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Stable isotopes reveal food web reliance on different carbon sources in a subtropical watershed in South China

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A B S T R A C T

Fish species in the Pearl River Watershed (PRW), the largest subtropical watershed in southern China, have declined dramatically in recent decades. To protect river habitats and maintain the integrity of river ecosystems, we need to know how different carbon sources contribute to aquatic food webs. We used information about stable carbon and nitrogen isotopes and IsoSource software to investigate the relative contributions of different carbon sources to the food web of the PRW. We found that the stable signatures of C_3 plants (C_3P), particulate organic matter (POM), submersed water grasses (SWG), and C_4 plants (C_4P) were significantly different and fell into four distinct groups. Of these, C_3P was a high and stable source of carbon to certain consumers, while C_4P was a low but stable carbon source. In comparison, the contributions from the POM and SWG groups were unstable and varied widely, between the 1st and 99th confidence intervals. The results suggest that the C_3P, POM, and SWG groups might be important carbon sources for the PRW food web. Analysis of the 99th confidence interval contribution of each group that provided more than 50% showed that consumers in the PRW food web might be divided into those that mainly relied on C_3P, those that mainly relied on SWG, those that potentially relied on C_3P-SWG, and those that potentially relied on POM-SWG. This study provides basic information that will help support the protection and management of the PRW ecosystem, and will be especially useful to help restore submersed macrophyte beds and riparian buffer strips in this watershed.

1. Introduction

Rivers are considered the cradle of human society and economic development. However, because of excessive exploitation and abuse, river water quality has deteriorated and river habitats are increasingly fragmented, with the result that river ecosystem functions are in decline worldwide (Nilsson et al., 2005; Azrina et al., 2006; Miyazono and Taylor, 2013). We therefore need to have information about the contribution of various sources of carbon that contribute to aquatic food webs to protect river habitats and maintain the integrity of river ecosystems. Three conceptual models can be used to describe the contributions of various carbon sources to river food webs. The river continuum concept holds that the majority of organic sources that support large river food webs originate from terrestrial plants in the headwater and middle reaches, while in-stream primary production is more important in the lower reaches of the river, where it may be limited by turbidity and light attenuation as depth increases (Vannote et al., 1980). The flood pulse concept proposes that lateral river floodplain exchanges, rather than organic matter subsidies from upstream, are the main source of carbon for the food web (Junk et al., 1989). The riverine productivity model highlights the importance of local autochthonous production, such as phytoplankton, benthic algae, water grasses, and direct organic inputs from the riparian zone (Thorp and Delong, 1994; Thorp et al., 1998). In recent decades, numerous studies have demonstrated that the contributions of carbon sources to food webs depend on a range of factors including hydrological conditions, latitude, habitat characteristics, and human activities (Caraco et al., 2010; Babler et al., 2011; Kaymak et al., 2015; Delong and Thoms, 2016) and that the contributions of various carbon sources may differ significantly between river systems (Pingram et al., 2012).

The Pearl River Watershed (PRW) is the largest watershed in southern China. Historically, the PRW has been known for its wide range of habitats that were home to more than 380 species of fish and other macroinvertebrates needed to support fish reproduction, growth, and fattening. In the early 1980s, the number of fish species in the lower reaches of the PRW had declined to 161 (Lu, 1990). However, in recent years, the number of fish species has declined further and now there are only 10 main species, the biomass of which accounts for 90% of the total fishing catches (Li et al., 2010). With these dramatic declines in fish species in the past 20 years, many species endemic to the

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PRW have gone extinct, and aquatic ecosystem function has decreased. The declines are most likely attributable to water pollution, and the high degree of river modification through dredging, channelization, and the construction of extensive levee systems that disrupt fish habitats and natural connections between the river and its floodplains (Li et al., 2010). Given the severity of the situation, we urgently need information about the contributions of different carbon sources and habitats to the food web of the PRW, as this information can be used to develop strategies to ensure fishery sources are protected, and that aquatic ecosystem function is maintained.

Stable isotopes have been frequently used to improve our understanding of aquatic food webs and aquatic ecosystems (Vander Zanden et al., 1999; Gu et al., 2011; Saigo et al., 2015), and, in particular, to support quantitative evaluations of the contributions of different carbon sources to various consumers in food webs (Zeug and Winemiller, 2008; Nyunja et al., 2009; Giarrizzo et al., 2011; Tue et al., 2014). However, because of a lack of studies in recent years, we have very little information about the stable carbon and nitrogen signatures in the PRW (Wei et al., 2008; Zhang and Ran, 2014). Statistical modules or packages, such as IsoSource software and Bayesian frameworks, that can be used to analyze the contributions of different carbon sources to consumers based on stable isotopes (Benstead et al., 2006; Cole and Solomon, 2012; Wang et al., 2014a) are increasingly available, though these different methods should be evaluated for their advantages and disadvantages before application (Moore and Semmens, 2008; Parnell et al., 2010; Fry, 2013).

The aim of the present study was therefore to estimate the contribution of potential carbon sources to the main consumers in the PRW food web using information about stable carbon and nitrogen isotopes and IsoSource software. From this study we hope to collect basic data that will support the management, protection, and restoration of the PRW and the maintenance of ecological function; we also hope that our data will supplement existing information on carbon fluxes in river systems worldwide.

2. Material and methods

2.1. Study area

The PRW drains an area of 442,100 km^2 and is the largest watershed in southern China. The Pearl River has an annual runoff of about 300 billion m^3 and an annual average flow of 11,000 m^3 per second. It has three main tributaries, the West River (Xijiang River), the North River (Beijiang River), and the East River (Dongjiang River). The present study was conducted in the lower reaches of the West and North Rivers (Fig. 1), where the main tributaries are anastomosing by forked channels of various widths. The lower reaches of the PRW, the key habitat for the fattening and growth of many riverine fishes and macroinvertebrates, are characterized by these linked channels. The PRW has a subtropical monsoon climate, with frequent light rain through spring, wet hot summers, and frequent typhoons in autumn and dry winters. The annual average water temperature ranges from 20 °C to 22 °C (Yan, 1989; Lu, 1990). Water pollution and engineering works, such as river dredging, channel expansion, and the construction of revetments and dams, have resulted in dramatic changes in the aquatic environment, habitats, and communities of the PRW, particularly in the spawning grounds of aquatic species (Li et al., 2010; Tan et al., 2012).

We established four sampling sites in the linked channel that flows through Xiaotang, Beijiao, Zuotan, and Waihai Counties (Fig. 1). The sampling sites had sandy sediment beds and habitats that were representative of the conditions in the lower reaches of the Pearl River. Beds of submersed macrophytes were dominated by, among others, *Vallisneria denseserrulata*, *Najas marina*, and *Hydrilla varticillata*, and riparian vegetation was made up of C_3 plants dominated by *Polygonim trigonocarpum* and *Callitriche palustris*, and C_4 plants dominated by *Cyperus rotundus* and *Hemarthria compressa*. There was very little woody debris on the river banks. More than 90% of the study watershed was wide-open-water channel, and less than 10% was submersed macrophyte beds and riparian buffer strip. The riparian buffer strips were usually on the sides of the river where the water was up to 1 m deep, and submersed macrophyte beds were usually on the sides of the river where the water was up to 3 m deep. Water deeper than 3 m was generally part of the wide water channel area, and ranged from 3 m to more than 10 m deep.

2.2. Sample collection and analysis

We collected samples of potential sources of organic carbon and of the main fish and macro-invertebrates present at the four sampling sites in March and August of 2015. The potential sources of organic carbon included particulate organic matter (POM) from the main stem of the wide-open water area; submersed water grasses (SWG) including *V. denseserrulata*, *N. marina*, *H. varticillata*, *Ceratophyllum demersum*, and *Potamogeton crispus* from the submersed macrophyte beds; emerged C_3 plants, such as *P. trigonocarpum*, *C. palustris*, *Oenanthe sinense*, and *Gratiola japonica*, and C_4 plants, such as *C. rotundus*, *H. compressa* and *Carex biuensis* from the riparian buffer strip.

Surface water was filtered through coarse sieves (100 μm) and then was further filtered onto Whatman GF/F glass fiber filters (0.7 μm) that had been pre-combusted at 450 °C for 5 h to collect the fine POM. Using this method we collected POM that mainly comprised planktonic algae (Wang et al., 2014a); our POM samples were made up mostly of diatoms, which dominate POM in the Pearl River (Wang et al., 2014b, 2016). The filters with the retained POM were acidified with hydrochloric acid vapor in a dryer system for 24 h to remove any carbonate, and then the filtered samples were dried at 55 °C and kept in a dryer until stable isotope analysis. The submersed macrophyte beds were usually under water that was between 0.5 and 1.5 m deep, so we hired a local fisherman to collect water grass samples from between three and five sampling frames at each (each with an area of 0.25 m^2), which were then combined into one sample each sampling site. The water grasses were classified and the dominant species were rinsed, packed separately, stored in a portable refrigerator, and transported back to the laboratory. In the laboratory, the algae or seston attached to the leaf were scraped off the water grasses, and then the grasses were rinsed and dried at 55 °C. The dominant C_3 and C_4 plant species were identified and collected from riparian buffer strips around each site and transported back to the laboratory, where they were treated in the same way as the SWG samples. The individual species collected at each site were combined into a single sample and ground, and then passed through a 60-mesh screen before stable isotope analysis.

During the first investigation in March, the dry season with relative high biomass of benthic algae and epiphytes to wet season of August, we scrapped the benthic algae and the epiphytes from the stones or SWG, and treated them in the same way as POM. Their stable carbon and nitrogen isotope values were in the same range as those of SWG and their biomass was negligible relative to the biomass of SWG. Given that the stable isotope values of benthic algae, epiphytes, and SWG overlapped, we just collected SWG samples during the second sampling in August 2015. We did not collect the coarse POM or benthic detritus as they may have been complex mixtures of algae, C_3P, C_4P, and submersed water grasses as previous study demonstrated (Wang et al., 2014a), and may have caused confusion in the IsoSource analysis.

Most of the fish and macroinvertebrates species were bought directly from local fishermen on their boats, who caught them around the sampling sites by various methods including seine nets, gillnets, bottom supporting nets, or shrimp traps. We hand-collected species of *Limnoperna fortunei*, an invertebrate that is attached to big stones, rocks, piers, wharves, and macrophytes, around the sampling sites, stored them in a portable refrigerator, and transported back them to the laboratory. The lengths and weights of the fishes were measured before dissection. The dorsal white muscle tissues from fishes, shrimp muscles