STABLE CARBON ISOTOPE COMPOSITION OF MOLLUSC TISSUES: EVIDENCE OF FOOD SOURCES

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Abstracts. Carbon isotope composition of the tissue of several mollusc species gathered from the Huaxi river, in China, a typical karst river, was analysed to determine their potential food sources. The results showed that $\delta^{13}C_{tissues}$ values of gastropod species *Cipangopaludina chinensis* are about 3‰ heavier than the values of bivalve species *Corbicula fluminea* and *Anodonta woodiana*, which indicates the different food and nutrition sources between species. According to the $\delta^{13}C$ values of mollusc's tissues and the potential food, bivalve species *C. fluminea* and *A. woodiana* mainly assimilate phytoplankton and terrestrial plant detritus as food, while gastropod species *C. chinensis* also utilize sediment organic matter as a food source excluding the phytoplankton and terrestrial plant detritus. Moreover, the preferential food selection behavior and varied metabolic intensity between different ages and individuals may lead to some variation of $\delta^{13}C_{tissues}$ values of *C. fluminea*. This study will be helpful in defining the role of molluscs in the energy flow of the karst river ecosystem, and to protect and manage those molluscs' resources.
Keywords: *nutrition, Corbicula fluminea, Anodonta woodiana, phytoplankton, terrestrial plant detritus*

Introduction

Molluscs, such as bivalves, and gastropods are important large benthic animals in river ecosystems, and they usually play an important role in the ecosystem through feeding and nutrient excretion activities as they often dominate the macrobenthic community (Boltovskoy et al., 1995; Nichols and Garling, 2000; Xu and Yang, 2007; Atkinson et al., 2010). Meanwhile, molluscs usually are important economic fishery resources. It is therefore essential to study the diet of those animals for better understanding the role of molluscs in carbon and nitrogen cycling in the freshwater ecosystems, and for the better management of molluscs resources (Paulet et al., 2006; Fukumoria et al., 2008).

There are several ways to better understand the diet of organisms, including direct observation of feeding behavior, gut contents analysis, and stable isotopes analysis of the soft tissues of organisms and the potential food sources (Raikow and Hamilton, 2001). Direct observation of the feeding behavior of bivalves are very complicated and

involved the measurement of filtering rates or examination of filtering morphology (Kryger and Riisgard, 1988; Ward et al., 1993; Silverman et al., 1997). Gut content analyses may be misleading because the method cannot distinguish ingested material that is not assimilated (Miura and Yamashiro, 1990; Raikow and Hamilton, 2001). In contrast to these conventional methods, stable isotope analysis has been used successfully in many studies of spatial and temporal variations in potential diets of invertebrates (DeNiro and Epstein, 1978; Stephenson and Lyon, 1982; Fry and Sherr, 1984; Zanden and Rasmussen, 1990; Kang et al., 1999; Cai et al., 2001; Kasai et al., 2006; Paulet et al., 2006; Fukumoria et al., 2008; Schlacher and Connolly, 2014; Graniero et al., 2016; Ishikawa et al., 2017; Zhao et al., 2019). The stable isotope techniques are based on the assumption that the isotopic composition of an organism is linked to its diet, such as it is generally accepted that the $\delta^{13}C$ value of an organism reflects the $\delta^{13}C$ value of its diet, with little ($\sim$1‰) or no change (DeNiro and Epstein, 1978; Fry and Sherr, 1984).

In this study we analyzed the stable carbon isotope composition of several molluscs tissues in the Huaxi river, China, which is a typical karst river, to determine what carbon sources were being used by those molluscs and to define the role of molluscs in the energy flow of the karst river ecosystem, which will be very helpful to protect and better manage the molluscs resources.

Materials and Methods

On August 10, 2007, a number of animals, bivalve species *Corbicula fluminea*, *Anodonta woodiana* and gastropod species *Cipangopaludina chinensis* were collected from Huaxi river, Guiyang city, China, which is a typical karst region (*Fig. 1*). The animals were brought back to the laboratory and the shell height was accurately measured using a caliper, the tissue is quickly removed with a scalpel. According to the size of shell height, *C. fluminea* tissue samples were divided into 6 groups (*Table 1*), and there were two kind of tissue samples in each group, one included individual tissue samples, and the other was a mixture of several animal tissue samples with the similar shell height (about 10 to 15 animals mixed together). Three individual tissue samples both of *A. woodiana* and *C. chinensis* were selected to determine the composition of organic carbon isotopes.

Tissues were dried in an oven at 60℃ for 24 hours, 5 drops of 2% hydrochloric acid was added to remove the inorganic carbonate (some *C. fluminea* mixed samples were not treated with hydrochloric acid) (*Table 1*). After acid treatment, *C. fluminea*, *A. woodiana* and *C. chinensis* tissue samples were dried again in an oven, and then ground into powder with a mortar and pestle, about 0.1 mg samples were placed in the tin cup, then wrapped the tin cup into cubes and no samples leakage was ensured. $\delta^{13}C_{tissues}$ are determined using EA-IRMS (Euro EA-3000) at the state key laboratory of

environmental geochemistry, CAS, China, IAEA C-3 cellulose (-24.91‰, PDB) was used for calibration, and the determination accuracy was 0.05‰.

Descriptive statistics and correlation analysis were used for data statistics, and SPSS13.0 software was used for data analysis.
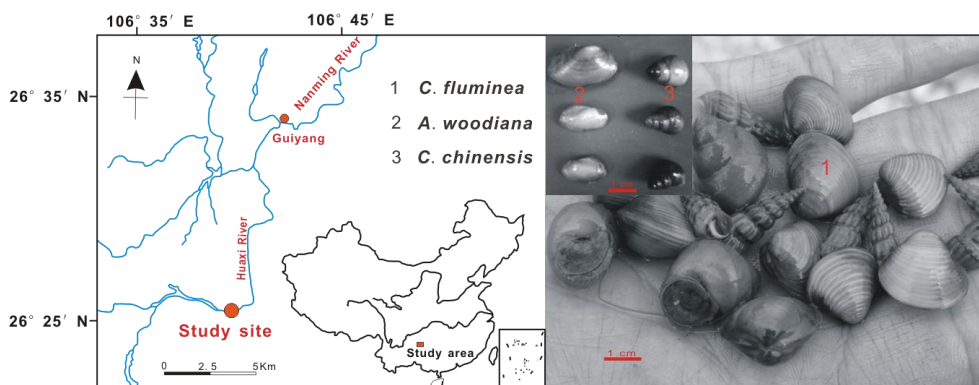
Figure 1. *Sampling locations and images of C. fluminea, A. woodiana and C. chinensis*

Table 1. *Different group of C. fluminea tissue samples*

| Group	Shell height (mm)	Individual sample	Mixed sample	
1	4.0～ 5.9	1-1	1-2	-
2	6.0～ 7.9	2-1	2-2	-
3	12.0～13.9	3-1	3-2	3-3*
4	15.0～16.9	4-1	4-2	4-3*
5	17.0～19.9	5-1	5-2	5-3*
6	20.0～22.9	6-1	6-2	6-3*

*the samples were not treated with hydrochloric acid

Results

$\delta^{13}C_{tissues}$ values of C. fluminea

From group 1 to group 6, $\delta^{13}C_{tissues}$ values of *C. fluminea* individual samples were -28.49‰, -28.67‰, -28.96‰, -29.00‰, -29.02‰ and -28.42‰, respectively, while $\delta13C_{tissues}$ values of mixed samples were -28.31‰, -28.60‰, -28.89‰, -28.93‰, -28.95‰ and -28.33‰, respectively. The results showed that there is no significant difference of $\delta^{13}C_{tissues}$ values between the individual samples and mixed samples in the same group ($R^2 = 0.98$, n=6) (*Fig. 2*), which may indicate that animals with similar shell height (similar age) assimilate similar food and nutrition. However, in the different groups (different ages), $\delta^{13}C_{tissues}$ values of *C. fluminea* showed some variation, the biggest amplitudes of variation are 0.60‰ and 0.65‰ for individual samples and mixed

samples, respectively, this variation may indicate that *C. fluminea* has certain changes of food resources or different preferable choices of food and nutrition during the growth process.
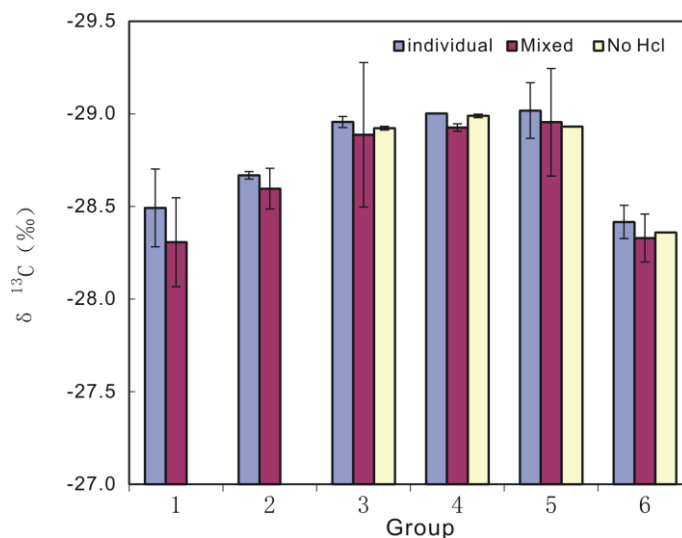
Figure 2. *$\delta^{13}C_{tissues}$ values of C. fluminea samples (The vertical line is the standard deviation)*

### Effect of sample treatment on $\delta^{13}C_{tissues}$ values of C. fluminea

From *Fig. 2*, $\delta^{13}C_{tissues}$ values of *C. fluminea* did not show significant differences between samples that were treated by hydrochloric acid and those with no-hydrochloric acid treatment, this result may indicate that inorganic carbonate in *C. fluminea* tissue samples are very low, and have little impact on the analysis of $\delta^{13}C_{tissues}$ values, so it seems unnecessary to treat *C. fluminea* tissues with hydrochloric acid before isotope analysis. While some studies found that the acid treatments can change both $\delta^{13}C_{tissues}$ and $\delta^{15}N_{tissues}$ values, specially the $\delta^{13}C_{tissues}$ because of the removal of the isotopically heavier inorganic carbonate (Schlacher and Connolly, 2014). Zhao et al. (2019) also reported that the acid-treated mussel's periostracum showed lower $\delta^{13}C$ values compared to those in untreated specimens, the offsets ranging from 0.35 to 5.13‰, which show a statistically significant difference in $\delta^{13}C$ values. Since it is impossible to determine the amount of inorganic carbonate in advance, and mechanical removal is unfeasible, acid treatment is needed in carbonate-rich sample.

$\delta^{13}C_{tissues}$ values between different species

$\delta^{13}C_{tissues}$ values of three *A. woodiana* samples were -28.92‰, -29.00‰ and -29.04‰, respectively (*Fig. 3a*), which are highly consistent with the $\delta^{13}C_{tissues}$ values of *Anodonta spp* (-28.7±0.8‰) in Congjiang county, Guizhou province (Zhang et al., 2010). Considering that Congjiang county is very close to Huaxi river area, and are both belong to typical southwest karst regions of China, and have the same geological

bedrock, vegetation types and soil types, this being consistent of $\delta^{13}C_{tissues}$ indicate that the carbon isotopes of molluscs tissues can really record the host environmental information.

$\delta^{13}C_{tissues}$ values of three *C. chinensis* samples were -26.27‰, -26.05‰ and -26.34‰, respectively (*Fig. 3a*). Comparation to *C. fluminea* and *A. woodiana* is also shown in *Fig. 3a*. $\delta^{13}C$ tissue values of *C. fluminea* and *A. woodiana* are very close to each other, while *C. chinensis* are about 3‰ heavier than bivalve species, this may indicate that both bivalve species *C. fluminea* and *A. woodiana* are relatively consistent in their food and nutrition sources, and are significantly different from gastropod species *C. chinensis*. This is also consistent with the previous study result, which found that $\delta^{13}C_{tissues}$ values of gastropod species are usually more positive than those of bivalves (*Fig. 3b*) (Cai et al., 2001).
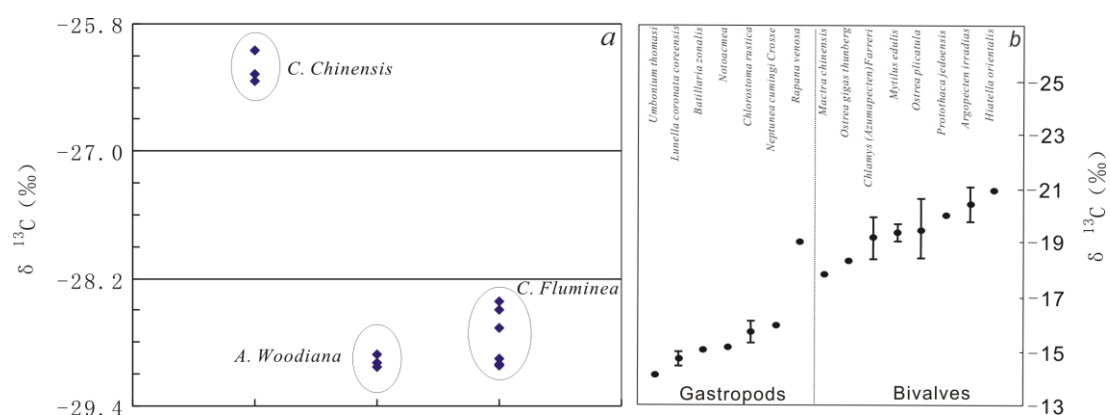
Figure 3. $\delta^{13}C_{tissues}$ *values of molluscs (a: $\delta^{13}C_{tissues}$ values of C. fluminea, A. woodiana and C. chinensis in this study; b: $\delta^{13}C_{tissues}$ values of several gastropods and bivalves species from Cai et al., 2001)*

Discussion

$\delta^{13}C_{tissues}$ *values of C. fluminea between different groups*

In the different groups (different ages), $\delta^{13}C_{tissues}$ values of *C. fluminea* show some variation both in individual samples and mixed samples, and the biggest amplitude of variation are 0.60‰ and 0.65‰ for individual samples and mixed samples, respectively. This variation of $\delta^{13}C_{tissues}$ values between different groups may indicate that *C. fluminea* has certain changes of food or different preferable choices of food during the growth process.

Studies have proved that bivalves have selective feeding characteristics at different ages, such as preferring different kinds of food or different sizes of particles in the same food (Ward and Shumway, 2004). They believed that filtering mollusc has two feeding mechanisms: (1) those that utilize mucous nets or strings (external to or within the

mantle cavity) to collect material, which known as hydrodynamic effect or passive feeding, and (2) those that rely on ciliated structures (proboscides, ctenidia) for particle collection, transport and processing, also known as mucilage ciliation effect or active feeding. There are also a few bivalves that rely on both of these mechanisms for particle collection (Ward and Shumway, 2004).

Ward and Shumway (2004) also found that the small size mollusc prefers to let the food flows through the main gill filaments directly into the back of the ventricle, along the back cilia swing into the lip, which known as hydrodynamic effect or passive feeding. The larger size of mollusc has strong active feeding ability, let the food flows enters the ventral edge of the gill under the action of the whole gill system, and enters the labial valve along the abdominal food delivery groove, also known as mucilage ciliation. Different ways of eating lead to absorb different or different sizes of food and nutrients, which can lead to differences in $\delta^{13}C_{tissues}$ values. Shumway et al. (1990) also found that the bivalve feeding behavior was related to the size of the food particles, and even related to the mucus action and chemical receptors of mollusc, which vary among individuals. Moreover, study on the scallop *Pecten maximus* in the field showed that tissue carbon isotopic composition can be also influenced by metabolic activity of the organism (Lorrain et al., 2002). Paulet et al. (2006) found that difference of $\delta^{13}C_{tissues}$ values between scallops and oysters could be due to differences in tissue growth rates between species. So the variation of $\delta^{13}C_{tissues}$ values of *C. fluminea* between different groups in this study may be caused by several reasons, such as the selective feeding characteristics and varied metabolic intensity at different ages.

$\delta^{13}C_{tissues}$ *values between different species: a tool to determine the food sources*

Gastropods are usually thought to be deposit-feeding, and food sources are mainly fluvial suspended material and sediment organic matter, while bivalves are defined as filter-feeding (Graney et al., 1984; Lorrain et al., 2002; Gillikin et al., 2006) and obtains suspended particles, such as plankton from the water through filtration by holding its inhalant siphon above the sediment surface (Britton and Morton, 1982; Kasai and Nakata, 2005; Kasai et al., 2006). While some studies found that filter-feeding alone cannot balance bivalve's energy budgets, the deposit feeding is also a significant source of nutrition for bivalve, especially in food-limiting situations (Aldridge and McMahon, 1978; Boltovskoy et al., 1995). For example, Hakenkamp and Palmer (1999) found that although *C. fluminea* is already known to be an important filterer of phytoplankton and seston from the water column, it has also been shown that it collects food within the stream through pedal-feeding, using cilia on the foot to collect subsurface organic matter. Moreover, Raikow and Hamilton (2001) found that potential food sources for bivalves also included epipsammon (detritus and possibly algae mixed with sand). An energy budget for the fingernail clam *Sphaerium striatinum* was reported by Hornbach et al. (1984), who estimated that 35% of its energy was derived from suspension feeding and the rest possibly from deposit feeding.

Determining the exact sources of food used by mollusc species can be difficult because of the heterogeneity of materials in suspension at any given time (Atkinson et al., 2010) and the co-existence of filter-feeding and deposit-feeding (McMahon and Bogan, 2001). However, from all the previous studies, we can still conclude that the main food sources of molluscs are POM (paticle organic matter) and SOM (sediment organic matter). Both the POM and SOM pool are a mixture of different sources of carbon, each with an often distinct $\delta^{13}C$ value, the POM pool are mainly including phytoplankton, terrestrial plant detritus and soil organic carbon, the SOM pool are mainly including benthic algae, bacteria and sediment organic matter (Langdon and Newell, 1990; Cranford and Grant, 1990; Kang et al., 1999; Nichols and Garling, 2000).

$\delta^{13}C$ of phytoplankton

Although it is well established that the carbon isotope fractionation between phytoplankton and DIC is variable (Rau et al., 1992; Hinga et al., 1994), a value between 18‰ and 22‰ is often used as an estimate (Cai et al., 1988; Hellings et al., 1999). Therefore, similar to Fry (2002) and Gillkin et al. (2006), an average value of 20‰ is used in this study ($\delta^{13}C_{DIC-20}$) to represent $\delta^{13}C$ of phytoplankton. $\delta^{13}C_{DIC}$ of Huaxi river range from -9.58‰ to -3.60‰, with an average of -7.15‰ (Yan et al., 2009), so $\delta^{13}C_{DIC-20}$ range from -29.58‰ ～ -23.60‰, with an average of -27.15‰. $\delta^{13}C_{DIC-20}$ of Huaxi river are consistent to the typical values of freshwater phytoplankton, which is between -24‰ and -42‰ (Zanden and Rasmussen, 1999), and also consistent to $\delta^{13}C$ of Guizhou freshwater phytoplankton, which ranges from -35.03‰ to -22.97‰ (Li, 2009).
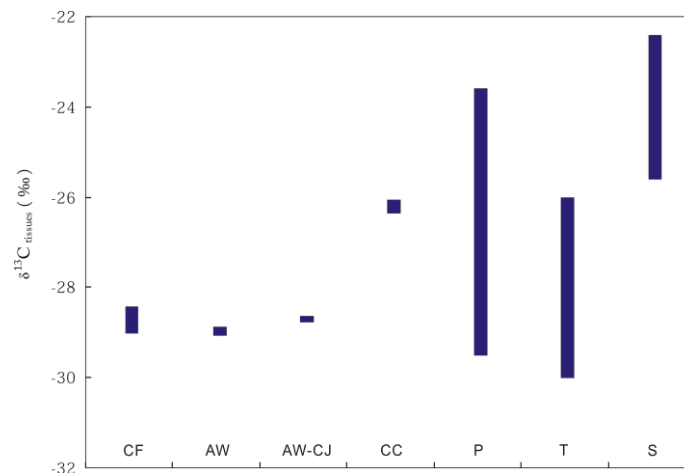
$\delta^{13}C$ of terrestrial plant detritus

Du et al. (2014) measured the $\delta^{13}C$ of common local plant species grown in Wangjiazhai catchments, a typical karst desertification area in Qingzhen City, which is very close to Huaxi river, $\delta^{13}C$ of plants ranged from -30.7‰～-26.5‰, with an average value of -28.1‰. Since the $\delta^{13}C$ value remains the same when a plant dies and becomes detritus (Smith and Epstein, 1971; Haines, 1977), so -30.7‰～-26.5‰, with an average value of -28.1‰ was used to represent the $\delta^{13}C$ of terrestrial plant detritus in this study.

$\delta^{13}C$ of sediment organic matter

Zhao et al. (2012) studied the geochemical characteristics in sediments in a small watershed of central Guizhou province, the results demonstrated that fluvial sediment are mainly derived from chemical weathering and soil physical erosion in the basin. The main soil types in Guizhou province were calcareous soil and yellow soil, and studies showed that the $\delta^{13}C$ values of calcareous soil and yellow soil were between -22.9‰ ~-21.5‰ and -25.6‰ ~-22.4‰ respectively (Li et al., 2012). The main soil type in Huaxi river area is yellow soil, so -25.6‰ ~-22.4‰ was used in this study.

Fig. 4 shows the $\delta^{13}C_{tissue}$ values of mollusks that were analyzed in this study and their potential main food sources. According to the *Fig. 4*, we can conclude that bivalve species *C. fluminea* and *A. woodiana* main food sources are phytoplankton and terrestrial plant detritus. Phytoplankton as a main food source for bivalves was proved by many studies (Ogilvie et al., 2000; Gillikin et al., 2006), while some research studies also found that terrestrial plant detritus contribution in bivalve diet is important during the periods when abundance of phytoplankton is too low to satisfy bivalve energy needs (Cranford and Grant, 1990; Langdon and Newell, 1990). Kasai et al. (2005) also found that the contribution of terrestrial organic matter is significantly important for the corbicula diet, although the contribution gradually changes among sampling sites. However, it's more complicated for gastropod species, according to the $\delta^{13}C_{tissues}$ values, besides of the phytoplankton and terrestrial plant detritus, *C. chinensis* must also assimilate sediment organic matter as food sources, which have a significantly heavier carbon isotope composition.

Figure 4. *$\delta^{13}C$ values of molluscs and potential food sources (CF is C. flminea; AW is A. woodiana; CC is C. chinensis; AW-CJ is Anodonta spp from Congjiang county; P, T, S are phytoplankton, terrestrial plant detritus and sediment organic matter, respectively)*

The relative abundance of multiple food sources in the diet of organisms can be quantified using a classical isotope mixing model, which is related to the $\delta^{13}C$ values of each food resource (Gannes et al., 1997; Raikow and Hamilton, 2001). As we did not have stable isotope values of some molluscs potential food sources in the Huaxi river, such as benthic algae, bacteria, we cannot quantitatively evaluate the mollusks' diet. However, this study still further illustrates the values of stable carbon isotope of mollusk's tissues as a tool in tracing organic carbon flow and recycle through a typical karst river ecosystem.

Conclusion

$\delta^{13}C_{tissues}$ values of bivalve species *C. fluminea* and *A. woodiana* are very close to each other, while *C. chinensis* are about 3‰ heavier than bivalve species, which indicate the different food and nutrition sources between species.

Comparing the $\delta^{13}C$ values of molluscs tissues and the potential food, bivalve species, *C. fluminea* and *A. woodiana* mainly assimilate phytoplankton and terrestrial plant detritus as food, while gastropod species *C. chinensis* also use sediment organic matter as a food source besides the phytoplankton and terrestrial plant detritus.

$\delta^{13}C_{tissues}$ values of *C. fluminea* between different sizes showed some variation, which may be caused by several reasons, such as the selective feeding characteristics and variational metabolic intensity at different ages.

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