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Use of stable isotopes to understand food webs in Macao wetlands

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Abstract In this study, components of the food-web in Macao wetlands were quantified using stable isotope ratio techniques based on carbon and nitrogen values. The $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter ($\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$, respectively) ranged from −30.64 ± 1.0 to −28.1 ± 0.7 ‰, and from −1.11 ± 0.8 to 3.98 ± 0.7 ‰, respectively. The $\delta^{13}C$ values of consumer species ranged from −33.94 to −16.92 ‰, showing a wide range from lower values in a freshwater lake and inner bay to higher values in a mangrove forest. The distinct dietary habits of consumer species and the location-specific food source composition were the main factors affecting the $\delta^{13}C$ values. The consumer ^{15}N-isotope enrichment values suggested that there were three trophic levels; primary, secondary, and tertiary. The primary consumer trophic level was represented by freshwater herbivorous gastropods, filter-feeding bivalves, and plankton-feeding fish, with a mean $\delta^{15}N$ value of 5.052 ‰. The secondary consumer level included four deposit-feeding fish species distributed in Fai Chi Kei Bay and deposit-feeding gastropods in the Lotus Flower Bridge flat, with a mean $\delta^{15}N$ value of 6.794 ‰. The tertiary consumers group consisted of four crab species, one shrimp species, and four fish species in the Lotus Flower Bridge Flat, with a mean $\delta^{15}N$ value of 13.473 ‰. Their diet mainly comprised organic debris, bottom fauna, and rotten animal tissues. This study confirms the applicability of the isotopic approach in food web studies.

Keywords Food web · Trophic level · Wetlands · Stable isotopes · Macao

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Introduction

Traditional approaches to delineate a food web include gut and stomach contents analyses, together with field and laboratory observations. These studies can identify what animals feed on with a high degree of taxonomic precision. However, these approaches are usually labor intensive and subject to errors because the identification of materials digested and assimilated by consumers is largely speculative (Fry and Sherr 1984; Persic et al. 2004; Sun et al. 2011). The alternative stable isotope method, which is based on the selective metabolic partitioning that results in the preferential waste of lighter isotopes during respiration and excretion, can overcome some of the above-mentioned difficulties. This integrative approach distinguishes assimilated

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rather than ingested food, reflecting the complexity of food webs over longer periods (Persic et al. 2004). Recently, analyses of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to trace the flow of organic matter in lake, estuarine, and marine food webs (Rodelli et al. 1984; Kendall et al. 2001; Gu et al. 2006, Bouillon et al. 2011). Therein, $\delta^{13}C$ can be used to evaluate the ultimate sources of carbon in an organism when the isotopic signatures of the sources are different. It has been demonstrated that $\delta^{13}C$ is enriched approximately 0.5–1 ‰ in the animal, relative to its diet (Kwak and Zedler 1997; Middelburg 2014). The basic assumption in estimating trophic positions is the conservative enrichment of 3–4 ‰ $\delta^{15}N$ in a consumer, relative to its diet (Persic et al. 2004). Stable isotope signatures based on $\delta^{13}C$ and $\delta^{15}N$ values can be used to determine the sources of food ingested by a consumer and food assimilation over a long period (Middelburg 2014).

Macao is a very densely populated modern city located on a peninsula in the south-west of the Pearl River Delta in China. The Macao wetlands are small but internationally important (Jin et al. 2008), because more than 50 individuals of black-faced spoonbills (*Platalea minor*), categorized as an endangered species by IUCN (IUCN, 2014), visit Macao for over-wintering (Zhang et al. 2012). These wetlands are also the only known habitat of the recently discovered bryophyte *Fissidens macaoensis*, which requires particular environmental conditions (Jin et al. 2008). These endangered and endemic species demonstrate the values of Macao's urbanized wetlands for conservation and ecological function. Although the phytoplankton, zooplankton, and benthic fauna of the Macao wetlands have been studied recently (Li et al. 2009; Chen et al. 2014, 2015), there have been few studies on the food web structure of this wetland. In this study, we present the first attempt to describe the food web in the Macao wetlands using a combined stable C and N isotope method. The aims were to characterize trophic relationships among the dominant species in Macao wetlands, and to determine which factors affect the trophic status of individual organisms whose feeding histories are poorly understood.

Materials and methods

Study area

The study was conducted in four typical wetlands in Macao; Fai Chi Kei Bay (22°12′29″N; 113°32′30″E), Nam Van Lake (22°11′21″N; 113°32′19″E), Wang de Notre Dame Bay (22°9′7″N; 113°33′47″E), and Lotus Flower Bridge Flat (22°8′29″; 113°33′6″E). Fai Chi Kei, located at the northern part of the Macao peninsula, is a semi-enclosed water body with poor exchange. Nam Van Lake is an artificial lake located at the southern end of the peninsula. Wang de Notre Dame Bay is a freshwater lake with a surface area of nearly 20 hectares, and covered with *Nelumbo nucifera* and water hyacinth. Lotus Flower Bridge Flat is the largest wetland in Macao, and consists of about 80 ha covered by mangroves. The most abundant mangrove species in Lotus Flower Bridge Flat are *Avicennia marina* and *Acanthus ilicifolius* (Fig. 1).

Sample collection and processing

Samples for isotopic analysis were collected from December 2012 to September 2013. Particulate organic matter (POM), mixed phytoplankton/zooplankton, and suspended organic matter samples were collected with a plankton net with a mesh size of 35 μm, washed with distilled water, filtered through Whatman GF/G filters (precombusted at 500 °C for 2 h), and then dried to constant weight at 55 °C (Thimdee et al. 2004). Because the aquatic community may vary seasonally, four replicate samples in spring (March 2013), summer (June 2014), autumn (September 2013), and winter (December 2012) were conducted to study the food web. Fish samples were collected by anglers around Fai Chi Kei Bay, and by using gill nets in Lotus Flower Bridge Flat. Additional benthic invertebrates such as crabs, shrimps, and mollusks were collected by hand picking or in nets during low tides from the mangrove inter-tidal zone and Wang de Notre Dame Bay. For the mollusks, the soft tissues of 20 individuals of each species were pooled as a sample. For the decapod crustaceans, muscle tissue of around 20 individuals was extracted as a composite sample from abdominal segments or pereiopods. For fish, muscle tissue was dissected from four to 10 individuals of each species and pooled as a sample. Only the dorsal muscle of fish was taken for analysis, because its $\delta^{13}C$ and $\delta^{15}N$ contents are less variable than those in other tissues (Pinnegar and Polunin 1999). The muscle tissues were dried at 60 °C in an oven to constant weight. The dry tissue was ground using a mortar and pestle.

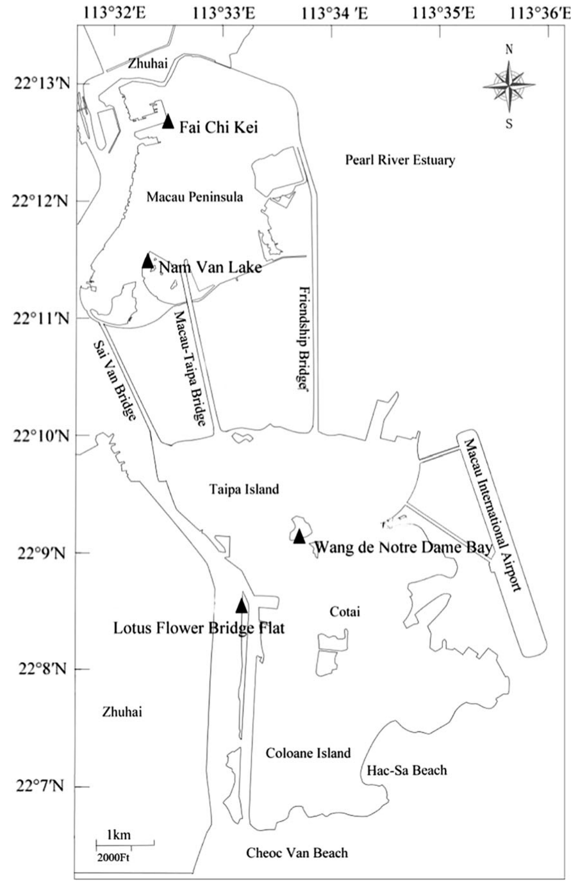


Fig. 1 Location of wetland study site (*triangle* sample collection site)

Isotopic value collection and data analysis

The $\delta^{15}N$ and $\delta^{13}C$ values were measured using gas chromatography–isotope ratio mass spectrometry (Thermo Finnigan Delta XL Plus GC-IRMS, Bremen, Germany). Isotopic values were expressed in the δ-unit notation as deviations from standards following the formula: $\delta^{13}C$ or $\delta^{15}N = [((R_{sample}/R_{standard}) - 1) \cdot 10^3]$, where R was the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for C was Peedee Belemnite, and for N was atmospheric diatomic nitrogen. Instrument precision was 0.1 ‰ for carbon and 0.3 ‰ for nitrogen, based on replicate analyses of standard reference materials.

Trophic level (TL) was calculated using the following formula (Fry 2006; Vander Zanden and Rasmussen 2001; Iken et al. 2010)

$$TL_{(consumer)} = \left(\left(\delta^{15}N_{consumer} - \delta^{15}N_{baseline}\right)/TEF\right) + \lambda$$

where TL $_{consumer}$ and $\delta^{15}N_{consumer}$ were the TL and the $\delta^{15}N$ value of the tested consumer, respectively, $\delta^{15}N_{\ baseline}$ was the $\delta^{15}N$ value of the baseline biota, and TEF represented the trophic enrichment factor (=3.4 ‰). All statistical analyses were performed with EXCEL (Microsoft, Redmond, WA, USA) and SPSS 17.0 for windows (SPSS Inc., Chicago, IL, USA). One-way ANOVA (analysis of variance) was used to test for statistically significant differences in $\delta^{15}N_{\ POM}$ and $\delta^{13}C_{POM}$ values among sites. Graphs were plotted using the ORIGIN 8.0 package (Origin Lab Corp., Northampton, MA, USA).

Results

Stable carbon and nitrogen isotope traits of POM in Macao wetlands

The $\delta^{13}C_{POM}$ values at the four wetlands ranged from −30.64 ± 1.0 to −28.1 ± 0.7 ‰, and the mean value was −28.76 ± 1.58 ‰. The $\delta^{15}N_{POM}$ values of each site ranged from −1.11 ± 0.8 to 3.98 ± 0.7 ‰, and the mean value was 2.314 ± 0.658 ‰ (Fig. 2). There was a significant difference ($p < 0.05$) in isotopic composition among the sampling sites.

$\delta^{13}C$ and $\delta^{15}N$ values of consumers in Macao wetlands

The $\delta^{13}C$ values of 21 consumers ranged from −33.94 (*Angulyagra polyzonata*) to −16.92 ‰ (*Uca arcuata*), indicating that $\delta^{13}C$ values varied widely among different species. Mollusk species (e.g. *Neritina violacea, Ellobium chinense*), which were distributed in inter-tidal areas with a certain amount of salinity, showed higher $\delta^{13}C$ values than those of freshwater species (e.g. *A. polyzonata, Pomacea canaliculata*) (Fig. 3). Crustaceans showed a higher $\delta^{13}C$ values than those of mollusks; *U. arcuata* showed the most enriched values (Figs. 3, 4). The $\delta^{13}C$ values of fishes ranged from −28.15 to −21.79 ‰. The depleted carbon signatures (−28.15 to −24.72 ‰) of fishes in the Bay of Fai Chi Kei contrasted with the more enriched signatures (−24.61 to −21.79 ‰) of those in the Lotus Flower Bridge Flat. Overall, $\delta^{13}C$ values of

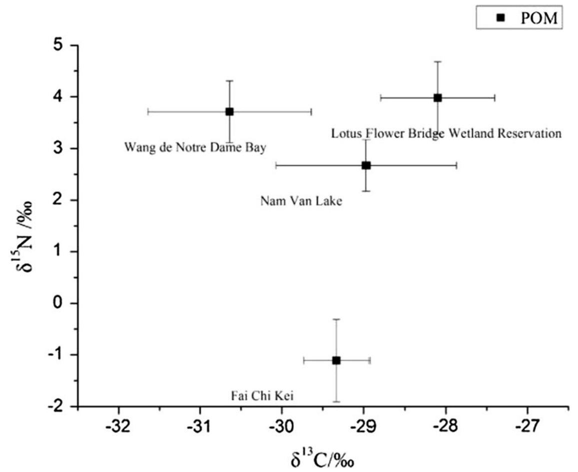


Fig. 2 δ^{13}C and δ^{15}N values of particulate organic matter in Macao wetlands

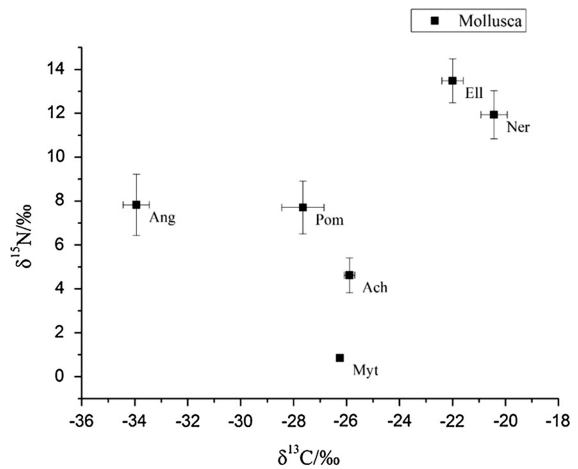


Fig. 3 Values of δ^{13}C and δ^{15}N for mollusks in Macao wetlands (Ang = *Angulyagra polyzonata*; Pom = *Pomacea canaliculata*; Ach = *Achatina fulica*; Myt = *Mytilopsis sallei*; Eli = *Ellobium chinense*; Ner = *Neritina violacea*)

N. violacea, *E. chinense*, *Exopalamon carincauda*, *Parasesarma plicatum*, *Perisesarma bidens*, and *Boleophthalmus pectinirostris* were very similar (−22.3 to −20.37 ‰) (Figs. 2, 3, 4 and 5).

The δ^{15}N values of macro-invertebrates ranged from 4.62 ‰ (*Achatina fulica*) to 14.69 ‰ (*E. carincauda*) (Figs. 2, 3). The δ^{15}N values of fishes ranged from 4.25 ‰ (*Mugil cephalus*) to 14.15 ‰ (*Sparus latus*) (Fig. 4). Among the mollusk taxa, compared with the freshwater species *A. polyzonata* (7.84 ‰) and *P. canaliculata* (7.71 ‰), the estuarine species *N. violacea* (11.94 ‰), *E. chinense* (13.48 ‰) showed more enriched δ^{15}N values (Fig. 3). Similarly, fishes distributed in the Fai Chi Kei Bay had more depleted δ^{15}N signatures than those in the mangroves (Fig. 5).

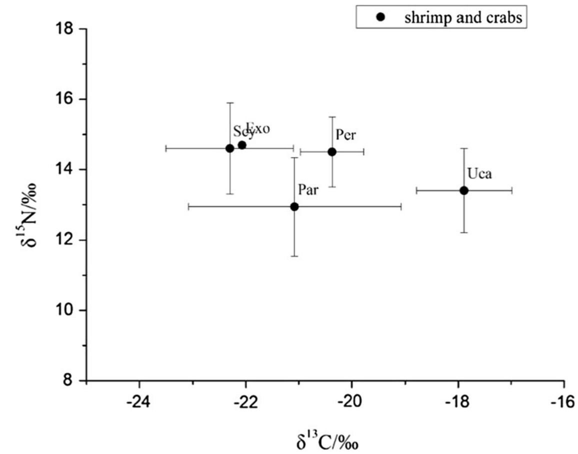


Fig. 4 Values of δ^{13}C and δ^{15}N for crustaceans in Macao wetlands (Scy = *Scylla paramamosain*; Exo = *Exopalamon carincauda*; Par = *Parasesarma plicatum*; Per = *Perisesarma bidens*; Uca = *Uca arcuata*)

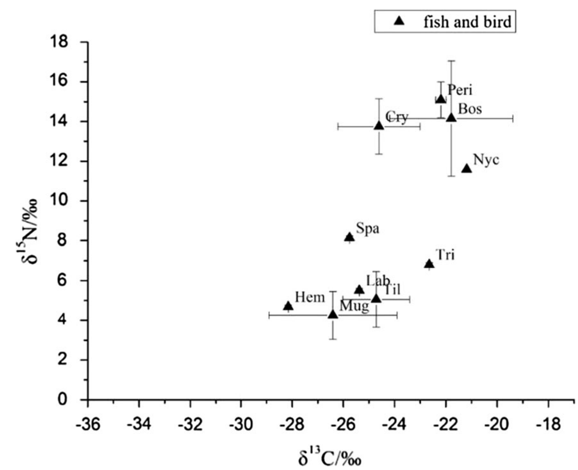


Fig. 5 Values of δ^{13}C and δ^{15}N for fishes and birds in Macao wetlands (Mug = *Mugil cephalus*; Til = *Tilapia*; Peri = *Periophthalmus cantonensis*; Hem = *Hemiculter leucisculus*; Spa = *Sparus latus*; Bos = *Bostrichthys sinensis*; Lab = *Labeo rohita*; Tri = *Tridentiger trigonocephalus*; Cry = *Cryprinus carpiod*; Nyc = *Nycticorax nycticorax*)

Food-web structure in Macao wetlands

It should be noted that POM, as the food-web baseline, is a heterogeneous food source comprising plankton, bacteria, and particulate matter, with large spatial and temporal variations in its isotopic signature. The variations arise from differences in biogeochemical processes (e.g., ammonium and nitrate availability) and in the reproductive cycle of plankton. In this study, POM at each sampling site was considered as the baseline. The trophic levels were ranked from 1.19 to 4.27 (Fig. 6). Eight species represented primary consumers, with mean TLs ranging from 1.19 to 2.94. Nine species were secondary consumers, with mean TLs ranging from 3.32 to 3.99. Four species were tertiary consumers, with mean TLs of >4.0. Ultimately, a scheme describing the feeding relationships of the analyzed dominant components of Macao wetlands trophic web (Fig. 7) was achieved by integrating the theoretical data with the results from the investigations described above.

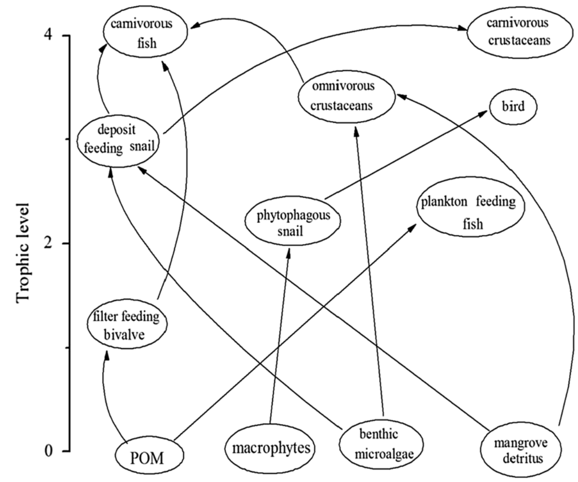


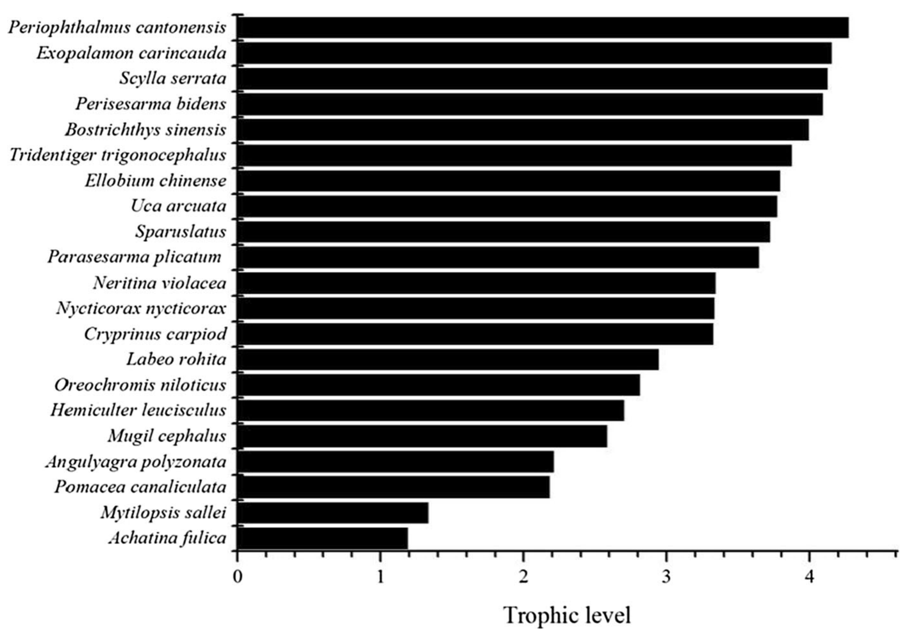
Fig. 7 Simplified diagram of food web in Macao wetlands

Discussion

Signatures of $\delta^{13}C$ and $\delta^{15}N$ for POM in Macao wetlands

The POM is composed of allochthonous and autochthonous organic materials, and can provide a detailed, integrated record of natural and anthropogenic activities in aquatic ecosystems (Hein et al. 2003; Zhang et al. 2007). Several lines of evidence indicate that POM mainly consists of phytoplankton in eutrophic water, but consists of autochthonous organic compounds in oligotrophic water (Junes et al. 1999; Gu 2009). Allochthonous and autochthonous organic matter usually have different $\delta^{13}C$ and $\delta^{15}N$ signatures, which results in different $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ signatures (Savoye et al. 2003; Kendall et al. 2001). In the oceanic food web at the

Fig. 6 Trophic level of consumers in Macao wetlands



northwestern gulf of Mexico, $\delta^{13}C$ values ranged from −30 to −23 ‰ for C_3 terrestrial plants and from −14 to −10 ‰ for C_4 plants, and the $\delta^{13}C_{POM}$ values ranged from −27 to −25 ‰ (Fry and Sherr 1984). In a mangrove-fringed estuary in Thailand, the $\delta^{13}C$ values of mangrove leaves ranged from −29.4 to −28.2 ‰ (Thimdee et al. 2004). Keely and Sandquist (1992) concluded that the $\delta^{13}C$ values of aquatic macrophytes were often less than −27 ‰ due to respiratory CO_2. In this survey, the mean $\delta^{13}C_{POM}$ value was relatively low (−28.76 ‰). The inadequate water exchange in the eutrophic conditions of the inner bay of Fai Chi Kei limited the input of allochthonous organic matter; consequently, the POM in the bay contained a large proportion of phytoplankton (Li et al. 2009). In contrast with Fai Chi Kei, Nan Vam Lake was an oligotrophic water body in which allochthonous organic matter from rainfall was the main source of POM. The most depleted $\delta^{13}C_{POM}$ was in Wang de Notre Dame Bay (−30.64 ± 1.0 ‰), a freshwater site extensively dominated by macrophytes. The POM at this site comprised mainly autochthonous organic matter.

Among the four wetlands, the Lotus Flower Bridge Flat showed the most enriched isotope signature with a mean $\delta^{13}C_{POM}$ of −28.1 ± 0.7 ‰ and a mean $\delta^{15}N_{POM}$ of 3.98 ± 0.7 ‰. A previous study demonstrated that POM in the intertidal mangrove forest consisted of both phytoplankton and mangrove detritus (Kon et al. 2007). Accordingly, the POM at the Lotus Flower Bridge Flat mainly consisted of mangrove detritus and phytoplankton. Meanwhile, the excreta of animals is known to have higher $\delta^{15}N$ values (Kendall et al. 2001; Vizzini and Mazzola 2002). As an estuarine wetland, the Lotus Flower Bridge Flat exhibited a high density and wide biological diversity of organisms (Chen et al. 2014). The utilization of $\delta^{13}C$ and δ^{15} N from animal excreta by phytoplankton may be the main reason for the high isotope signatures at this site.

$\delta^{13}C$ values of consumers in Macao wetlands

The $\delta^{13}C$ composition of consumers typically reflects the composition of assimilated food, plus a slight enrichment, and is often used to discriminate between consumer use of pelagic (depleted in $\delta^{13}C$) and littoral (near-shore) benthic resources (enriched in $\delta^{13}C$), with terrestrial allochthonous $\delta^{13}C$ being intermediate (Fry and Sherr 1984; Larson et al. 2011). Tue et al. (2012) found that the $\delta^{13}C$ value of invertebrates in an estuarine mangrove ecosystem in Vietnam ranged from −26.8 to −14.5 ‰. The wide ranges of $\delta^{13}C$ signatures indicated that the invertebrates had heterogeneous diets, comprising benthic microalgae, marine phytoplankton, POM, sediment organic matter, mangrove detritus, meiofauna, and rotten animal tissues. In Laoshan Bay, China, the carbon isotope data confirmed that POM was the main food source of benthic filter feeders (e.g., bivalves, crustaceans). These filter feeders had a wide range of $\delta^{13}C$ values, reflecting the diversity of their food sources. Benthic diatoms are an important part of the diet of most gastropods (Cai et al. 2011). In this study, the $\delta^{13}C$ values of consumers ranged from −33.45 ‰ (*A. polyzonata*) to −16.92 ‰ (*U. arcuata*), and showed a wide range from lower values in the freshwater lake and inner bay to higher values in the mangrove forest. The wide variation in values was mainly because of the distinct dietary habits of the consumers and the diverse food sources in the ecosystem. Benthic microalgae in estuarine ecosystems are considered to be ^{13}C-enriched (Doi et al. 2005). In this study, the $\delta^{13}C$ values of gastropods distributed in mangrove mudflats (e.g., *N. violacea* and *E. chinense*) were higher than those of gastropods living in freshwater habitats (e.g., *A. polyzonata* and *P. canaliculata*). The gastropods in mangrove mudflats consumed mainly sediment organic matter and benthic microalgae, whereas those in freshwater habitats consumed mainly leaves and stems of aquatic plants. The macro-invertebrates collected from the Lotus Flower Bridge Flat (*E. chinense*, *N. violacea*, *E. carincauda*, *P. plicatum*, *P. bidens*, and *P. cantonensis*) had similar $\delta^{13}C$ values (−22.3 to −20.37 ‰). This result suggested that the food sources were restricted in this area, and that those different types of benthic animals had similar omnivorous diets.

Food-web structure and trophic level of consumers

Naturally occurring stable isotopes of nitrogen and carbon display a stepwise enrichment between prey and consumer tissues. In particular, the heavier nitrogen isotope becomes progressively enriched from prey (3 ‰) to predator (5 ‰). The values of $\delta^{15}N$, therefore, provide a continuous variable that can quantify the relative trophic positioning of biota (Van der Zanden et al. 1998; Persic et al. 2004). Nitrogen isotope distributions have been shown to be robust indicators of trophic position in estuary ecosystems, where ^{15}N enrichment increases predictably with the TL of consumers. The number of trophic levels (food chain length) and consumer trophic positions were approximated in previous studies (Minagawa and Wada 1984; Kwak and Zedler 1997). Quan et al. (2010) examined the ^{15}N isotope distributions of consumers in the Yangtze River Delta, and estimated that there were three trophic levels in that system: primary consumers (debris/phytoplankton feeders, $TL2 < 2.6$), secondary consumers (omnivorous, $2.6 < TL3 < 3.4$) and tertiary consumers (carnivorous animals, $TL4 > 4.0$). In the present study, invertebrates except for *E. carincauda* and *S. paramamosain* occupied the secondary TL and mainly fed on organic debris and phytoplankton, while *E. carincauda* and *S. serrata* may have fed on small animals and rotten animal tissues. Yu et al. (2008) reported that the trophic levels of macrobenthic fauna distributed in Chongming Island Flat ranged from 2.0 to 3.7, with *P. cantonensis* showing the highest value and *Glaucomya chinensis* the lowest. The trophic levels ranging from 2.0 to 3.0 were occupied by filter-feeding bivalves, deposit-feeding gastropods, and crustaceans. *Exopalaemon modestus*, *E. carincauda*, *S. serrata*, and *P. cantonensis* represented the secondary trophic level ($TL > 3.0$), suggested that they could feed on some animal baits. In this study, the TL of the consumers ranged from 1.19 (*A. fulica*) to 4.27 (*P. cantonensis*). There was a wide variation in trophic levels among different species. According to the TL division by Quan et al. (2010), the consumer ^{15}N isotopic enrichment detected in this study indicated that there were three trophic levels. The primary consumer TL (mean $\delta^{15}N$ value, 5.052 ‰) was represented by freshwater herbivorous gastropods (*P. canaliculata* and *A. polyzonata*), filter-feeding bivalves (*Mytilopsis sallei*) and plankton-feeding fish (*M. cephalus*). The secondary consumer level (mean $\delta^{15}N$ value, 6.794 ‰) included four deposit-feeding fish species (*Hemiculter leucisculus*, *Tilapia, Labeo rohita,* and *Cryprinus carpiod*), which were distributed in Fai Chi Kei Bay, and a deposit-feeding gastropod in Lotus Flower Bridge Flat. The tertiary consumer level (mean $\delta^{15}N$ value, 13.473 ‰) consisted of four crab species (*P. plicatum*, *U. arcuata*, *P. bidens*, *S. paramamosain*), one shrimp species (*E. carincauda*), and four fish species (*S. latus*, *Tridentiger trigonocephalus*, *Bostrichthys sinensis*, *P. cantonensis*) in the Lotus Flower Bridge Flat. The main diet of these species was organic debris, bottom fauna, and rotten animal tissues.

In conclusion, we quantified trophic relationships between common species that were collected from representative wetlands using a stable isotopic approach. Based on these results, we proposed food web structure and their site variations in wetlands of Macao. This study also confirms the applicability of the isotopic approach in food web studies where direct quantification is not easy. Collectively, this is the first quantitative study of food web in Macao wetlands, highly urbanized wetlands that still support endangered and endemic animal and plant species. Thus this study provides important knowledge that can contribute wetland management and conservation in Macao.

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