Reply to Reviewer Comments

General Remarks from authors

We received two reviews for the first version of this manuscript, one from Jonathan Holmes and another one from an anonymous reviewer. We followed the suggestions of the reviewers and revised the manuscript carefully. Most of the reviewer suggestions were adopted as requested and further modification was carried out to make the manuscript more coherent for reading.

Preliminary comments on reviewer remarks were already given point by point as authors’ comments during the open discussion process. The final changes that were made can differ from the authors’ comments in particular points (e.g., additional information are not given as commented in the discussion, but were added in the methods or results).

Here we provide an overview about the most important changes that were made.

Enclosed to the following reply we send the revised manuscript with tracked modifications.

The following changes were made:

Many parts of the manuscript were restructured and therefore paragraphs and sentences are added, changed, shifted or deleted throughout the whole manuscript to make it more coherent.

Title

The title of the manuscript was changed to highlight the most important outcome of this study as suggested by both reviewers.

Abstract

The abstract was adapted according to changes that were made in the manuscript. More details on the included data were given as suggested by both reviewers.

Introduction

The whole introduction was rewritten to better point out the aims of the study. This includes the idea of single shell measurements as indication for a seasonal restriction of molting periods and many ecological details on *C. ilosvayi* as requested by the anonymous reviewer.

Study area

The whole chapter was restructured to support assumptions for the presented model approach in this study.

Climate

Here we point out the position of South Florida at the boundary of a tropical and subtropical climate and give specific information on the climatic conditions of region and more details on the conditions during the sampling period 2013/2014.

Hydrology and Study sites

These sections were combined to one with emphasis to relevant processes on the seasonal environmental variation of the investigated sites.

Information on the deeper aquifer systems was abridged as this is only of limited importance.

Some of the details on the study sites were added to table 1. These and other details that are not relevant for the further discussion were deleted from that section.

Seasonal variation of water isotopes

This section was restructured for a coherent presentation of available data and to emphasize important information on surface water variation to prove the plausibility of the subsequent model.

Material and Methods

Details on sampling and ostracod material used for isotopic analyses were given in the Methods and Results and supplementary material (adult, juvenile, number of valves per measurements).

The required number of isotopic measurements in one sample was tested to be representative for environmental variation to exclude ‘bulking of shells’ as reason to cause reduced variability.

The assumptions for the calculation of calcification periods were clearly formulated

Results

Seasonal variation of theoretical equilibrium calcite for river habitats

The section was separated into smaller sections

Isotopic signatures of ostracod calcite

Information on the isotopic composition of juvenile measurements were added and the outcome of the required number of isotopic measurements were added.

Discussion

Physico-chemical and isotopic characteristics of surface water habitats

The whole section was rewritten to signify environmental parameters influencing the isotopic composition of habitats.

Additional information of potential influences on $\delta^{13}C_{DIC}$ is given.

Stable isotope compositions of *C. ilosvayi*

Some paragraphs were shifted

Conclusion

Conclusions were rewritten to answer the formulated research questions in the introduction.

Minor points

Spelling mistakes and other small mistakes were corrected within the whole manuscript.

Number of decimals was adapted to one decimal place. Minus and plus marks were placed in front of isotopic values, while isotopic ranges were not be marked with any sign.
Details on the number of valves and the development stage and sex are added to the supplementary material.

A further supplementary file was added with results of isotopic signatures from further water samples of Pease River and sites within this river basin, Loxahatchee River and Caloosahatchee River.

Tables

Table 1: details on sample sites were added.

In all tables the number of decimals was adapted to one decimal place.

Figures

Figure 2 was changed to give separated information on the climate 2013 and 2014; the respective figure caption was corrected

Figure 1 (Study area) and 2 (Climate) were swapped

In Figure 7 one missing data point was added

In Figure 8 the sample CAL-1 was excluded from all charts

Modelling calcification periods of *Cytheridella ilosvayi* from Florida based on isotopic signatures and hydrological data ~~Also tropical freshwater ostracods show a seasonal life cycle~~

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Abstract. Isotopic signatures of ostracod shells ~~became common proxies for the reconstruction of paleo environmental conditions. Their isotopic composition is~~are the result of the composition of their host water and the phenology and ecology of the target species. ~~The sum of spatial and temporal variations from environmental factors in the species habitat defines the maximum isotopic variation of a population during the time of their shell formation.~~ Investigations addressing the influence of site specific environmental variations on isotopic ranges of ostracod shells are still rare, but can provide important information on habitat dependent variations and may signify a seasonal restricted timing of calcification periods.

Here we present isotopic signatures ($\delta^{18}O_{ostr}$, $\delta^{13}C_{ostr}$) of living *Cytheridella ilosvayi* (Ostracoda) and physical, chemical and isotopic (δD, $\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$) compositions of 14 ~~simultaneously sampled~~ freshwater habitats (rivers, lakes, canals, marshes, sinkholes) in South Florida from winter 2013 and summer 2014 and instrumental data of river temperatures and $\delta^{18}O$ of precipitation ($\delta^{18}O_{prec}$) from this~~e~~ region. The physico-chemical and isotopic compositions of the selected sites characterize the different habitats and show the influence of the source water, biological activity and the duration of exposure to the surface. Mean $\delta^{18}O_{ostr}$ and $\delta^{13}C_{ostr}$ ~~Isotopic~~ signatures of *C. ilosvayi* shells correlate well with the isotopic composition of their host waters. Within-sample variabilities~~y~~ of repeated isotopic measurements of single ostracod shells reflect habitat dependent ranges~~–~~. The similar high range of ostracod $\delta^{18}O$ in rivers and one marsh sample~~and~~ indicates that both, temperature and the ~~$\delta^{18}O$ composition of precipitation (~~$\delta^{18}O_{prec}$~~)~~ are responsible for their variation in the whole study area~~as regional environmental factors responsible for the population variation~~. Rivers and canals which are predominantly influenced by input and mixing of inorganic carbon from the catchment show smaller $\delta^{13}C_{ostr}$ ranges than the marsh dominated by local fluctuations in biological activities.

Based on these observations, background ~~Instrumental~~ data of water temperatures and $\delta^{18}O_{prec}$ were used to calculate ~~the~~ monthly $\delta^{18}O$ variations of a theoretical calcite formed in rivers of Florida assuming a direct reaction on precipitation changes. Calculated values showed a high variation coupled with low mean values during the summer wet season while during the winter dry season the variation remains small and mean values increased. ~~showing distinct seasonal variations in values and ranges. Different configurations of the theoretical calcite were compared to the within-sample variability to identify possible calcification periods of *C. ilosvayi*.~~ Inferred configurations were used to approximate possible calcification periods of *C. ilosvayi*. For a plausible calcification period ~~the~~ mean values and ranges of $\delta^{18}O_{ostr}$ had to be equal to ~~ostracod~~

~~isotopic range has to correlate with mean values of~~ the theoretical calcite with a slight positive offset (vital effect)~~ and the extension of the theoretical calcite range~~. The applied~~tested~~ model suggests a seasonal calcification period of *C. ilosvayi* in early spring that is ~~. The surprising seasonality of a tropical ostracod life cycle is~~ probably coupled to the hydrologic cycle of Florida. ~~The results of this study contribute to the application of ostracod isotopes in modern calibration studies and their potential use in paleontology.~~

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1 Introduction

~~Ostracod shells are discrete archives for biogenic carbonate and their chemical composition, including stable oxygen and carbon isotopes, are used routinely as proxies for environmental reconstructions (Von Grafenstein et al., 2000; Alvarez Zarikian et al., 2005; Anadón et al., 2006; Medley et al., 2008; Wrozyna et al., 2010; Escobar et al., 2012; Marco-Barba et al., 2012; Pérez et al., 2013; Börner et al., 2013). Their shell is formed within a short time (hours to days) and reflect the conditions of the surrounding water ($\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$, temperature) at the time of formation with species specific vital effects (Xia et al., 1997a; Von Grafenstein et al., 1999; Keatings et al., 2002; Decrouy et al., 2011). The calibration of isotopic signatures of ostracods from modern water systems is necessary to understand the complex interaction of processes for a proper interpretation of the paleontological record.~~

~~In modern aquatic environments the stable oxygen and carbon isotopes are influenced by a variety of interlinked environmental factors that vary locally and seasonally (Schwalb, 2003; Leng and Marshall, 2004). These spatial and temporal changes in the environment result in an isotopic range of an ostracod population that can be characteristic for a specific site (Decrouy, 2012; Van der Meeren et al., 2011). Modern studies showed that changes in the within-sample variability of ostracods can be coupled to temporal (Heaton et al., 1995; Xia et al., 1997a; Meyer et al., 2016) and local (Von Grafenstein et al., 1999; Meyer et al., 2016; Marco-Barba et al., 2012) differences of their environment.~~

~~The temporal environmental variation of a habitat reflected by isotopic range of ostracods is restricted to the duration of the calcification period of the target species. When seasonal shifts in the isotopic composition are indicative for certain time frames they can reveal insights on the annual timing and duration of the calcification period of an ostracod species. Thus, this information about the life cycle of an ostracod species can enhance the paleontological interpretation.~~

~~Here we present hydrological data and isotopic measurements ($\delta^{18}O$, $\delta^{13}C$) of *C. ilosvayi* from 14 modern freshwater habitats in South Florida. The aim of this study was to calculate potential calcification periods for the ostracod species *Cytheridella ilosvayi* Daday based on its $\delta^{18}O$ within-sample variability. Therefore, habitats were characterized by their chemical and~~

~~stable isotope composition and compared to the isotopic signatures of *C. ilosvayi*. In addition, the isotopic within-sample variability of *C. ilosvayi* was compared between sites to identify parameters influencing their site specific isotopic range. Further, instrumental hydrologic data of South Florida were used to set different seasonal scenarios for a theoretical calcite formed in equilibrium with the surrounding water. These settings were used to approximate potential calcification periods for *C. ilosvayi* based on the isotopic within-sample variability.~~

Ostracods molt up to eight times before they reach their adult stage. Molting periods of the single stages can be restricted to certain time periods within populations resulting in species specific annual population structures (Cohen and Morin, 1990). Known life cycles include seasonal cycles, multiple molting periods with or without overlapping generations and non-seasonal continuous life cycles (Cohen and Morin, 1990). In temperate and boreal regions temperature is regarded as the main abiotic factor controlling seasonal ostracod population dynamics (Horne, 1983; Cohen and Morin, 1990). This may be of minor importance in tropical and subtropical regions where temperatures remain comparably high during the whole year. But other factors like variation of food supply, water conditions or competition that are linked to other abiotic variables have also been suggested to influence the periodicity of ostracods (Horne, 1983; Kamiya, 1988; Martens, 1985). Still, life cycle information of freshwater ostracods and environmental parameters influencing their occurrence is scattered.

Commonly life cycle information is gained by repeated sampling of living ostracod material (seasonally, monthly) during a one or two year period for specific sites (e.g., Schweitzer and Lohmann, 1990; Cronin et al., 2005; Decrouy et al., 2012, Marco-Barba et al., 2012). Thereby, uncertainties of time lags between ostracod sampling and the actual molting can be reduced to seasonal or monthly periods, because species with seasonal life cycles can exhibit strong changes in the population structure between the repetition of two samplings. Still, this is only an approximation for a whole population and the actual calcification timing for single individuals remains unknown.

With every molt ostracods form a new calcite shell whose stable isotopes (δ^{18}O, δ^{13}C) reflect the conditions of the surrounding water at the moment of their calcification (Xia et al., 1997a; Von Grafenstein et al., 1999; Keatings et al., 2002; Wetterich et al., 2008; Decrouy et al., 2011b; Van der Meeren et al., 2011). The stable isotopic variation of shells within an ostracod population depends on the timing and duration of species specific molting periods. Especially, in seasonally varying environments fast changes of water conditions are possible and probably do not reflect annual mean isotopic values. Hence, a seasonally restricted shell calcification may be indicative for certain days, month or seasons. Thus, isotopic signatures of single shells can provide short term information on molting and changes of the population structure.

How strong single abiotic parameters influence certain ostracod habitats depends on regional conditions (e.g., climate, geology, geography) as well as site specific environmental constraints (e.g., morphology of the water body, water input and output, vegetation) (e.g., Schwalb, 2003; Leng and Marshall, 2004). For instance, ostracod populations living in the littoral zone reflect large δ^{18}O variations depending on seasonal precipitation/evaporation and temperature changes while conditions in the profundal zone remain almost constant and the $\delta^{18}O_{ostr}$ range of the same species remains small (e.g., von Grafenstein et al., 1999).

Cytheridella ilosvayi is a widespread neotropical ostracod taxon which occurs in aquatic systems from South Brazil and North Argentina to Yucatan and South Florida (Wrozyna et al., under revision) at water temperatures between 16 °C and 30 °C (Purper, 1974; Alvarez-Zarikian et al., 2005; Pérez et al., 2010). It prefers shallow water habitats with slow movement; dense macrophyte cover (Pérez et al., 2010) and sandy substrate (Higuti et al., 2010). It can be found in a variety of freshwater environments but can also tolerate higher conductivity (<5960µS/cm) and salinity (<3.2 psu) conditions (Alvarez-Zarikian et al., 2005; Pérez et al., 2010). Its latitudinal distribution covers tropical and subtropical climates that differ in their seasonal temperature ranges and precipitation amounts. These parameters importantly influence the seasonal variation of habitats and may affect the spatial and seasonal occurrence of *C. ilosvayi* and also its isotopic shell signatures. As for many other ostracods species, the knowledge of the life cycle of *C. ilosvayi* is poor. Indications for seasonal as well as permanent occurrences of the species are known only from single sites (Purper, 1974; Higuti et al., 2007; Pérez et al., 2010).

Here we present regional hydrological data from 14 freshwater habitats in South Florida and appendant single shell δ^{18}O and δ^{13}C signatures of *C. ilosvayi* to evaluate the following questions: (1) Do mean isotopic signatures of *C. ilosvayi* from single sampling reflect the general conditions of surface waters in South Florida? (2) Are there site specific differences in the isotopic ranges of single shell measurements and can we identify environmental parameters responsible for these ranges? (3) Can the δ^{18}O variation of *C. ilosvayi* signify the annual range or seasonally restricted variations of the suggested parameters for certain habitats? (4) And, is it possible to deduce the calcification period out of isotopic composition of shell calcite and hydrological data?

2 Study area

2.1 Climate

South Florida is located at the boundary between two climatic zones: a warm temperate fully humid climate with hot summers in the north and an equatorial monsoonal climate with dry winters in the south (Kottek et al., 2006). The transition between these climatic regions is gradual and a comparison of the 55-year average of air temperatures and precipitation from the southern and southwestern watershed of Florida did not show significant differences (Adler et al., 2013). The whole study area is characterized by a summer wet season from May to October with highest air temperatures in August (22.6 – 33.0 °C) and a winter dry season from November to April with lowest air temperatures in January (10.4 – 23.0 °C) (Fig. 2). South Florida has a mean annual precipitation of 1400 mm. About 60-70 % of the rainfall occurs during the summer wet season from May to October with precipitation amounts higher than 95 mm (Black, 1993). In the beginning and end of the wet season thunderstorms and hurricanes appear that may drop tens of centimeters of precipitation in a single event (Price et al., 2008). The major moisture source for precipitation in South Florida is evaporated seawater from the southeast of Florida. Sea-born vapor arises from the tropical North Atlantic dominating the weather during the wet season while within the winter dry season Maritime-Tropical Air alternate with modified Continental-Polar Air from a high latitude North-American source

(Price et al., 2008). Additionally, during the cool season westerlies can bring moisture from the Gulf of Mexico, Caribbean, or the tropical Western Pacific (Price et al., 2008). When cold fronts from the north pass the peninsula sometimes intense precipitation occurs.

Temperatures during the sampling period in 2013 and 2014 were higher than expected from the 55-year average. Additionally, annual temperature ranges were smaller (8.9 °C and 9.4 °C for 2013 and 2014, respectively) than the 55-year average (11.1 °C) mainly caused by mild winters. There was one unusual temperature drop that occurred during March 2013. Temperatures during sampling in November 2013 were higher (25.1 °C) than in the following year (22.6 °C) while temperatures during July and August were similar in both years (28.0 °C and 28.8 °C in 2013 and 28.8 °C and 29.4 °C in 2014, respectively). Annual precipitation during 2013 was slightly higher than in 2014 with 1790 mm and 1615 mm, respectively. The wet season started early in 2013 with precipitation twice as high as expected for April (130 mm) and May (280 mm). Rain amounts were also higher in July and from September until December 2013. Precipitation in 2014 was similar to the 55-year average except for July where rain amounts were twice as high as usual with ~500 mm.

~~2.1 Sample locations~~

~~Numerous sinkholes, ponds, lakes, rivers, wetlands and marshes are widely spread over the whole Floridian peninsula. For this study 15 water and surface sediment samples were taken at 14 locations from different surface water bodies within the southern and southwestern watersheds in November 2013 and July/August 2014 (Fig. 1, Tab. 1).~~

~~In the southwestern watershed of Florida five samples were taken: four within the Peace River basin and a further one at Little Salt Spring in the Myakka River basin.~~

~~Saddle Creek is one of the head streams of Peace River that originates close to the City of Lakeland and then passes through Lake Hancock where the first sample was taken (LH) at a stillwater area close to the inflow of the lake. Onward, Saddle Creek empties into Peace River where it flows southwards and passes on to Charlotte Harbor estuary at Punta Gorda. South of the City of Bartow, in the center of Polk County, at one of numerous boat ramps of Peace River a second sample was taken in a stillwater area (PR). The southernmost tributary stream of Peace River is Shell Creek, which originates in the northwest of Charlotte County and flows westward where it converges with the Peace River at Punta Gorda. Sampling was performed two times (November 2013 and August 2014) in the littoral zone of an artificial dead end branch of the river at Hathaway Park (SC-3, SC-15).~~

~~Close to the City of North Port in the lower part of the Myakka River watershed Little Salt Spring (LSS) is located. It is a sinkhole of about 72 m depth discharging warm (~28 °C) slightly saline groundwater (Sacks and Tihansky, 1996). A sample was taken in the littoral zone of LSS.~~

~~The ten remaining locations are distributed within the southern watershed of Florida. Boundaries of the southern watershed are poorly defined. Lake Okeechobee is a central part of the hydrological region and receives water from precipitation and different surrounding river watersheds mainly from Kissimmee River in the north (Abtew, 2001).~~

~~The lake itself is surrounded by a complex system of drainage canals (Smith et al., 1989; Harvey and McCormick, 2009). This system is alternately used for controlled irrigation or drainage depending on the weather and season. Agriculturally used water can enter the lake at numerous locations of the canal system and increase the concentrations of nutrients of the lake (Gain, 1997). Sample CAL-1 and CAL-2 were taken from the littoral zone of drainage canals in the south of Lake Okeechobee. In the southwest of the lake, two canals converge at Moor Haven and flow into Caloosahatchee River where a further sample was taken in the littoral zone (CAL-4). Approximately 25 km downstream sample CAL-5 was taken from the littoral zone close to a boat ramp.~~

~~Much of the water leaving Lake Okeechobee to the south (surface and subsurface) forms huge areas of wetlands and marshes including the Everglades extending to the south of Florida (Meyers et al., 1993). At Rock Reef Pass Trail, a trail in the Everglades National Park located between the Shark River Slough and Taylor Slough in Dade County, periphyton was taken from the marsh (EG). Throughout the whole central Everglades conservation areas have been constructed using a levee and canal system for flood control and water supply which impedes the natural overland flow (Smith et al., 1989; Meyers et al., 1993; Harvey and McCormick, 2009). Water allowances to the park are made through spillways along the Tamiami Canal in the north of the Everglades National Park. The Tamiami Trail Highway is one of the biggest impediments crossing the Everglades and adjacent areas from east to west. There, a further sample was taken at a marsh close to the trail in the Big Cypress National Preserve in Collier County (BiC).~~

~~Loxahatchee River is located in the east of the southern watershed and empties into the Atlantic Ocean at the City of Jupiter in Palm Beach County. There, four samples (LX-1, LX-2, LX-3 and LX-5) were taken in the littoral zone of Northwest Fork in short intervals within 2 km distance. In the sampling area Loxahatchee Rive receives its water from local shallow groundwater input, direct precipitation and runoff in the catchment (Swarzenski et al., 2006).~~

2.2 Hydrology of sample locations

The study area includes 14 locations within the southern and southwestern watersheds of Florida sampled in November 2013 and July/August 2014 (Table 1). Single samples were taken in shallow water areas from a variety of surface water bodies including rivers, canals, marshes, one lake and one sinkhole (Fig. 1).

The major water source for any surface or subsurface water in South Florida is precipitation (Meyers et al., 1993; Sacks, 2002; Wilcox et al., 2004; Price and Swart, 2006; Harvey and McCormick, 2009). Further,~~T~~ the whole area of South Florida is pervaded by a complex groundwater system that can be divided into three hydrogeological units: the surficial, the intermediate and the Floridian aquifer system (Maddox et al., 1992). The surficial aquifer system (SAS) is a shallow (less than 150 m) unconfined unit bounded by the land surface~~. It is a freshwater aquiferand is locally used for agricultural and domestic water supplies, public water supply or municipal and industrial supplies (Maddox et al., 1992).~~ and the most important groundwater source for most surface waters. ~~The SAS~~It is not only recharged ~~primarily~~ by seasonally fluctuating precipitation, but also by seepage from canals, other surface water bodies and the upward leakage of the intermediate aquifer system (IAS) (Wolansky, 1983; Alvarez Zarikian et al., 2005; Price and Swart, 2006). The general flow direction of groundwater is from north to southern coastal regions (e.g., Meyers et al., 1993). Close to the coast s~~S~~altwater intrusions into the SAS are possible ~~along the coasts~~ depending on the porosity and the permeability of the aquifer building a gradual mixing zone (Meyers et al., 1993; Wicks and Herman, 1995). ~~From north to south the~~Along its flow path the carbonate content of the aquifer increases and the calcium-carbonate water type dominates while in the coastal mixing zone sodium-chloride waters are dominant (Maddox et al., 1992).

The southwestern watershed of Florida can be separated into smaller catchment areas of several rivers including Peace River and Myakka River. Sampling within the Peace River Basin was performed at the inflow of Lake Hancock (LH); at Peace River itself (PR) close to the City of Bartow; and at Shell Creek, a southern tributary of Peace River. Sampling at Shell Creek was performed twice in November 2013 and August 2014 (SC-3, SC-15). Local karst processes can form open sinkholes or collapse features like Little Salt Spring in the lower part of the Myakka River watershed were a further sample was taken. Such sinkholes transport water from deeper groundwater to the surface (Maddox et al., 1992; Alvarez-Zarikian, 2005).

The ten remaining locations are distributed within the southern watershed of Florida that has poorly defined boundaries. Lake Okeechobee is the central part of this hydrological region discharging water to the south (surface and subsurface) forming huge areas of wetlands and marshes including the Everglades and Big Cypress Swamp (e.g., Meyers et al., 1993). At Rock Reef Pass Trail, a trail in the Everglades National Park, a periphyton sample was taken from a marsh (EG) and a further one at a marsh close to the Tamiami Trail Highway in the Big Cypress National Preserve (BiC). Further, sampling was performed at canals of the water control system around the lake. Sample CAL-1 and CAL-2 were taken from canals in the south of Lake Okeechobee and in the southwest, where two canals converge and flow into the Caloosahatchee River (CAL-4). Approximately 25 km downstream at Caloosahatchee River another sample was taken (CAL-5).

In the remaining southern watershed short rivers appear close to the coasts that are recharged by local precipitation and shallow groundwater, like Loxahatchee River in the east (Swarzenski et al., 2006). There, four samples (LX-1, LX-2, LX-3 and LX-5) were taken at the Northwest Fork of the river within 2 km distance.

Water levels in South Florida vary with seasonally fluctuating rain amounts. This results in increasing river runoff from June to September and the dry up of great areas of South Florida during the end of the dry season (Duever et al., 1994). Periodic water supply to conservation areas in the central part of the Everglades may modify natural water level variations south of Lake Okeechobee (e.g., Harvey and McCormick, 2009). ~~The base of the SAS is made up by an impermeable confining layer to the IAS. Water of the IAS is slightly saline with a chemical composition varying from bicarbonate-dominated waters or mixed-ion type in the inland to sodium and chloride-dominated waters in coastal areas (Sacks and Tihansky, 1996). Its composition is controlled by the downward leakage from the SAS, the upward leakage from the Floridian aquifer system (FAS), aquifer mineral reactions and saltwater mixing (Sacks and Tihansky, 1996). The deepest unit is the FAS that can be separated locally into the upper and lower Floridian aquifer. Water of the FAS is generally brackish to saline in coastal areas. Its chemical composition ranges from calcium- and bicarbonate-dominated in central recharge areas, to calcium-, magnesium- and sulfate-rich waters down gradient, and to a sodium-chloride type near the coast (Sacks and Tihansky, 1996).~~

~~This aquifer unit is recharged by the IAS inland while in the coastal area upward discharge into the IAS is driven by the proximity to the sea (Sacks and Tihansky, 1996). Water from the IAS and the Floridian aquifer system (FAS) is locally pumped to the surface for public water supply and agricultural usage (Wolansky, 1983; Alvarez Zarikian et al., 2005).~~

~~Ut rutrum, sapien et vulputate molestie, augue velit consectetur lectus, bibendum porta justo odio lobortis ligula. In in urna nec arcu iaculis accumsan nec et quam. Integer ut orci mollis, varius justo vitae, pellentesque leo. Vestibulum eu finibus nisl. Cras ac arcu urna. Duis ut pellentesque urna.~~

~~2.3 Climate~~

~~The South Florida subtropical climate is characterized by highest air temperatures in August (22.6 – 33.0 °C) and lowest in January (10.4 – 23.0 °C) in the fifty year average (Fig. 2). Single digit temperatures during winter are possible, but frost is rare (Price et al., 2008). About 60-70 % of the rainfall in Florida occurs during the summer wet season from May to November in the course of thunderstorms and hurricanes with precipitation higher than 95 mm (Black, 1993).~~

~~The surface water level varies seasonally with the amount of precipitation. This results in increasing river runoff from June reaching its maximum in September. This also results in the drying up of great areas of South Florida, including the Everglades, at the end of the wet season during spring (Duever et al., 1994).~~

~~The variation in evaporation follows a seasonal pattern, with lowest monthly evaporation occurring from December to February, and highest evaporation occurring from May to August (German, 2000). In the Everglades 70-90 % of water gets evaporated with highest evaporation rates during summer while in spring evaporation is limited due to the low availability of water (Duever et al., 1994; Price et al., 2008). Evaporation of surface water contributes 7 – 12 % of the local atmosphere vapor (Price and Swart, 2006).~~

~~The major moisture source for precipitation in South Florida is evaporated seawater from the southeast of Florida. Sea-born vapor arises from the tropical North Atlantic dominating south Florida weather during the wet season by diurnally forced sea-breeze, occasional easterly waves that originate from Africa and tropical cyclones that may drop tens of centimeters of precipitation in a single event as they make landfall (Price et al., 2008). Within the winter dry season Maritime Tropical Air alternate with modified Continental-Polar Air from a high latitude North-American source (Price et al., 2008). When cold fronts from the north pass the peninsula sometimes intense precipitation occurs. Additionally, during the cool season westerlies can bring moisture from the Gulf of Mexico, Caribbean, or the tropical Western Pacific (Price et al., 2008).~~

~~Daily relative humidity in South Florida is about 70-80 % (Abtew, 2001; Price and Swart, 2006). The minimum humidity occurs midday and generally exceeds 50 % while maximum values occur by dawn and often approaches 90 % (Black, 1993; Abtew, 2001).~~

2.3 Seasonal variations of water isotopes

Precipitation determines the isotopic composition (δD, $\delta^{18}O$) of surface water and groundwater in Florida, as it is the major water source of the whole region (Meyers et al., 1993; Sacks and Tihansky, 1996; Sacks, 2002; Wilcox et al., 2004; Price and Swart, 2006; Harvey and McCormick, 2009). It has an annual weighted mean value of -12.9 ‰ for δD_{prec} and -2.98 ‰

for $\delta^{18}O_{prec}$ (Price et al., 2008). Seasonal δD_{prec} and $\delta^{18}O_{prec}$ values show generally high values during the winter dry season and lower values during the wet season in June through August. Further, intense excursions to very low values occur at the beginning and end of the summer wet-season in May and September/October (Price et al., 2008) (Fig. 3a). These low values are linked to the high proportion of the oceanic vapor source due to fractionation by upstream rainout and greater disequilibrium between larger hydrometeors (Price et al., 2008) and the formation of tropical cyclones (Lawrence et al., 2004). Low isotopic values can also be observed during winter when cold fronts from middle-latitude North America pass Florida (Price et al., 2008). During the wet season, when surface water level increases and recycling of evaporated Everglades water increases the δD_{prec} and $\delta^{18}O_{prec}$ from June through October (Price and Swart, 2006).

Additionally to precipitation, evaporation alters the composition of surface water. Water bodies affected by evaporation show increased δD and $\delta^{18}O$ values along a local evaporation line (e.g., Meyers et al., 1993; Sacks, 2002; Harvey and McCormick, 2009). The variation in evaporation follows a seasonal pattern, with lowest monthly evaporation occurring from December to February, when solar radiation is lowest, and highest evaporation occurring from May to August (German, 2000). Additionally, evaporation rates can be limited during the end of the wet season when the availability of water at the surface is low (Duever et al., 1994; Price et al., 2008).

Especially waterbodies with a long exposure to the surface respond to changes in net precipitation (rainfall minus evaporation). In a study of central Florida, it has been shown that lake waters get enriched in ^{18}O at the end of the wet season and during summer when evaporation exceeds rainfall (Sacks, 2002). Also water from wells, canals and shallow groundwater in the Everglades varies seasonally with increasing $\delta^{18}O$ values from January to the end of the dry season and suddenly drop when rain sets in during spring (Price and Swart, 2006).

Also rivers of Florida react directly to rain events which can be seen in the increase of overland flow in the beginning of the wet season. This will result in a direct reaction to isotopic changes of precipitation (e.g., Fritz, 1981; Criss 1999; Clark and Fritz, 1997). A regional analysis showed that rivers in Florida are generally slightly enriched in ^{18}O compared to precipitation (Dutton et al., 2005). The addition of isotopically heavy water from the catchment (e.g., by lake water) can cause such enrichment of heavy isotopes in river waters (Gremillion and Wanielista, 2000). Overall, the influence of evaporation is small in rivers and the seasonal variation is buffered by mixing of different source waters.

The $^{13}C_{DIC}$ content in freshwater habitats depends on its source of dissolved CO_2 in the water from carbonate rock weathering, mineral springs, the atmosphere or respired organic matter (Peterson and Fry, 1987; Clark and Fritz, 1997; Leng and Marshall, 2004). Carbonate dissolution dominates the deeper groundwater system of Florida and results in values close to ocean water (~ 0 ‰). Values from deeper groundwater in Florida vary widely all over Florida from -14.9 to 0.54 ‰ (Sprinkle, 1989; Sacks and Tihansky, 1996). Groundwater values become lighter close to the surface where soil water processes dominate the system. Soil CO_2 mainly reflects $\delta^{13}C$ values of accumulated dead vegetation with values between -23 ‰ (C3 plants) and -9 ‰ (C4 plants) (Clark and Fritz, 1997). The seasonal variation of $\delta^{13}C$ caused by carbonate dissolution is low, while processes at the surface and subsurface are controlled by the proportion of photosynthesis and respiration that can vary strongly with the biological activity on a daily to seasonal scale (e.g.; Leng and Marshall, 2004).

~~Several studies used the oxygen and deuterium isotopic composition of surface water and groundwater to assess the origin and movement of water in Florida (Meyers et al., 1993; Sacks and Tihansky, 1996; Sacks, 2002; Wilcox et al., 2004; Price and Swart, 2006; Harvey and McCormick, 2009). These studies showed that precipitation is the major water source for surface and groundwater in Florida and their isotopic composition is mainly affected by the seasonal isotopic variation of precipitation.~~

~~Seasonal δD and $\delta^{18}O$ values of precipitation show high values during winter and low values at the start and end of the summer wet-season and slightly higher values in June through August (Price et al., 2008). The low isotopic values in the beginning of the summer suggest a more oceanic vapor source, fractionation by upstream rainout and greater disequilibrium between larger hydrometeors as they fall through lower-tropospheric isotope ratios (Price et al., 2008). In September and October tropical cyclones transport oceanic vapor to Florida and values decrease during that time again (Lawrence et al., 2004). Low isotopic values can also be observed in winter when cold fronts from middle-latitude North America pass Florida (Price et al., 2008). During the wet season surface water level increases and recycling of evaporated Everglades water increases the $\delta^{18}O_{prec}$ from June through October (Price and Swart, 2006).~~

~~The annual variability of $\delta^{18}O$ values of waters in Florida is highest in surface waters and decreases with depth in the groundwater system (Meyers et al., 1993; Price and Swart, 2006). Evaporation of surface waters results in the accumulation of heavy isotopes along a local evaporation line (Swart et al., 1989; Meyers et al., 1993; Sacks, 2002; Harvey and McCormick, 2009). The $\delta^{18}O$ values of shallow groundwater tend to vary with those of surface waters and net precipitation (rainfall minus evaporation) (Sacks, 2002; Price and Swart, 2006). When evaporated surface water enters the groundwater system it shows enriched in ^{18}O values (Wilcox et al., 2004).~~

~~Data on the $\delta^{13}C$ composition of inorganic carbon of water in Florida is rare. There are only data available from deeper groundwater that vary widely all over Florida from -14.9 to 0.54 ‰ (Sprinkle, 1989; Sacks and Tihansky, 1996). In general, $\delta^{13}C$ values from the IAS are lighter compared to the FAS. Heavier $\delta^{13}C$ values indicate dissolution of dolomite, which is more common in the deeper aquifer, while calcite precipitation removes some of the isotopically heavy isotopes (Sacks and Tihansky, 1996).~~

3 Material and Methods

3.1 ~~Sediment s~~Sampling~~es~~ and water analyses

Sampling and ~~analysis~~analytical methods are similar to Meyer et al. (2016). ~~Surface sediment s~~Sampling was performed in the littoral zone and stillwater areas ~~es were taken~~by scratching over the substrate or moving through water plants with a hand net to receive living ostracod material. Samples were stored in ethanol (96%) to preserve soft tissues of living animals. Simultaneously, water samples were taken and~~to water sampling,~~ field ~~variables~~parameters (electrical conductivity, water temperature and pH) were measured *in situ* at all sample sites. Water samples were promptly filtrated using a syringe filter (pore size of 0.45 µm) and stored in polyethene bottles until analysis. Major ions, the isotopic composition of the water

($\delta^{18}O$, δD) and dissolved inorganic carbon ($\delta^{13}C_{DIC}$) were measured at the laboratory center of JR-AquaConSoL in Graz. Total dissolved solids were calculated from major ion concentrations. The analytical procedure that was used in this study is similar to the method described by ~~(~~Brand et al.~~,~~ (2009). A classic CO_2–H_2O equilibrium technique (Epstein and Mayeda, 1953) with a fully automated device adapted from ~~(~~Horita et al.~~,~~ (1989) coupled to a Finnigan DELTA^{plus} Dual Inlet Mass Spectrometer was used for the measurement of oxygen isotopes. The stable isotopes of hydrogen in water were measured using a Finnigan DELTA^{plus} XP mass spectrometer working in continuous flow mode by the chromium reduction method (Morrison et al., 2001). Isotopic composition of DIC was analyzed using a Gasbench II device (Thermo) connected to a Finnigan DELTA^{plus} XP isotope ratio mass spectrometer comparable to setups in other studies (Spötl, 2005). Results of isotopic measurements are given in per mil (‰) with respect to Vienna Mean Ocean Water (V-SMOW) and Vienna Peedee Belemnite (V-PDB), respectively, using the standard delta notation. The analytical precision for stable isotope measurements is ±0.8 ‰ for δD, ±0.08 ‰ for $\delta^{18}O$ in water and ±0.1 ‰ $\delta^{13}C$ in DIC.

3.2 Isotopic analyses of *Cytheridella ilosvayi*

Ostracods were picked from the ~~sediment~~ samples under a binocular (Zeiss Discovery V8) and shells of *Cytheridella ilosvayi* (Daday, 1905) were separated and stored in micro slides for isotopic measurements. *C. ilosvayi* was identified by morphological features of the shell in accordance with the description of ~~(~~Purper~~,~~ (1974).

Prior to isotopic analyses soft part tissues and contaminations were removed from all ostracod valves with deionized water, brushes and entomological needles. If necessary, single valves were cleaned with H_2O_2 (10%) for five to ten minutes at room temperature.

Stable isotopic measurements of *C. ilosvayi* were performed at the Institute of Earth Sciences, University of Graz. One to sixteen ~~Per sample 1-16~~ measurements per sample were performed for carbon and oxygen stable isotopes (supplementary material 1). In most cases, one to two valves of adult females and males contained sufficient calcite material for isotopic measurements. Whenever possible, valves of the same individual were used for isotopic analyses. In single cases, valves were fragmented and up to nine fragments were used for measurements. Further, LX-3, CAL-1, CAL-2, SC-3 and BiC also include separate measurements of the juvenile A-1 stage (four to six valves per measurement).~~ containing two to eight valves of *C. ilosvayi* (female, male and A-1) depending on the valve size and if fragments were missing. Adult and juvenile shells were analyzed separately.~~

The samples were reacted with 100% phosphoric acid at 70 °C in a Kiel II automated reaction system and measured with a Finnigan DELTA^{plus} isotope-ratio mass spectrometer. Reproducibility of replicate analyses for standards (in-house and NBS 19) was better than ±0.08 ‰ for $\delta^{13}C$ and ±0.1 ‰ for $\delta^{18}O$. All carbonate isotopic values are quoted relative to V-PDB.

Isotopic values of *C. ilosvayi* were compared with their host water. Within-sample variability was evaluated for samples with more than four adult measurements. This included LX-1, LX-2, LX-3, LX-5, ~~CAL-1,~~ CAL-2, CAL-4, CAL-5, PR, SC-3, SC-15 and EG.

The required number of isotopic measurements in one sample to distinguish environmental variation was calculated as suggested by Holmes (2008) with a 10% acceptable error and a confidence level of 90%.

3.3 Calculation of the isotopic composition of calcite grown in equilibrium

The isotopic composition of water and its temperature dependent fractionation (Kim and O'Neil, 1997) can be used to calculate the isotopic composition of a theoretical calcite precipitated in equilibrium as shown in the following equation:

$$\delta^{18}O_{calcite} = 1000 - \left(e^{\left(\frac{18.03*\left(\frac{1000}{T}\right)-32.42}{1000}\right)} * \left(1000 + \delta^{18}O_{water}\right) \right) \qquad (1)$$

where T is the measured water temperature in Kelvin.

Generally, $\delta^{18}O_{water}$ values are expressed relative to V-SMOW, whereas $\delta^{18}O_{calcite}$ values are expressed relative to V-PDB. To convert the $\delta^{18}O_{water}$ values in the equation relative to V-PDB , the expression of ~~(~~Coplen et al.~~,~~ (1983) was used.

3.4 Calculation of calcification periods

River (LX-1, LX-2, LX-3, LX-5, CAL-5, SC-3, SC-15) and canal samples (~~LX-1, LX-2, LX-3, LX-5,~~ CAL-1, CAL-2~~, CAL-4, CAL-5, SC-3, SC-15~~) were selected to compare the $\delta^{18}O_{ostr}$ ranges~~within-sample variability of *C. ilosvayi*~~ with calculated isotopic compositions of a calcite precipitated under equilibrium with river water.~~the calculated monthly range of a theoretical calcite grown under equilibrium conditions.~~

The following assumptions were made for the comparison between $\delta^{18}O_{ostr}$ and $\delta^{18}O_{calcite}$: (1) changes in the $\delta^{18}O_{water}$ composition of rivers are induced by changes of the $\delta^{18}O_{prec}$ composition (Meyers et al., 1993; Sacks and Tihansky, 1996; Sacks, 2002; Wilcox et al., 2004; Price and Swart, 2006; Harvey and McCormick, 2009), (2) evaporation is of minor importance for river water and seasonally constant (Gremillion and Wanielista, 2000; Dutton et al., 2005), (3) the $\delta^{18}O_{ostr}$ variation is environmentally induced, (4) if calcification is seasonally restricted, the last molting period for a whole *C. ilosvayi* population to reach the adult stage lasts one month maximum, (5) it exists a constant positive vital effect for the species of 1.04 ‰ (Escobar et al., 2012).

Hence, ~~T~~the calculation of ~~the~~ monthly ranges of an equilibrium calcite of a certain site is ~~was~~ performed as following:

(a) Monthly values of $\delta^{18}O_{prec}$ ~~ranges~~ and ~~monthly ranges of~~ water temperature ~~data~~(min, max, mean) are ~~were~~ used in equation (1) to calculate the maximum variation of a theoretical equilibrium calcite for one year.

(b) Measured $\delta^{18}O_{water}$ values from the investigated site and the mean monthly temperature from the sampling month of the corresponding river are~~were~~ used to calculate the mean isotopic value of an equilibrium calcite precipitated in the particular aquatic system.

(c) The difference between the mean $\delta^{18}O_{prec}$ value from the sampling month and the river $\delta^{18}O$ value are calculated and all monthly $\delta^{18}O_{prec}$ values (min, max, mean) are corrected by that offset individually for each river (Fig. 3).

Daily temperature data were obtained from the National Water Information System Mapper (NWIS) of the US Geological Survey (http://maps.waterdata.usgs.gov/mapper/) for Loxahatchee River (Station: 265906080093500) and Shell Creek (Station: 02297635) during the sampling period 2013/14 and for Caloosahatchee River (Station: 02292900) from May 2014 to April 2016. Data for Caloosahatchee River before 2014 are not available. Monthly minimum, maximum and mean temperature values are calculated from the respective~~included data~~ years. Canal samples from Lake Okeechobee (CAL-2 and CAL-4) were related to Caloosahatchee River as no other data were available.

The $\delta^{18}O_{prec}$ composition was obtained from the Global Network for Isotopes in Precipitation (GNIP). ~~Isotopic data (δD and $\delta^{18}O$) of precipitation are available between 1997 and 2006 from 5 GNIP stations in South Florida. Spatially, inland sites are influenced by evaporated Everglades surface water while at the lower Keys precipitation is influenced by a more maritime vapor source (Price and Swart, 2006; Price et al., 2008).~~

For this study all available data from Rosenstiel School of Marine and Atmospheric Sciences (RSMAS), Biscayne National Park (BNP) and Redlands GNIP stations between October 1997 and December 2006 were summarized and used to calculate monthly $\delta^{18}O_{prec}$ values~~ between October 1997 and December 2006~~.

The location of the GNIP and NWIS sites are displayed in Figure 1.

For a plausible calcification time inferred from the $\delta^{18}O_{ostr}$ range of *C. ilosvayi* two requirements have to be complied: (1) the $\delta^{18}O_{calcite}$ range formed in the ostracod host water has to correspond with the $\delta^{18}O_{ostr}$ range and (2) considering a positive vital effect mean $\delta^{18}O_{ostr}$ values have to be positively offset to $\delta^{18}O_{calcite}$ considering a positive vital effect (Xia et al., 1997b; von Grafenstein et al., 1999; Decrouy et al., 2011b).

~~We excluded the two coastal sites to minimize spatial influences assuming that recycling of Florida surface water influences precipitation in the whole study area equally. To estimate plausible annual calcification times of *C. ilosvayi* a calcification period of one month was assumed.~~

4 Results

4.1 Physico-chemical and stable isotope characteristics of the study sites

The results of all parameters measured in the field (temperature, pH, electrical conductivity (EC)), total dissolved solids (TDS) and salinity, as well as ~~analysed in the ~~laboratory analyses (major ions, δD, $\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$) are summarized in Table 2 and Figures 4 to 6.

All investigated sites contain freshwater with salinity lower than 0.6 psu except for Little Salt Spring (LSS) which has a salinity of 2.6 psu (Tab. 2). The TDS of Loxahatchee River are similar between sampling locations (~ 275 mg/l) while samples of Lake Okeechobee Canal and Caloosahatchee River have higher values and cover a wider range (310.1-503.3 mg/l). The TDS value of BiC (464.0 mg/l) is most similar to CAL samples. Within the Peace River basin TDS values are lower for LH and PR (187.0 and 125.8 mg/l) than for Shell Creek in winter (655.8 mg/l) and in summer (490.2 mg/l). The TDS value of EG is most similar to PR.

Samples can be split into three groups~~separated~~ by their major anion and cation composition~~ into three groups~~, calcium-bicarbonate-dominated, sodium-chloride-dominated and mixed waters (Fig. 4). Waters of the calcium-bicarbonate-type include EG, BiC, LH, CAL-5 and all Loxahatchee River samples. Samples belonging to the sodium-chloride-type are LSS and SC-15. The remaining samples (CAL-1, CAL-2, CAL-4, PR, SC-3) lie in a zone of mixing between these types. Measured pH values range from 6.1 to 8.6. The majority of samples provide values between 7.0 and 7.9 except PR and LX-3 with lower values of 6.5 and 6.1, and EG and CAL-1 with higher values of 8.1 and 8.6, respectively.

~~All observed temperature ranges are in agreement with literature data for each site and season.~~ Temperature measurements during winter provided values around 20 °C. One exception is Little Salt Spring showing raised winter temperatures of 26.8 °C. Temperatures range during summer from 28.3 to 35.5 °C. Values of Loxahatchee River show a variation of 1.3 °C between the locations during the sampling day. Samples from Lake Okeechobee Canal and Caloosahatchee River were also taken within one day and have a~~ higher~~ range of 5 °C.

The isotopic values of the water samples range from -6.0 to +16.9 ‰ for δD, from -1.74 to +2.4~~35~~ ‰ for $\delta^{18}O$ and from -12.4~~36~~ to -2.3~~28~~ ‰ for $\delta^{13}C$ (Fig. 5 and 6, Tab. 2). The δD and $\delta^{18}O$ values deviate negatively to the global meteoric water line (GMWL) and describe a local evaporation line (LEL) similar to the one described by Meyers et al. (1993) (δD= +4~~,~~.67 $\delta^{18}O$(±0~~,~~.52) ~~$\delta^{18}O$~~ + 2~~,~~.68(±3~~,~~.86). The samples can be divided into three groups by their δD and $\delta^{18}O$ values (Fig. 5). The first group includes the samples SC-15/SC-3 and LSS-1 with the lowest isotopic values (-6.0 to -4.1~~2~~ ‰ for δD and~~ -~~ -1.3~~1.74~~ to ~~-1.28~~1.7 ‰ for $\delta^{18}O$) that lie closest to the GMWL. The second group contains all other river samples and EG-3 and BiC-1 with values ranging from -2.4 to +4.9 ‰ and -0.7~~3~~ to +0.4~~0~~ ‰ for δD and $\delta^{18}O$, respectively. LH, CAL-1 and CAL-2 form the third group with the highest isotopic values (+9.5 to +16.9 ‰ for δD and +1.7~~1~~ to +2.4~~35~~ for $\delta^{18}O$).

~~-~~Concerning $\delta^{13}C_{DIC}$, the values ~~can ~~differ strongly between sites (Fig. 6a). Within the Loxahatchee River values are very similar (-10.6~~0~~ to -9.7~~0~~ ‰) while in Caloosahatchee River/Lake Okeechobee (-9.0~~8.98~~ to -5.5~~2~~ ‰) and the Peace River Basin (-12.4~~36~~ to -6.6~~2~~ ‰) the variation is strong. LSS has by far the highest value with -2.3~~28~~ ‰.

4.2 Seasonal variation of theoretical equilibrium calcite for river habitats

The isotopic composition of the theoretical calcite grown under equilibrium was inferred using available background data of $\delta^{18}O_{prec}$ and river water temperatures from Loxahatchee River, Caloosahatchee River and Shell Creek.

The increase of $\delta^{18}O_{prec}$ ~~results in the same~~causes an increase in the calculated calcite, while an increase in temperature results in a decrease in the $\delta^{18}O_{calcite}$ (Fig. 3). Thus, highest isotopic values for $\delta^{18}O_{calcite}$ can be calculated from minimum temperatures (T_{min}) and maximum $\delta^{18}O_{prec}$ (O_{max}) ~~while the~~and the opposite combination of T_{max} and O_{min} results in the lowest values (Fig. 3c). Temperatures and $\delta^{18}O_{prec}$ vary differently throughout the year which leads to differences in the monthly values and ranges of equilibrium calcites.

4.2.1 Influence of temperature

Measured water temperatures of the investigated site lie all in the temperature range of the according NWIS stations except for SC-15 where the temperature exceeds the maximum value about 1 °C. However, the determined temperature ranges of the NWIS stations are suitable to approximate the range of an equilibrium calcite for the studied sites.

The annual variation of water temperatures at NWIS stations varies from 17.0 °C to 31.0 °C for Loxahatchee River (LX), from 15.5 °C to 30.3 °C for Shell Creek (SC), and from 17.0 °C to 37.0 °C for Caloosahatchee River (CAL) (Tab. 3) and ~~of Shell Creek (SC), Caloosahatchee River (CAL) and Loxahatchee River (LX) at NWIS stations~~ is correlated to annual air temperatures of Florida with highest values during the summer wet season and lowest during the winter dry season. But, the annual river temperature range is smaller than for air temperatures. ~~Temperatures vary from 17.0 °C to 31.0 °C for LX, from 15.5 °C to 30.3 °C for SC, and from 17.0 °C to 37.0 °C for CAL (Tab. 3).~~ Hence, calculated $\delta^{18}O_{calcite}$ values are~~is~~ high during winter and decreases until August ~~where~~when temperatures reach their maximum values in all rivers.

The annual temperature variation (T_{max}-T_{min}) is similar for LX (14.0 °C) and SC (14.8 °C) and about 6 °C greater for CAL (20 °C). Temperatures in winter are similar for CAL and LX while SC~~H~~ has lower temperatures. In summer SC and LX are more similar while CAL has higher temperatures. This results in lowest $\delta^{18}O_{calcite}$ for CAL during summer and highest $\delta^{18}O_{calcite}$ for SC during winter.

The monthly temperature range for CAL is higher (5.0 to 8.6 °C) than for LX (4.4 to 7.6 °C) or SC (3.0 to 6.4 °C). A temperature increase of 1 °C can be translated into a decrease of 0.2 ‰ in the theoretical calcite (Craig, 1965; Kim and O'Neil, 1997~~Chivas et al., 1986~~). Thus, the monthly isotopic range for a theoretical calcite caused by temperature differences within the rivers varies from +0.9~~88~~ to +1.5~~2~~ ‰ for LX, from +0.6 to +1.3~~28~~ ‰ for SC and from +0.8~~2~~ to +1.7~~2~~ for CAL.

A distinct annual pattern in the monthly temperature ranges was not found. For instance, the range of Shell Creek is highest in October and lowest in May while the highest range of Caloosahatchee River was found in August and the lowest in December (Tab. 3).

4.2.2 Influence of $\delta^{18}O_{prec}$

The annual range~~mean value~~ for $\delta^{18}O_{prec}$ ~~is -1.94 ‰ and the total annual range~~ varies from -10.3~~1~~ ‰ in October to +1.5~~3~~ ‰ in December (Fig. 3a; Tab. 4). Mean monthly values range from -3.4~~3~~ ‰ in May to -0.8~~75~~ ‰ in January. During the whole year values are relatively constant (-1.7~~-0.75~~ to -0.8~~-1.73~~ ‰). Only in the beginning (May/June) and the end (October/November) of the wet season values fall below the annual mean value (-3.4~~3~~ to -2.8 ‰). Maximum monthly $\delta^{18}O_{prec}$ values vary less then mean values, but~~,~~ show also slightly lower values in the beginning and end of the wet season. The strongest variation can be seen in minimum values with a similar development throughout the year as mean and maximum values. The monthly $\delta^{18}O_{prec}$ range is correlated to minimum $\delta^{18}O_{prec}$ values. Negative excursions of $\delta^{18}O_{prec}$ cause ~~the~~ greater isotopic range. The lowest variation has been observed at the end of the wet season during April (3.0~~2.99~~ ‰)

while the highest variation appears in October (10.2~~18~~ ‰). The strong monthly variation of $\delta^{18}O_{prec}$ can also be seen in $\delta^{18}O_{calcite}$.

4.2.3 Maximum monthly variation of $\delta^{18}O_{calcite}$

The increase of $\delta^{18}O_{prec}$ and the decrease of temperatures during winter both increase $\delta^{18}O_{calcite}$ values, while from July through September the temperature increase is contrary to the $\delta^{18}O_{prec}$ increase. This effect can be seen clearly in the maximum $\delta^{18}O_{calcite}$ where the variation of precipitation is small and changes in temperature are more dominant. The variation of minimum $\delta^{18}O_{calcite}$ values is dominated by the variation of $\delta^{18}O_{prec}$. This results in highest values of $\delta^{18}O_{calcite}$ from December to March and lowest in the beginning and the end of the wet season in May/June and October. The lowest range was observed ~~in the same month as for precipitation~~ during April before the beginning of the wet season (4.0~~3.97~~ ‰ for SC, 4.2~~3~~ ‰ for CAL and 4.4~~38~~ ‰ for LX) and the highest range in October (11.44 ‰ for SC, 11.3~~25~~ ‰ for CAL and 11.3~~2~~ ‰ for LX).

The enrichment of heavy isotopes of the investigated sites is considered to be constant during the year and seasonal changes of evaporation are neglected for the correction of their values. Hence, corrected $\delta^{18}O_{calcite}$ values of the study sites have the same seasonal variation as the theoretical equilibrium calcite and differ only in their annual offsets to each other.

4.3 Isotopic signatures of ostracod calcite

~~Isotopic values of *C. ilosvayi* ($\delta^{18}O_{ostr}$, $\delta^{13}C_{ostr}$) range from -3.05 to 2.28 ‰ for $\delta^{18}O_{ostr}$ and from -10.31 to -2.71 ‰ for $\delta^{13}C_{ostr}$, respectively (Tab. 5; Fig. 6b to 6f).~~ There is a positive correlation between the mean $\delta^{18}O_{ostr}$ and their host waters (R²=0.66) and the mean $\delta^{13}C_{ostr}$ and the dissolved inorganic carbon (R²=0.90) (Fig. 7). This correlation for $\delta^{18}O_{ostr}$ becomes even more significant excluding ~~the strongly anthropogenic influenced~~ CAL-4 from the statistical analyses (R²=0.83).

The highest mean $\delta^{18}O_{ostr}$ values were found in canal samples of Lake Okeechobee CAL-1, CAL-2 and CAL-4 (+0.1~~05~~ to +1.3~~25~~ ‰). River and marsh samples from the southern watersheds show mean values in a similar range (-1.3~~-0.22~~ to -0.2~~-1.30~~ ‰). Within the Peace River watershed values decrease from north to south. Mean $\delta^{18}O_{ostr}$ values of SC-15 and LSS are the lowest with -2.1~~09~~ ‰.

All river samples show equally low mean $\delta^{13}C_{ostr}$ values (-9.1~~0~~ to -8.0~~7.95~~ ‰) while canal samples and Lake Hancock exhibit higher values (-7.0~~3~~ to -6.1~~08~~ ‰). Both marsh samples have distinct different $\delta^{13}C_{ostr}$ values, whereat BiC is similar to river samples (-9.04 ‰ $\delta^{13}C_{ostr}$) and EG is more similar to canals and rivers (-6.0~~1~~ ‰ $\delta^{13}C_{ostr}$). LSS has by far the highest $\delta^{13}C_{ostr}$ value (-2.6~~1~~ ‰).

~~Within-sample variability (max-min) of rivers and canals is relatively high for $\delta^{18}O_{ostr}$ and lower for $\delta^{13}C_{ostr}$.~~ Isotopic values of *C. ilosvayi* ($\delta^{18}O_{ostr}$, $\delta^{13}C_{ostr}$) range from -3.1 to +2.3 ‰ for $\delta^{18}O_{ostr}$ and from -10.3 to -2.7 ‰ for $\delta^{13}C_{ostr}$, respectively (Tab. 5; Fig. 6b to 6f). Loxahatchee River, Shell Creek, Caloosahatchee River and the canal sample CAL-4 show similar isotopic variations between 2.0 and 3.0 ‰ (Table 5, Fig. 6). ~~Canal samples CAL-1 and CAL-2 show the lowest $\delta^{18}O_{ostr}$ variation (1.04 ‰ and 1.21 ‰), while CAL-4 (2.44 ‰) has a variation similar to Loxahatchee River, Shell Creek and Caloosahatchee~~

~~River (1.97 to 3.00 ‰).~~ PR is the only river sample with a distinct lower $\delta^{18}O_{ostr}$ range (0.4~~36~~ ‰) than all other samples. The $\delta^{18}O_{ostr}$ variation of the marsh sample EG is slightly higher (3.1~~08~~ ‰) than for the highest river variation~~.~~ and CAL-2 shows the lowest $\delta^{18}O_{ostr}$ within-sample variation of 1.2 ‰.

The pattern for $\delta^{13}C_{ostr}$ is similar with lowest values for ~~CAL-1 (0.47 ‰) and~~ CAL-2 (+0.7~~65~~ ‰), while CAL-4 (+1.6~~2~~ ‰) is more similar to most river samples. The $\delta^{13}C_{ostr}$ variation of river samples ranges from +1.4~~1~~ to +1.7~~67~~ ‰ for Loxahatchee River, CAL-5 and Shell Creek in summer, while PR is more similar to canal samples (+0.8~~76~~ ‰). The range of the Shell Creek winter sample is twice as high as the summer sample (+3.0~~2.95~~ ‰). EG has by far the highest $\delta^{13}C_{ostr}$ variation (+5.2~~16~~ ‰). ~~Although there are just two measurements for BiC available, the $\delta^{13}C_{ostr}$ variation from the marsh sample is already higher (2.55 ‰) than for most river and canal samples.~~

Additionally, measurements of juvenile (A-1) individuals show no difference in the isotopic composition except for CAL-2 where $\delta^{18}O_{ostr}$ of both juvenile measurements were slightly lower and $\delta^{13}C_{ostr}$ was higher than values of adult measurements and BiC with a difference of 2.6 ‰ of the $\delta^{13}C_{ostr}$ value.

Isotopic measurements of single shells define the maximum range in a sample. Additional measurements combined of two or more shells from different individuals did not exceed this variation. Further, we tested the required sample-size for isotopic measurements (Holmes, 2008) and found the number of single shell measurements from LX, CAL, SC samples and EG to be sufficient to be representative for habitat variations, while in PR the number of measurements may be too small.

5 Discussion

5.1 Physico-chemical and isotopic characteristics of surface water habitats

~~The major water source for any surface or subsurface water in South Florida is precipitation (Price and Swart, 2006). Precipitation has a generally low δD and $\delta^{18}O$ composition and a low ion concentration with a composition similar to seawater of a sodium-chloride water type (Price and Swart, 2006).~~

~~Florida surface waters results from mixing between these two extreme water types (Price and Swart, 2006; Harvey and McCormick, 2009). Water from the SAS can seep into the lower groundwater aquifers. In the IAS and FAS also intrusions of seawater are possible and the ionic composition can change again to a sodium-chloride composition while isotopic composition again remains low.~~

~~The sodium-chloride-dominated water type, a salinity of ~ 2.6 psu, an elevated sulfate concentration (>250 mg/l), a nearly neutral pH value (7.5) (Fig. 4, Tab. 2) and the depth of ~70 m suggest that LSS is in contact with the lower part of the IAS (Alvarez Zarikian et al., 2005). The ion concentrations of deeper groundwater are higher than in LSS, but mixing with shallow groundwater and surface water runoff entering the sinkhole can lead to the observed values of LSS. In addition, groundwater in this region has annual temperatures of 24 to 28°C (Maddox et al., 1992), which correspond with measured temperatures of LSS (26.8 °C) in winter while temperatures from other surface water during that time had values about 6 °C lower (Tab. 2).~~

~~The only sample with a similar chemical composition is SC-15 which suggests a similar groundwater source. In contrast, SC-3 has a different chemical composition similar to PR. Within the Peace River Basin groundwater flows from the central part in the north to the coastal area in the south-west (Sacks and Tihansky, 1996). Lake Hancock in the north reflects the bicarbonate-dominated composition of the SAS in central Florida. Its low TDS value of <200 mg/l and the neutral pH (7.0) are typical for water from the SAS where carbonate dissolution already takes place but water gets periodically diluted by precipitation. Groundwater entering the rivers mixes with water from surface water runoff in the watershed shifting the ion proportion to a mixed water type which is reflected by PR and SC-3. The shift in the chemical composition of Shell Creek between summer and winter displays seasonal differences in the groundwater source of the river. In this region near the coast a change in the potentiometric surface of the FAS can cause a reversal between recharge and discharge of FAS and IAS and saltwater intrusions from the south appear periodically (Sacks and Tihansky, 1996).~~

~~Samples, that are located in the center of the southern watershed (CAL-1, CAL-2, CAL-4) receive water from Lake Okeechobee. The chemical composition of CAL-1 corresponds very well with water from Lake Okeechobee with an electrical conductivity of 400-500 µS/cm, a mixed water type (Harvey and McCormick, 2009). The higher conductivity of CAL-2 and CAL-4 implies additional water input from agricultural areas (median values of 946 µS/cm) surrounding the lake (Harvey and McCormick, 2009). In addition, CAL-4 has raised phosphate (0.2 mg/l) and nitrate (0.48 mg/l) values compared to other canal samples that indicate agricultural usage.~~

~~Canal water gets discharged into Caloosahatchee River, but the change in the chemical composition to a bicarbonate-dominated water type of CAL-5 portrays the change in the watershed and increasing inflow of shallow groundwater to the river. The Everglades also receive water from the direction of Lake Okeechobee as surface and groundwater flow and from local precipitation. The long water pathways from Lake Okeechobee to the Florida Keys cause big spatial and seasonal differences in the water composition (Meyers et al., 1993; Price and Swart, 2006; Harvey and McCormick, 2009).~~

~~The bicarbonate-dominated water type and the low TDS of EG differs from samples influenced by Lake Okeechobee water. Thus, local precipitation has a higher influence on the coastal area than water from Lake Okeechobee. BiC shows a distinct higher TDS concentration than EG and an ionic composition shifted to a more mixed water type. Big Cypress Swamp has a different watershed then the Everglades what may explain the difference between BiC and EG. Both watersheds show similar hydrological conditions, and long water pathways and the local input of precipitation probably also causes spatial difference within Big Cypress Swamp. The bicarbonate-dominated water type, the low TDS, a neutral pH and isotopic values of Loxahatchee River are typical for a river receiving water from the SAS recharged by local precipitation and without the influence of inflowing water from Lake Okeechobee. The similarity of LX samples show a regional equal water input from precipitation and groundwater along the river, that can also be seen in the isotopic composition.~~

~~The δ^{18}O composition of surface waters in Florida evolves through time as a response of evaporation. The longer the exposure of water to the surface, the higher is the accumulation of ^{18}O$_{water}$ (Fig. 5). Samples that are dominated by groundwater inflow have the lowest δ^{18}O$_{water}$ values and include LSS and SC-3 and SC-15. These samples have higher~~

~~isotopic values than water from the FAS, SAS or precipitation indicating also a low influence by evaporated water from the watershed. The change in the groundwater source between SC-3 and SC-15 is not reflected in the $\delta^{18}O_{water}$ values of this site. High $\delta^{18}O_{water}$ values are characteristic for lakes like Lake Hancock. CAL-1 and CAL-2 are strongly influenced by water from Lake Okeechobee showing also high $\delta^{18}O_{water}$ values. The chemical composition of CAL-4 corresponds with CAL-1 and CAL-2 but the sample has a much lower $\delta^{18}O_{water}$ value. Great differences can be seen in the isotopic composition between several canals around Lake Okeechobee. Canals with high isotopic values range in the dimension of the lake while other canals have lower isotopic values and reflect additional components of runoff by flushing of rainfall and input of groundwater through agricultural area (Harvey and McCormick, 2009). This may explain the difference in the investigated canal samples and would also be consistent with higher conductivity values of CAL-4 (Harvey and McCormick, 2009). CAL-5 has a much lower $\delta^{18}O_{water}$ value (-0.73 ‰) indicating the addition of groundwater and the low influence of Lake Okeechobee water on the river. The remaining river and marsh samples lie between values of CAL-4 and CAL-5 and range from -1 to 1 ‰ for $\delta^{18}O_{water}$. The through flow of rivers replaces water permanently and the accumulation of heavy isotopes is low. Varying retention times and periodic input of precipitation at BiC and EG can lead to big spatial and seasonal differences in the isotopic composition.~~

~~The $\delta^{13}C_{DIC}$ composition of the investigated sites differs widely (-12.36 to -2.28 ‰, Fig. 6a). In freshwater habitats the ^{13}C content depends on its source of dissolved CO_2 in the water from carbonate rock weathering, mineral springs, the atmosphere or respired organic matter (Peterson and Fry, 1987). Inflowing shallow groundwater and river waters have typically low values of -15 to -10 ‰ for $\delta^{13}C_{DIC}$ deriving from plant respiration and production of CO_2 in the soil. Loxahatchee River, CAL-5, PR, SC-15 and SC-3 reflect low values (-12.36 ‰ to -8.88 ‰) of incoming shallow groundwater. Discrepancies between the rivers are probably a result of the relative strength of CO_2 production in soil within the catchment areas or the exchange of CO_2 with the atmosphere (Atekwana and Krishnamurthy, 1998). Higher values in groundwater (-3 ‰ to +3 ‰) occur when limestone dissolution from the catchment is more dominant (Leng and Marshall, 2004). LSS is the only sample reflecting dissolution of older marine limestone from the deep groundwater aquifer with the highest observed $\delta^{13}C_{DIC}$ value (-2.28 ‰). The change to a deeper groundwater source in Shell Creek between summer and winter may also explain the shift to a more positive $\delta^{13}C_{DIC}$ value at SC-3. Long exposure of surface water leads to the exchange of CO_2 with the atmosphere until equilibrium at ~2.5 ‰ for $\delta^{13}C_{DIC}$ (e.g., Leng and Marshall 2004). None of the investigated samples has reached equilibrium with the atmosphere, but samples of Lake Hancock and CAL-1 and CAL-2 that are strongly connected to Lake Okeechobee, are more enriched in $\delta^{13}C_{DIC}$ indicating the longer residence time compared to rivers. BiC and EG are both characterized by a low water level, stagnant water and a dense aquatic vegetation. In such habitats the $\delta^{13}C_{DIC}$ composition depends strongly on the proportion of photosynthesis and respiration of aquatic organisms. In general, during photosynthesis ^{12}C concentration is reduced in the water by the preferential uptake of organisms resulting in high $\delta^{13}C_{DIC}$ values, while respiration has the opposite effect (e.g., Leng and Marshall 2004). The lower value of BiC may be the result of a lower photosynthetic activity of aquatic plants during winter. Further, EG was marked by a dense cover of periphyton. Algal fractionation during carbon uptake is higher than for aquatic plants (Rounick and Winterbourn, 1986) resulting in higher~~

~~$\delta^{13}C_{DIC}$ values of the water and may explain the higher value of EG.~~ The wide range of chemical compositions along an ion mixing line of the investigated sites (Fig. 4) reflects dissolution of carbonates in the underground and mixing with sea water that is typical for Florida surface waters (Price and Swart, 2006; Harvey and McCormick, 2009). Differences in the ionic composition and concentration are independent from habitat types.

Samples with a bicarbonate-dominated composition are distributed in the whole study area and reflect the typical composition of the SAS (Sacks and Tihansky, 1996; Price and Swart, 2006; Swarzenski et al., 2006; Harvey and McCormick, 2009). The similarity of LX samples (major ions and stable isotopes) shows equal water input from groundwater along the river within its catchment (Fig. 5 and 6).

Canal samples have a mixed water type similar to values reported for Lake Okeechobee receiving water mainly from Kissimmee River in the north (Harvey and McCormick, 2009). The higher ion concentrations of CAL-2 and CAL-4 are probably caused by additional water input from agricultural areas surrounding Lake Okeechobee (Harvey and McCormick, 2009). The different chemical composition of samples located in the discharge area of Lake Okeechobee (CAL-5, EG and BiC) indicates a local water input from precipitation and shallow groundwater and a low influence by transported lake water to the south (e.g., Meyers et al., 1993).

The change from bicarbonate to a mixed water type from LH to PR can be explained by the addition of surface water along the catchment and soil-water interactions in that area. This may also account for the composition of Shell Creek in winter. The composition of Shell Creek in summer corresponds with seawater, but with a lower concentration. Changes in the potentiometric surface of groundwater aquifers can cause saltwater intrusions to surface water in this area (Sacks and Tihansky, 1996). This may assert the shift in the chemical composition of Shell Creek between summer and winter and would also correspond with the sodium-chloride-dominated composition of LSS which is in direct contact with the FAS (Sacks and Tihansky, 1996).

LSS is the only sample with a higher temperature value compared to other winter samples. The temperature variation in LSS is small due to its direct contact with deeper groundwater resulting in average temperatures of ~28 °C (Sacks and Tihansky, 1996; Alvarez-Zarikian et al., 2005). A seasonal temperature variation of 10.9 °C was observed in Shell Creek which is similar to values reported from NWIS (Table 2 and 3). A similar temperature variation can also be expected in samples from other rivers, canals, lakes and marshes.

The $\delta^{18}O$ composition of surface waters in Florida evolves through time as a response of evaporation (Fig. 5). High $\delta^{18}O_{water}$ values are characteristic for lakes like Lake Hancock and canal samples CAL-1 and CAL-2 that are strongly influenced by water from Lake Okeechobee. The longer the exposure of water to the surface, the higher is the accumulation of $^{18}O_{water}$ in these habitats. Lower $\delta^{18}O_{water}$ values can be observed in all rivers, both marshes, LSS and CAL-4. The low $\delta^{18}O_{water}$ value of CAL-4 is probably caused by additional water input to the canal from agricultural area (Harvey and McCormick, 2009). Water from Caloosahatchee River has lower $\delta^{18}O_{water}$ values indicating the addition of groundwater and the low influence of Lake Okeechobee water on the river which can also be seen in the chemical composition. Differences in the $\delta^{18}O_{water}$ values between different rivers are probably caused by differences in the addition of groundwater, precipitation and surface runoff in the catchments. Shell Creek shows the lowest river $\delta^{18}O_{water}$ values. This site is located closest to Little Salt Spring which shows similar low $\delta^{18}O_{water}$ values of -1.3 ‰ and is highly affected by deeper groundwater input (Sacks and Tihansky, 1996). The remaining river sites and marshes show higher values and may be affected by evaporation from the water surface or input of shallow groundwater that is enriched in ^{18}O when it gets recharged by evaporated surface water (e.g., Wilcox et al., 2004). Isotopic measurements along Loxahatchee River, Caloosahatchee River and Peace River did not show any enrichment along their courses (supplementary material 2). Thus, this indicates that water has been enriched in ^{18}O before entering the river. Varying retention times of water and direct evaporation from the water surface in marshes can lead to big spatial and seasonal differences in the isotopic composition and it is unclear how evaporation, input of groundwater and precipitation affects EG and BiC.

The $\delta^{13}C_{DIC}$ composition of the investigated sites is typically low for freshwater habitats (Clark and Fritz, 1996) but differs widely (-12.4 to -2.3 ‰, Fig. 6a). LSS is the only site reflecting dissolution of older marine limestone from the deep groundwater aquifer with the highest observed $\delta^{13}C_{DIC}$ value (-2.3 ‰). Lowest values can be observed in rivers (-12.4 ‰ to -8.9 ‰) while lake and canal samples are more enriched in ^{13}C (-8.3 ‰ to -5.5 ‰). Higher $\delta^{13}C_{DIC}$ values in these samples co-occur with high $\delta^{18}O_{water}$ values indicating the long exposure of water to the surface and the exchange of CO_2 with the atmosphere (e.g., Leng and Marshall 2004). EG has a $\delta^{13}C_{DIC}$ value similar to Lake Hancock while its $\delta^{18}O_{water}$ value is low. Both marsh samples are characterized by a low water level, stagnant water and dense aquatic vegetation. In such habitats the $\delta^{13}C_{DIC}$ composition depends strongly on the biological activity and the proportion of photosynthesis and respiration of aquatic organisms while $\delta^{18}O_{water}$ evolves independently. During photosynthesis ^{12}C concentration is reduced in the water by the preferential uptake of organisms resulting in high $\delta^{13}C_{DIC}$ values, while respiration has the opposite effect (e.g., Leng and Marshall, 2004). The lower $\delta^{13}C_{DIC}$ value of BiC may be the result of a lower photosynthetic activity of aquatic plants during winter. Further, EG was marked by a dense cover of periphyton. Algal fractionation during carbon uptake is higher than for aquatic plants (Rounick and Winterbourn, 1986) resulting in higher $\delta^{13}C_{DIC}$ values of the water and may explain the higher value of EG.

The river $\delta^{13}C_{DIC}$ is also affected by biological activity and represents the mixed DIC composition of input from the whole catchment area. Differences between the rivers are probably a result of the relative strength of CO_2 production in soil within the catchment areas (Atekwana and Krishnamurthy, 1998). The change to a deeper groundwater source in Shell Creek between summer and winter may explain the shift to a more positive $\delta^{13}C_{DIC}$ value due to the input of dissolved carbon from limestone while $\delta^{18}O_{water}$ remains low.

5.2 Stable isotope compositions of *Cytheridella ilosvayi*

~~Simultaneous sampling of ostracods and water can be used to relate them to each other on a regional scale when the number of samples and the isotopic range of host waters are high enough (Wetterich et al., 2008). For this study, the $\delta^{18}O$ and $\delta^{13}C$ composition of *C. ilosvayi* from 15 surface waters in Florida is strongly correlated to the $\delta^{13}C_{DIC}$ ($R^2=0.90$) and to a minor degree to the $\delta^{18}O$ of their host water ($R^2=0.66$). Thus, the number of sites and the isotopic range of surface waters were~~

~~sufficient to connect mean isotopic values of *C. ilosvayi* with simultaneously taken water samples. The correlation of *C. ilosvayi* to $\delta^{18}O_{water}$ gets even more significant (R²=0.83) excluding sample CAL-4 from the statistics (Fig. 7).~~ Numerous studies showed that the isotopic composition of ostracod shells is connected to the conditions of their host water during the time of their shell formation (e.g., Xia et al., 1997b; von Grafenstein et al., 1999; Decrouy et al., 2011b). This relationship has been established also in regional studies, comparing the water chemistry of various sites with the isotopic composition of widely distributed ostracod species (Wetterich et al. 2008; Van der Meeren et al, 2011). For this study, the mean $\delta^{18}O$ and $\delta^{13}C$ compositions of *C. ilosvayi* also exhibit the general isotopic composition of surface waters in the region of South Florida (Fig. 7). One outlier is CAL-4, which probably is ~~As discussed above, CAL-4 is~~ the only sample directly influenced by the temporal anthropogenic input of agricultural water which results in a distinct different isotopic composition compared to other canal samples with the same chemical composition. Further~~Contrary~~, the isotopic composition of *C. ilosvayi* from CAL-4 is ~~very~~ also more similar to that of CAL-1 (Tab. 5, Fig. 6d). Thus, the input of agricultural water was probably initiated ~~in the period between~~ after valve calcification and before sampling, resulting in a great difference between $\delta^{18}O_{water}$ and $\delta^{18}O_{ostr}$.

It can be also expected from all other sites that the water conditions (temperature, $\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$) changed between valve calcification and sampling time. But, the duration of this time lag is unclear, because of missing information on the calcification time of *C. ilosvayi*. In open water systems input and output can be complex and different water bodies may behave seasonally different depending on the hydrologic factors dominating the system (DeDeckker and Forester, 1988; Leng and Marshall, 2004). As discussed above, the chemical and isotopic composition of the investigated water samples exhibit different environmental factors influencing the system. For instance, the $\delta^{13}C_{DIC}$ of LSS is influenced by carbonate dissolution in deeper groundwater that is seasonally constant while other sites depend on biological activities that can vary strongly. Then, the good correlation of ostracod and water isotopes may indicate molting and shell calcification close to sampling (Van der Meeren et al., 2011).

However, sampling was performed in two different seasons and sampling close to molting would imply calcification times for *C. ilosvayi* in different habitats during autumn 2013 and summer 2014. Such a difference in the calcification time of a species independent from habitats seems unlikely except strongly differing climatic conditions alter habitat conditions in the whole area between the two years. Temperature and precipitation amounts did not show variations similarly high between 2013 and 2014 (Fig. 2) to cause extreme differences in surface waters.

Further interpretation on the exact timing of calcification is not possible comparing mean ostracod values with single water measurements of different habitats. ~~Thus, CAL-4 is probably the only sample where the natural variation of the habitat gets strongly altered by the anthropogenic water input.~~~~Further information on the anthropogenic usage of the canal system and the calcification time of *C. ilosvayi* would be needed to verify that.~~

~~It can be also expected from all~~ other ~~sites that the water conditions (temperature, $\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$) changed between valve calcification and sampling time. In open systems water input and output can be complex and different water bodies may behave seasonally different depending on the hydrologic factor dominating the system (DeDeckker and Forester, 1988; Leng~~

~~and Marshall, 2004). The good correlation of ostracod and water isotopes then can indicate a calcification time close to sampling, a low seasonal variation of the habitats, or a similar seasonal habitat independent development of the sites between calcification and sampling. Thus, CAL-4 is probably the only sample where the natural variation of the habitat gets strongly altered by the anthropogenic water input. At the repeatedly sampled site of Shell Creek (SC-3, SC-15) a shift can be observed in the water $\delta^{13}C_{DIC}$ from -8.88 ‰ to -10.73 ‰ between summer and winter, while the $\delta^{13}C$ of *C. ilosvayi* remains almost the same between seasons. This may indicate a calcification period during a time of the year where the hydrological conditions in the underground were similar. Still, it is unclear at which time *C. ilosvayi* formed their valve and at which point the hydrological conditions of Shell Creek changed.~~ To overcome the uncertainty of time lags between valve calcification and sampling more information about the life cycle including molting periods of *C. ilosvayi* is~~are~~ necessary. But, if samples provide enough ostracod material to perform repeated isotopic measurements for one or more ostracod species, the intra- and interspecific variations can be useful to identify major changes in the environment during the time of calcification. The combination of mean isotopic values and ranges may be more characteristic for certain habitats or time periods and can possibly imply potential molting periods of a species.

5.2.1 Within-sample variability

Two major factors are important for the isotopic composition of a single ostracod shell: the ostracod biology, determining the time (calcification period) and place (micro-habitat) of calcification, and the general characteristics of the environment itself, responsible for the seasonal variations (Decrouy, 2012). Small-scale differences of these factors result in the isotopic variation of an ostracod population at a specific site during the period of their valve calcification (within-sample variability). Thus, the isotopic variation of a population is controlled by (1) the duration of the calcification period (Decrouy, 2012), (2) the seasonal environmental variation of a waterbody (Xia et al., 1997a; von Grafenstein et al., 1999; Decrouy, 2012), and (3) the response of the micro-habitat to certain environmental changes (Decrouy, 2012).

The within-sample variability of *C. ilosvayi* was investigated from eight river sites, ~~three~~two canals and one marsh sample. All investigated ~~micro-~~habitats are characterized by shallow water areas and a dense macrophyte cover (Tab. 1). EG is the only lentic water body and accumulations of heavy or light isotopes are more probable in the marsh with a longer residence time than in rivers and canals with a permanent through~~-~~-flow.

EG exhibits a similar $\delta^{18}O_{ostr}$ variation as all river samples (except PR) and CAL-4, with variations of +2.0~~1.97~~ to +3.1~~08~~ ‰ (Tab. 5; Fig. 6). In contrast, ~~canal samples CAL-1 and~~ CAL-2 has~~ve~~ a smaller $\delta^{18}O_{ostr}$ range with ~~1.04 ‰ and~~ 1.2~~1~~ ‰. The similar variation of the $\delta^{18}O_{ostr}$ ranges in Loxahatchee River can only be explained by a ~~seasonally~~ homogeneous isotopic development of the host water within the catchment area of the river during the calcification period of *C. ilosvayi*. Furthermore, a regional influence that is independent from the catchment area seems to be a reasonable explanation for the similar ranges of LX, other river samples and EG. The seasonal temperature variation is similar at the whole peninsular and can vary strongly within hours in aquatic habitats with small water volumes or a low water level. This will lead to a high variation in the ostracod calcite within a short time (Leng and Marshall, 2004), but it cannot explain the similarity of EG and rivers while the canal samples show lower ranges. It is more likely that another regional important factor, like the source water, causes the difference between the canal~~s~~ and other sites. Rivers and EG are mainly fed by precipitation or surficial groundwater. The water source for the SAS is also precipitation and exhibits a similar isotopic variation~~range~~ (Price and Swart, 2006). The permanent replacement of water in rivers results in a direct reaction to changes in the isotopic composition of precipitation. Contrary, canal samples receive their water mainly from Lake Okeechobee ~~and their $\delta^{18}O_{water}$ composition corresponds with the $\delta^{18}O$ range of the lake water of about 1 ‰~~ (Harvey and McCormick, 2009). In ~~Lake Okeechobee~~lakes, incoming rainwater gets mixed with a great volume of older evaporated water buffering the $\delta^{18}O_{water}$ variation (e.g., Leng and Marshall, 2004) and explains the low $\delta^{18}O_{ostr}$ variation in canals. This is also in accordance with the assumption that CAL-4 receives water not only from Lake Okeechobee but also from agricultural areas that obtain water from precipitation. Lentic water bodies with a smaller volume and a low water level (like marshes) have a much smaller buffering capacity and react similarly strong as rivers to changes in precipitation. ~~This indicates the regional influence of precipitation as source water for surface waters in Florida (Price and Swart, 2006).~~ Interestingly, the winter and summer samples of Shell Creek (SC-3 and SC-15) have a similar $\delta^{18}O_{ostr}$ range of 2.5~~2~~ ‰ and 2.04 ‰. Together with a similar mean value, this indicates similar water and temperature conditions during the valve calcification time in both years and, thus, hint to a seasonal calcification time of *C. ilosvayi*.

Cytheridella~~.~~ *ilosvayi* exhibits clear differences in the $\delta^{13}C_{ostr}$ range (Tab. 5; Fig. 6) between samples with a through~~-~~-flow and marsh samples indicating complex interactions of biological characteristics, input from external sources and mixing. ~~Depending on the dominant source of carbon t~~The $\delta^{13}C_{DIC}$ can vary widely within different time scales depending on the dominant source of carbon. Photosynthetic activity will remove ^{12}C from the system, while respiration has the opposite effect. The proportion of respiration and photosynthesis varies between day and night and affects aquatic systems strongly with a high biological activity. Strong biological activity can be expected from all investigated habitats. The population of EG shows a higher variation~~ higher~~ (5.2~~16~~ ‰) than for canals and rivers (0.5~~47~~ ‰ to 3.0~~2.95~~ ‰). ~~The hydrological conditions at BiC are similar to EG and although there are just two measurements for this sample available, the $\delta^{13}C_{ostr}$ variation from the marsh is already higher (2.55 ‰) than for most river and canal samples.~~ High residence time in marshes enables the accumulation and consumption of organic matter in the system which is probably reduced in rivers and canals by their permanent flow. In addition, exchange of CO_2 with the atmosphere will increase the $\delta^{13}C_{DIC}$ over time. This process is also more important in marshes than in flowing water systems.

In rivers and canals large-scale processes like the input and mixing of inorganic carbon from different sources in the catchment is more important than local small scale processes (Atekwana and Krishnamurthy, 1998). This results in the small $\delta^{13}C_{ostr}$ range of 1.14 to 1.7~~67~~ ‰ in Loxahatchee River, CAL-5 and SC-15. But, changes in the influx, e.g., increased input of water from tributary ~~streams~~creeks after a rain event~~shower~~, can cause shifts in the $\delta^{13}C_{DIC}$ composition. At SC-3 the $\delta^{13}C_{ostr}$ range is higher (3.0~~2.95~~ ‰) than in other river samples what is probably connected to the seasonal change in the groundwater source in the watershed that is not reflected in the $\delta^{18}O_{water}$ variation (Sacks and Tihansky, 1996). Further, ~~CAL-1 and~~ CAL-2 has~~ve~~ a lower $\delta^{13}C_{ostr}$ range than rivers (0.5~~47~~ ‰ and 0.7~~65~~ ‰). The low variation is probably related to the dominance of inflowing Lake Okeechobee water with a more stable $\delta^{13}C_{DIC}$ composition than in rivers, which have multiple tributary ~~streams~~creeks. CAL-4 has a~~n~~ $\delta^{13}C_{ostr}$ range similar to river samples (1.6~~2~~ ‰). Mixing of Lake Okeechobee water with agricultural water probably increases the variation of $\delta^{13}C_{ostr}$~~.~~ compared to other canals.~~PR is the only sample with an $\delta^{13}C_{ostr}$ range as small as CAL-1 and CAL-2 (0.76 ‰). Possibly the variation of the source water is small at this site or a higher flow rate stabilizes the $\delta^{13}C$ variation. It would also be possible that the number of measurements is too low to reveal statistically significant information from that site (Holmes, 2008).~~

Information on the life-history of *C. ilosvayi* is almost non-existent. It is unclear if this species has preferential molting periods for different development stages or if the population structure remains the same over the year. At Rio Grande do Sul (Brazil) *C. ilosvayi* did not show great seasonal variation in its distribution during a one year period (Purper, 1974) while its occurrence in the Parana River floodplain (Brazil) varied as a result of seasonal recruitment caused by flood pulses (Higuti et al., 2007). ~~(~~Pérez et al.~~,~~ (2010~~1~~) stated that surface sediment samples collected in November 2005 from Lago Petén Itzá (Guatemala) contained mainly valves of *C. ilosvayi* without soft parts while samples retrieved in February and March 2008 had both carapaces with soft parts, mostly from females. In Shell Creek we found a similar population variation with living *C. ilosvayi* ~~very~~highly abundant in summer and less in winter. This indicates a seasonal life cycle and a temporal restricted calcification period of *C. ilosvayi*~~.~~ in Florida. It is possible that climatic differences can cause discrepancies in the population structure of a species from different sites (Schweitzer and Lohmann, 1990). But~~,~~ within the region of South Florida climatic variation is negligible and calcification periods of *C. ilosvayi* should be equal at all sites. ~~Then~~Therefore, it can be expected that the within-sample variability from a single species provide information of a similar time frame. Consequently, when the seasonal variation of a habitat is strong enough the within-sample variability of *C. ilosvayi* contains information on the time and duration of its calcification period.

5.3 Reconstruction of *C. ilosvayi* calcification times ~~from rivers using within-sample $\delta^{18}O$ variability~~

To calculate a plausible calcification time for *C. ilosvayi* during a year we used instrumental data of water temperatures and $\delta^{18}O_{prec}$ to determine possible monthly compositions of an equilibrium calcite ($\delta^{18}O_{calcite}$) precipitated in rivers and canals of Florida and compared it to the within-sample range of ostracods from rivers and canals (Fig. 8).

~~Two requirements have to be complied for a plausible calcification time: (1) the $\delta^{18}O$ range of the equilibrium calcite has to correspond with the $\delta^{18}O_{ostr}$ range and (2) a positive vital effect has to be considered for the $\delta^{18}O_{ostr}$ (Xia et al., 1997b; Von Grafenstein et al., 1999; Decrouy et al., 2011).~~

The calculated monthly mean $\delta^{18}O_{calcite}$ values and ranges vary seasonally distinctly ~~among each other~~ and ~~can be~~are characteristic for certain months. In general, calcite ranges exceed the $\delta^{18}O$ range of *C. ilosvayi* in every sample and month (Fig. 8). From the beginning of the wet season in May until December the range is up to three times higher than for *C. ilosvayi*. A shorter calcification period of half a month will not reduce the isotopic range of the theoretical calcite significantly. For instance, within October the temperature range of CAL in the first half of the month is 2.3 °C smaller than for the whole month. For $\delta^{18}O_{prec}$ the range will be reduced about 1 ‰. Thus, the $\delta^{18}O_{calcite}$ range will be reduced from

11.3~~25~~ ‰ to 9.8~~79~~ ‰ and remain much higher than the ostracod range. Hence, months with a high $\delta^{18}O_{calcite}$ range can be excluded as calcification period for *C. ilosvayi*. From January onward the range decreases constantly until it reaches its minimum in April. During April the $\delta^{18}O_{calcite}$ range varies between 4.0~~3.97~~ ‰ in SC and 4.4~~38~~ ‰ in LX and is most similar to the ostracod $\delta^{18}O$ ranges.

For a plausible calcification time it can be expected, that values of *C. ilosvayi* lie within the range of the theoretical calcite and tend to more positive values compared to mean calcite values due to a vital effect (Xia et al., 1997a; ~~Xia et al., 1997~~b; ~~V~~von Grafenstein et al., 1999; Decrouy et al., 2011b). One~~A~~ vital effect for modern *C. ilosvayi* of +1.04 ‰ is reported from Lake Petén Itzá (Escobar et al., 2012). Currently, it has been shown, that the vital effect within a species can differ between sites and the ionic composition of the water may change vital effects (Marco-Barba et al., 2012; Decrouy and Vennemann, 2013). The most recent study could explain differences in vital effects with changes in the $[CO_3^{2-}]/[DIC]$ in closed basins (Devriendt et al. 2017). These authors stated that the carbonate ion effect on $\delta^{18}O$ is negligible for ostracods in permanent freshwater lakes because of very low $[CO_3^{2-}]/[DIC]$ in these environments. This is probably also the case for other freshwater environments. Thus~~However~~, the chemical composition of the investigated sites is considered to be stable enough for a constant vital effect within a sample.~~ Investigations on possible seasonal changes of the vital effect from a single site lie beyond the methodical approach of this study.~~

During the wet season from May until October the positive offset of *C. ilosvayi* to the mean calcite value exceeds 1.04 ‰ by far and even exceeds maximum values of the theoretical calcite at nearly all sites excluding these months as calcification time (Fig. 8). In November, values of the theoretical calcite increase until April and the $\delta^{18}O_{ostr}$ values converge to mean calcite values with a lower positive offset.

The combination of a small range and a ~1 ‰ positive vital offset between *C. ilosvayi* and the theoretical calcite assuming a maximum one month calcification period indicates April as the most plausible calcification time for *C. ilosvayi* (Fig. 8). This would also fit with the finding of *C. ilosvayi* from Guatemala where it molts in spring (Pérez et al., 2010~~1~~).

The applied model is restricted to the variation of extreme values of two components influencing the final composition of river waters. ~~Other f~~Factors like mixing of different source waters, the variation of evaporation, or the anthropogenic regulation of a surface water~~ body~~ probably influence the actual isotopic range of aquatic habitats. Further, large rain amounts with low $\delta^{18}O_{prec}$ values from thunderstorms may have a stronger influence on the isotopic composition of surface water then small amounts of precipitation with high values. But, low $\delta^{18}O_{prec}$ values are not exclusively connected to heavy rainfalls and can also occur during the winter dry season. Thus, the listed factors cannot be included in the calculation without further investigations. However, assuming a lower isotopic variation of precipitation during the sampling years would result in a lower variation of the theoretical calcite and other months become also plausible as calcification period. This would be the case for the period from January to March where the isotopic variation would decrease to a range comparable to *C. ilosvayi* and vital offsets are also reasonable. Nevertheless, calcification will remain seasonal and can be excluded to take place during the summer wet season in Florida.

Adding isotopic measurements of juvenile stages would be helpful to enhance the interpretation of seasonal calcification. Isotopic signatures of different development stages with temporal restricted calcification periods can reveal information on the seasonal development of temperatures or variations of the isotopic composition of their habitat (Xia et al., 1997a; von Grafenstein et al., 1999; Decrouy et al. 2011a, 2012). In five samples single juvenile measurements are included and showed similar values as adults, except for CAL-2 values were slightly lower and BiC with a difference of 2.6 ‰ of the $\delta^{13}C_{ostr}$ value. The overall amount of juvenile shell material, however, was not high enough for sufficient isotopic measurements and the comparison with adults.

~~Various kinds of life cycles are known from freshwater ostracods (Cohen and Morin, 1990). These include seasonal cycles, multiple cycles with or without overlapping generations and non-seasonal continuous life cycles of ostracods.~~ However, ~~T~~the isotopic range of *C. ilosvayi* indicates clearly a restricted seasonal calcification period. ~~However, almost nothing is known about the controlling factors for life cycles of tropical freshwater ostracods. In temperate and boreal regions temperature is regarded as the main abiotic factor controlling seasonal ostracod population dynamics (Horne, 1983; Cohen and Morin, 1990).~~ In cooler climates it is necessary to overcome sub-zero temperatures during the cold season and this can result in seasonally low abundances (Horne, 1983; Cohen and Morin, 1990). ~~, b~~But this is not the case in warm regions like Florida or~~f~~ Guatemala. Further, low temperatures slow down the development of ostracods and increase inter-molt periods which could be the case in Florida winter (Martens, 1985). But this would not explain a restriction of the calcification period to a certain season. ~~Other factors like v~~Variation of food supply, water conditions or competition ~~have also been suggested to~~can also influence the periodicity of ostracods (Horne, 1983; Kamiya, 1988; Martens, 1985). These factors are possibly coupled to other abiotic factors than temperature. In both regions, Florida and Guatemala, the wet season lasts from May to October. The initiation of the rainy season in spring leads to flooding of dried up areas and higher surface runoff which has an essential influence on seasonal habitat conditions and input of organic matter as food source. This also coincides with findings from the Parana River floodplain (Brazil) where flood pulses caused seasonal changes in *C. ilosvayi* abundances (Higuti et al., 2007).

The connection to the hydrological cycle is a plausible explanation for the seasonality of the life cycle of~~ a tropical~~ ostracods. *Cytheridella*~~.~~ *ilosvayi* possibly overwinters the dry season in a juvenile stage and maturation is initiated during early spring when rain sets in, water level and food supply rise and, thus, conditions for reproduction are more advantageous.

~~In a paleontological application the within-horizon stable isotope variability of *C. ilosvayi* from lake sediment cores on the Yucatan Peninsula has been successfully used as proxy for high-frequency climate variability (Escobar et al., 2010). In that study a low within-sample δ^{18}O variability of ostracod shells could be connected with periods of constant aridity when the ratio of evaporation/precipitation was also low and supports the suggestion of a life cycle connected to the hydrological cycle.~~

6 Conclusions

~~In this study we present a model to calculate the calcification period of the ostracod species *Cytheridella ilosvayi* from South Florida. This model is based on the comparison of instrumental hydrological background data and simultaneously taken water samples and living ostracods.~~

~~The combination of the isotopic and chemical composition of surface waters shows distinct patterns that characterizes the different habitats. The variation of the chemical composition of surface waters differed from a bicarbonate-dominated to a sodium-chloride-dominated water type as a result of solution processes at the surface and in the underground and illustrates the origin of the source waters for the study sites. Contrary, the δD and δ^{18}O composition differs as a function of evaporation. Sites with the longest exposure to the surface showed the most positive isotopic values. Further, the δ^{13}C composition differs widely caused by the combination of different processes such as biological processes, limestone dissolution and degassing.~~

~~Simultaneous sampling of water and ostracods was useful to correlate the δ^{18}O and δ^{13}C signature of *C. ilosvayi* to their host water and expose the general isotopic characteristics ($\delta^{13}C_{DIC}$ and $\delta^{18}O_{water}$) of their habitats. Different isotopic offsets between the sites can be addressed to changes in the water input and output of the complex hydrologic systems between valve calcification and sampling. The within-sample variability of *C. ilosvayi* (δ^{18}O, δ^{13}C) from all samples provide information on the same time duration and, thus, identify changes in the different environments during their shell calcification period. Similar $\delta^{18}O_{ostr}$ ranges of the samples show the regional equal influence of precipitation on marshes and rivers. The variation is smaller in canals due to the influence of older evaporated source water. High $\delta^{13}C_{ostr}$ variability in marsh samples is caused by rapid differences in the biological activity. In contrast, rivers and canals are dominated by input and mixing of inorganic carbon from different sources in the catchment leading to small $\delta^{13}C_{ostr}$ ranges.~~

In this study we compared site specific hydrological data with isotopic signatures (δ^{18}O and δ^{13}C) of the common ostracod species *Cytheridella ilosvayi* from 14 water bodies in South Florida to evaluate habitat dependent differences caused by seasonal environmental variabilities. Mean δ^{18}O and δ^{13}C of *C. ilosvayi* signified the general isotopic characteristics of their host water on a regional base. Further, the isotopic range signified habitat dependent differences that could be connected to specific environmental parameters. The $\delta^{18}O_{ostr}$ ranges of nearly all river samples and the marsh sample were similar. Their high variation could only be explained by a seasonal influence of both, temperature and $\delta^{18}O_{water}$ in the whole area. Contrary, canal samples reflected low lake water variations caused by mixing of inflowing water and older lake water. The $\delta^{13}C_{ostr}$ variation separated habitats with a permanent through flow that reflect mixed $\delta^{13}C_{DIC}$ from the catchment from lentic sites where variation of the $\delta^{13}C_{DIC}$ is caused by high frequency variations of photosynthesis/respiration within the water body. We assume that $\delta^{18}O_{water}$ variations in rivers are caused by the $\delta^{18}O_{prec}$ composition. Monthly maximum ranges of $\delta^{18}O_{calcite}$ from a theoretical calcite in equilibrium with the surrounding water were calculated from instrumental data of river water temperatures and $\delta^{18}O_{prec}$. The composition of the theoretical equilibrium calcite varied seasonally with high mean values in winter and low values in summer. Ranges were highest in the beginning and end of the wet season and lowest in April at~~in~~ the end of the dry season. These monthly variations~~ranges~~ were compared to the isotopic range of *C. ilosvayi* to test a new approach to identify possible calcification times during the year. Using ~~this~~these scenarios, the most plausible calcification period for *C. ilosvayi* is in April when water temperatures are high enough and the $\delta^{18}O_{prec}$ range is lowest to cause the isotopic signatures observed from *C. ilosvayi*. ~~A seasonal calcification period is surprising for a tropical ostracod. However, t~~This seasonality is probably connected to strong seasonal changes of habitat conditions caused by an annual weathering cycle of Florida.

~~This model contributes to the use of ostracod isotopes as indicator for the phenology of modern ostracods and the potential extension of interpretations in paleontological studies using ostracod isotopes.~~

Data availability

All relevant data are presented within the manuscript or in supplementary material

Author contribution

J. Meyer, C. Wrozyna and W.E. Piller carried out sampling of all water and sediment material and measurements of field data. In addition,~~-~~ J. Meyer and C. Wrozyna prepared ostracod material for isotopic analyses and J. Meyer carried them out. A. Leis carried out water analyses. J. Meyer prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1 Location and characterization of the studied sites

Sample	Date	Latitude	Longitude	Location	Water body type	Habitat	Sample depth (m)
LSS	26.11.2013	27° 4' 29.33"	082° 14' 0.37"	Little Salt Spring, North Port	sinkhole	littoral zone	1.0
SC-3	28.11.2013	26° 58' 27.04"	081° 53' 21.55"	Shell Creek, Hatheway Park	artificial river branch	littoral zone	0.3
BiC	29.11.2013	25° 53' 29.53"	081° 16' 14.52"	Big Cypris National Reserve, Tamiami Train Highway	marsh	swamp	0.2
LX-1	31.07.2014	26° 56' 03.00"	080° 10' 36.40"	Loxahatchee River, at Jupiter	river	littoral zone	1.0
LX-2	31.07.2014	26° 56' 32.50"	080° 10' 19.20"	Loxahatchee River, at Jupiter	river	littoral zone	0.2
LX-3	31.07.2014	26°56'40.28"	080° 10' 15.94"	Loxahatchee River, at Jupiter	river	littoral zone	0.2
LX-5	31.07.2014	26° 56' 49.80"	080° 10' 12.40"	Loxahatchee River, at Jupiter	river	littoral zone	0.2
EG	02.08.2014	25° 26' 2.00"	080° 45' 12.30"	Rock Reef Pass Trail, Everglades National Park	marsh	swamp, periphyton	0.3
CAL-1	06.08.2014	26° 43' 37.90"	080° 42' 10.50"	Canal south of Lake Okeechobee	artificial canal	littoral zone	0.25
CAL-2	06.08.2014	26° 45' 41.50"	080° 55' 11.70"	Canal south of Lake Okeechobee	artificial canal	littoral zone	1.2
CAL-4	06.08.2014	26° 50' 09.80"	081° 05' 14.40"	Canal southwest of Lake Okeechobee ~~Canal~~ at the inflow to Caloosahatchee River	artificial canal	littoral zone	0.3
CAL-5	06.08.2014	26° 47' 21.70"	081° 18' 33.60"	Caloosahatchee River	river	boat ramp, stillwater area	0.1-0.4
LH	07.08.2014	28° 0' 7.310"	081° 51' 4.22"	Lake Hancock, at Lakeland	lake	lake inflow, stillwater area	0.05-0.2
PR	07.08.2014	27° 48' 46.20"	081° 47' 36.90"	Peace River, at Bartow	river	Boat ramp, stillwater area	0.5
SC-15	08.08.2014	26° 58' 26.99"	081° 53' 21.81"	Shell Creek, Hatheway Park	artificial river branch	littoral zone	0.3

Table 2 Physicochemical and stable isotope characteristics of the studied sites

Sample	**Field parameters**					**Cations**				**Anions**					**Stable Isotopes**		
	Temp [°C]	**pH**	**EC [µS/cm]**	**Sal [psu]**	**TDS [mg/l]**	**Na [mg/l]**	**K [mg/l]**	**Mg [mg/l]**	**Ca [mg/l]**	**Cl [mg/l]**	**NO_3 [mg/l]**	**SO_4 [mg/l]**	**HCO_3 [mg/l]**	**Br [mg/l]**	**δD [‰]**	**$\delta^{18}O$ [‰]**	**$\delta^{13}C$ [‰]**
LSS	26.8	7.5	5110	2.6	3193.4	766.6	22.3	135.8	167.5	1430	<0.25	498.9	172.1	<0.5	-4.1~~2~~	-1.3~~28~~	-2.3~~28~~
SC-3	20.3	7.9	938	0.56	655.8	71.9	5.1	13.3	103.1	145.0	<0.25	59.8	257.5	0.6	-5.9	-1.4~~0~~	-8.9~~88~~
BiC	20.8	7.6	586	0.35	464.0	32.8	1.6	6.2	87.3	56.9	<0.25	4.8	274.6	<0.5	-0.9~~89~~	-0.5~~4~~	-9.6~~55~~
LX-1	30.6	7.3	363	0.22	270.1	22.8	1.4~~38~~	2.6~~4~~	50.1~~09~~	35.8~~4~~	0.4~~2~~	6.6~~4~~	150.7	0.07	2.4	0.3~~28~~	-9.7~~0~~
LX-2	30.5	7.2	375	0.23	276.6	23.0	1.4~~36~~	2.6~~2~~	51.7~~3~~	36.3~~26~~	0.4~~4~~	7.4~~36~~	154.4	0.04	2.1	0.3~~2~~	-10.6~~0~~
LX-3	31.7	6.1*	375	0.23	275.8	23.1	1.4~~36~~	2.6~~3~~	52.1~~3~~	36.1~~08~~	0.5~~47~~	7.3~~2~~	153.2	0.06	2.1	0.3~~28~~	-10.4~~2~~
LX-5	30.4	7.1	375	0.23	276.0	22.8	1.4~~39~~	2.5~~0~~	51.5~~0~~	36.1~~4~~	<0.01	7.3~~3~~	154.4	0.07	0.3	0.1~~1~~	-9.9~~2~~
EG	33.1	8.1	189	0.11	152.8	5.2	0.3~~25~~	0.7~~65~~	33.3~~0~~	7.2~~0~~	<0.01	<0.1	106.2	<0.01	5.8	0.2~~19~~	-6.1~~3~~
CAL-1	31	8.6	444	0.27	310.1	32.8	5.9~~2~~	9.4~~3~~	42.3~~1~~	57.8~~4~~	<0.01	30.1~~06~~	131.8	0.16	16.9	2.4~~35~~	-5.5~~2~~
CAL-2	30.5	7.4	676	0.4	466.1	51.7	9.5~~46~~	13.1~~4~~	65.8~~1~~	85.7~~2~~	0.2~~17~~	47.5~~46~~	192.8	0.21	13.0	1.7~~2~~	-7.8~~1~~
CAL-4	35.5	7.5	724	0.43	503.3	52.3	8.3~~1~~	13.1~~2~~	77.9~~3~~	90.5~~49~~	0.5~~48~~	57.4~~3~~	203.8	0.24	4.9	0.40	-8.3~~1~~
CAL-5	34.7	7.4	550	0.33	394.4	25.8	6.2~~1~~	7.0~~0~~	74.8~~78~~	45.4~~35~~	0.2~~3~~	30.8~~77~~	204.4	0.14	-1.3	-0.7~~3~~	-9.0~~8.98~~
LH	28.3	7.0	247	0.14	187.0	12.2	3.3~~27~~	5.4~~4~~	29.0~~1~~	20.0~~2~~	0.5~~46~~	2.9~~0~~	114.1	0.01	9.5	1.7~~1~~	-6.6~~2~~
PR	28.3	6.5	189	0.11	125.8	14.4	5.4~~36~~	4.3~~0~~	14.3~~27~~	20.6~~4~~	1.5~~45~~	15.0~~4.96~~	51.9	0.05	-2.4	-0.3~~28~~	-12.4~~36~~
SC-15	31.2	7.1	297*	0.18	490.2	105.3	5.4~~35~~	14.3~~1~~	41.0~~0.99~~	193.8	0.1~~1~~	34.7~~4~~	95.8	0.59	-6.0	-1.7~~4~~	-10.7~~3~~

*- accuracy of *in situ* measurement uncertain

Table 3 Monthly temperature data from NWIS stations: 02297635 (Shell Creek), 02292900 (Caloosahatchee River) and 265906080093500 (Loxahatchee River)

	Shell Creek				**Caloosahatchee River**				**Loxahatchee River**			
	Mean	Max	Min	Max-Min	Mean	Max	Min	Max-Min	Mean	Max	Min	Max-Min
Jan	17.7	20.4	15.5	4.9	20.6	25.0	17.6	7.4	20.5	23.3	17.0	6.3
Feb	18.1	21.1	16.2	5.0	19.3	22.4	17.0	5.4	21.7	25.0	17.4	7.6
Mar	19.8	21.7	18.3	3.4	24.3	28.0	19.4	8.6	21.5	25.0	18.1	6.9
Apr	22.7	24.7	19.9	4.8	27.1	30.8	24.5	6.3	25.2	28.3	21.4	6.9
May	25.1	26.7	23.7	3.0	28.9	31.7	26.7	5.0	26.6	29.3	22.7	6.7
Jun	27.3	29.8	24.9	4.9	30.6	34.3	28.3	6.0	28.2	30.7	25.7	5.1
Jul	28.0	30.3	25.8	4.5	30.9	34.5	28.7	5.9	28.5	30.9	26.2	4.7
Aug	28.4	30.3	26.2	4.1	31.0	37.0	28.4	8.6	29.3	31.0	26.7	4.4
Sep	27.1	29.1	24.6	4.5	29.7	33.8	27.6	6.2	27.8	30.4	26.0	4.4
Oct	25.0	28.2	21.8	6.4	27.7	31.0	25.4	5.6	26.2	29.0	23.2	5.9
Nov	20.6	22.9	17.9	5.1	24.6	28.0	21.7	6.3	22.5	25.5	18.9	6.6
Dec	18.5	21.0	16.4	4.6	22.2	24.4	20.3	4.1	21.3	23.7	18.2	5.6

Table 4 Monthly values of $\delta^{18}O$ from precipitation of southeast Florida including data from GNIP stations: Biscayne National Park BNP, Rosensteil School of Marine and Atmospheric Sciences (RSMAS) and Redland 1998-2005

	$\delta^{18}O$					**Number of measurements**			
Month	Mean	Max	Min	Max-Min	SD	BNP	RSMAS	Redland	total
Jan	-0.8~~75~~	0.6~~2~~	-3.8~~1~~	4.4~~3~~	1.5~~45~~	6	5	1	12
Feb	-1.40	1.1~~1~~	-3.4~~36~~	4.5~~47~~	1.2~~18~~	2	10	2	14
Mar	-1.2~~17~~	1.5~~47~~	-4.1~~08~~	5.6~~55~~	1.4~~4~~	2	11	3	16
Apr	-1.1~~4~~	0.8~~75~~	-2.2~~4~~	3.02~~2.99~~	1.2~~2~~	0	7	0	7
May	-3.4~~3~~	-0.5~~49~~	-7.6~~2~~	7.1~~3~~	2.01~~1.97~~	1	6	5	12
Jun	-2.8~~0~~	0.1~~2~~	-8.7~~2~~	8.8~~4~~	2.1~~1~~	5	8	5	18
Jul	-1.7~~69~~	0.9	-4.5~~46~~	5.4~~36~~	1.6~~58~~	5	17	2	24
Aug	-1.6~~56~~	0.9~~86~~	-4.2~~2~~	5.1~~08~~	1.4~~2~~	4	18	4	26
Sep	-2.8~~1~~	0.9~~4~~	-5.6~~57~~	6.5~~1~~	1.7~~0~~	4	9	3	16
Oct	-3.4~~37~~	-0.1~~3~~	-10.3~~1~~	10.2~~18~~	2.7~~0~~	12	13	5	30
Nov	-1.7~~3~~	0.6~~1~~	-6.0~~3~~	6.6~~4~~	1.5~~3~~	15	10	1	26
Dez	-1.4~~0~~	1.5~~3~~	-7.4~~3~~	9.08~~8.96~~	1.9~~88~~	28	15	5	48

Table 5 Isotopic data of *Cytheridella ilosvayi* in comparison to its host water

Sample	**Water**			***Cytheridella ilosvayi***										
					$\delta^{18}O_{valve}$ (‰ V-PDB)					**$\delta^{13}C_{valve}$** (‰ V-PDB)				
	$\delta^{18}O$ (‰ V-SMOW)	**$\delta^{13}C_{DIC}$** (‰ V-PDB)	**Temp** (°C)	**n**[a]	**Mean**	**STD**	**Min**	**Max**	**Max-Min**[b]	**Mean**	**STD**	**Min**	**Max**	**Max-Min**[b]
LSS	-1.3~~28~~	-2.3~~28~~	26.8	1	-2.1~~09~~	-	-	-	-	-2.6~~1~~	-	-	-	-
SC-3	-1.4~~0~~	-8.9~~88~~	20.3	8	-1.8~~0~~	1.0~~0.96~~	-3.1~~05~~	-0.5~~3~~	2.5~~2~~	-8.2~~17~~	1.0~~0.98~~	-9.6~~59~~	-6.6~~4~~	3.0~~2.95~~
BiC	-0.5~~4~~	-9.6~~55~~	20.8	2	-1.3~~27~~	0.0~~2~~	-1.3~~29~~	-1.3~~26~~	(0.0~~3~~)	-9.0~~4~~	1.8~~0~~	-10.3~~1~~	-7.8~~76~~	(2.5~~5~~)
LX-1	0.3~~28~~	-9.7~~0~~	30.6	8	-0.2~~2~~	0.8~~77~~	-1.8~~78~~	0.6~~58~~	2.4~~36~~	-8.0~~7.95~~	0.7~~65~~	-8.7~~1~~	-7.0~~4~~	1.7~~67~~
LX-2	0.3~~2~~	-10.6~~0~~	30.5	9	-1.3~~26~~	0.7~~67~~	-2.2~~4~~	-0.3~~27~~	1.9~~1.97~~	-8.5~~1~~	0.5~~46~~	-9.2~~4~~	-7.8~~77~~	1.4~~47~~
LX-3	0.3~~28~~	-10.4~~2~~	31.7	8	-0.8~~76~~	0.7~~67~~	-1.5~~4~~	0.6~~55~~	2.1~~09~~	-8.4~~4~~	0.5~~3~~	-9.5~~47~~	-8.0~~7.96~~	1.5~~1~~
LX-5	0.1~~1~~	-9.9~~2~~	30.4	7	-1.1~~1~~	1.2~~16~~	-2.9~~87~~	0.1~~3~~	3.0~~0~~	-8.2~~1~~	0.6~~1~~	-8.8~~75~~	-7.3~~29~~	1.5~~46~~
EG	0.2~~19~~	-6.1~~3~~	33.1	7	-1.3~~0~~	1.3~~2~~	-2.4~~2~~	0.7~~66~~	3.1~~08~~	-6.0~~1~~	2.1~~2~~	-7.9~~87~~	-2.7~~1~~	5.2~~16~~
CAL-1	2.4~~35~~	-5.5~~2~~	31	4	1.3~~25~~	0.5~~49~~	0.8~~78~~	1.8~~2~~	1.0~~4~~	-6.2~~27~~	0.2~~2~~	-6.5~~3~~	-6.1~~06~~	0.4~~47~~
CAL-2	1.7~~2~~	-7.8~~1~~	30.5	6	0.1~~05~~	0.5~~45~~	-0.7~~68~~	0.5~~3~~	1.2~~1~~	-6.1~~08~~	0.3~~25~~	-6.3~~4~~	-5.7~~69~~	0.6~~5~~
CAL-4	0.4~~0~~	-8.3~~1~~	35.5	16	1.1~~2~~	0.8~~76~~	-0.2~~16~~	2.3~~28~~	2.5~~44~~	-7.0~~3~~	0.5~~1~~	-7.9~~86~~	-6.2~~4~~	1.7~~62~~
CAL-5	-0.7~~3~~	-9.0~~8.98~~	34.7	8	-1.0~~3~~	1.2~~16~~	-2.3~~28~~	0.3~~0~~	2.6~~58~~	-8.1~~06~~	0.5~~46~~	-8.5~~2~~	-7.4~~38~~	1.1~~4~~
LH	1.7~~1~~	-6.6~~2~~	28.3	1	-0.3~~29~~	-	-	-	-	-6.4~~36~~	-	-	-	-
PR	-0.3~~28~~	-12.4~~36~~	28.3	4	-1.0~~2~~	0.2~~16~~	-1.3~~25~~	-0.9~~89~~	0.4~~36~~	-9.1~~0~~	0.3~~3~~	-9.4~~2~~	-8.7~~66~~	0.7~~6~~
SC-15	-1.7~~4~~	-10.7~~3~~	31.2	7	-2.1~~09~~	0.7~~3~~	-3.0~~2.99~~	-1.0~~0.95~~	2.0~~4~~	-8.8~~77~~	0.5~~1~~	-9.6~~59~~	-8.2~~18~~	1.4~~1~~

[a] n- number of measurements

[b] numbers in brackets are excluded from the discussion of the within-sample variability

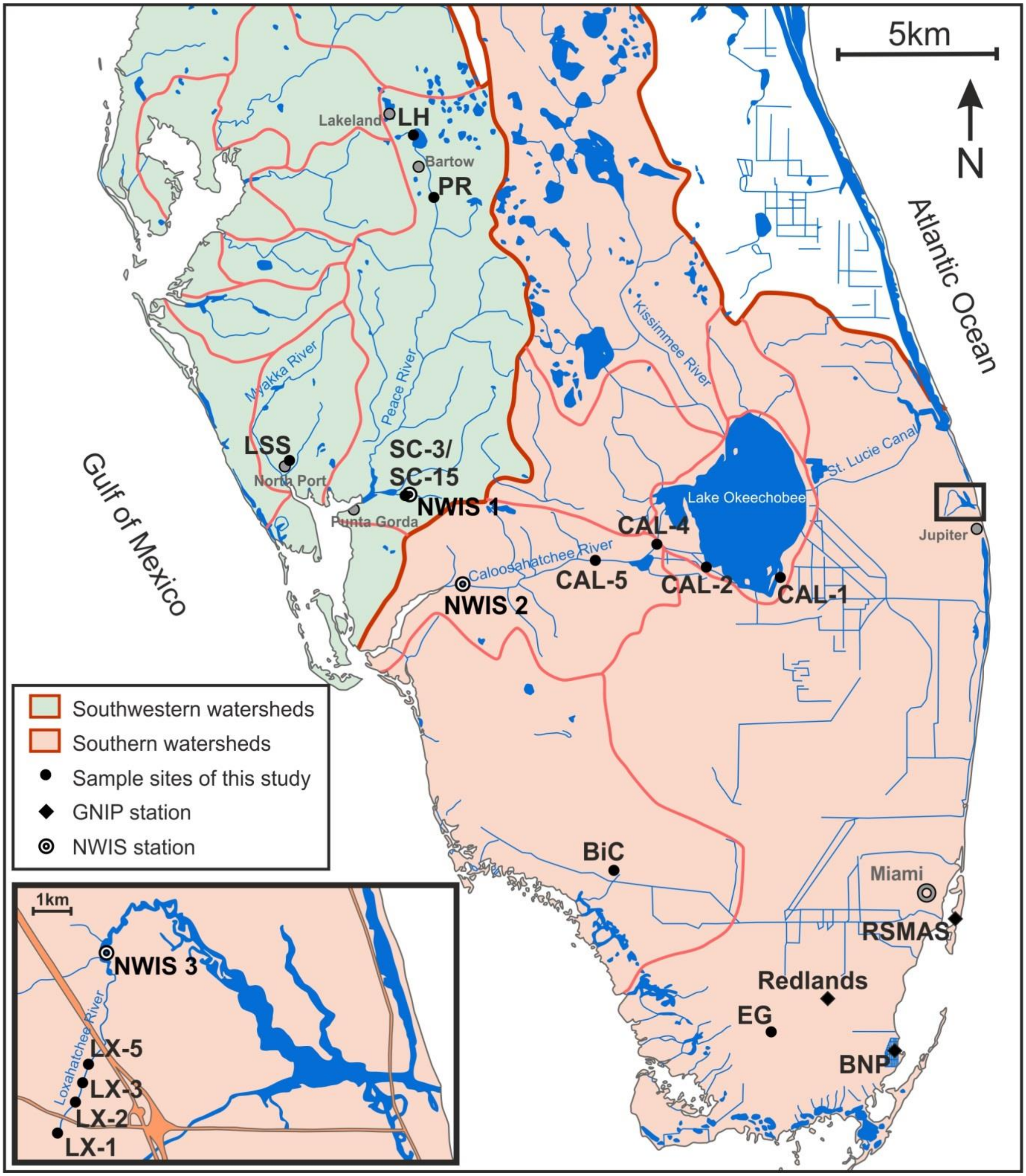

Figure 1: Location of sample sites (BiC=Big Cypress Swamp, CAL=Caloosahatchee River, EG=Everglades, LH= Lake Hancock, LSS=Little Salt Spring, LX= Loxahatchee River, PR= Peace River, SC=Shell Creek). Also included are GNIP stations (Redlands, RSMAS, BNP) and NWIS stations (NWIS 1=02297635; NWIS 2=02292900; NWIS 3=265906080093500).

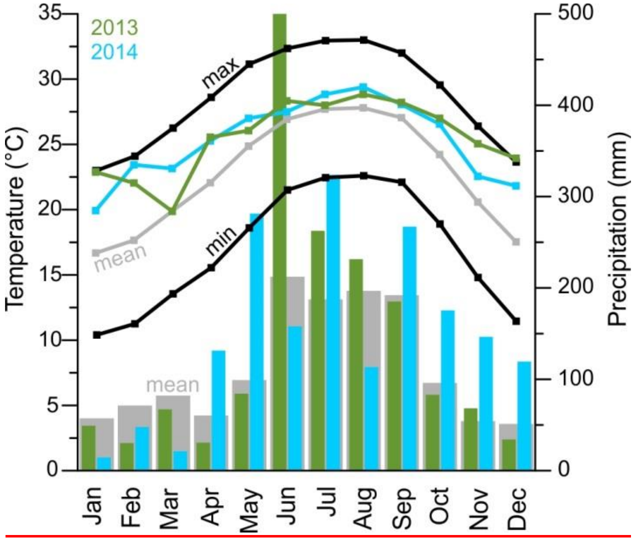

Figure 2: Fifty-years average (1955-2005) of maximum and minimum temperatures and mean precipitation of the southwestern and southern catchment area of Florida in comparison to mean temperature and mean precipitation of Miami 2013 (green) and~~/~~ 2014(blue) (National Climate Change Viewer, U.S. Geological Survey; http://www2.usgs.gov/climate_landuse/clu_rd/nccv.asp; Adler and Hostetler, 2013)

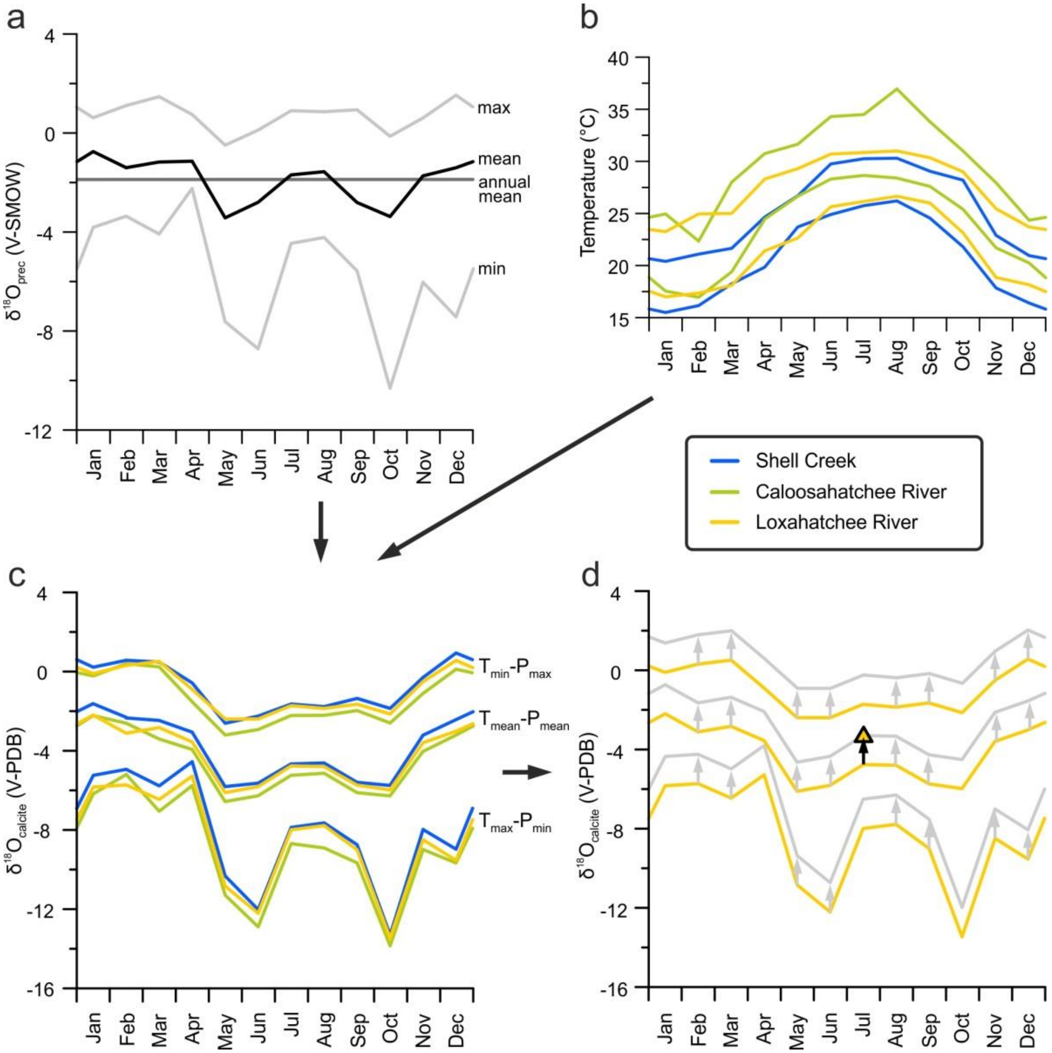

Figure 3: Modeling of the monthly composition of a theoretical calcite formed in equilibrium in Florida rivers (a) precipitation $\delta^{18}O$; (b) water temperature from rivers in Florida; (c) calculated calcite ranges using equation (1); (d) example for the offset correction from LX-4. For detailed explanations see text (chapter 3.4).

5

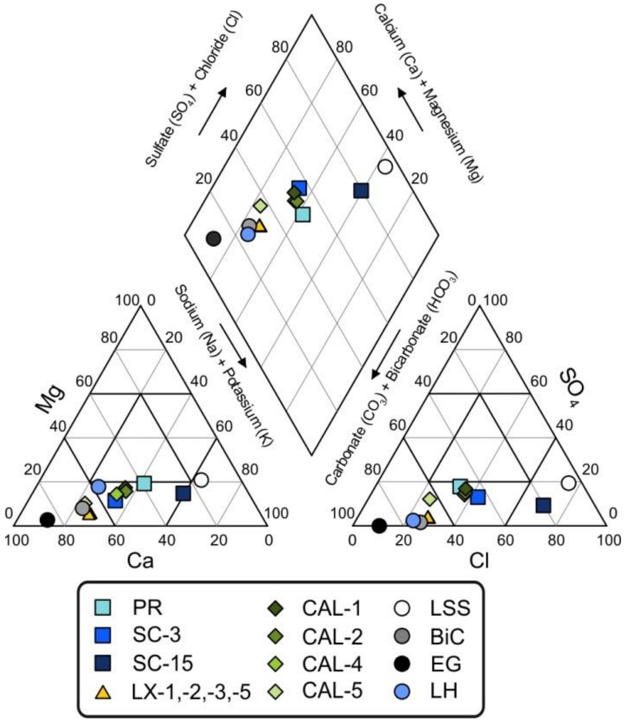

Figure 4: Piper diagram illustrating the major ion composition of the investigated sites.

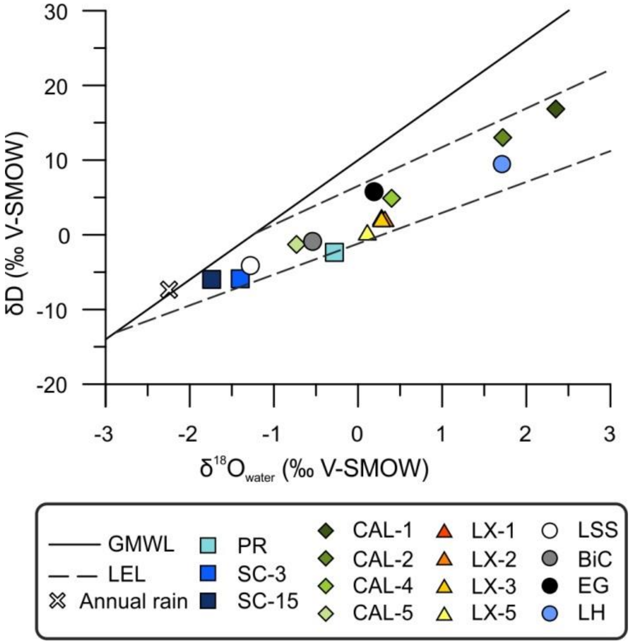

Figure 5: Stable oxygen and deuterium isotope composition of all water samples in comparison to the Global Meteoric Water Line (GMWL) (Craig 1961) and Local Evaporation Lines (LEL) from Meyers et al. (1993). Also included is the annual rainfall calculated from GNIP stations.

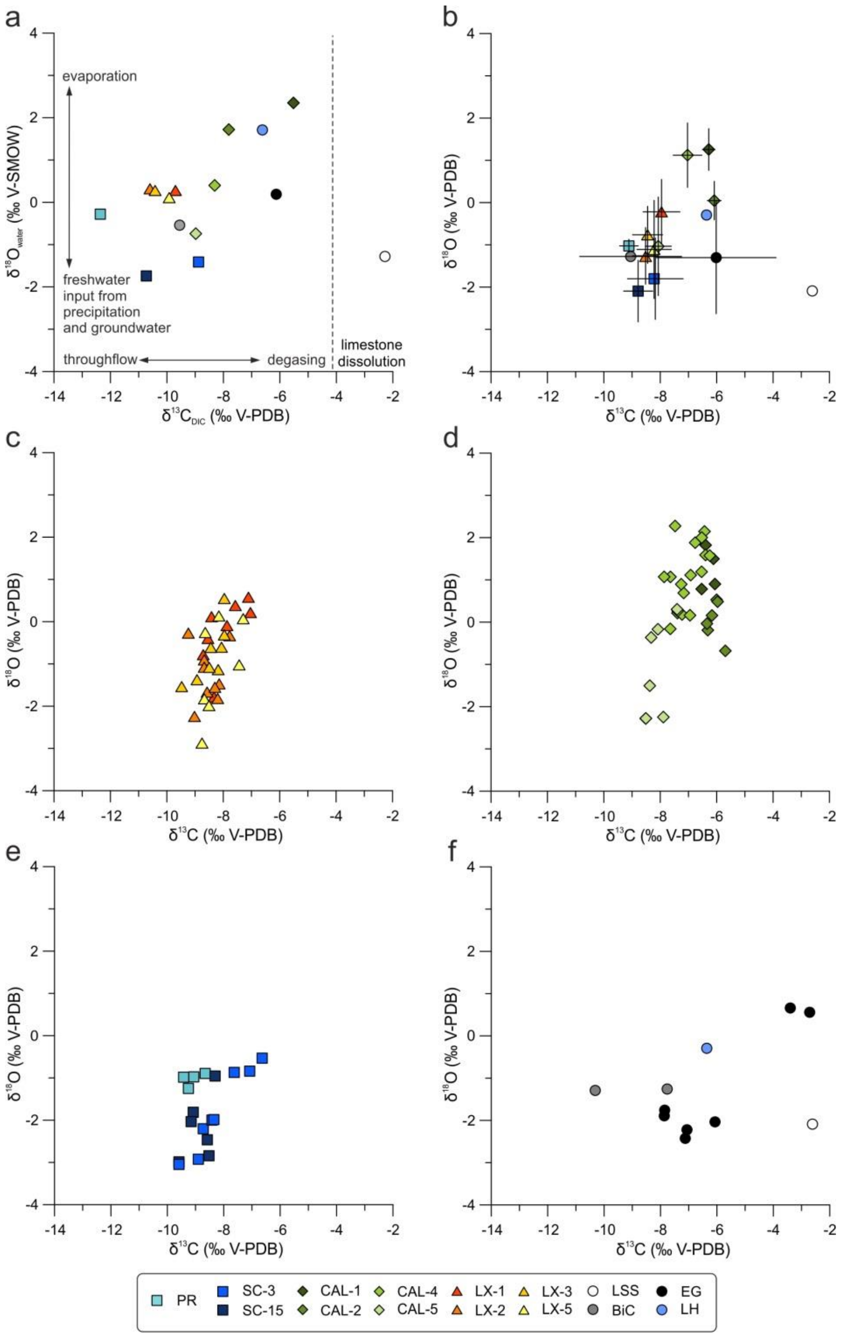

Figure 6: δ13C and δ^{18}O values of water samples and *C. ilosvayi*. (a) water samples, controls on the isotopic composition of the sites are indicated; (b)-(f) *C. ilosvayi*: (b) mean values of all sites with standard deviation; (c) *C. ilosvayi* from Loxahatchee River; (d) *C. ilosvayi* from Lake Okeechobee Canal and Caloosahatchee River; (e) *C. ilosvayi* from Peace River and Shell Creek; (f) *C. ilosvayi* from Everglades, Big Cypress Swamp, Lake Hancock and Little Salt Spring

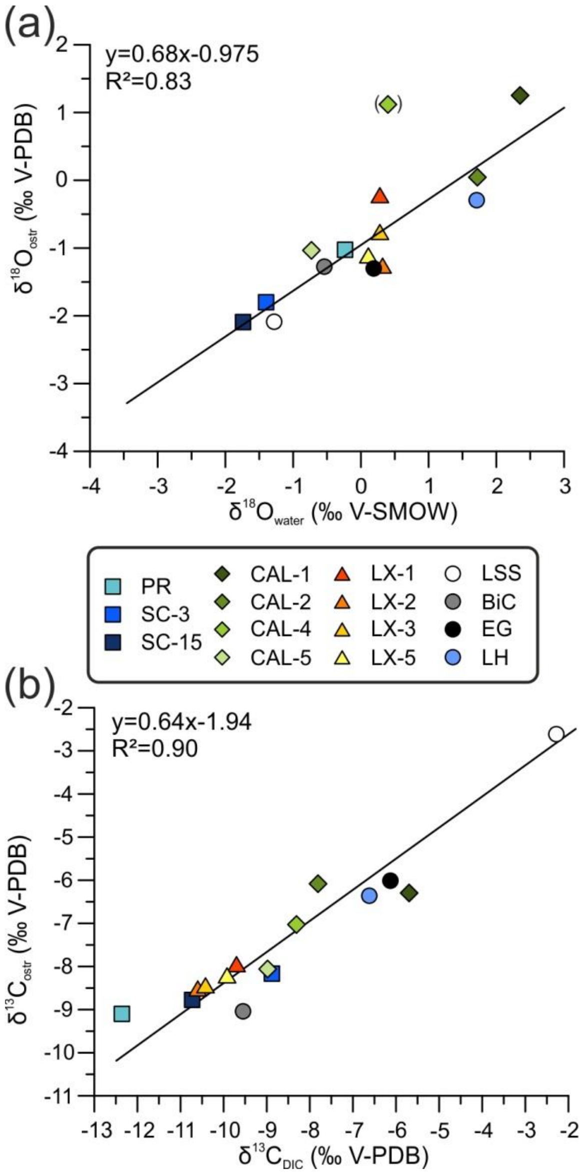

Figure 7: Correlation of the isotopic composition of ostracod valves of *C. ilosvayi* vs. the water in which they evolved: (a) ostracod δ^{18}O vs. water δ^{18}O, sample in brackets is excluded from the correlation statistics, for further explanations see text; (b) ostracod δ^{13}C vs. $\delta^{13}C_{DIC}$

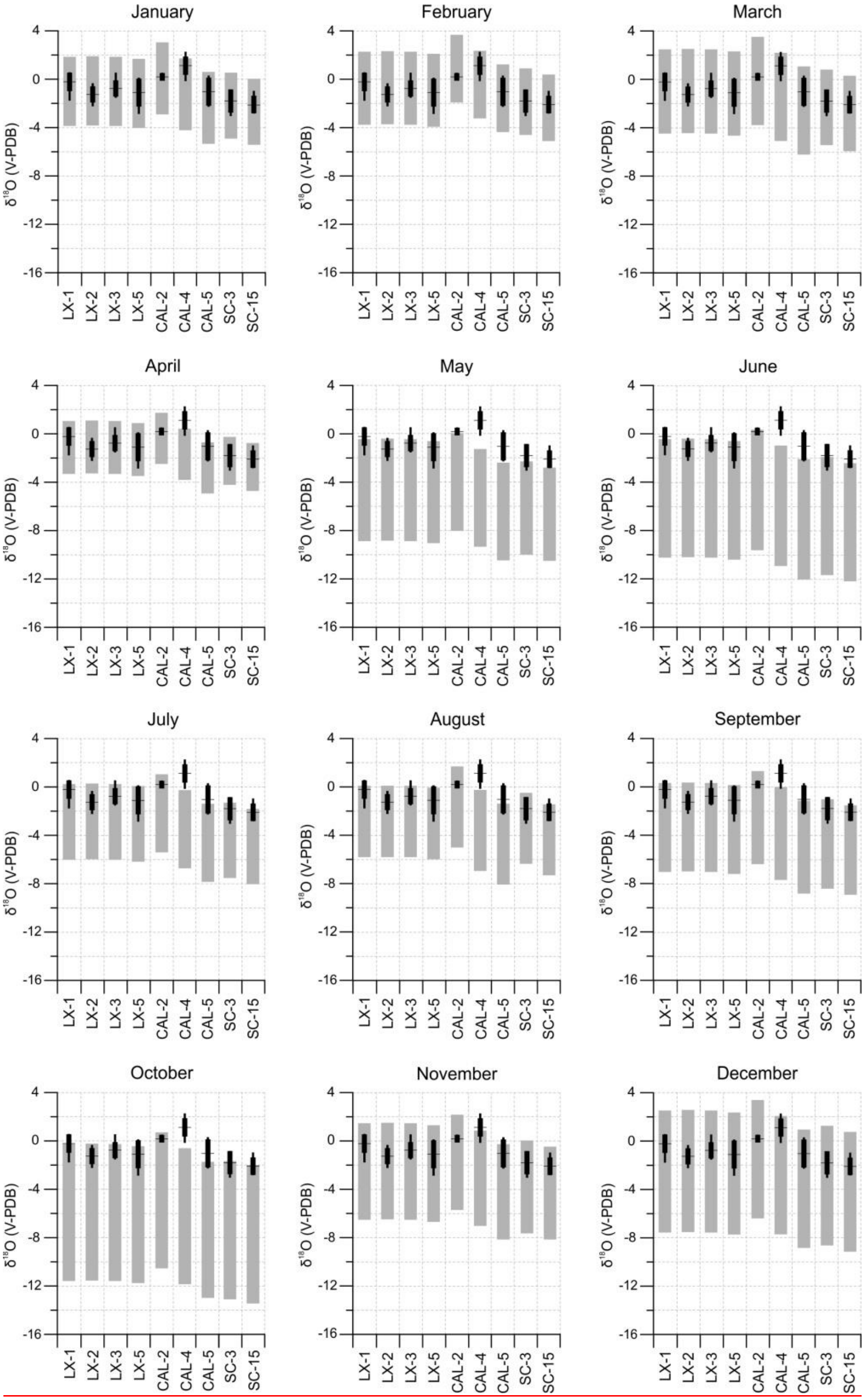

Figure 8: $\delta^{18}O$ of *C. ilosvayi* compared to $\delta^{18}O$ range of a calcite in isotopic equilibrium calculated from water temperatures obtained from NWIS stations and precipitation $\delta^{18}O$ from GNIP stations. Horizontal black lines indicate mean values of *C. ilosvayi* $\delta^{18}O$, black vertical lines indicate maximum and minimum values and black vertical bars show the standard deviation. Gray bars indicate the maximum range of $\delta^{18}O$ of a calcite in isotopic equilibrium during the particular month.