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Original Research

Nutrients Affecting the Characteristics of Food-Web Structure in Aquatic Ecosystem of Pearl River

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Abstract

The water quality has degraded with economic development globally. However, the relationship between food-web structure and nitrogen and phosphorus is of rare concern. To study food chain in this ecosystem, we measured the concentrations of stable isotope value $\delta^{13}C$ and $\delta^{15}N$ in aquatic matter and organisms in Pearl River. The $\delta^{13}C$ and $\delta^{15}N$ concentrations ranged from −41.2‰ to −19.4‰ and from 0.81‰ to 25.4‰, respectively. The $\delta^{13}C$ concentrations in consumers were significantly higher than the particulate organic matter (POM), periphyton, phytoplankton, and higher aquatic plants. The $\delta^{13}C$ of POM was likely derived from phytoplankton and exogenous organic detritus entering from a tributary, rather than from endogenous phytoplankton in the main river channel. The $\delta^{13}C$ of phytoplankton was derived from eutrophic water with high nitrogen and phosphorus concentrations. Total $\delta^{13}C$ was significantly higher in fish than in POM, phytoplankton, higher aquatic plants, and zooplankton, indicating that those components were the main carbon sources for fish. The carbon sources tended to be the same for different fish species in the same season at the same site, but different for a given fish species among seasons and sampling sites. This finding suggested that the feeding habits of different fish species converge as an adaptation to change environment. The food chain was longer (trophic level = 4.4) in river subsections with more carnivorous fish, such as *Erythroculter pseudobrevicauda* and *Coilia grayii*, and shorter in areas with more omnivorous fish. The total nitrogen and total phosphorus concentrations in the water were negatively correlated with food-chain length (R^2 = 0.67, P<0.05; R^2 = 0.40, P<0.05). These results suggested that limiting nitrogen and phosphorus inputs into the water body would reduce the ecological risk in this area.

Keywords: nutrients, stable isotope, trophic level, food web, the Pearl River

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Introduction

Stable isotope technology has been used to study food-network structure and trophic levels in aquatic ecosystems. Studies have shown that the average carbon stable isotope $\delta^{13}C$ value of consumers is 1‰ higher than that of their food, while the average nitrogen stable isotope $\delta^{13}N$ value of consumers is 3.4 ±1‰ higher than that of their food [1-7]. Based on this principle, food sources and trophic levels in the ecosystem can be established. Fetahi et al. [8] reported that the proportion of $\delta^{13}C$ was −22.57‰ in *Tilapia*, compared with −23.28‰ in particulate organic matter (POM) in their habitat, a difference of 0.71‰, this result indicated that POM was the carbon source for *Tilapia*. Wada et al. [4] established the following food-chain structure from $\delta^{15}N$ values: diatoms ($\delta^{15}N$: 0‰) – shrimp ($\delta^{15}N$: 3‰) – small fishes (body length <2 cm, $\delta^{15}N$: 10‰) – small fishes (8–20 cm, $\delta^{15}N$: 13‰) – large fishes (body length >100 cm, $\delta^{15}N$: 17‰), based on the distribution of food in the consumer body. Trophic levels (TL) are usually determined to characterize the food web of aquatic ecosystems, ecosystems and food webs are structured into trophic levels of who eats whom, and species that occupy higher trophic levels have less available energy and higher energetic costs than species at lower trophic levels [9]. Usually, $\delta^{15}N_{base}$ refers to the primary producer, whose TL is one, while the TL of consumers is greater than 2. Numerous studies have shown that the TL of the food chain in marine ecosystems is 5-6. The higher TL in marine ecosystems is mainly due to the presence of carnivorous fishes, while the higher TL in lakes and reservoirs is mainly due to predominantly omnivorous fish species [10]. Some studies have shown that estuaries where seawater and freshwater meet have a TL of 4.5, significantly longer than the TL in inland waters with mean 3.180.06 to 3.340.07 [11-12], mainly because there are more carnivorous fish in the former environment. Studies have suggested that the TL in water ecosystems is related to water temperature and regional climate. A change in water temperature will lead to changes in primary producers, which directly affects food consumption and changes the structure of consumer communities. This indirectly affects the nutrition-level features in the water ecosystem [13]. In addition, TL of consumers was limited by production space and primary producers, because of diversity and community structure of species were controlled by production space and primary producers, main reason was the first possible explanation is that primary producers are the basic food sources for consumers, Additionally, the contributions of primary food sources to consumers will change in different water habitats. The second possible explanation is the baseline of $\delta^{15}N$ values, the isotopic values of food source after assimilated diet over a period of time can be reflected in consumers. Each food source item possesses an individual stable isotopic value [11, 14].

The Pearl River is an important economic development region in the Pearl River delta, and its health is directly related to the region's social stability and prosperity. In this study, we determined the characteristics of stable carbon and nitrogen isotopes to analyze food network and TL relationships in the water ecosystem from in the section of the Pearl River from Guangzhou to Humen. We analyzed the contribution of different carbon sources such as POM, periphyton, phytoplankton and higher aquatic plants to consumers in the food network. We also studied the impact of environmental factors on TL. The ultimate aims of our study were to explore ecosystem relationships in this region and to determine the factors that control the quality of the water environment. At present, it is not clear how to control food-web structure and trophic level in aquatic ecosystem of Pearl River when there are changes in nitrogen and phosphorus concentration increased. So, we also focused on changes in $\delta^{13}C$ and $\delta^{15}N$ in aquatic matter and organisms and nitrogen and phosphorus concentration in Pearl River. This study tested the hypothesis that higher nitrogen and phosphorus concentration can affect food-web structure and trophic level in aquatic ecosystem of Pearl River.

Materials and Methods

Study Area

The Pearl River is the largest river in southern China. It is 2217 km long and is located at E97°39'-E117°18', N3°41'-N29°15' [15]. The Pearl River is an important area for sustainable wild fishery resources due to the convergence of fresh and seawater creating a brackish environment. The Pearl River is the largest river system in South China and the primary water source for the Pearl River Delta (PRD) with the largest urban agglomeration on a global scale. The PRD accounts for 0.57% of the total national land area (54,754 km^2) in China, which raises 4.27% of the total population (58.74 million people in 2015). This indicates the high population density in the PRD and the great importance of water source security in the Pearl River. Due to the rapid economic development and population growth in the PRD, the excessive discharge of domestic and industrial sewages poses a remarkable threat to the local water environment [16]. The Pearl River catchment, situated at tropical latitudes, is strongly influenced by the East Asia Summer Monsoon. The average annual temperature ranges from 14 to 22ºC, with extreme values up to 42ºC and down to –10ºC. Mean annual precipitation is 1.5 m and decreases markedly westward, with a maximum of 2.4 m in the southeast and a minimum of 0.7 m in the northwest [17]. As the 13th largest river in the world, the Pearl River has an annual total discharge of about 10,000 m^3/s, pouring into the South China Sea through eight gates, namely Humen, Jiaomen, Hongqimen, Hengmen, Modaomen,

Jitimen, Hutiaomen, and Yamen. And almost 60% (5700 m^3/s) of the Pearl River water discharges into the Pearl River estuary, through four major gates of Humen, Jiaomen, Hongqimen, and Hengmen [18]. In the present work, location of the sampling sites along Pearl River in Guongdong Province, P.R. China (S1, 23.25°N, 113.22°E; S2, 23.22°N, 113.20°E; S3, 23.15°N, 113.22°E; S4, 23.11°N, 113.25°E; S5, 23.06°N, 113.33°E; S6, 23.07°N, 113.38°E; S7, 23.09°N, 113.42°E; S8, 23.07°N, 113.49°E; S9, 23.02°N, 113.52°E; S10, 22.99°N, 113.58°E; S11, 22.95°N, 113.54°E; S12, 22.88°N, 113.52°E (Supplement Fig. S1) [19-20].

Sample Collection and Procedure

Environmental Sampling

Water temperature and pH were measured when each sample was collected using a 6600 multi-sensor sonde (Yellow Springs Inc., Yellow Springs, OH, USA). Approximately 100 mL of combined water sample was used for the analysis of total nitrogen (TN) and total phosphorus (TP). After the water sample is collected, acidify it with sulfuric acid to pH<2 and determine it within 24 hours. TN and TP concentrations in each sample were determined using the alkaline potassium persulfate oxidation method [19-20]. Method for determination of total nitrogen, the 5 ml alkaline potassium persulfate solution and the water sample (10 mL) was added into 25 ml graduated tube with plug and wrap with gauze, after 120ºC digestion 30 min, the absorbance values were determined by UV spectrophotometry at 220 nm and 275 nm, respectively. Method for determination of total phosphorus, the water sample (25 mL) was filtrated to use 0.45 μm microporous membrane, and was added into the graduated tube with plug (50 mL), then oxidative decomposition with alkaline potassium persulfate solution 5% (4 mL), after 120ºC digestion 30 min, add 1mL ascorbic acid (10%) and 2 mL molybdate solution, the absorbance values were determined by UV spectrophotometry at 700 nm.

Collection of Samples for Isotope Analysis

Sampling along the Pearl River was conducted three times (in September 2016, January 2017 and July 2017), at sites (S1-S12) between Guangzhou and Humen. Samples of POM, periphyton along the river edge, phytoplankton, floating and emergent plants, zooplankton, benthic animals, and fishes were collected.

POM was collected by filtering two liter of surface water with a pre-combusted at 450ºC for 6h and pre-weighed GF/F glass fiber filter and frozen dry at −80ºC in polypropylene and polystyrol containers. The filters were weighed again and dry weight (POM) concentration determined [21], and packed in aluminum foil. Next, the samples were fumigated in an airtight drier filled with a strong hydrochloric acid atmosphere, for 24-36 h, to remove inorganic carbon. The samples were rinsed with deionized water, until the pH returned to 7, then freeze-dried and stored until analysis.

To sample epiphytic algae at each site, we randomly collected pebbles or gravel and used a clean toothbrush and river water to gently scrub algae from the stones into a tray. Visible impurities were removed with forceps. The water was poured into a 250 mL glass beaker, and stirred with a glass rod to further remove impurities by the principle of differences in settlement rate among objects with different densities. Each sample was packed into a 50 mL centrifugal tube with cover (EP). In the laboratory, the samples were frozen at −80ºC and then freeze dried (−80ºC). The freeze-dried phytoplankton samples were wrapped in aluminum foil and stored in 0.5 mL EP tubes until analysis.

Phytoplankton: small plankton net in deep water (mesh length: 280 cm, mesh diameter: 90 cm, mesh ring: stainless steel, mesh clothing: aperture 64 μm) was adopted to ensure that more phytoplankton will be collected [19-20]. When the phytoplankton is collected, a net of phytoplankton is placed in the water, and the boat needs to move forward at a certain speed to keep the phytoplankton from sinking to the bottom. Next, the procedure is as follows: Collect the phytoplankton with trawl, and place them in a 20 L sample bottle. And then collect the phytoplankton repeatedly until the dry weight of the phytoplankton meets the requirements. Add a small amount of formaldehyde reagent added to water samples kills zooplankton in the water. Then, the sampling will be settled for 20 minutes, so that zooplankton and particulate matter will sink to the bottom of the barrel. Filter the surface water sample by a phytoplankton net (64 μm mesh), the phytoplankton samples were transferred into a 50 mL measuring cylinder and thoroughly rinsed with Milli-Q water to remove other impurities, and then keep stationary again, so that the larger particles and the first filtered zooplankton sank to the bottom of the glass measuring cylinder. Transferr the surface phytoplankton samples into a 50 mL EP tube, and place in an ice box. Subsequently, freeze the samples at −80ºC in polypropylene and polystyrol containers and store in a vacuum desiccator prior to analysis [19-20].

Higher aquatic plants were represented by collecting one species each of a floating and emergent species. Common water hyacinth *Eichhornia crassipes* was gathered with a net, rinsed, and then the blades were removed with scissors. The blades were washed, again with ultrapure water and then sealed in bags. The same procedure was followed for *Scirpus yagara* growing along the river's edge, except that the leave, stem, and tuber were collected. The higher plant samples were oven dried at 60ºC, ground to pass through 80 mm sieve, wrapped in aluminum foil, and finally stored in 2 mL EP tubes until isotopic analysis.

Benthic organisms were collected from areas suitable for wading, using a D-net and Surber sampler.

The collection intensity was determined by habitat, and the collection area did not exceed 2 m^2. In the deeper parts of the river, shrimp cages were positioned to collect zoobenthos. Digestive tissue was removed from collected animals (snails, crabs, shrimp), and only the muscle was retained. The zoobenthos samples were freeze-dried (−80ºC), ground, wrapped in aluminum foil, and stored in 2 mL EP tubes until analysis.

Large quantities of zooplankton were collected with multiple tows using a 160μm nylon mesh net from 0.5 m above the bottom to the surface at each sampling site [23]. Each tow was put into a 20 L sample bottle; subsequent tows at each site were repeated until the zooplankton dry weight was estimated to be >3.0 g. To clean the sample, the bottle was static for 20 minute to concentrate and remove particulate matter from the bottom of the jars. Zooplankton and phytoplankton in the suspended water were transferred to another sample bottle (20 L), and formaldehyde reagent added. The fixed (dead) zooplankton was then concentrated at the bottom of the jar and the remaining suspended phytoplankton and soluble organic matter in the supernatant removed. The zooplankton was transferred into a 100mL measuring cylinder and thoroughly rinsed with Milli-Q water to remove other impurities. Again, the zooplankton sample was settled for 20 minutes and supernatant removed. This helped to remove dead phytoplankton and other impurities. The zooplankton sample was then transferred into sealed 50 mL EP tubes and placed in an ice box. Subsequently, the samples were frozen at −80ºC in polypropylene and polystyrol containers and stored in a vacuum desiccator prior to analysis [19-20].

Dorsal muscle tissue of fish was removed from the fish, frozen, freeze-dried (−80ºC), then ground, sieved, wrapped in aluminum foil, and stored in 2 mL EP tubes until analysis.

Isotopic Determination for Aquatic Matter and Organisms

The isotopic composition of the sampled aquatic organisms and POM were determined at the Third Institute of Oceanography. Samples were analyzed through an elemental analyzer and stable isotope mass spectrometer (Flash EA 1112 HT-Delta V Advantage, Thermo Fisher Scientific, Bremen,Germany), coupled with a Delta V Advantage isotope ratio mass spectrometer with a Confo IV interface (Termo Scientifc, Bremen, Germany) at the Littoral, Environment and Societies Joint Research Unit stable isotope facility (LIENSs) at the University of La Rochelle (France). Isotope compositions were expressed in the δ notation as parts per mil (‰) as deviations from an international standard (Vienna Pee Dee Belemnite for carbon and atmospheric N2 for nitrogen) following the formula:

$$\delta X = [R_{sample})/Rstandard)\text{-}1]\ 1000$$

Where X is ^{13}C or ^{15}N, R is the corresponding ration ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Calibration was done using reference materials (USGS-24, -61, -62, IAEA-CH6, -600 for carbon; USGS-61, -62, IAEA-N2, –NO-3, -600 for nitrogen). The analytical precision of the measurements was 0.1‰ for carbon and <0.15% for nitrogen based on analyses of USGS-61 and USGS-62 use as laboratory internal standards [11].

The TL of consumers was calculated using the formula of Renaud et al. (2011), as follows:

$$TL = ((^{15}N_{consumer} - ^{15}N_{primary\ production})) / 3.4 + 1$$

Where $\delta^{15}N_{consume}$ is the stable nitrogen isotope of the consumer; $\delta^{13}N_{primary\ production}$ is the stable nitrogen isotope of the primary producers; and 3.4 indicates that the $\delta^{15}N$ of the consumer is 3.4‰ higher than the $\delta^{15}N$ content in their food [22].

Results and Discussion

Physical and Chemical Characteristics of Water

Water temperature, pH, TN, TP concentrations in the Pearl River from Sept. 2016 to Jul. 2017 are shown in Table 1. The mean water temperature was 24.67, and the mean pH during the study was 7.530.21. TP and TN concentrations were significantly higher (ten times) than regional reservoir waters [23]. TP ranged from 0.130.06 mg L^{-1} to 0.800.61 mg L^{-1}, with an average of 0.350.22 mg L^{-1}, and TN from 2.410.76 mg L^{-1} to 8.491.29 mg L^{-1} (average mean 5.771.01 mg L^{-1}). TP and TN concentrations showed a decreasing trend from S1 to S12 with high nutrient loading in the Pearl River [19-20]. In this study, we detected a weak positive correlation between the $\delta^{13}C$ value of POM and the pH ($R^2 = 0.2$, $P<0.05$) (Fig. 1a), in addition, we observed that the $\delta^{13}C$ value of POM decreased with the increasing total nitrogen (TN) ($R^2 = 0.3$, $P<0.05$) (Fig. 1b).

The results the study suggested that an increase TN will boost phytoplankton growth, the TN content in this section of the Pearl River exceeded that requirement for phytoplankton growth (Table 1), a higher POM content affected the growth of phytoplankton, and the $\delta^{13}C$ values of phytoplankton was significantly higher than that of POM, and the $\delta^{13}C$ value of POM was significantly affected by TN. The TN content was higher in the river with more tributary isthmuses than in main channel of the Pearl River, the phytoplankton density and biomass in the water-body were higher in part of the river with more tributary isthmuses than main river channel. This result proved that the $\delta^{13}C$ value of POM in this area was mainly affected by the phytoplankton and exogenous organic debris in the water-body in the isthmuses of tributaries, rather than by endogenous phytoplankton in main river channel.

Table 1. Water temperature, pH, total nitrogen, total phosphorus, in the Pearl River between September 2016 and July 2017 (Mean±standard error)..

	TN (mg L^{-1})	TP (mg L^{-1})	WT (ºC)	pH
S1	6.761.37	0.800.61	25.303.46	7.570.47
S2	8.001.10	0.310.26	24.774.40	7.640.06
S3	8.491.29	0.740.65	25.333.87	7.340.04
S4	7.410.47	0.260.14	24.872.81	7.800.14
S5	7.261.58	0.440.22	24.054.22	7.140.10
S6	6.021.80	0.190.10	24.872.58	7.450.11
S7	6.271.53	0.340.21	24.502.36	7.580.04
S8	5.590.53	0.280.18	24.473.27	8.020.26
S9	4.300.64	0.280.13	24.473.27	7.320.48
S10	4.070.87	0.140.03	24.133.29	7.850.04
S11	2.660.19	0.130.06	24.60	7.400.45
S12	2.410.76	0.250.11	24.703.01	7.290.35
Mean	5.771.01	0.350.22	24.673.28	7.530.21

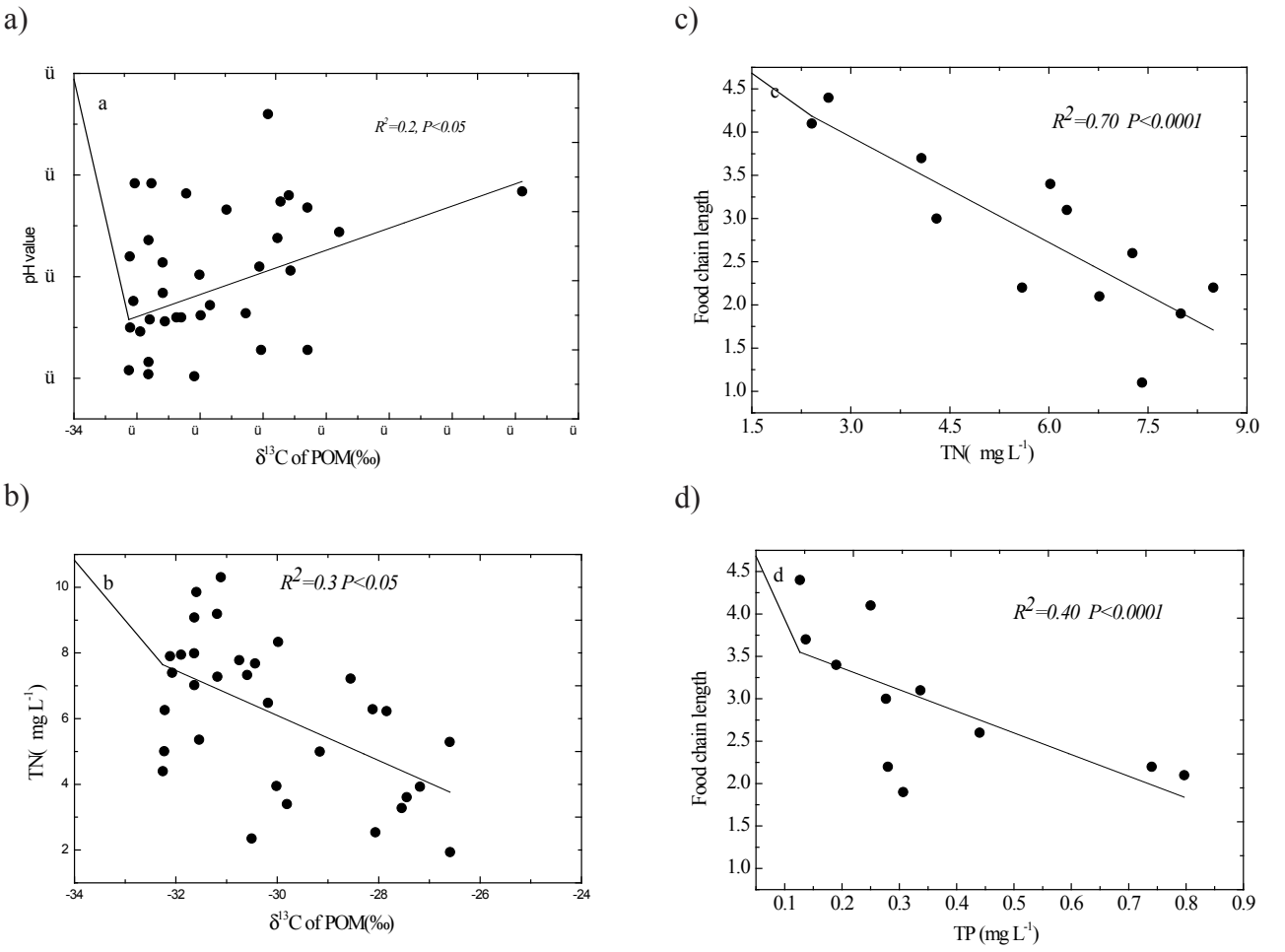

Fig. 1. Correlations between ^{13}C of particulate organic matter and pH, TN (a and b), and correlations between top consumer food chain length and TN, TP (c and d).

Variations of $\delta^{13}C$ and $\delta^{15}N$ in POM and Organisms in the Pearl River

The POM in the water is comprised mainly of fine sediment or organic debris. There were significant differences in POM $\delta^{13}C$ and $\delta^{15}N$ between Sept. 2016 and July 2017, the POM $\delta^{13}C$ value ranged from −32.3‰ to −19.7‰. The $\delta^{13}C$ values generally decreased and the increased from S1 to S12. The POM $\delta^{15}N$ value ranged from 4.2‰ to 13.8‰ (Supplement Fig. S2). Studies have shown that the $\delta^{13}C$ value in the aquatic ecosystem increases with the increase in consumer trophic level. Generally, $\delta^{13}C$ enrichment values increase by 0.05‰±0.063‰ with increases in $\delta^{13}C$ levels. However, most research has shown that the average increase in $\delta^{13}C$ enrichment value is 1‰–2.0‰, while the fractionation of $\delta^{15}N$ enrichment value is 3.2‰-3.4‰ [24]. There was evidence that the $\delta^{13}C$ value of POM in water was affected by pH, and the average $\delta^{13}C$ value of POM was reported to –31.4‰ [25]. The $\delta^{13}C$ value of POM was significantly higher at S9~S12 in Jan. 2017, while the spatial and temporal variations at other sampling sites were significantly lower in this study, our result suggested that the $\delta^{13}C$ and $\delta^{15}N$ values were significantly higher in fish consumers than in POM, and indicating that these components were the carbon sources for consumers.

In river ecosystems, periphyton is the main carbon source for consumers, [26], there were studies showing that the $\delta^{13}C$ values of periphyton are relatively low, ranging from −37.34‰ to −19.19‰ [27-29], and have noted that these values can be affected by other primary producers in the water along the rock belt [30-32]. The $\delta^{13}C$ value of periphyton is significantly related to their growth environment. The $\delta^{13}C$ value of periphyton growing on rocks (−18‰ to −10‰) is obviously affected by the calcium in the substrate [30-32]. The $\delta^{13}C$ value of periphyton increased with greater calcium contents in the contaminating substances. In this study, the range of $\delta^{13}C$ values in periphyton was −37.2‰ to −18.8‰, and the $\delta^{15}N$ value ranged from 0.9‰ to 25.4‰. The $\delta^{13}C$ values of periphyton were higher at S4 and S5 than at other sites in Jul. 2017 and Jan. 2017 (−22.0‰), The $\delta^{13}C$ values showed unimodal trend from S1 to S12 (Supplement Fig. S3). The results suggested that the $\delta^{13}C$ values of periphyton at S4 and S5 in Jul. 2017 and Jan. 2017 might be affected by contamination by calcium in the rocks they were growing on. The $\delta^{13}C$ values of periphyton in this study were similar to those reported by previous studies [27]. Previous studies demonstrate the importance of periphyton for shrimp growth, including postlarvae. Those studies showed that determined a 54% contribution of benthic organisms to the marine shrimp *F. paulensis*, and the microbial communities from the periphyton and shallow sediments contributed more in the treatment without a feed (50%) than in the treatment with feed supply (22.6%) [33-35]. In this study showed that the $\delta^{13}C$ values of periphyton was lower than the benthic organisms, and our results suggested that the periphyton in this area was among the most important carbon sources for benthic species (shrimps, crabs, snails, *L. lacustris*, *Corbicula fluminea*), consistent with the results of previous studies [33-37].

Phytoplankton is an important primary producer in water ecosystems, and a main food source. In previous studies, the reported range of $\delta^{13}C$ values of phytoplankton (Chrysophyta and Chlorophyta) was −37.2‰ to −23.6‰ [37-42]. These values are slightly different from that of zooplankton (−36.2‰), suggesting that phytoplankton are the main carbon source for zooplankton [29, 39, 43]. There was study showed that the higher $\delta^{13}C$ values of phytoplankton in eutrophic water (−28.45‰ to 23.63‰) are mainly due to the higher growth rate and abundance of Cyanophyta in warm, nitrogen-rich eutrophic water [44-45]. Cyanophyta accumulate in large amounts at the water surface, thus blocking the light to planktonic algae and large phytoplankton growing below. Therefore, the $\delta^{13}C$ value of phytoplankton is affected by the abundance of planktonic algae in the surface water. In this study, the $\delta^{13}C$ value of phytoplankton range form −34.8‰ to −25.7‰, the $\delta^{13}C$ value of phytoplankton was a regular and significant increase from Sept. 2016 to Jul. 2017. The $\delta^{15}N$ values range from 1.04‰ to 18.8‰. The $\delta^{15}N$ value of phytoplankton increased regularly from S1 to S12 (Supplement Fig. S4), which are intermediate compared with previously reported values. The main phytoplankton in the study area was *Granulodesma*, Bacillariophyta, *Microcystis,* and Cyanophyta. High nitrogen and phosphorus concentrations can increase the stock of Cyanophyta. The nitrogen and phosphorus contents were high in the study area, leading to abundant Cyanophyta in the water [46]. In the study, river water is washed into the open water area, thereby increasing the abundance of cyanobacteria in open water. The phytoplankton species composition and high nutrient concentrations in the water were key factors explaining differences between the phytoplankton $\delta^{13}C$ values in our study and those reported in other studies. Some studies shown that in lakes with low or high forest cover, the main carbon source for zooplankton was endogenous phytoplankton ($\delta^{13}C$ value less than −30.0‰), rather than exogenous phytoplankton ($\delta^{13}C$ value: −28.0‰±1.0‰). In this study, the $\delta^{13}C$ value of phytoplankton was significantly lower than −30.0‰ at S9 and S12 in Jan. 2017, while the $\delta^{13}C$ values of phytoplankton at other sampling points were around −28.0‰±1.0‰. The results showed that the main carbon source for planktonic invertebrates in this area was exogenous phytoplankton, which may have been introduced via stored water or drainage water. The $\delta^{13}C$ values of zooplankton ranged from −32.1‰ to −24.2‰, similar to the range of $\delta^{13}C$ values of phytoplankton. The $\delta^{13}C$ value of zooplankton was slightly lower than that of phytoplankton, but was lower than that of POM. This result indicated that phytoplankton and particulate matter were the main carbon sources for zooplankton, the primary consumers. It has been reported that large

aquatic plants are also a carbon source for zooplankton in lakes with high coverage of large aquatic plants [47-48]. In this study, the current stock of *Eichhornia crassipes* floating in the Pearl River in Guangzhou was relatively high, and there was little difference between $\delta^{13}C$ value of *Eichhornia crassipe* and zooplankton, which suggested that the *Eichhornia crassipes* floating in this area is also a source of carbon for zooplankton. The main reason for this is that rotten fragments of *E. crassipes* floating in the water are filtered by zooplankton, or the roots of floating *E. crassipes* are eaten by zooplankton. The abundance of *E. crassipes* floating in the Pearl River in Guangzhou was relatively high. The changes in $\delta^{13}C$ values were similar in *E. crassipes* and zooplankton, suggesting that *E. crassipes* was a carbon source for zooplankton. Zooplankton consumed rotten fragments or roots of *E. crassipes* floating in the water. The life cycle of zooplankton such as copepods is usually 1 month to 1 year. Studies have shown that the $\delta^{13}C$ value of zooplankton is significantly related to its species, body length, and life cycle. Generally, the $\delta^{13}C$ values are lower for larger zooplankton than for smaller ones, because larger zooplankton has a longer life cycle and a more complex pattern of food intake [44, 48]. In this study, the $\delta^{13}C$ value of zooplankton at S7 was higher in Sept. 2016 (−24.2‰) than at the other two sampling times. The biomass of small phytoplankton rotifers with a shorter life cycle is obviously greater than that of Cladocera and Copepoda with longer life cycles. The results of this study are similar to those of previous studies.

The main floating plant in the Pearl River was *Eichhornia crassipes*, and the main emergent plant is *Scirpus yagara*. The $\delta^{13}C$ and $\delta^{15}N$ values of higher plants clear spatial and temporal variation among sampling sites. The $\delta^{13}C$ values ranged from −31.5‰ to −13.9‰, and the $\delta^{15}N$ values from range 2.6‰ to 19.1‰ in higher plants (Supplement. Fig. S5). In this study, higher aquatic plants were represented by two species: the C_3 plant *E. crassipes*, and the C_4 plant *S. yagara*. The $\delta^{13}C$ values of *E. crassipes* and *S. yagara* ranged from −31.6‰ to −23.3‰ and from −19.6‰ to −13.9‰, respectively. The $\delta^{13}C$ values of *E. crassipes* were similar to those reported in other studies [37-38, 40-42]. The $\delta^{13}C$ values of *S. yagara* were lower than those reported in previous studies [37-38, 40-42], similar to those reported for higher aquatic plants [43, 49]. Eutrophication strongly affects the $\delta^{13}C$ value of higher aquatic plants. Higher plant roots floating in the water are convenient food for invertebrates [50-51]. Leady & Gottgens [52] and Carlsson & Bronmarck [53] found that the main carbon source for *Ampullaria gigas* was floating aquatic higher plants. In this study, *E. crassipes* was the carbon source for shrimp, snail, *L. lacustris* in the Pearl River. In the Pearl River, *E. crassipes* floats in open water and grows in the shallows along the banks. The main carbon sources for benthic organisms in the Pearl River were periphyton and higher aquatic plants.

Zooplankton is the most abundant and widely distributed aquatic organisms. The $\delta^{13}C$ values of zooplankton ranged from −32.1‰ to −24.2‰, and the differences of $\delta^{13}C$ values in zooplankton were relatively small in this study, there was evidence showed that the $\delta^{13}C$ values of zooplankton were range from −36.74‰ to 20.46‰, which was similar with previous studies [48]. The zooplankton $\delta^{13}C$ values showed obvious seasonal variations in a wave shape. The $\delta^{13}C$ value of zooplankton from S1 to S12 showed a single peak trend. The $\delta^{15}N$ value of zooplankton ranged from 2.9‰ to 21.4‰, the $\delta^{15}N$ value of zooplankton significantly increased between S1 and S12 (Supplement Fig. S6). In this study showed that carbon signature of the pelagic baseline tends towards less ^{13}C depleted values. this observation probably reflects changes in phytoplankton isotopic signature, phytoplankton exhibits $\delta^{13}C$ values less negative, because of reduced isotopic carbon fractionation at high cell densities and/or a shift on exploitation of HCO_3^- as carbon source instead of CO_2 [26]. The dominant benthic animals along the river edge were shrimps, crabs, snails, the mussel *Limnoperna lacustris* and *Corbicula fluminea*. The $\delta^{13}C$ and $\delta^{15}N$ values of the benthos showed clear spatial and temporal variations in this area. The $\delta^{13}C$ values of the benthos ranged from −29.9‰ to −22.5‰, the $\delta^{15}N$ values range from 1.4‰ to 17.9‰, the $\delta^{13}C$ value of shrimp significantly decreased in S1 to S12, the $\delta^{13}C$ value of crab were significantly higher at S4 (Supplement. Fig. S7). Fish are the highest aquatic organism in the aquatic ecosystems, we determined the values of $\delta^{13}C$ and $\delta^{15}N$ for 38 species was determined in the Pearl River. The results showed that the changes in $\delta^{13}C$ were relatively stable, while the changes in $\delta^{15}N$ were more obvious. The $\delta^{13}C$ values of fish range from −29.8‰ to −19.5‰ in Sept. 2016. The $\delta^{15}N$ values of fish range from 1.3‰ to 20.04‰ in Sept. 2016, and the $\delta^{15}N$ values tended to increased alomg the S1 to S12 (Fig. 2). The highest $\delta^{15}N$ value was in *Parabramis pekinensis* at S12. The $\delta^{15}N$ values of *Ctenopharyngodon idellus*, *Tilapia zillii*, *Hypostomus plecostomus*, *Coilia grayi* and *Odontamblyopus rubicundus* increased regularly (Fig. 2). The $\delta^{13}C$ values of fish in the Pearl River ranged from −28.7‰ to −19.4‰ in Jan. 2017. The $\delta^{15}N$ values in fish range from 4.1‰–21.3‰ in Jan. 2017, the spatial distribution characteristics of $\delta^{15}N$ value of the fish were similar in Jan. 2017 and in Sept. 2016, and both increased regularly from S1 to S12, the $\delta^{15}N$ values of fish were generally higher at S11 (Fig. 3). The $\delta^{13}C$ value of fish range from −41.2‰ to −20.0‰ in Jul. 2017. The $\delta^{15}N$ values in fish range from 0.81‰–16.6‰ in Jul. 2017 (Fig. 4).

Many studies have shown that fish have four main food sources: plants, debris or algae, plants/animals, and animals. We found that the $\delta^{13}C$ values of fish were significantly higher than those of POM, phytoplankton, higher aquatic plants, and zooplankton, except for the $\delta^{13}C$ value of *H. plecostomus* in Jul. 2017, which was significantly lower (−41.2‰). The results showed

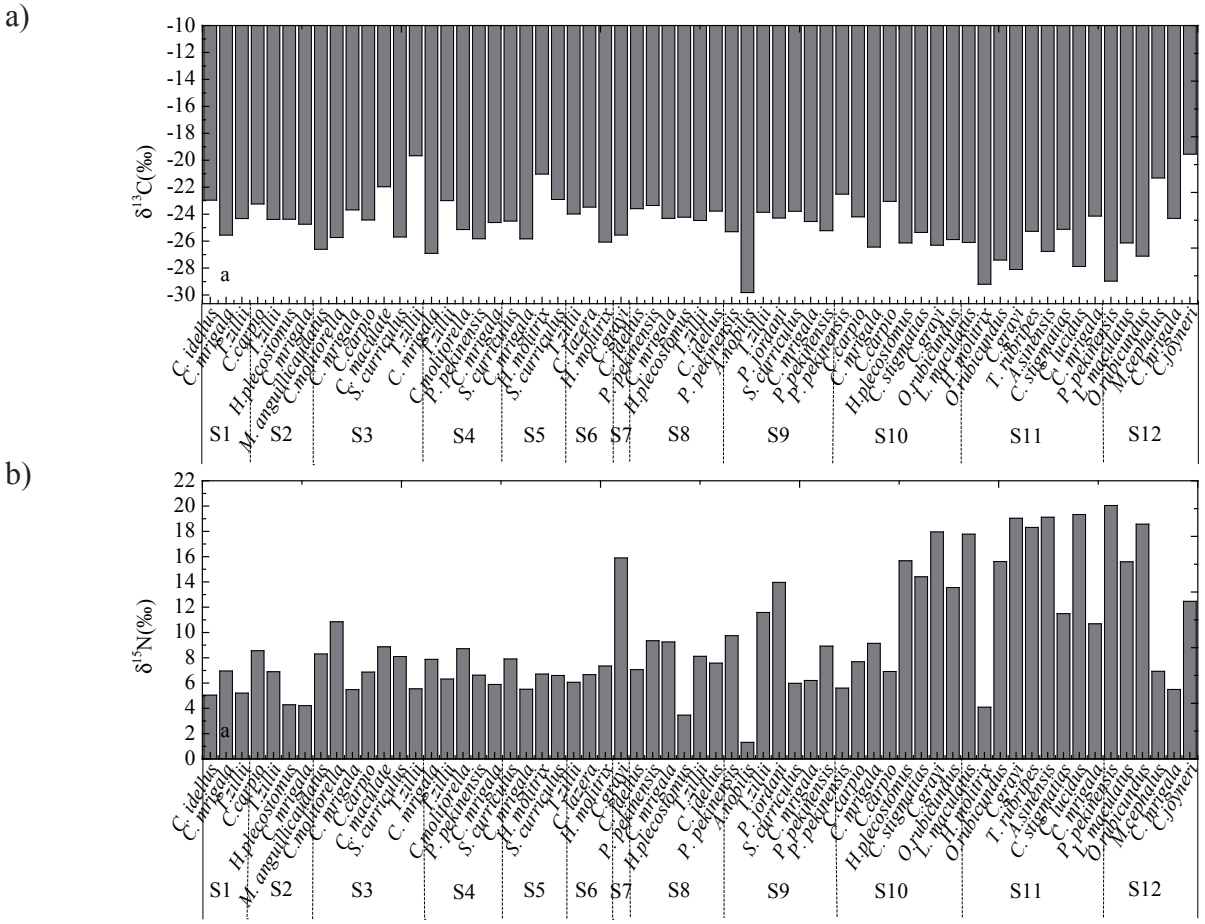

Fig. 2.Variations of ^{13}C a) and ^{15}N b) in different fish species collected from Pearl River between Guongzhou and Humen in Sept. 2016 (S1-S12: sampling sites see Fig. S1).

that the carbon source for *H. plecostomus* was materials other than POM, phytoplankton, higher aquatic plants, zooplankton, and benthos, as the $\delta^{13}C$ values of these five sources were much higher than the value of −41.2‰ detected for *H. plecostomus*. A previous study reported that the $\delta^{13}C$ value of the water lily *Nymphaea* sp. was very low (−41.3‰). The results suggested that *H. plecostomus* feed on *Nymphaea* sp. in Jul. 2017. The carbon source of *H. plecostomus* may have been exogenous to S4 and deposited at the bottom of the river.

However, non-native individuals of *H. plecostomus* migrated from other points to S4, so it is possible that artificially released fish entered the sampling point. Further research on this topic could include analyses of fish swimming ability, migration distance, living area difference, life cycle length, and feeding habits. This species was selected to monitor the source and direction of pollution sources in surface water by means of capture, release, and recapture. As shown in Fig.5, the feeding behavior of fish was complex, and they did not simply ingest any carbon source. In this study, there were 38 main species of fish in the studied, and their carbon sources were POM, periphyton, phytoplankton, higher aquatic plants, zooplankton, and benthic organisms. Some studies have shown that *Tilapia* is mainly herbivore: its main food sources are reported to be algae and plant-based substances in water, and sometimes zooplankton [54-55]. However, Fetahi et al. [8] detected a significant relationship between the carbon source and body length of *Tilapia*. On the basis of analyses of intestinal contents, they reported that the main carbon sources of *Tilapia* < 6 cm were fixed organisms and planktonic algae, while that of larger individuals was aquatic plants.

The results of Adámek and Mareš [56] and Chapman and Fernando [57] were consistent with those of Fetahi et al. [8]. Other studies found that the carbon sources of *Tilapia* in eutrophic lakes were phytoplankton and zooplankton. In a lake with low nutrient levels, the carbon source of *Tilapia* was POM, because the plankton stock was very low [58-59]. The main food of *Tilapia* is large aquatic higher plants, if they are available. Researchers have found that aquatic higher plants increase in biomass as the phytoplankton biomass decreases. Comparisons of $\delta^{13}C$ values among *Tilapia* (−23.5‰ to −21.56‰), POM (−23.71‰), and zooplankton (−20.13‰) showed that POM, zooplankton, and aquatic higher plants were the main carbon sources for *Tilapia*, and that aquatic higher plants contributed

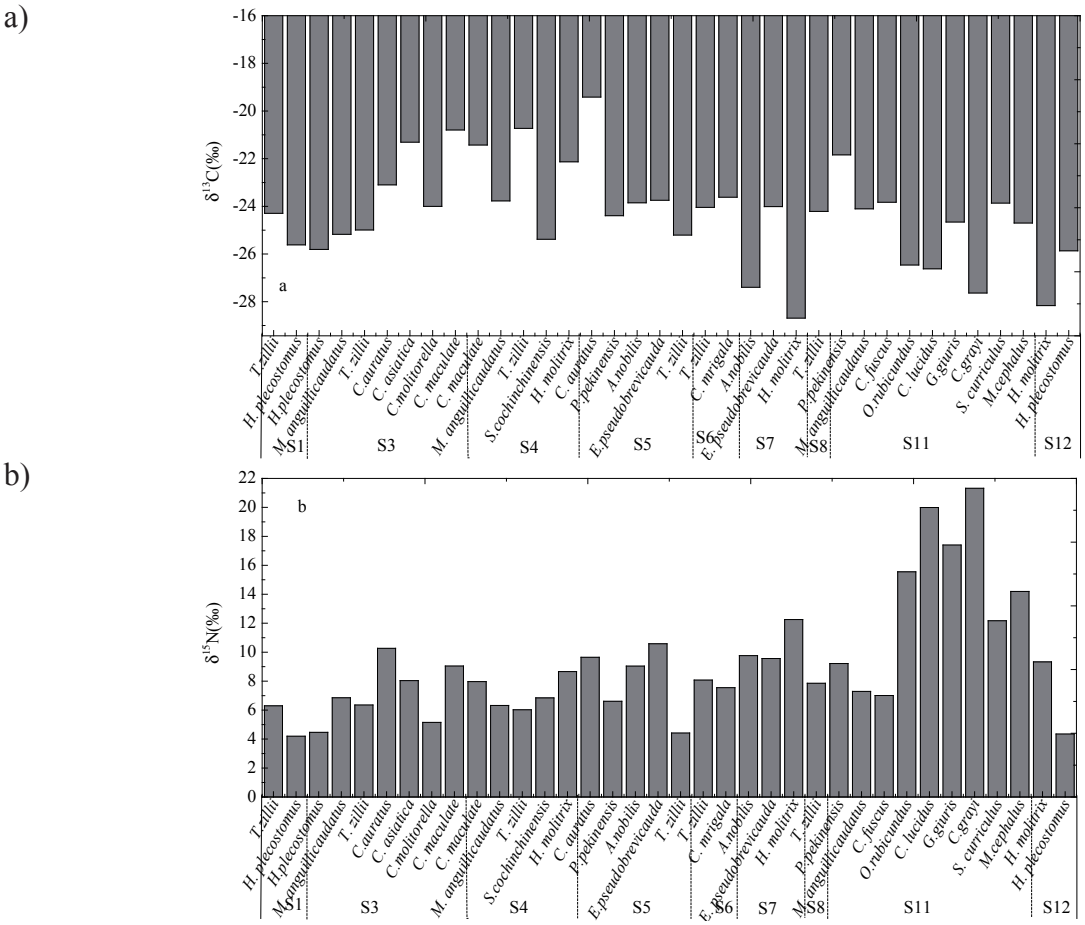

Fig. 3.Variations of ^{13}C a) and ^{15}N b) in different fish species collected from Pearl River between Guongzhou and Humen Jan. 2017 (S1-S12: sampling sites see Fig. S1).

significantly (56%) [8]. In this study, *Tilapia* was distributed throughout the study area. According to the enrichment characteristics of $\delta^{13}C$ in fish, *Tilapia* at the same sampling sites and season tended to share the same carbon sources as other fish, such as in Sept. 2016, the carbon sources for *C. idellus*, *P. pekinensi*s, *C. mrigala*, *H. Plecostomus,* and *Tilapia* were periphyton and benthic organisms at S8, and the carbon source for *A. nobili*s, tilapia, *P. jordani*, *S. curriculus* and *C. mrigala* was periphyton at S9 in July 2017. Carbon is very important to organisms in natural ecosystems. Generally, the change of relative abundance of isotopes was little from primary producers to consumers in ecosystem, and the average accumulation is 0.10‰-1.0‰, this result was used to analyze consumer sources [60]. Fig. 5 illustrates the carbon sources and TL of primary producers and consumers in the ecosystem.

The average $\delta^{13}C$ values at S1 to S12 were as follows: primary producers (POM, −29.6‰; periphyton, −26.5‰; phytoplankton, −28.3‰; and higher aquatic plants, −29.1‰; zooplankton, −29.4‰; benthonic organism −26.4‰. The average $\delta^{13}C$ value of fish was −24.91‰ in this area. According to the fluctuations of $\delta^{13}C$ values of fish and the $\delta^{13}C$ values of food accumulation characteristics in the range of 1‰-2‰, the $\delta^{13}C$ value of fish mainly appear at the right in Fig. 5.

The $\delta^{13}C$ values of fish were significantly higher than that those of primary organisms (organic particles, periphyton, phytoplankton and higher aquatic plants), zooplankton and benthic organisms, the carbon sources for fish, were POM, periphyton, phytoplankton and higher aquatic plants, zooplankton and benthic organisms (Fig. 5). Fig.5 shows that the carbon sources for some fish species were similar among seasons and sampling sites, such as, carbon sources of *Clupanodon thrissa* were both associated with POM in September 2016 (Fig. 5a), periphyton was the main carbon sources for *Odontamblyopus rubicundus* and *Chaeturichthys stigmatias* in Sept. 2016 and Jul. 2017 (Fig. 5a and 5c), and periphyton was the carbon sources for *Parabramis pekinensis*, benthic organisms were the carbon source for *Cyprinus carpio* at all samping sites and in all seasons. The carbon sources for *Coilia grayi* were zooplankton and benthic organisms, and those for *Tilapia zillii* were related to benthic organisms and periphyton, the carbon source for *Cirrhinus molitorella* were periphyton, phytoplankton and benthic organisms, the carbon sources for *Hypostomus plecostomus* was benthic organisms at all sampling sites. The carbon

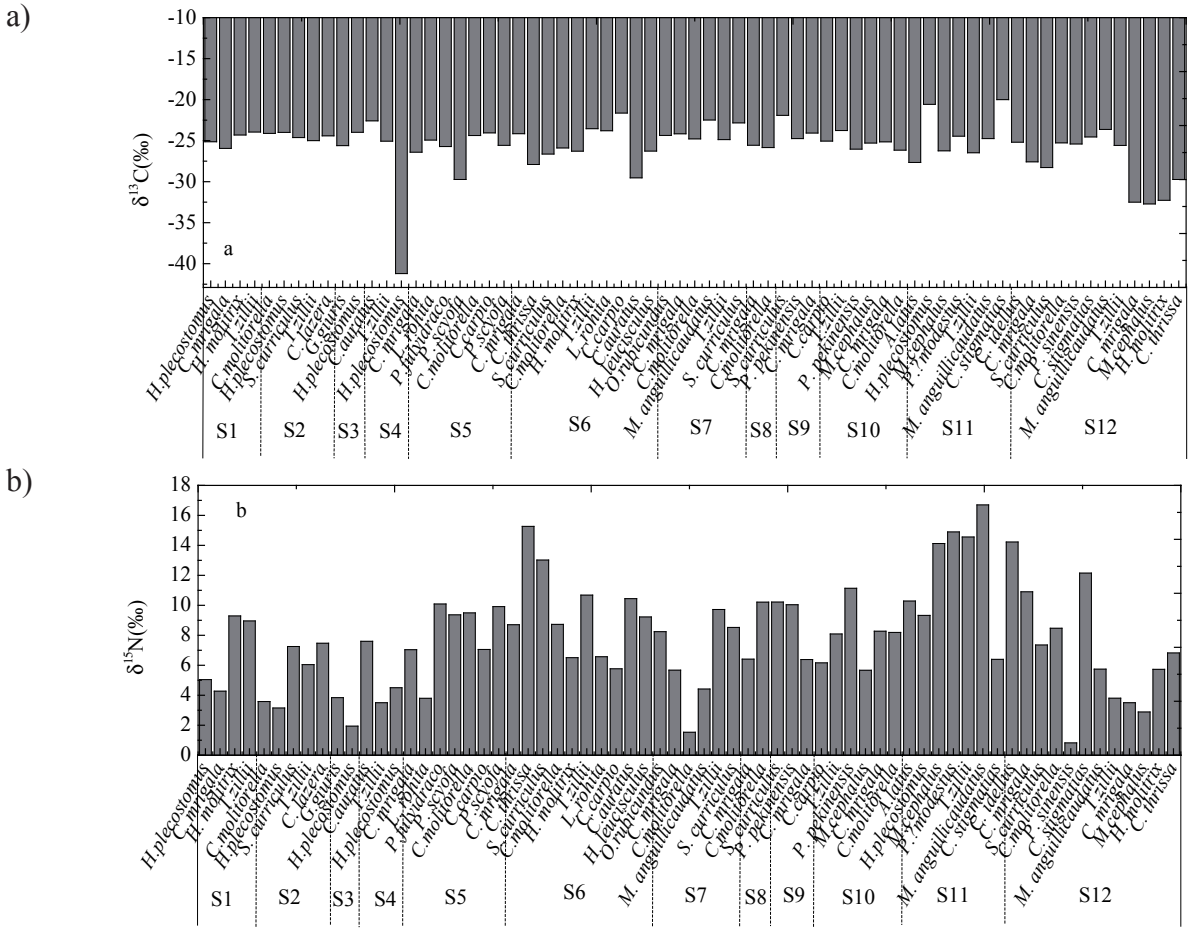

Fig. 4.Variations of ^{13}C a) and ^{15}N b) in different fish species collected from Pearl River between Guongzhou and Humen Jul. 2017 (S1-S12: sampling sites see Fig. S1).

source for *C. mrigala* was periphyton and benthic organisms (Fig. 5).

Consumer Food-Web Structure and Trophic Level

Stable isotope analysis is an important method to study the relationships within consumer food web [24]. The $\delta^{15}N$ value of consumer is 3.4±1.1‰ higher than that of their food [1-7]. According to this characteristic, we studied the food chain and its nutritional level characteristics in the aquatic ecosystem in the Pearl River. The results showed that the aquatic ecosystem food chain was mainly composed of the phytoplankton food chain and the detritus food chain, the higher aquatic plant food chain and the epiphyte food chain. The top fish were mainly the filter-feeding *Hypophthalmichthys molitrix*, omnivorous tilapia, *Cyprinus carpio*, *C. mrigala*, *Carassius auratus*, *Cirrhinus molitorella*, *P. jordani*; the carnivorous species *Erythroculter pseudobrevicauda*, *Coilia grayi*, *Collichthys lucidus*; and the herbivorous *Parabramis pekinensi*s, and the warm-water species closest to the shore. The TL for sites S1 to S12 were 2.1, 1.9, 2.2, 1.1, 2.6, 3.4, 3.1, 2.2, 3.0, 3.7, 4.4 and 4.1, respectively. Thus, the TLs of the ecosystem gradually increased from S1 to S12 in this study, the results showed that the aquatic ecosystem in this area was complex. Analysis of $\delta^{15}N$ values is the most common method to study the food network and TL in water ecosystems [61]. Previous studies have found that the $\delta^{15}N$ value of a predator will be 2.4‰-4.4‰ higher than that of its prey, with an average value of 3.4‰ [1-7]. There was evidence showing that the TL of POM (phytoplankton) is set to one, the structure of the food web in the system is usually at four TL. Some studies have also used $\delta^{15}N$ values to establish a baseline for primary consumers (TL2) [61]. In this study, we found that the food-chain length from TL1 to the top fish in this water ecosystem was 4.4, significantly lower than those in marine ecosystems (TL5-6) [10], and similar to the reported for estuary ecosystems (TL4.5) [11-12]. Some researchers found that phytoplankton (Bacillariophyta) are the main food providers for top organisms in marine ecosystems, thus making their food chains longer [62]. In this study, the dominant phytoplankton species studied in water were *Melosira granulate* spp and *Cyclotella* spp in the Bacillariophyta; these are important primary producers, and our results are relatively similar to those of previous studies. Other research has shown that the food chain

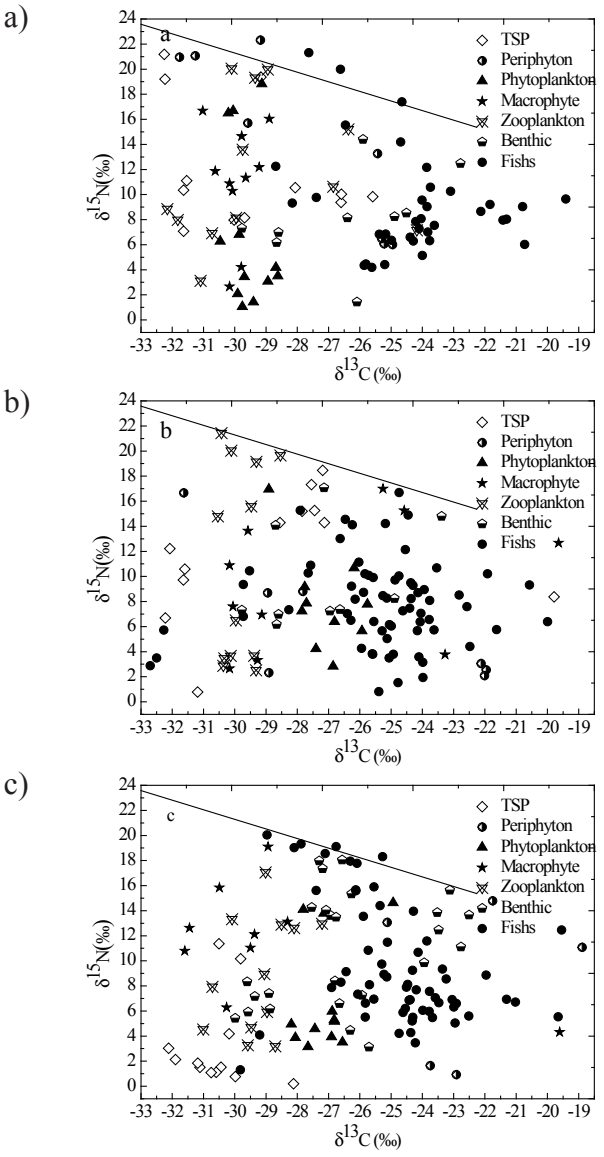

Fig. 5. Carbons source and trophic levels of primary producers and consumers in Pearl River between Guongzhou and Humen in Sept. 2016 to July. 2017.

is longer when the amount of nutrient exchange is larger [13]. Nutrient transfer in this water ecosystem is relatively sufficient. Hoeinghaus et al. [13] reported that the TL in the aquatic ecosystem of the Paraná River Basin, Brazil, was 4.0-4.5, with a significance of 0.35. Vander Zanden and Fetzer [61] reported that the length difference of a food chain was 0.5. In this study, the difference in the food-chain length in this water section was 3.3, which indicated that there were large differences in the food network in this area. Differences of food-chain length were greater in larger water ecosystems than in smaller ones, mainly because there were more carnivorous fish, such as salmon, in larger water ecosystems [61-62]. In this study, carnivorous fish like *E. pseudobrevicauda*, *C. grayi,* and *C. lucidus* were dominant species. The main reason for smaller differences in food-chain length was found to be the larger size of omnivorous fish in water ecosystems [63]. In this study, the TL differences were significantly smaller at S1, S2, S3, S5, and S8 (range: 0.1-0.7) than at other sites. The omnivorous fish *Tilapia*, *C. carpio*, *C. mrigala*, *C. molitorella*, and *C. auratus* were the dominant species at those sampling points. A previous study similarly showed that large catches of fish and the introduction of fishes were main contributors to changes in TLs in aquatic ecosystems [13]. The same study found that water temperature significantly affected the length of the food chain, but energy gain did not [13]. The researchers proposed that water temperature and climate indirectly affect food-chain length by affecting the stock of primary producers in the water. However, we did not detect a significant correlation between food-chain length and water temperature in our study area. The water temperature of the Pearl River is relatively low (average, 24.6ºC) and was not related to the abundance of primary producers. Therefore, the results suggested the food-chain length in this section was not affected by the small seasonal differences in water temperature. We found that POM content (average dry weight: 0.034 mg L^{-1}) was negatively correlated with the abundance of phytoplankton, the primary food source for the aquatic ecosystem. The TN and TP concentrations were high in this section of the river, and were negatively correlated with the length of the food chain in the water ($R^2 = 0.70$, $P<0.05$; $R^2 = 0.40$, $P<0.05$) (Fig. 1c, d). High nutrient concentrations may promote the growth and reproduction of Cyanophyta and weaken the ecosystem via their effects on primary producers, thereby directly affecting the feeding behavior of primary consumers. Alternatively, high nutrient concentrations might indirectly change the feeding behavior of top consumers, thereby affecting the nutrient level structure in the water ecosystem. The food network at S8 had a simple structure and short trophic level, which was obviously greatly affected by variations in the environment.

Conclusion

This study showed the $\delta^{13}C$ and $\delta^{15}N$ concentrations ranged from −41.2‰ to −19.4‰ and from 0.81‰ to 25.4‰, respectively. The $\delta^{13}C$ concentrations in consumers were significantly higher than the particulate organic matter (POM), periphyton, phytoplankton, and higher aquatic plants. The $\delta^{13}C$ of phytoplankton was derived from eutrophic water with high nitrogen and phosphorus concentrations. Total $\delta^{13}C$ was significantly higher in fish than in POM, phytoplankton, higher aquatic plants, and zooplankton, indicating that those components were the main carbon sources for fish. The carbon sources tended to be the same for different fish species in the same season at the same site, but different for a given fish species among seasons and sampling sites. This finding suggested that the feeding habits of different fish species converge as an adaptation

to changes in environment. The food chain was longer (trophic level = 4.4) in river subsections with more carnivorous fish, such as *Erythroculter*, *pseudobrevicauda* and *Coilia grayii*, and shorter in areas with more omnivorous fish. The total nitrogen and total phosphorus concentrations in the water were negatively correlated with food-chain length (R^2 = 0.67, P<0.05; R^2 = 0.40, P<0.05). These results suggested that limiting nitrogen and phosphorus inputs into the water body would reduce the ecological risk in this area.

Author Contributions

G. X. and L. S. designed and wrote the research; G. X., L. S. and A. D. performed the experiments and data analysis.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interest

The authors declare that they have no competing interests.

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Supplementary Material

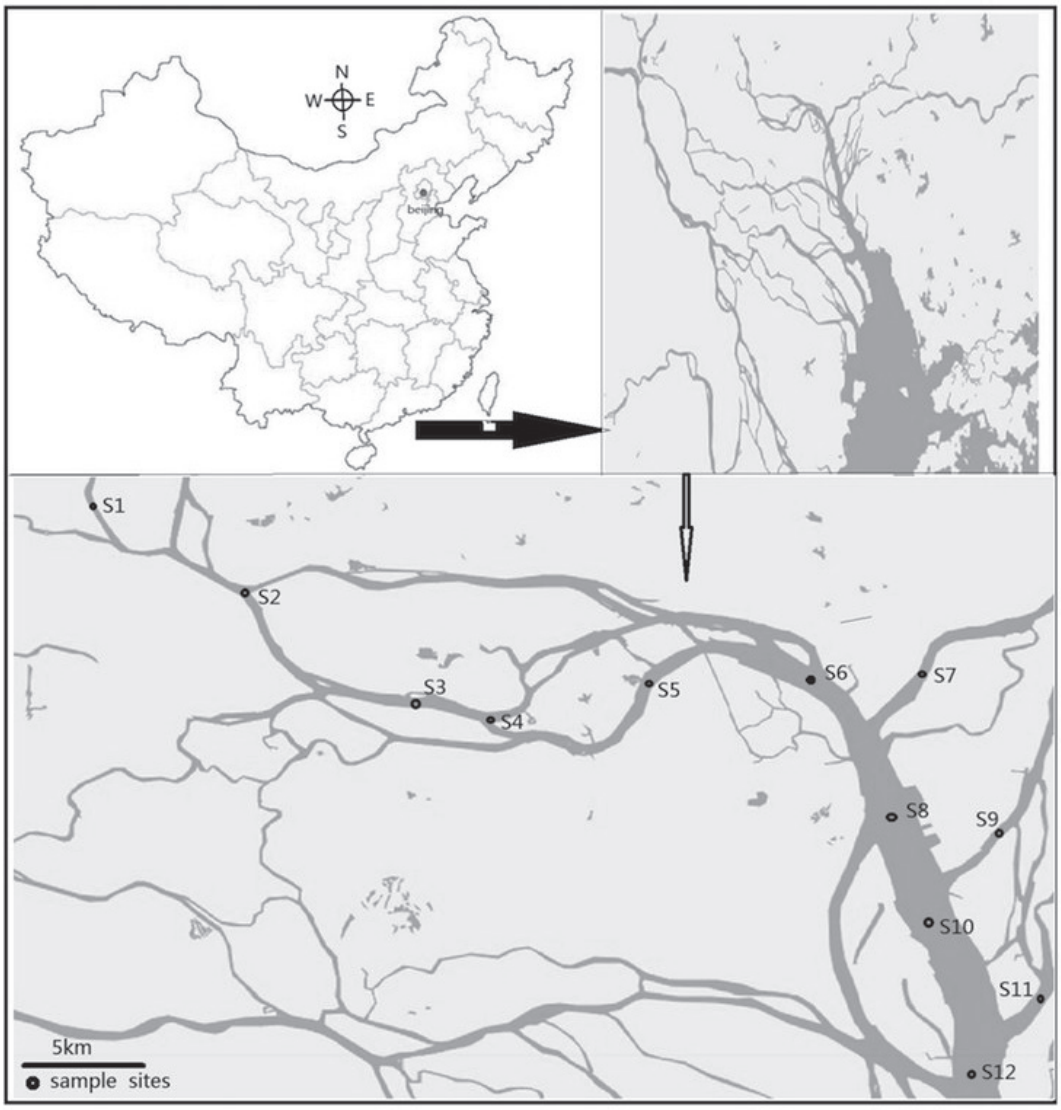

Fig. S1. Location of the sampling sites along Pearl River in Guongdong Province, P.R. China.

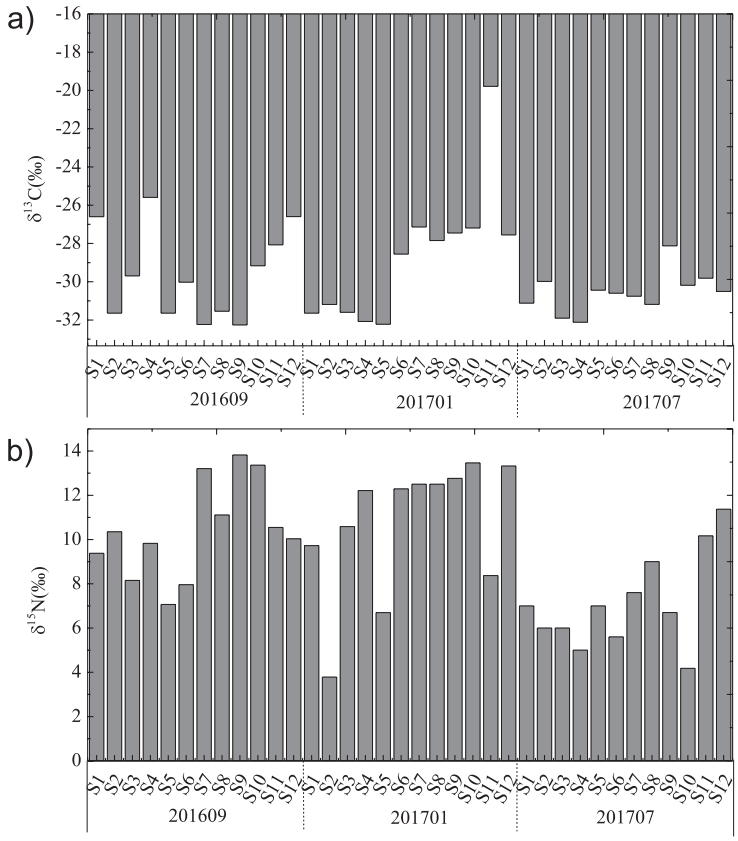

Fig. S2. Variations of ^{13}C a) and ^{15}N b) in suspended particulate organic matter (POM)collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).

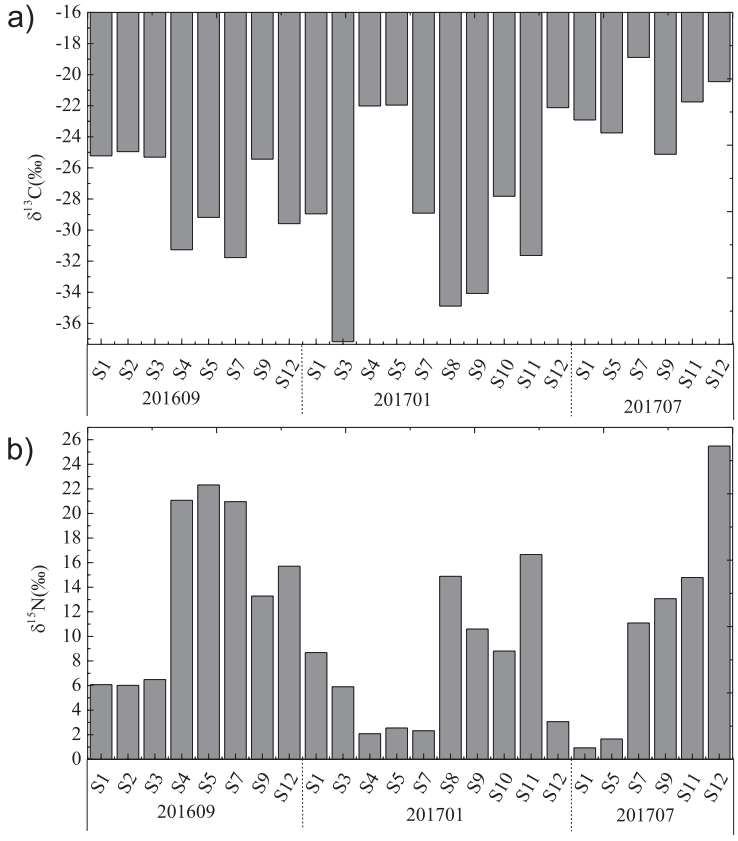

Fig. S3. Variations of ^{13}C a) and ^{15}N b) in periphyton collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).

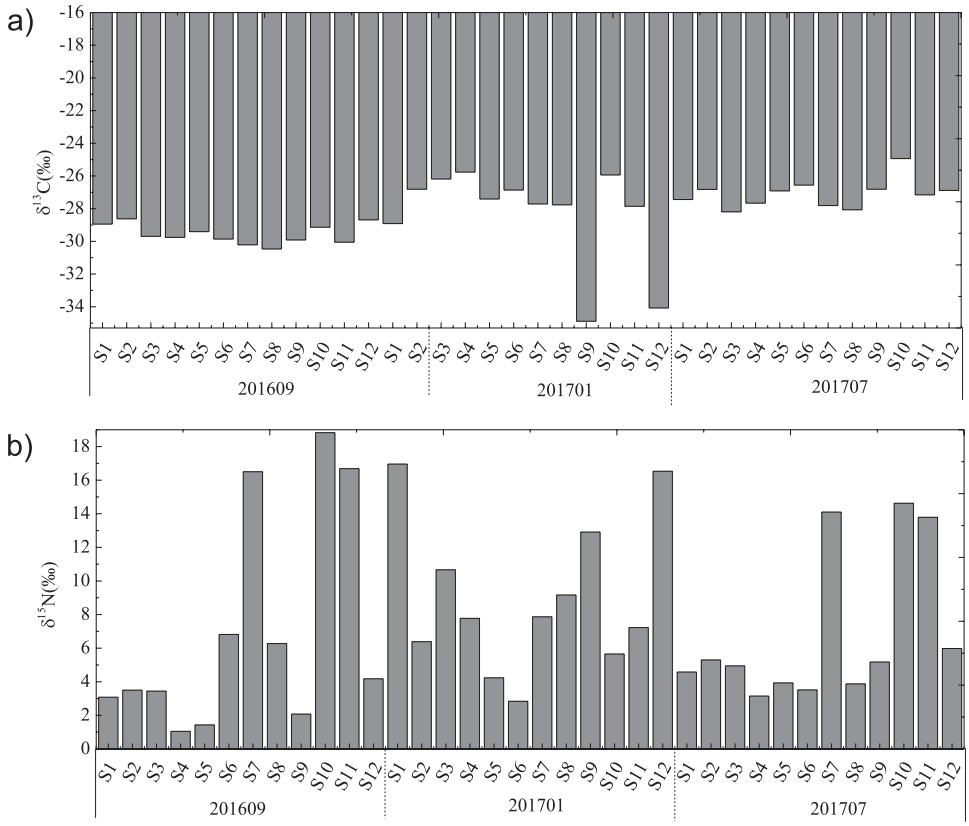

Fig. S4. Variations of ^{13}C a) and ^{15}N b) in phytoplankton collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).

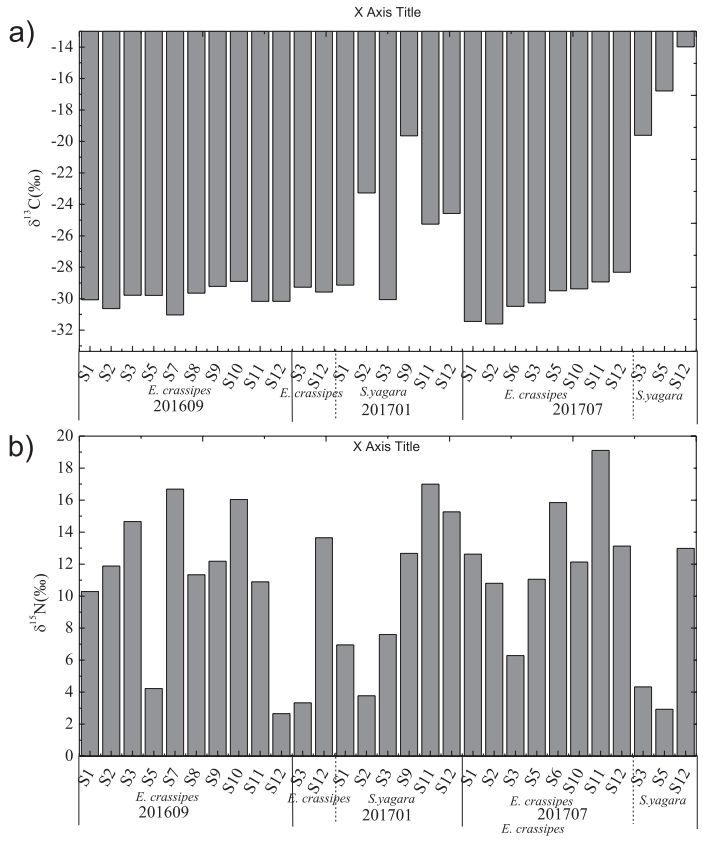

Fig. S5. Variations of ^{13}C a) and ^{15}N b) in macrophytes collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).

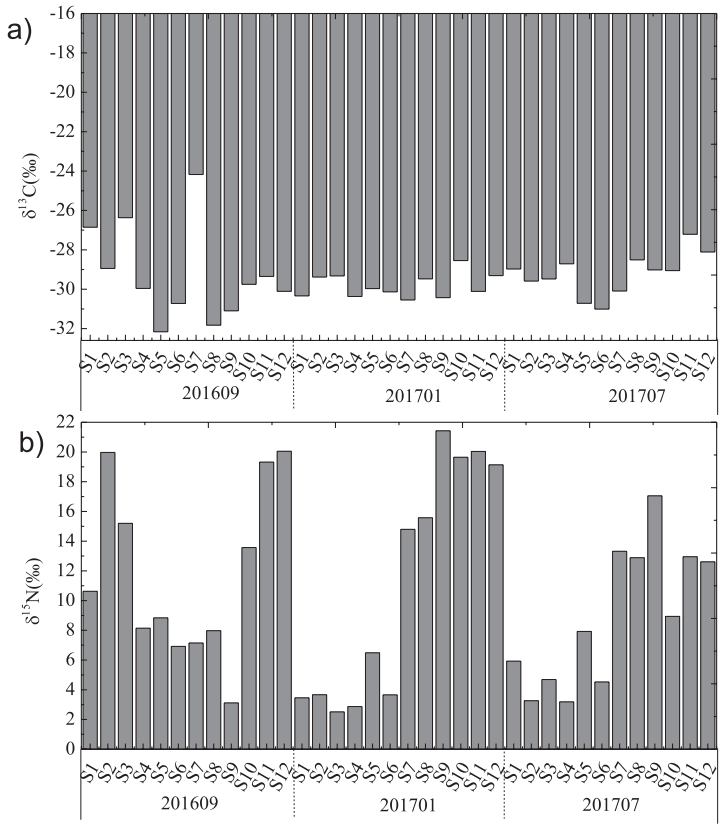

Fig. S6. Variations of ^{13}C a) and ^{15}N b) in zooplankton collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).

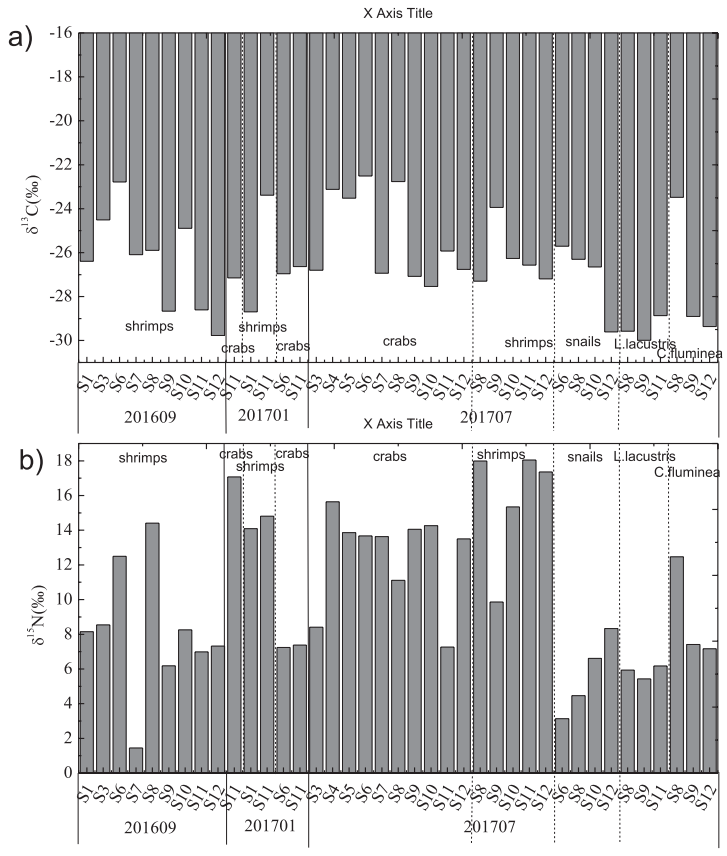

Fig. S7. Variations of ^{13}C a) and ^{15}N b) in benthic organisms collected from Pearl River between Guongzhou and Humen (S1-S12: sampling sites see Fig. S1).