.rchy.gov.cn])

Cultured species	Cultured area (km^2), total per group	Stocking period	Harvesting period	Culture period	Production ($t\ yr^{-1}$)
Shellfish (SF)					
Chlamys farreri (Chinese scallop)	32	May	March	1–2 yr	$\sim 60 \times 10^3$
Crassostrea gigas (Pacific oyster)		May	March	1–2 yr	$\sim 15 \times 10^3$
Seaweed (SW)					
Saccharina japonica (kelp)	40	November	April	6 mo	$\sim 84 \times 10^3$
Gracilaria lemaneiformis (Gracilaria)		June	October	5 mo	$\sim 25 \times 10^3$
Fish (F)					
Paralichthys olivaceus (Japanese flounder)	0.36	May	October	6 mo	$\sim 24 \times 10^3$

Bottom sediment samples were collected with a Van Veen grab (Hydro-bios) from a few stations and then frozen at −20°C until analysis. Salinity and chlorophyll *a* (chl *a*) were measured *in situ* with a multi-parameter instrument (Model: YSI Professional plus USA) and an ACLW-RS chlorophyll sensor, respectively. Cultured fish, shellfish, seaweed and trash fish samples were collected by local fishermen at some sampling sites. Phytoplankton (60 µm) and zooplankton (200 µm) nets were used to collect plankton samples. Plankton samples were filtered through Whatman GF/F filter papers, then frozen at −40 °C until analysis. All samples of fish, shellfish and trash fish were rinsed carefully with filtered seawater and guts were removed to reduce bias. Muscle of cultured fish, trash fish and shellfish, as well as sediments and particulate samples, were dried at 60°C for at least 24 h prior to stable isotope analysis. Cultured fish, trash fish and bivalve samples were soaked in 1.2 N HCl for 30 min, rinsed with distilled water, dried at 60°C and ground to a powder. The bottom sediment samples were ground and sieved through a 0.2 µm mesh, and then both the sediments and particulate samples were digested with 1 M HCl to remove carbonates and dried at 60°C for 12 h. Samples for total nitrogen concentration and isotopes were directly measured without the acid treatment (Cui et al. 2012).

Organic carbon, total nitrogen content and isotopes of carbon and nitrogen were measured using a Finnigan EA-1112 elemental analyzer interfaced with a Finnigan Delta plus XP continuous flow isotope ratio mass spectrometer. Carbon and nitrogen isotope ratios are expressed in the delta notation $\delta^{13}C$ and $\delta^{15}N$ relative to Vienna Pee Dee Belemnite and atmospheric nitrogen, respectively, and expressed as (Hayes 2004):

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000\ (‰) \quad (1)$$

where, $X = {}^{13}C$ or ${}^{15}N$, and $R = {}^{13}C:{}^{12}C$ for $\delta^{13}C$ or ${}^{15}N:{}^{14}N$ for $\delta^{15}N$.

Internal standards of caffeine and cellulose were used for calibration during the measurements. The average precision for organic carbon and total nitrogen measurements during this study was ±0.1%.

Trophic levels among the cultured species were calculated using the following formula (Wan et al. 2010):

$$\text{Trophic level} = [(\text{consumer } \delta^{15}N - \text{phyto } \delta^{15}N)/3.2] + 1 \quad (2)$$

where 3.2 represents the average enrichment of $\delta^{15}N$ among trophic levels in the present study, obtained by calculating the average value of $\delta^{15}N$ of each trophic level. This value is close to the enrichment factor of 3.1 reported by Wan et al. (2010) in a YS trophic level study.

Statistical analysis

SPSS 17.0 and Golden Software Grapher 9 were used to perform data analysis. Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of POM and SOM were examined using 1-way ANOVA. Difference of $\delta^{13}C$ and $\delta^{15}N$ values of POM and SOM were analyzed by a paired *t*-test (Cui et al. 2012).

RESULTS

Hydrographic parameters

A negative correlation between salinity and chl *a* was observed in the wet season ($r^2 = -0.82$; $p < 0.05$). The coastal region was dominated by low salinity

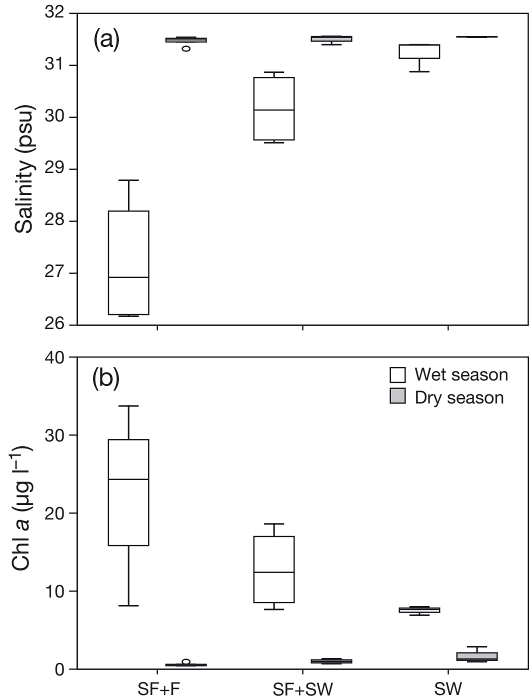

Fig. 2. Surface distribution of (a) salinity and (b) chlorophyll *a* in the 3 culture areas (see Fig. 1) in Sanggou Bay during the wet and dry seasons. Box plots show the median value (line), 25 and 75% quantiles (box), 5 and 95% quantiles (whiskers), and outliers (circles)

and high chl *a* concentration. Slightly lower salinity and higher chl *a* concentrations were found in the SF+F culture area of the bay compared to SF+SW and SW culture areas. The other 2 culture areas showed high salinity and low chl *a* concentrations. The maximum salinity and minimum chl *a* values were observed in the SW culture region (Fig. 2). The average values of salinity and chl *a* during the wet season in SGB were 29.4 ± 2.0 psu and 15.5 ± 10.9 µg l^{-1} (Fig. 2), respectively. There was no significant variation in salinity among the aquaculture areas during the dry season (Fig. 2), due to low freshwater input into the bay. Considering all culture areas of SGB in the dry season, salinity ranged between 31 and 32 psu, with an average (±SD) of 31.5 ± 0.07 psu. During the dry season, the average (±SD) chl *a* concentration was 1.0 ± 0.63 µg l^{-1}. Chl *a* was significantly higher in the SW culture area in the offshore region than in the SF+F area in the coastal region of the bay (Fig. 2).

Stable isotope analysis of biological samples

The weight percentages of organic carbon in cultured fish and shellfish were higher than in plankton and seaweed. The maximum values of nitrogen (% dry wt) were found in cultured fish and minimum values in plankton (Fig. 3). The C/N ratios of cultured fish, oysters, scallops and trash fish were in the range of 2.7–2.8, 3.6–4.0, 5.2–5.4 and 4.4–4.5, respectively, which were lower than the C/N ratios of phytoplankton (9.9), zooplankton (11.6) and *Gracilaria* spp. (hereafter simply *Gracilaria*) (10.0). $\delta^{13}C$ versus $\delta^{15}N$ values of SOM, POM, biological samples and the trophic level of the cultured species are shown in Fig. 4. The respective average values (±SD) of $\delta^{13}C$ and $\delta^{15}N$ were −21.1 ± 0.1‰ and 9.2 ± 0.4‰ for scallops, −21.1 ± 0.2‰ and 11.2 ± 0.3‰ for oysters, −20.9 ± 0.1‰ and 6.7‰ for *Gracilaria*, −19.0 ± 0.2‰ and −21.0 ± 0.6‰ for cultured fish, and 11.1 ± 0.3‰ and 9.6 ± 1.2‰ for trash fish.

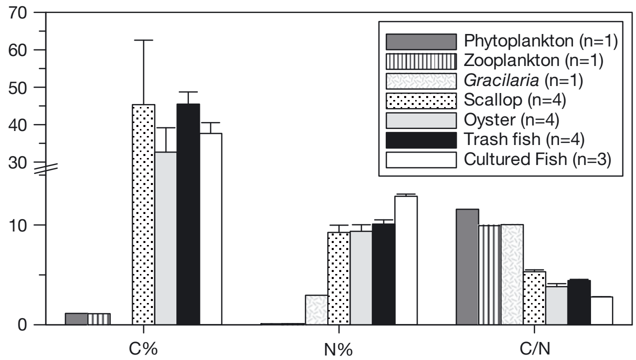

Fig. 3. Carbon and nitrogen contents (% dry wt) and C/N ratios of cultured species (seaweed, shellfish and fish; see Table 1) and of phyto- and zooplankton and input feed (i.e trash fish) in Sanggou Bay during the wet season. Means ± SD

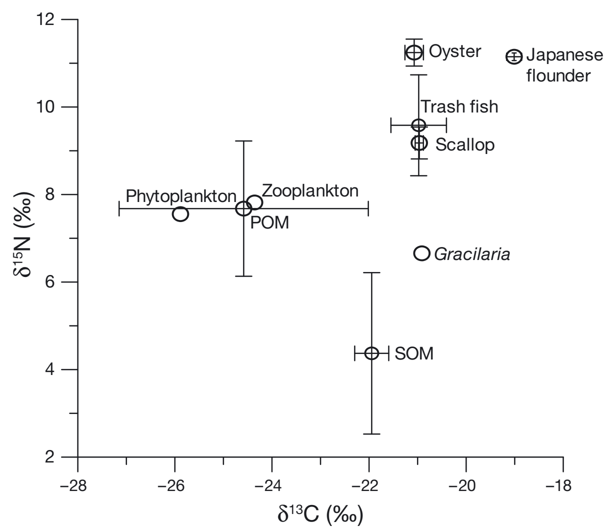

Fig. 4. $\delta^{15}N$‰ versus $\delta^{13}C$ (‰) isotopic signatures of plankton, cultured species, trash fish, and particulate and sediment organic matter from Sanggou Bay during the wet season. Means given ±SD, if n > 1

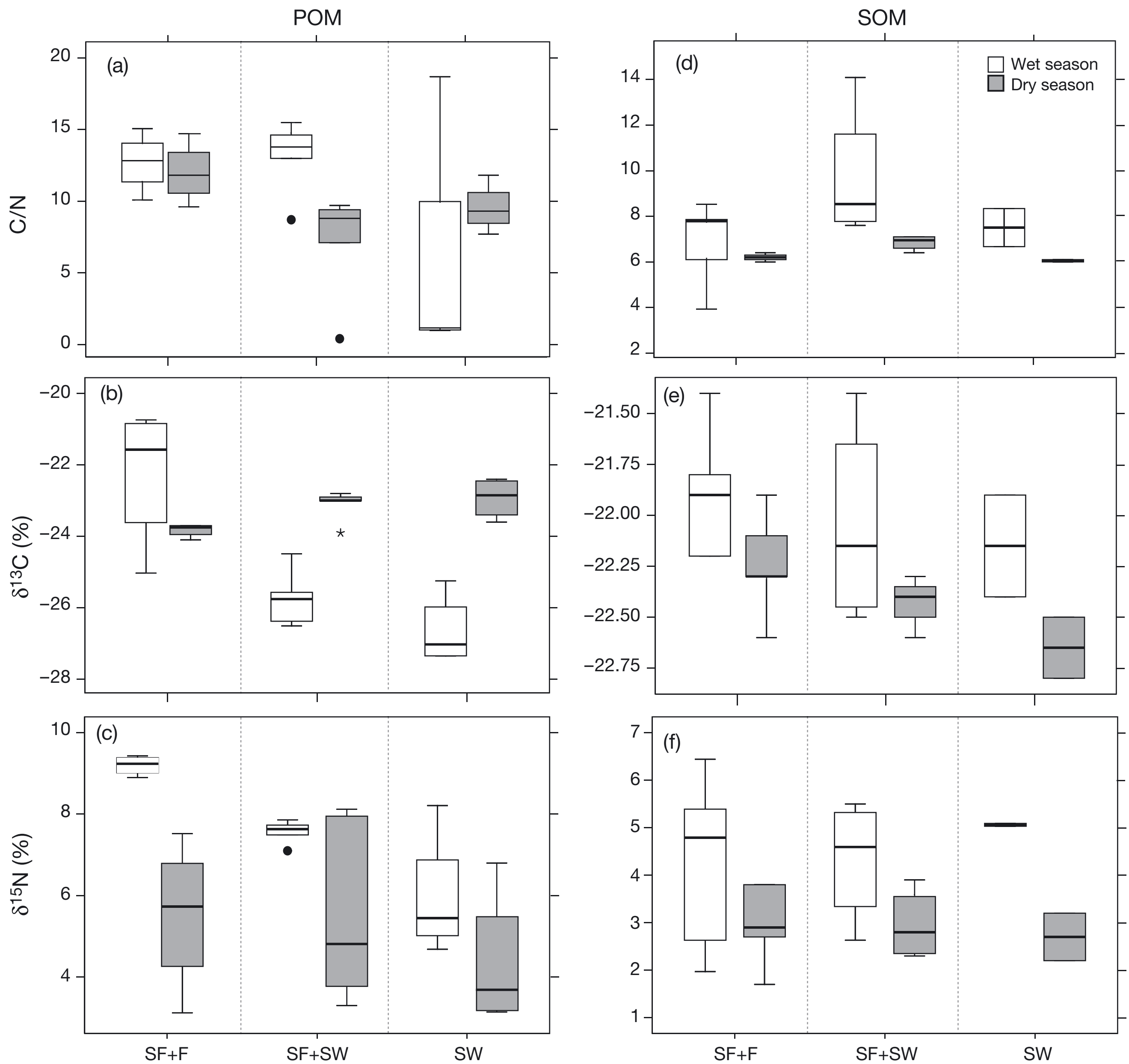

Fig. 5. Distribution of C/N, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of (a–c) particulate (POM) and (d–f) sediment organic matter (SOM) in the 3 culture areas (see Fig. 1) of Sanggou Bay during the wet and dry seasons. Box plots show the median value (line), 25 and 75% quantiles (box), 5 and 95% quantiles (whiskers), outliers (black dots) and extremes (stars)

Stable isotope analysis of SOM and POM in culture areas

The distribution of C/N, $\delta^{13}C$ and $\delta^{15}N$ of SOM (n = 26) and POM (n = 28) in the 3 culture areas of SGB during the wet and dry seasons is shown in Fig. 5. The fish cage culture and long-line culture of *Gracilaria* in SGB are performed during the wet season. Mixing of the bay water with the YS is higher in the SW culture area compared to the central (SF+SW) area. In the wet season, the lowest (1.16) and highest (18.68) C/N values of POM were observed in the SW culture area (Fig. 5a). For SOM, the lowest C/N value (3.93) was found in the SF+F culture area and the highest (14.09) in the SF+SW area (Fig. 5d). Highest values of $\delta^{13}C$ and $\delta^{15}N$ of POM were found in the SF+F culture area (−20.74‰ and 9.43‰, respectively) and the lowest in the SW culture area (−27.35‰ and 4.68‰, respectively) (Fig. 5b,c). The $\delta^{15}N$ values of POM showed a decreasing trend from SF+F to seaward (Fig. 5c). In contrast, no significant difference was found in the distribution of $\delta^{13}C$ of SOM among the 3 culture areas in the wet season (Fig. 5e). $\delta^{15}N$ values of SOM also showed no significant difference among the 3 culture areas in the wet season (Fig. 5f).

In the dry season, POM maximum and minimum C/N ratios were observed in SF+F (14.77) and SF+SW (0.39) culture areas, respectively, whereas for SOM no significance difference was found in C/N ratios among the 3 culture areas (Fig. 5a,d). The $\delta^{13}C$ values of POM and SOM were in the range of −24.06‰ to −21.88‰, with only minor variations being observed between POM and SOM (Fig. 5b,e). The lowest value (3.12‰) of $\delta^{15}N$ of POM was found in the SF+F culture area and the highest (8.12‰) in SF+SW (Fig. 5c). A slight, though non-significant, decrease in SOM $\delta^{15}N$ was observed from SF+F to SW culture areas (Fig. 5f).

Within SGB overall, significant differences in $\delta^{13}C$, $\delta^{15}N$ and C/N values of SOM and POM between wet and dry seasons ($p < 0.05$) were found. In both seasons, SOM had slightly higher values of $\delta^{13}C$ than POM. In contrast, SOM had lower values of $\delta^{15}N$ and C/N compared to POM in both seasons.

DISCUSSION

Trophic relationships among the cultured species

In the present study, the C/N ratio (>11) of phytoplankton (being a major fraction of POM) indicates that terrestrial material from the rivers is a major source of carbon, since these values are higher than those previously reported for marine phytoplankton (range: 6.7–10) and closer to vascular plants (>12) (Redfield et al. 1963, Holligan et al. 1984, Meyers 1994, Hedges & Oades 1997, Bale & Morris 1998, Bates et al. 2005, Lamb et al. 2006). The $\delta^{13}C$ values of plankton (range: −25.4‰ to −25.9‰) in this study were lower than those reported for Narragansett Bay, USA (mean ± SD: −22 ± 0.6‰) and Osaka Bay in Japan (range: −18.0‰ to −24.0‰) (Gearing et al. 1984, Mishima et al. 1996). The $\delta^{15}N$ values of phyto- and zooplankton (range: 7.6–7.8‰) were within the range reported for marine phytoplankton (3.0–10‰) (Wada et al. 1991). For oysters, we determined relatively lower values of $\delta^{13}C$ (mean ± SD: −20.03 ± 0.18‰) and higher values of $\delta^{15}N$ (8.27 ± 0.13‰) compared to values reported from oysters around a fish cage area in Ailian Bay, China (mean ± SD: −20.03 ± 0.18‰; Jiang et al. 2012), indicating that river runoff has been a source of carbon and nitrogen in oysters of the present study. The $\delta^{13}C$ and $\delta^{15}N$ values (−19.0 ‰ and 11.1 ‰) we determined in cultured fish were lower than the average values (−17‰ and 13‰, respectively) observed for marine fishes (Mays 2000).

The wet season in SGB is characterized by peak IMTA activities, when fish cage culture occurs in conjunction with shellfish and seaweed. In addition, maximum freshwater inputs influence the sources and flow of OM (carbon and nitrogen) among cultured species and other organisms at various trophic levels. In the wet season, along with integrated aquaculture, primary production is a large carbon source for higher trophic levels. In the summer months, SGB usally experiences comparatively high light intensity and water temperature, which promote phytoplankton growth. This is reflected by the high chl *a* concentrations we observed in this season and the positive correlation between chl *a* and the $\delta^{13}C$ of POM that is dominated by phytoplankton (Fig. 6). Similar findings were reported by Lehmann et al. (2004), who showed that an increase in $\delta^{13}C$ values of POC is associated with increasing primary productivity due to the seasonal environmental conditions, including water temperature and light intensity. The enhanced primary production is connected to the high input of nutrients by freshwater inflow, as indicated by depleted $\delta^{13}C$ values of POM in the present study. It is possible that zooplankton in this bay feed on terrestrial detritus, which has $\delta^{13}C$ and $\delta^{15}N$ values similar to POM. Shellfish are usually considered to derive a large proportion of organic carbon from phytoplankton (Xu & Yang 2007). By identifying the relative con-

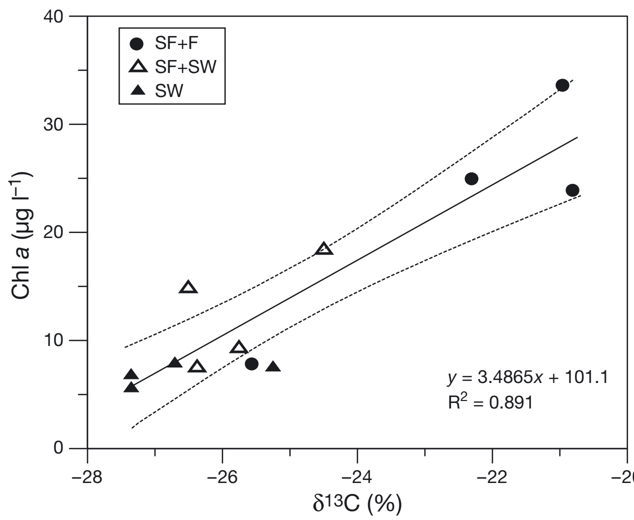

Fig. 6. Relationship between $\delta^{13}C$ (‰) of particulate organic matter and chlorophyll *a* concentration in 3 different aquaculture areas (see Fig. 1) of Sanggou Bay during the wet season

tribution of aquaculture-derived OM and its impact on water quality, the present study shows that shellfish can be considered to function as biological filters in coastal integrated aquaculture, as was reported previously for land-based integrated aquaculture (Shpigel et al. 1991, Shpigel & Neori 1996). In the coastal area different kinds of POM are present that may serve as a food source for shellfish (oyster and scallop) (Dame 1996). The observed increase in $\delta^{15}N$ from phytoplankton and POM to omnivorous fish was indicative of the trophic position of the cultured species in SGB: $\delta^{15}N$ ranged from 6.7‰ for autotrophs to up to 11.2‰ for heterotrophs, reflecting the enrichment in $\delta^{15}N$ with increasing trophic level. The $\delta^{15}N$ signatures of primary producers (phytoplankton and seaweed) clearly separated the filter feeders (shellfish) from omnivorous fish (Japanese flounder) (Fig. 4). Some species shared the same trophic level, such as cultured fish and oyster (2.16), but differed in $\delta^{13}C$ values (fish: −19.0 ± 0.2‰, oyster: −21.1 ± 0.2‰; mean ± SD), indicating that these species are up-taking carbon from different sources. In spite of this, cultured fish showed 2% enrichment in $\delta^{13}C$ from its primary input source of feeding, i.e. trash fish, while oysters also showed a $\delta^{13}C$ signature similar to trash fish with 0% enrichment. In contrast, some species, such as scallop and oyster, showed similar $\delta^{13}C$ values (−21.0 ± 0.1‰ and −21.1 ± 0.2‰ [mean ± SD], respectively) indicating the same carbon source, but the difference in their $\delta^{15}N$ values revealed that they belong to different trophic levels (1.52 and 2.16, respectively). Similar findings of the same trophic relationship (i.e. different carbon sources with same trophic level and similar carbon sources with different trophic levels) were reported in Jinghai Bay, China (Feng et al. 2014).

Table 2. Carbon and nitrogen isotopes signatures of fish faecal material reported in previous literature and resulting average value that was adopted as reference value for this study

Study area	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	References
Gokasho Bay, Japan	−24.3	6.3	Yokoyama et al. (2006)
Gokasho Bay, Japan	−24.7	5.6	Yokoyama et al. (2010)
Gokasho Bay, Japan	−20.6	6.2	Yokoyama (2013)
Kat O Bay, Hong Kong	−21.6	4.4	Wai et al. (2011)
Nansha Bay, China	−17.5	7.5	Jiang et al. (2012)
Average	−21.8	6.0	

In the present study, scallop showed low $\delta^{15}N$ isotopic fractionation compared to the average fractionation factor reported elsewhere (3.4; Minagawa & Wada 1984). Several studies have reported low nitrogen fractionation values for shellfish (Raikow & Hamilton 2001, Post 2002, Marin-Leal et al. 2008), suggesting that low $\delta^{15}N$ enrichment may be due to the specific physiological characteristics of scallops. Moreover, the $\delta^{13}C$ values of trash fish, seaweed and shellfish were close to each other. We did not collect faeces samples but used an average ($\delta^{13}C$ = −21.8‰) of respective values from the literature (Table 2). This average value was close to the $\delta^{13}C$ value of shellfish, suggesting that shellfish in SGB may also use carbon sources from faecal material released from fish cages, uneaten particles of trash fish and rotten seaweed. Therefore, shellfish cultured in SGB possibly not only help in reducing OM but may also be able to increase the economic benefit and production and survival rate of other species in the IMTA system by maintaining water quality. Based upon stable isotope analysis, a conceptual model of OM flow among the integrated aquaculture species in SGB was established (Fig. 7). The trophic level efficiency was calculated by dividing the $\delta^{13}C$ and $\delta^{15}N$ values of one trophic level to the next. POM integrated both phytoplankton and zooplankton and acted as a large source of OM that could be transferred to all the upper trophic levels in the integrated food web structure of SGB. Stable isotope results indicate that scallop and oyster are taking up >80% of the OM from these sources in SGB. Bivalves accumulated approx. 90% of their carbon and 60% of the nitrogen from fish faeces and uneaten particles of trash fish, but during the wet season only; as opposed to the dry season, when shellfish mostly relied on POM, phytoplankton and zooplankton. Feeding on faeces and trash fish remains during the warm wet season probably helped to meet the high metabolic demand of the shellfish in warmer water temperatures. Alternative sources of OM in the dry season at low temperature may be provided through large-scale cultivation of kelp. Kelp culture produces a considerable amount of rotten kelp particles that can serve as a source of OM to shellfish, whereas shellfish would only be provided a minute amount (1%) of OM from *Gracilaria* culture. Omnivorous cultured fish obtained most of their carbon (90%) and nitrogen (60%) from trash fish, while other sources were OM from producers and herbivores. In the food web structure of the cultured species of the present study, shellfish played a crucial role in OM accumulation from various sources. In summary, the water quality of SGB is not impacted by OM generated by caged fish and shellfish culture activities; on the contrary, shellfish

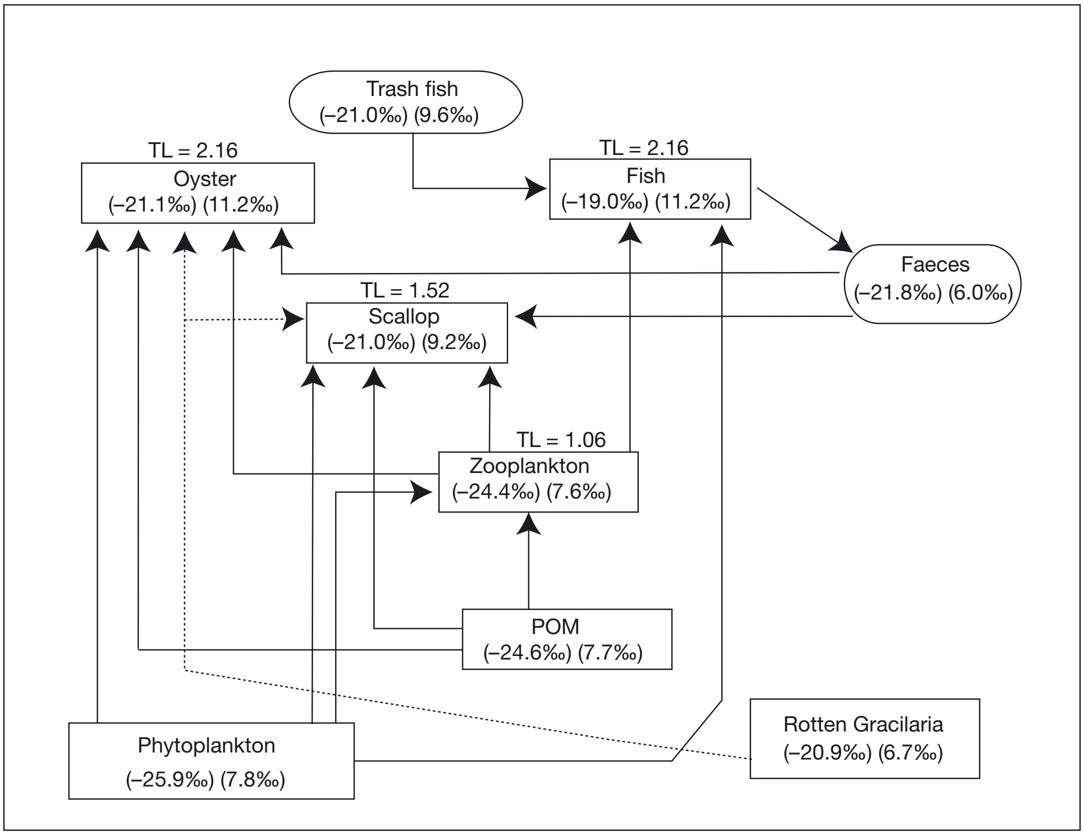

Fig. 7. Conceptual model of carbon flow among the aquaculture species in Sanggou Bay on the basis of stable isotope analysis. Values in parentheses are $\delta^{13}C$ (first) and $\delta^{15}N$ (second) isotopic signatures (‰) of various species and represent the flow (arrows; dotted arrows originate from rotten *Gracilaria*) from one trophic level (TL) to the next, showing the trophic efficiency. The TL is given for each cultured species

co-culture combined with proximity to the YS to allow for water mixing may be helpful in maintaining the water quality of the bay.

Sources of suspended and sedimentary OM across the bay

In the wet season, higher C/N values (>10) of POM in the SF+F (near coast) and SF+SW (central bay) culture areas indicate the influence of terrestrial OM. The lower C/N ratio in the *Gracilaria* monoculture area (near YS) may indicate the high consumption of nitrogen in this area or mixing with YS water. In the wet season, POM in the SF+F culture area showed higher values of $\delta^{13}C$ with a decreasing trend towards offshore, indicating OM load in the SF+F near-coast area compared to the other 2 areas. The lower values of $\delta^{13}C$ towards offshore may have resulted from the presence of degraded OM (Khodse et al. 2007). Another reason could be that high freshwater discharge during the wet season may have resulted in the rapid distribution of OM to offshore waters, preventing utilization and deposition of OM in the bay. By contrast, the higher values of $\delta^{15}N$ of POM in the SF+F culture area (near-shore area) compared to the central SF+SW and outer SW culture areas may be attributed to nitrate derived from human activities coupled with increased denitrification (Michener & Schell 1994, McClelland et al. 1997, Chanton and Lewis 1999, Miller et al. 2010). The decreasing trend of $\delta^{15}N$ in POM towards the sea suggests an offshore source of nitrogen (Miller et al. 2011). In the wet season, higher values of $\delta^{13}C$ (–22.4 ‰ to –21.4‰) in SOM of 3 culture areas displayed the isotopic signature of marine-derived OM (Wada et al. 1987, Tan et al. 1991, Mishima et al. 1996, Barros et al. 2010). Similar results for SOM $\delta^{13}C$ were found by Meksumpun et al. (2005) (avg. $\delta^{13}C$ = –21.0‰) in the Gulf of Thailand, as well as in an earlier study by Gearing et al. (1984), who reported $\delta^{13}C$ values indicative of a plankton source in SOM, ranging from –22.2 ± 0.6‰ to –20.3 ± 0.6‰ in Narragansett Bay, USA and an average value of –21.0‰ in Malaysian waters. Relatively low values of δ^{15}N in SOM of all culture areas in the present study indicate a marine source of the deposited OM. This is supported by an increasing trend of δ^{15}N in SOM of the 3 aquaculture areas from shellfish to polyculture to seaweed, suggesting the import of OM from the sea.

In the present study, δ^{13}C, δ^{15}N and C/N of POM are applied to describe OM sources The δ^{13}C of POM in the wet season has either lower or higher values than SOM in the 3 culture areas. Therefore, in the wet season, due to maximum freshwater discharge into the bay, as indicated by a decreasing inshore salinity trend, fluctuations in δ^{13}C and δ^{15}N values of POM among the stations imply different sources of OM. The results of the present study suggest that during the wet season, OM in SGB originates from 2 sources; marine and terrestrial. Hence to quantify the relative contribution of each source, a 2 end-member mixing model has been applied to the wet season data, using terrestrial and marine end-members values based on the model by Calder & Parker (1968).

The equation used in this model is given as:

$$\text{TC}\ (\%) = \delta^{13}\text{C}_{\text{mar}} - \delta^{13}\text{C}_{\text{sam}} / \delta^{13}\text{C}_{\text{mar}} - \delta^{13}\text{C}_{\text{ters}} \times 100 \quad (3)$$

where TC is the terrestrial carbon, $\delta^{13}C_{mar}$ is the marine end-member, $\delta^{13}C_{ters}$ is the terrestrial end-member, and $\delta^{13}C_{sam}$ is the measured value of the samples at each station. Generally, terrestrial OM has relatively low values of δ^{13}C and δ^{15}N. Therefore, in our study, δ^{13}C (−27.4‰) and δ^{15}N (4.7‰) values of POM were selected as terrestrial end-members, which are closer to terrestrial end-member values of δ^{13}C and δ^{15}N identified in a number of previous studies (Peters et al. 1978, Wada et al. 1987, Middleburg & Nieuwenhuize 1998, Barros et al. 2010). In the present study, mean δ^{13}C (−19.0‰) and δ^{15}N (9.4‰) values of cultured fish and oyster, respectively, have been selected as marine end-members and are close to the values of Middleburg & Nieuwenhuize (1998). Model results indicated that during the wet season in SGB, an average of ~72% of OM in POM is derived from the land.

In contrast to the wet season, during the dry season the range and average values of δ^{13}C and δ^{15}N of POM in all culture areas were within the range of marine-derived OM reported in previous studies (Gearing et al. 1984, Wada & Hattori 1991, Meyers 1997, Lamb et al. 2006). The high C/N values observed among SF+F culture stations might have resulted from the presence of degraded OM (Khodse et al. 2007) due to limited river inflow during the dry season, while in the other 2 culture areas, C/N values were in the range of marine-derived OM (Meyers 1994). SOM of all culture areas was assumed to be derived from suspended matter during the dry season, as indicated by their mean values of δ^{13}C (SOM = −22.4 ± 0.3‰ and POM = −23.2 ± 0.6‰; ANOVA, $p < 0.05$), revealing material exchange between the 2 different OM pools (Meksumpun et al. 2005).

Comparing both seasons, significant differences were found between δ^{13}C and δ^{15}N values of SOM and POM (ANOVA, $p < 0.05$). The relatively high values of δ^{13}C in SOM showed that SOM in SGB was derived from the same marine source in both seasons. The reason for this could be that sediments were receiving OM from autochthonous sources originating from diatoms, bacteria, and green macroalgae (Gao et al. 2012). The significant difference between SOM and POM in the wet season shows less exchange between the 2 OM pools, the reason being either high freshwater inflow or assimilation of terrestrial-derived OM in the upper water column. In both seasons, the δ^{15}N values were also close to those reported for marine-derived OM in previous studies (Gearing et al. 1984, Wada et al. 1991). The comparison of our carbon and nitrogen isotopic signatures of the POM in SGB with that of other bays (Table 3) suggests that the water quality of SGB is not significantly impacted by land-based sources of OM. The

Table 3. Ranges of carbon and nitrogen isotope values of the present study compared to previous values reported in the literature from different coastal areas having aquaculture activities or being impacted by various sources of organic matter. nd: not determined

Study area	Activity / source of impact	δ^{13}C (‰)	δ^{15}N (‰)	References
Southwestern Thailand	Land-based aquaculture	−27.3 to −20.6	3.1–8.4	Kuramoto & Minagawa (2001)
Gaeta Gulf (Mediterranean)	Bivalve and cage culture	−25.0 to −19.8	nd	Mazzola & Sarà (2001)
Kat O Bay, Hong Kong	Land-based aquaculture	−21.2 to −20.1	8.5-10.2	Wai et al. (2011)
Simon Bay, South Africa	Anthropogenic	−24.8 to −19.3	nd	Filgueira & Castro (2011)
Kosirina Bay, Croatia	Anthropogenic	nd	4.3–8.3	Dolenec et al. (2011)
Sanggou Bay	Bivalve and cage culture	−27.4 to −19.0	4.7–9.4	Present study

high production of phytoplankton and the δ^{13}C values in all cultured species indicate that the bay acts as source of carbon, and that this carbon is utilized by cultured species and removed from the bay at their harvest. However, the high C/N values indicate that SGB may act as a sink for anthropogenic material (river input).

CONCLUSIONS

In SGB, phytoplankton production is one of the main sources of OM to higher trophic levels during the wet season, as indicated by a positive correlation between δ^{13}C and POM, the latter of which containing a large proportion of phytoplankton. Trophic relationships showed that cultured fish and oyster take up carbon from different sources while sharing the same trophic level (2.16). On the other hand, oyster and scallop used the same carbon sources in spite of different trophic levels (2.16 and 1.52 respectively). Based on the results of the stable isotope analysis, our conceptual model for the wet season suggested that ~80% of the OM including faecal material and riverine OM in the form of POM is extracted by oyster and scallop. In the dry season, these species still mainly rely on POM but to some extent also use rotten kelp. C/N values >11 for POM indicate the partly terrestrial origin of OM in SGB; however, in the wet season the bay also functions as a source of carbon due to the high phytoplankton production and aquaculture activities, while high C/N values indicate that SBG may also be a sink of anthropogenic material (river input). Therefore, both culture areas SF+F (avg. C/N = 12.69) and SF+SW (avg. C/N = 13.11) in SGB are highly impacted by OM from river inflow and human activities in the wet season, as indicated by average C/N ratios in POM. In the dry season, POM in the near-shore SF+F culture area showed high C/N values of 11.97 ± 2.08 (mean ± SD) relative to the other 2 areas with C/N values (<10) indicative of more marine-derived OM. The outer SW culture area (near YS) is highly impacted by YS water. However, C/N values (<10, typical of a marine source) indicate the influence of YS, but the δ^{13}C values show the signature of terrestrial OM that may result from river input and degraded OM. Results from the 2 end-member mixing model revealed that for POM an average of 72% OM is derived from land during the wet season. δ^{13}C and δ^{15}N signatures show that OM in SOM during both the wet and dry seasons is mostly of marine origin. However, a detailed study on terrestrial organic input from rivers into the SGB is required to better understand the sources of OM and its influence on the water quality of the bay. In addition, studies investigating the role of benthic, non-aquaculture organisms and seagrass could further the understanding of the detailed food web structure of the bay.

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