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Modeling calcification periods of *Cytheridella ilosvayi* from Florida based on isotopic signatures and hydrological data

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Abstract. The isotopic signatures of ostracod shells are the result of the temperature and composition of their host water and the phenology and ecology of the target species. Investigations addressing the influence of site-specific environmental variations on the isotopic ranges of ostracod shells are still rare but can provide important information on habitat-dependent variations and may signify a seasonally 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 freshwater habitats (rivers, lakes, canals, marshes, sinkholes) in South Florida from winter 2013 and summer 2014. We also present instrumental data of river temperatures and $\delta^{18}O$ of precipitation ($\delta^{18}O_{prec}$) from this region. The physicochemical and isotopic compositions of the selected sites characterize the different habitats and show the influence of the source water, biological activity, and duration of exposure to the surface. Mean $\delta^{18}O_{ostr}$ and $\delta^{13}C_{ostr}$ signatures of *C. ilosvayi* shells correlate well with the isotopic composition of their host waters. Within-sample variabilities in repeated isotopic measurements of single ostracod shells reflect habitat-dependent ranges. The similarly high range of ostracod $\delta^{18}O$ in rivers and one marsh sample indicates that both temperature and $\delta^{18}O_{prec}$ are responsible for their variation in the whole study area. Rivers and canals, which are predominantly influenced by the 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 data of water temperatures and $\delta^{18}O_{prec}$ were used to calculate monthly $\delta^{18}O$ variations in a theoretical calcite formed in rivers in Florida assuming a direct reaction on precipitation changes. The 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. Inferred configurations were used to approximate possible calcification periods of *C. ilosvayi*. For a plausible calcification period, mean values and ranges of $\delta^{18}O_{ostr}$ had to be equal to the theoretical calcite with a slight positive offset (vital effect). The applied model suggests a seasonal calcification period of *C. ilosvayi* in early spring that is probably coupled to the hydrologic cycle of Florida.

1 Introduction

Ostracods are small aquatic crustaceans, which produce shells composed of low-Mg calcite. They molt up to eight times before they reach their adult stage. The 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 nonseasonal 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 in 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 the environmental parameters influencing their occurrence is scattered.

Commonly, life cycle information is gained through repeated sampling of living ostracod material (seasonally, monthly) during a 1- or 2-year period for specific sites (e.g., Schweitzer and Lohmann, 1990; Cronin et al., 2005; Decrouy, 2012; Marco-Barba et al., 2012). Thereby, uncertainties in 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 containing stable isotopes (δ^{18}O, δ^{13}C) that 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 in shells within an ostracod population depends on the timing and duration of species-specific molting periods. Especially in seasonally varying environments, fast changes in water conditions are possible and probably do not reflect annual mean isotopic values. Hence, a seasonally restricted shell calcification may be indicative of certain days, months, or seasons. Thus, the isotopic signatures of single shells can provide short-term information on molting and changes in the population structure.

How strong single abiotic parameters influence certain ostracod habitats depends on regional conditions (e.g., climate, geology, geography) and 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 that occurs in aquatic systems from southern Brazil and northern Argentina to Yucatán and South Florida (Wrozyna et al., 2017) at water temperatures between 16 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 ($< 5960\,\mu S\,cm^{-1}$) 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 in habitats and may affect the spatial and seasonal occurrence of *C. ilosvayi* and its isotopic shell signatures. As for many other ostracods species, the knowledge of the life cycle of *C. ilosvayi* is poor. Indications of seasonal and 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 the 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 the environmental parameters responsible for these ranges? (3) Can the δ^{18}O variation in *C. ilosvayi* signify the annual range or seasonally restricted variations in the suggested parameters for certain habitats? (4) Is it possible to deduce the calcification period from the 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 the highest air temperatures in August (22.6–33.0 °C) and a winter dry season from November to April with the lowest air temperatures in January (10.4–23.0 °C; Fig. 1). 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. Seaborne vapor arises from the tropical North Atlantic, dominating the weather during the wet season, while within the winter dry season maritime tropical air alternates 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.,

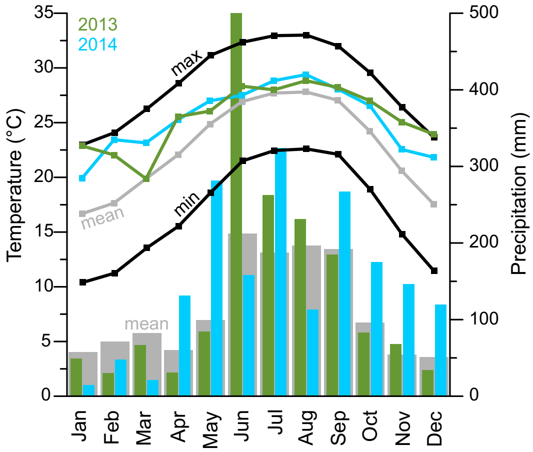

Figure 1. Fifty-year average (1955–2005) of maximum and minimum temperatures and mean precipitation of the southwestern and southern catchment area of Florida in comparison to the mean temperature and mean precipitation of Miami in 2013 (green) and 2014 (blue; National Climate Change Viewer, US Geological Survey; http://www2.usgs.gov/climate_landuse/clu_rd/nccv.asp; Adler and Hostetler, 2013).

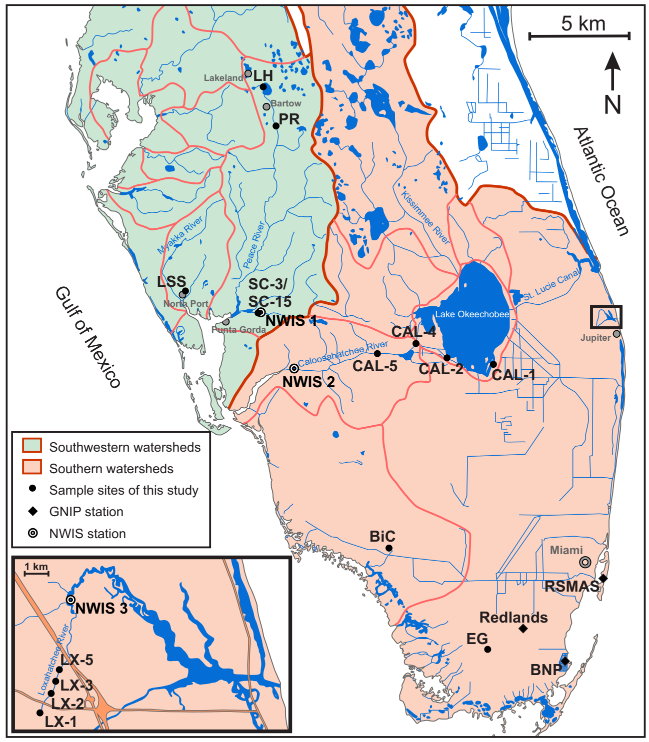

Figure 2. Location of sample sites (BiC: Big Cypress Swamp, CAL: Caloosahatchee River, EG: Everglades, LH: Lake Hancock, LSS: Little Salt Spring, LX: Loxahatchee River, PR: Peace River, SC: Shell Creek). Also included are GNIP stations (Redlands, RSMAS, BNP) and NWIS stations (NWIS 1 = 02297635; NWIS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

2008). When cold fronts from the north pass the peninsula, intense precipitation sometimes 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 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 and 28.8 °C in 2013 and 28.8 and 29.4 °C in 2014, respectively). Annual precipitation during 2013 was slightly higher than in 2014 with 1790 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 when rain amounts were twice as high as usual with ∼ 500 mm.

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. 2).

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, 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 and the most important groundwater source for most surface waters. It is not only recharged 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, saltwater intrusions into the SAS are possible depending on the porosity and the permeability of the aquifer, building a gradual mixing zone (Meyers et al., 1993; Wicks and Herman, 1995). Along its flow path the carbonate content of the aquifer increases and the calcium-carbonate water type dominates, while in

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 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, still-water area	0.1–0.4
LH	07.08.2014	28°0′7.310″	081°51′4.22″	Lake Hancock, at Lakeland	Lake	Lake inflow, still-water area	0.05–0.2
PR	07.08.2014	27°48′46.20″	081°47′36.90″	Peace River, at Bartow	River	Boat ramp, still-water 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

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 where a further sample was taken. Such sinkholes transport water from deeper groundwater to the surface (Maddox et al., 1992; Alvarez-Zarikian et al., 2005).

The 10 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) that forms 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 was taken 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. Samples 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 of distance.

Water levels in South Florida vary with seasonally fluctuating rain amounts. This results in increasing river runoff from June to September and the drying 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).

2.3 Seasonal variations in water isotopes

Precipitation determines the isotopic composition (δD, δ^{18}O) of surface water and groundwater in Florida, as it is the major water source for 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 from 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 the surface water level increases and the 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 the lowest monthly evaporation occurring from December to February when solar radiation is the lowest, and the 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).

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

Rivers in Florida react directly to rain events, which can be seen in the increase in overland flow in the beginning of the wet season. This will result in a direct reaction to isotopic changes in 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., 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 the 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 lower close to the surface where soil water processes dominate the system. Soil CO_2 mainly reflects the $\delta^{13}C$ values of accumulated dead vegetation with values between -23 ‰ (C_3 plants) and -9 ‰ (C_4 plants; Clark and Fritz, 1997). The seasonal variation in $\delta^{13}C$ 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).

3 Material and methods

3.1 Sampling and water analyses

Sampling and analytical methods are similar to Meyer et al. (2016). Sampling was performed in the littoral zone and still-water areas 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 the soft tissues of living animals. Simultaneously, water samples were taken and field 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 through the chromium reduction method (Morrison et al., 2001). The 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). The results of isotopic measurements are given in per mil (‰) with respect to Vienna Standard Mean Ocean Water (VSMOW) and Vienna Peedee Belemnite (VPDB), 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 samples under a binocular (Zeiss Discovery V8) and shells of *Cytheridella ilosvayi* (von Daday, 1905) were separated and stored in micro slides for isotopic measurements. *C. ilosvayi* was identified by the morphological features of the shell in accordance with the description of Purper (1974).

Prior to isotopic analyses, soft-part tissues and contamination were removed from all ostracod valves with deionized

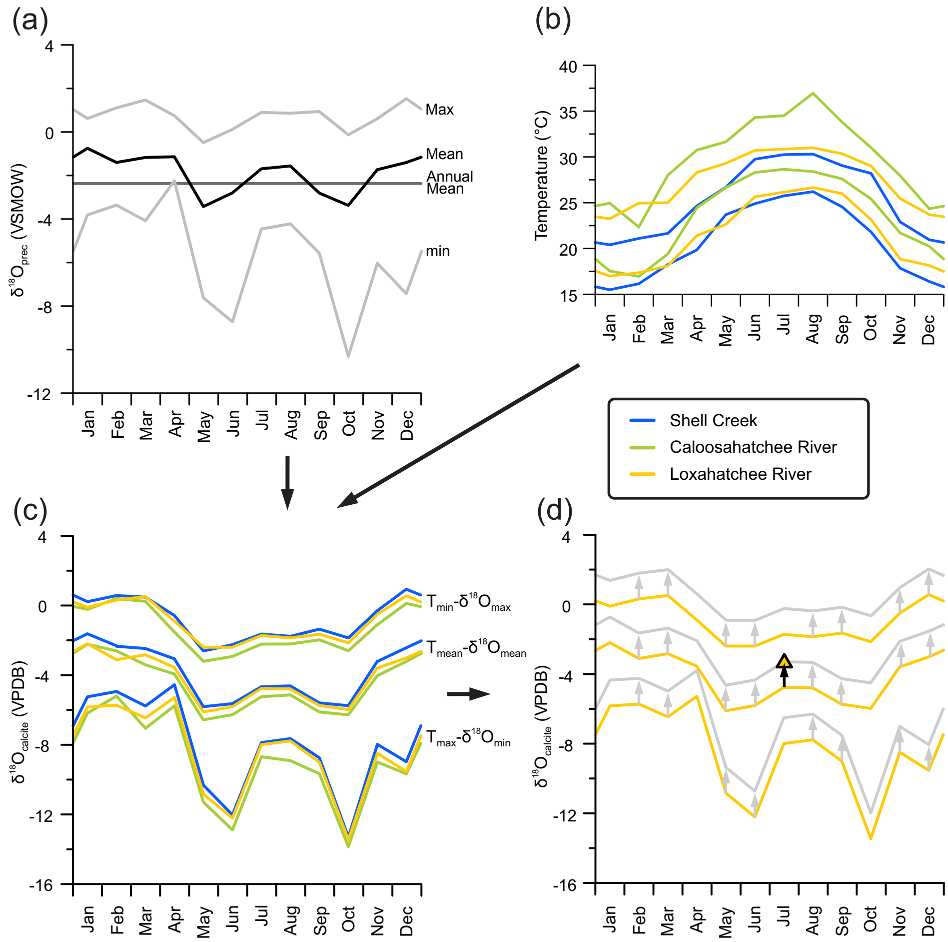

Figure 3. Modeling of the monthly composition of a theoretical calcite formed in equilibrium in Florida rivers: **(a)** precipitation $\delta^{18}O$; **(b)** water temperature from rivers in Florida; **(c)** calculated calcite ranges using Eq. (1); **(d)** example for the offset correction from LX-4.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

water, brushes, and entomological needles. If necessary, single valves were cleaned with H_2O_2 (10 %) for 5 to 10 min at room temperature.

Stable isotopic measurements of *C. ilosvayi* were performed at the Institute of Earth Sciences, University of Graz. One to 16 measurements per sample were performed for carbon and oxygen stable isotopes (see the Supplement). 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).

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. The 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 VPDB.

The 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-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:

$$\delta^{18}\mathrm{O}_{\mathrm{calcite}} = 1000 - \left(e^{\left(\frac{18.03 \times \left(\frac{1000}{T} \right) - 32.42}{1000} \right)} \times \left(1000 + \delta^{18}\mathrm{O}_{\mathrm{water}} \right) \right), \quad (1)$$

where T is the measured water temperature in Kelvin.

Generally, $\delta^{18}O_{water}$ values are expressed relative to VSMOW, whereas $\delta^{18}O_{calcite}$ values are expressed relative to VPDB. To convert the $\delta^{18}O_{water}$ values in the equation relative to VPDB, 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 (CAL-1, CAL-2) were selected to compare the $\delta^{18}O_{ostr}$ ranges with the calculated isotopic compositions of a calcite precipitated under equilibrium with river water.

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 in 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 is 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 1 month at maximum; (5) and there is a constant positive vital effect for the species of about +1 ‰ (Escobar et al., 2012).

Hence, the calculation of the monthly ranges of an equilibrium calcite from a certain site is performed as follows.

a. Monthly values of $\delta^{18}O_{prec}$ and water temperature (min, max, mean) are used in Eq. (1) to calculate the maximum variation in a theoretical equilibrium calcite for 1 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 used to calculate the mean isotopic value of an equilibrium calcite precipitated in the particular aquatic system.

c. Referring to Eq. (1), the enrichment of heavy isotopes of the investigated sites is considered to be constant during the year and seasonal changes in evaporation are neglected for the correction of their values. Hence, the 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. Thus, 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 2013–2014 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 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). For this study all available data from the 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.

The locations of the GNIP and NWIS sites are displayed in Fig. 2.

For a plausible calcification time inferred from the $\delta^{18}O_{ostr}$ range of *C. ilosvayi*, two requirements have to be met: (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) 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).

4 Results

4.1 Physicochemical 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), salinity, and laboratory analyses (major ions, δD, $\delta^{18}O_{water}$, $\delta^{13}C_{DIC}$) are summarized in Table 2 and Figs. 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 (Table 2). The TDS values of Loxahatchee River are similar between sampling locations ($\sim 275\,\mathrm{mg\,L^{-1}}$), while samples of Lake Okeechobee Canal and Caloosahatchee River have higher values and cover a wider range (310.1–503.3 $\mathrm{mg\,L^{-1}}$). The TDS value of BiC (464.0 $\mathrm{mg\,L^{-1}}$) is most similar to CAL samples. Within the Peace River basin, TDS values are lower for LH and PR (187.0 and 125.8 $\mathrm{mg\,L^{-1}}$) than for Shell Creek in winter

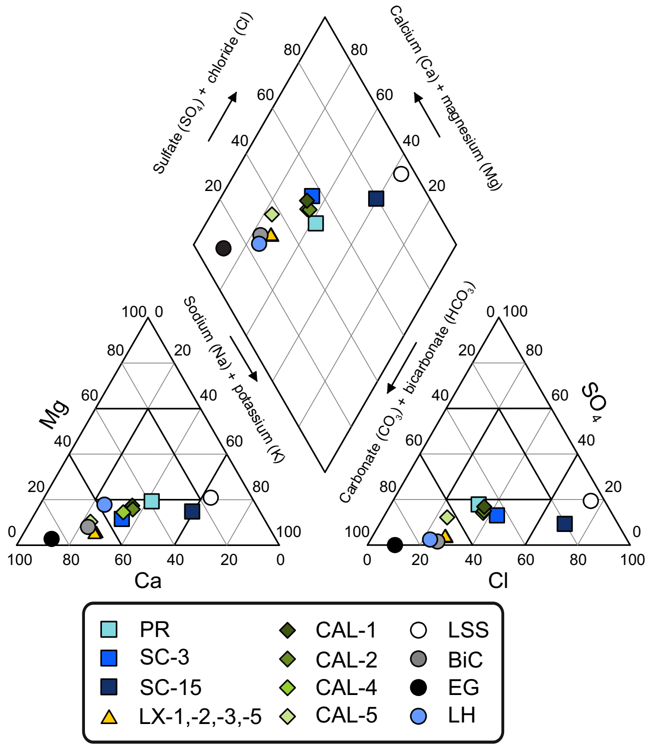

Figure 4. Piper diagram illustrating the major ion composition of the investigated sites.

Table 2. Physicochemical and stable isotope characteristics of the studied sites.

Sample	Field parameters					Cations				Anions					Stable isotopes		
	Temp (°C)	pH	EC ($\mu S\,cm^{-1}$)	Sal (psu)	TDS ($mg\,L^{-1}$)	Na ($mg\,L^{-1}$)	K ($mg\,L^{-1}$)	Mg ($mg\,L^{-1}$)	Ca ($mg\,L^{-1}$)	Cl ($mg\,L^{-1}$)	NO_3 ($mg\,L^{-1}$)	SO_4 ($mg\,L^{-1}$)	HCO_3 ($mg\,L^{-1}$)	Br ($mg\,L^{-1}$)	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	−1.3	−2.3
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	−8.9
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	−0.5	−9.6
LX-1	30.6	7.3	363	0.22	270.1	22.8	1.4	2.6	50.1	35.8	0.4	6.6	150.7	0.07	2.4	0.3	−9.7
LX-2	30.5	7.2	375	0.23	276.6	23.0	1.4	2.6	51.7	36.3	0.4	7.4	154.4	0.04	2.1	0.3	−10.6
LX-3	31.7	6.1*	375	0.23	275.8	23.1	1.4	2.6	52.1	36.1	0.5	7.3	153.2	0.06	2.1	0.3	−10.4
LX-5	30.4	7.1	375	0.23	276.0	22.8	1.4	2.5	51.5	36.1	< 0.01	7.3	154.4	0.07	0.3	0.1	−9.9
EG	33.1	8.1	189	0.11	152.8	5.2	0.3	0.7	33.3	7.2	< 0.01	< 0.1	106.2	< 0.01	5.8	0.2	−6.1
CAL-1	31	8.6	444	0.27	310.1	32.8	5.9	9.4	42.3	57.8	< 0.01	30.1	131.8	0.16	16.9	2.4	−5.5
CAL-2	30.5	7.4	676	0.4	466.1	51.7	9.5	13.1	65.8	85.7	0.2	47.5	192.8	0.21	13.0	1.7	−7.8
CAL-4	35.5	7.5	724	0.43	503.3	52.3	8.3	13.1	77.9	90.5	0.5	57.4	203.8	0.24	4.9	0.40	−8.3
CAL-5	34.7	7.4	550	0.33	394.4	25.8	6.2	7.0	74.8	45.4	0.2	30.8	204.4	0.14	−1.3	−0.7	−9.0
LH	28.3	7.0	247	0.14	187.0	12.2	3.3	5.4	29.0	20.0	0.5	2.9	114.1	0.01	9.5	1.7	−6.6
PR	28.3	6.5	189	0.11	125.8	14.4	5.4	4.3	14.3	20.6	1.5	15.0	51.9	0.05	−2.4	−0.3	−12.4
SC-15	31.2	7.1	297*	0.18	490.2	105.3	5.4	14.3	41.0	193.8	0.1	34.7	95.8	0.59	−6.0	−1.7	−10.7

* Accuracy of in situ measurement is uncertain.

($655.8\,mg\,L^{-1}$) and in summer ($490.2\,mg\,L^{-1}$). The TDS value of EG is most similar to PR.

Samples can be split into three groups by their major anion and cation composition as calcium-bicarbonate-dominated, sodium-chloride-dominated, or 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.

Temperatures measured during winter were 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. The values in 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 1 day and have a range of 5 °C.

The isotopic values of the water samples range from −6.0 to +16.9 ‰ for δD, from −1.7 to +2.4 ‰ for $\delta^{18}O$, and from −12.4 to −2.3 ‰ for $\delta^{13}C$ (Figs. 5 and 6, Table 2). The δD and $\delta^{18}O$ values deviate negatively to the global meteoric

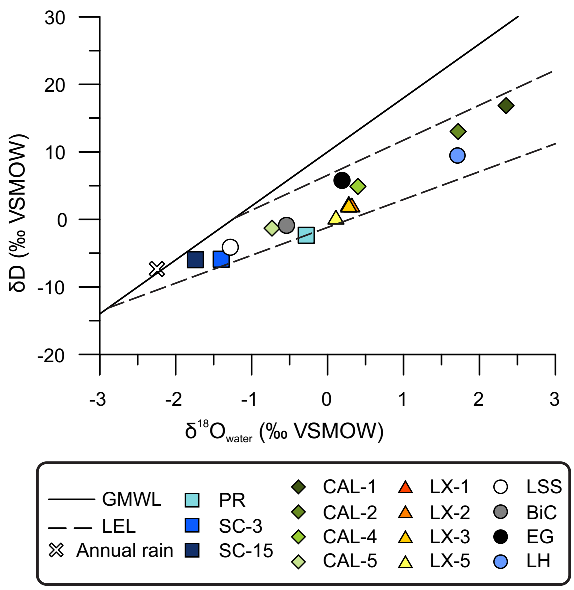

Figure 5. Stable oxygen and deuterium isotope composition of all water samples in comparison to the global meteoric water line (GMWL; Craig, 1961) and local evaporation lines (LELs) from Meyers et al. (1993). Also included is the annual rainfall calculated from GNIP stations.

water line (GMWL) and describe a local evaporation line (LEL) similar to the one described by Meyers et al. (1993); $\delta D = 4.67\ \Delta^{18}O\ (\pm 0.52) + 2.68\ (\pm 3.86)$. 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 ‰ for δD and −1.3 to 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 to +0.4 ‰ 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 to +2.4 for $\delta^{18}O$).

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

4.2 Seasonal variation in 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 in $\delta^{18}O_{prec}$ 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, the highest isotopic values for $\delta^{18}O_{calcite}$ can be calculated from minimum temperatures (T_{min}) and maximum $\delta^{18}O_{prec}$ ($\delta^{18}O_{max}$), and the opposite combination of T_{max} and $\delta^{18}O_{min}$ 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 at the investigated sites all lie in the temperature range of the corresponding NWIS stations except for SC-15 where the temperature exceeds the maximum value by 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 in water temperatures at NWIS stations varies from 17.0 to 31.0 °C for Loxahatchee River (LX), from 15.5 to 30.3 °C for Shell Creek (SC), and from 17.0 to 37.0 °C for Caloosahatchee River (CAL; Table 3) and is correlated with annual air temperatures in Florida with the highest values during the summer wet season and the lowest during the winter dry season. However, the annual river temperature range is smaller than for air temperatures. Hence, calculated $\delta^{18}O_{calcite}$ values are high during winter and decrease until August when temperatures reach their maximum values in all rivers.

The annual temperature range ($T_{max} - T_{min}$) 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 has lower temperatures. In summer, SC and LX are more similar, while CAL has higher temperatures. This results in the lowest $\delta^{18}O_{calcite}$ for CAL during summer and the 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 about 0.2 ‰ in the theoretical calcite (Craig, 1965; Kim and O'Neil, 1997). Thus, the monthly isotopic range for a theoretical calcite caused by temperature differences within the rivers varies from +0.9 to +1.5 ‰ for LX, from +0.6 to +1.3 ‰ for SC, and from +0.8 to +1.7 for CAL.

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

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

The annual range for $\delta^{18}O_{prec}$ varies from −10.3 ‰ in October to +1.5 ‰ in December (Fig. 3a; Table 4). Mean monthly values range from −3.4 ‰ in May to −0.8 ‰ in January. During the whole year values are relatively constant (−1.7 to −0.8 ‰). Only in the beginning (May–June) and the end

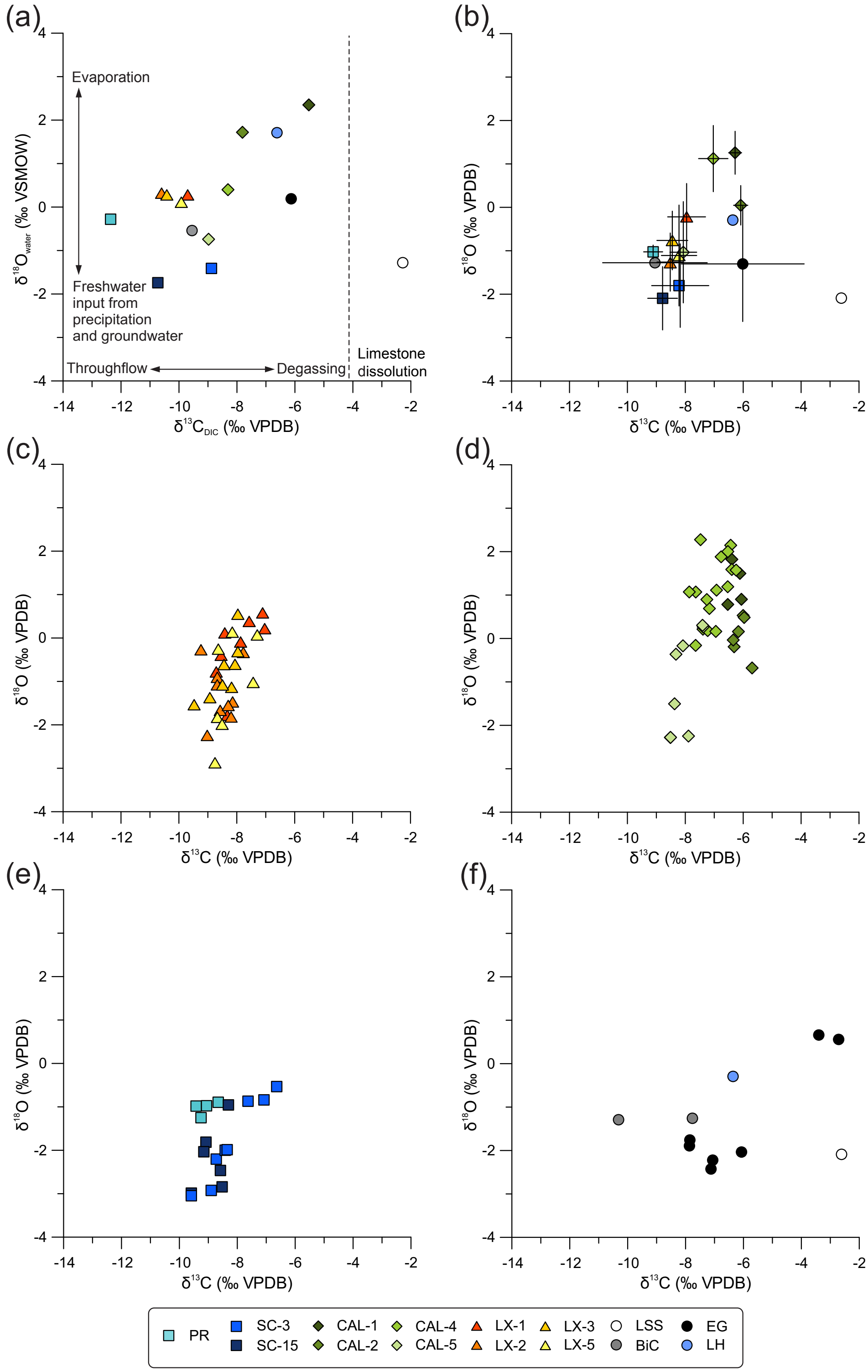

Figure 6. The $\delta^{13}C$ and $\delta^{18}O$ values of water samples and *C. ilosvayi*. **(a)** Water samples; controls on the isotopic composition of the sites are indicated. Panels **(b–f)** *C. ilosvayi*: **(b)** mean values of all sites with standard deviation; **(c)** *C. ilosvayi* from Loxahatchee 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.

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

(October–November) of the wet season do values fall below the annual mean value (−3.4 to −2.8 ‰). Maximum monthly $\delta^{18}O_{prec}$ values vary less than mean values but also show 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 with minimum $\delta^{18}O_{prec}$ values. Negative excursions of $\delta^{18}O_{prec}$ cause greater isotopic range. The lowest variation has been observed at the end of the wet season during April (3.0 ‰), while the highest variation appears in October (10.2 ‰). The strong monthly variation in $\delta^{18}O_{prec}$ can also be seen in $\delta^{18}O_{calcite}$.

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

The increase in $\delta^{18}O_{prec}$ and the decrease in 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 $\delta^{18}O_{calcite}$ for which the variation in precipitation is small and changes in temperature are more dominant. The variation in minimum $\delta^{18}O_{calcite}$ values is dominated by the variation in $\delta^{18}O_{prec}$. This results in the highest values of $\delta^{18}O_{calcite}$ from December to March and the lowest in the beginning and the end of the wet season in May–June and October. The lowest range was observed during April before the beginning of the wet season (4.0 ‰ for SC, 4.2 ‰ for CAL, and 4.4 ‰ for LX) and the highest range in October (11.4 ‰ for SC, 11.3 ‰ for CAL, and 11.3 ‰ for LX).

4.3 Isotopic signatures of ostracod calcite

There is a positive correlation between the mean $\delta^{18}O_{ostr}$ and their host waters ($R^2 = 0.66$) and the mean $\delta^{13}C_{ostr}$ and the dissolved inorganic carbon ($R^2 = 0.90$; Fig. 7). This correlation for $\delta^{18}O_{ostr}$ becomes even more significant by excluding CAL-4 from the statistical analyses ($R^2 = 0.83$).

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 to +1.3 ‰). River and marsh samples from the southern watersheds show mean values in a similar range (−1.3 to −0.2 ‰). Within the Peace River watershed values decrease from north to south. The mean $\delta^{18}O_{ostr}$ values of SC-15 and LSS are the lowest with −2.1 ‰.

All river samples show equally low mean $\delta^{13}C_{ostr}$ values (−9.1 to −8.0 ‰), while canal samples and Lake Hancock exhibit higher values (−7.0 to −6.1 ‰). Both marsh samples have distinct different $\delta^{13}C_{ostr}$ values, whereas BiC is similar to river samples (−9.0 ‰ $\delta^{13}C_{ostr}$) and EG is more similar to canals and rivers (−6.0 ‰ $\delta^{13}C_{ostr}$). LSS has by far the highest $\delta^{13}C_{ostr}$ value (−2.6 ‰).

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 (Table 5; Fig. 6b to f). 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). PR is the only river sample with a distinctly lower $\delta^{18}O_{ostr}$ range (0.4 ‰) than all other samples. The $\delta^{18}O_{ostr}$ variation in the marsh sample EG is slightly higher (3.1 ‰) 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 the lowest values for CAL-2 (+0.7 ‰), while CAL-4 (+1.6 ‰) is more similar to most river samples. The $\delta^{13}C_{ostr}$ variation in river samples ranges from +1.4 to +1.7 ‰ for Loxahatchee River, CAL-5, and Shell Creek in summer, while PR is more similar to canal samples (+0.8 ‰). The range of the Shell Creek winter sample is twice as high as the summer sample (+3.0 ‰). EG has by far the highest $\delta^{13}C_{ostr}$ variation (+5.2 ‰). Additionally, measurements of juvenile (A-1) individuals show

Table 4. Monthly values of δ^{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.

	δ^{18}O (‰ VSMOW)					Number of measurements			
Month	Mean	Max	Min	Max–min	SD	BNP	RSMAS	Redland	Total
Jan	−0.8	0.6	−3.8	4.4	1.5	6	5	1	12
Feb	−1.40	1.1	−3.4	4.5	1.2	2	10	2	14
Mar	−1.2	1.5	−4.1	5.6	1.4	2	11	3	16
Apr	−1.1	0.8	−2.2	3.0	1.2	0	7	0	7
May	−3.4	−0.5	−7.6	7.1	2.0	1	6	5	12
Jun	−2.8	0.1	−8.7	8.8	2.1	5	8	5	18
Jul	−1.7	0.9	−4.5	5.4	1.6	5	17	2	24
Aug	−1.6	0.9	−4.2	5.1	1.4	4	18	4	26
Sep	−2.8	0.9	−5.6	6.5	1.7	4	9	3	16
Oct	−3.4	−0.1	−10.3	10.2	2.7	12	13	5	30
Nov	−1.7	0.6	−6.0	6.6	1.5	15	10	1	26
Dec	−1.4	1.5	−7.4	9.0	1.9	28	15	5	48

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

Sample	Water			*Cytheridella ilosvayi*										
					$\delta^{18}O_{valve}$ (‰VPDB)					$\delta^{13}C_{valve}$ (‰VPDB)				
	δ^{18}O (‰VSMOW)	$\delta^{13}C_{DIC}$ (‰VPDB)	Temp (°C)	n[a]	Mean	SD	Min	Max	Max–min[b]	Mean	SD	Min	Max	Max–min[b]
LSS	−1.3	−2.3	26.8	1	−2.1	–	–	–	–	−2.6	–	–	–	–
SC-3	−1.4	−8.9	20.3	8	−1.8	1.0	−3.1	−0.5	2.5	−8.2	1.0	−9.6	−6.6	3.0
BiC	−0.5	−9.6	20.8	2	−1.3	0.0	−1.3	−1.3	(0.0)	−9.0	1.8	−10.3	−7.8	(2.5)
LX-1	0.3	−9.7	30.6	8	−0.2	0.8	−1.8	0.6	2.4	−8.0	0.7	−8.7	−7.0	1.7
LX-2	0.3	−10.6	30.5	9	−1.3	0.7	−2.2	−0.3	1.9	−8.5	0.5	−9.2	−7.8	1.4
LX-3	0.3	−10.4	31.7	8	−0.8	0.7	−1.5	0.6	2.1	−8.4	0.5	−9.5	−8.0	1.5
LX-5	0.1	−9.9	30.4	7	−1.1	1.2	−2.9	0.1	3.0	−8.2	0.6	−8.8	−7.3	1.5
EG	0.2	−6.1	33.1	7	−1.3	1.3	−2.4	0.7	3.1	−6.0	2.1	−7.9	−2.7	5.2
CAL-1	2.4	−5.5	31	4	1.3	0.5	0.8	1.8	1.0	−6.2	0.2	−6.5	−6.1	0.4
CAL-2	1.7	−7.8	30.5	6	0.1	0.5	−0.7	0.5	1.2	−6.1	0.3	−6.3	−5.7	0.6
CAL-4	0.4	−8.3	35.5	16	1.1	0.8	−0.2	2.3	2.5	−7.0	0.5	−7.9	−6.2	1.7
CAL-5	−0.7	−9.0	34.7	8	−1.0	1.2	−2.3	0.3	2.6	−8.1	0.5	−8.5	−7.4	1.1
LH	1.7	−6.6	28.3	1	−0.3	–	–	–	–	−6.4	–	–	–	–
PR	−0.3	−12.4	28.3	4	−1.0	0.2	−1.3	−0.9	0.4	−9.1	0.3	−9.4	−8.7	0.7
SC-15	−1.7	−10.7	31.2	7	−2.1	0.7	−3.0	−1.0	2.0	−8.8	0.5	−9.6	−8.2	1.4

[a] n is the number of measurements.
[b] Numbers in brackets are excluded from the discussion of the within-sample variability.

no difference in the isotopic composition except for CAL-2 for which the $\delta^{18}O_{ostr}$ values 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 from 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, and 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 Physicochemical and isotopic characteristics of surface water habitats

The wide range of chemical compositions along an ion mixing line of the investigated sites (Fig. 4) reflects the dissolution of carbonates underground and the mixing with seawater that is typical for Florida surface waters (Price and

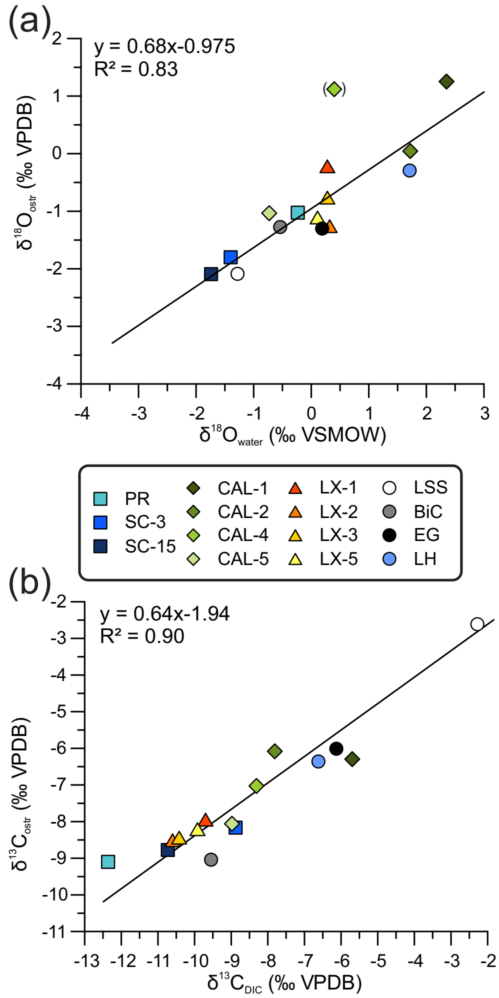

Figure 7. Correlation of the isotopic composition of ostracod valves of *C. ilosvayi* vs. the water in which they evolved: **(a)** ostracod δ^{18}O vs. water δ^{18}O (the sample in parentheses is excluded from the correlation statistics; for further explanations, see the text); **(b)** ostracod δ^{13}C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

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 (Figs. 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 ionic 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 enhance 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 $\sim 28\,°C$ (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 (Tables 2 and 3). A similar temperature variation can also be expected in samples from other rivers, canals, lakes, and marshes.

The δ^{18}O composition of surface waters in Florida evolves through time as a response to 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 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 areas (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 similarly 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 (see the Supplement). 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 affect 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 the dissolution of older marine limestone from the deep groundwater aquifer with the highest observed $\delta^{13}C_{DIC}$ value (−2.3 ‰). The 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 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*

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 also been established 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 is probably the only sample directly influenced by the temporal anthropogenic input of agricultural water. This results in a distinctly different isotopic composition compared to other canal samples with the same chemical composition. Further, the isotopic composition of *C. ilosvayi* from CAL-4 is also more similar to that of CAL-1 (Table 5, Fig. 6d). Thus, the input of agricultural water was probably initiated after valve calcification and before sampling, resulting in a great difference between $\delta^{18}O_{water}$ and $\delta^{18}O_{ostr}$.

It can also be 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. However, 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 differently 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 the 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 that 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 enough between 2013 and 2014 (Fig. 1) to cause extreme differences in surface waters.

Further interpretation of the exact timing of calcification is not possible by comparing mean ostracod values with single-water measurements of different habitats. To overcome the uncertainty in time lags between valve calcification and sampling, more information about the life cycle that includes the molting periods of *C. ilosvayi* is necessary. However, if samples provide enough ostracod material to perform repeated isotopic measurements for one or more ostracod species, the intraspecific 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 (microhabitat) of calcification, and the general characteristics of the environment itself, responsible for the seasonal variations (Decrouy, 2012). Small-scale differences in these factors result in isotopic variation in an ostracod population at a specific site during the period of their valve calcification (within-sample variability). Thus, the isotopic variation in a population is controlled by (1) the duration of the calcification period (Decrouy, 2012), (2) the seasonal environmental variation in a water body (Xia et al., 1997a; von Grafenstein et al., 1999; Decrouy, 2012), and (3) the response of the microhabitat to certain environmental changes (Decrouy, 2012).

The within-sample variability in *C. ilosvayi* was investigated from eight river sites, two canals, and one marsh sample. All investigated habitats are characterized by shallow water areas and a dense macrophyte cover (Table 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 throughflow.

EG exhibits a similar $\delta^{18}O_{ostr}$ variation as all river samples (except PR) and CAL-4, with variations of $+2.0$ to $+3.1$ ‰ (Table 5; Fig. 6). In contrast, CAL-2 has a smaller $\delta^{18}O_{ostr}$ range with 1.2 ‰. The similar variation in the $\delta^{18}O_{ostr}$ ranges in Loxahatchee River can only be explained by a 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 on the whole peninsula 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 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 (Price and Swart, 2006). The permanent replacement of water in rivers results in a direct reaction to changes in the isotopic composition of precipitation. In contrast, canal samples receive their water mainly from Lake Okeechobee (Harvey and McCormick, 2009). In 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. 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 and 2.0 ‰. Together with a similar mean value, this indicates similar water and temperature conditions during the valve calcification time in both years and thus points to a seasonal calcification time of *C. ilosvayi*.

Cytheridella ilosvayi exhibits clear differences in the $\delta^{13}C_{ostr}$ range (Table 5; Fig. 6) between samples with a throughflow and marsh samples, indicating complex interactions of biological characteristics, input from external sources, and mixing. The $\delta^{13}C_{DIC}$ can vary widely within different timescales 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 high biological activity. Strong biological activity can be expected from all investigated habitats. The population of EG shows a higher variation (5.2 ‰) than for canals and rivers (0.5 to 3.0 ‰). A 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 1.1 to 1.7 ‰ in Loxahatchee River, CAL-5, and SC-15. However, changes in the influx, for example the increased input of water from tributary creeks after a rain event, can cause shifts in the $\delta^{13}C_{DIC}$ composition. At SC-3 the $\delta^{13}C_{ostr}$ range is higher (3.0 ‰) than in other river samples, which 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-2 has a lower $\delta^{13}C_{ostr}$ range than rivers (0.5 and 0.7 ‰). 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 creeks. CAL-4 has a $\delta^{13}C_{ostr}$ range similar to river samples (1.6 ‰). The mixing of Lake Okeechobee water with agricultural water probably increases the variation in $\delta^{13}C_{ostr}$ compared to other canals.

Information on the life history of *C. ilosvayi* is almost nonexistent. 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 1-year period (Purper, 1974), while its occurrence in the Paraná River floodplain (Brazil) varied as a result of seasonal recruitment caused by flood pulses (Higuti et al., 2007). Pérez et al. (2010) 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 both had carapaces with soft parts, mostly from females. In Shell Creek we found a similar population variation with living *C. ilosvayi* highly abundant in summer and less in winter. This indicates a seasonal life cycle and a temporally restricted calcification period of *C. ilosvayi* in Florida. It is possible that climatic differences can cause variations in the life cycle 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. Therefore, it can be expected that the within-sample variability from a single species provides information of a similar time frame. Consequently, when the seasonal variation in 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

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 the rivers and canals of Florida and compared it to the within-sample range of ostracods from rivers and canals (Fig. 8).

The calculated monthly mean $\delta^{18}O_{calcite}$ values and ranges vary distinctly seasonally and 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 3 times higher than for *C. ilosvayi*. A shorter calcification period of 2 weeks 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 lower 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 to 9.8 ‰ and remain much higher than the ostracod range. Hence, months with a high $\delta^{18}O_{calcite}$ range can be excluded as a 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 ‰ in SC and 4.4 ‰ 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.

Further, measured mean $\delta^{18}O$ values of *C. ilosvayi* should be similar to the calculated $\delta^{18}O_{calcite}$ and tend to more positive values due to a positive vital effect (Xia et al., 1997a, b; von Grafenstein et al., 1999; Decrouy et al., 2011b). One vital effect for modern *C. ilosvayi* of about +1 ‰ is reported from Lake Petén Itzá (Escobar et al., 2012). Recently, 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, the chemical composition of the investigated sites is considered to be stable enough for a constant vital effect within a sample. During the wet season from May until October, the positive offset of *C. ilosvayi* to the mean calcite value exceeds +1 ‰ by far and even exceeds the maximum values of the theoretical calcite at nearly all sites, excluding these months as calcification times (Fig. 8). In November, the 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 1-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).

As shown above, the $\delta^{13}C_{DIC}$ of rivers depends strongly on the biological activity within the catchment. Variation in the proportion of photosynthesis to respiration may also lead to seasonally characteristic $\delta^{13}C_{DIC}$ compositions and ranges that can be reflected by the ostracod $\delta^{13}C$. When seasonal data on the $\delta^{13}C_{DIC}$ compositions of aquatic environments are available it is possible to compare them to the ostracod ranges and to confine calcification times. Unfortunately, this was not the case for this study. The applied model is restricted to variation in the extreme values of two components influencing the final composition of river waters. Factors like the mixing of different source waters, the variation in evaporation, or the anthropogenic regulation of a surface water 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 than small amounts of precipitation with high values, but low $\delta^{18}O_{prec}$ values are not exclusively connected to heavy rainfall events 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 in precipitation during the sampling years would result in a lower variation in the theoretical calcite, and other months also become plausible as calcification periods. This would be the case for the period from January to March when the isotopic variation would decrease to a range comparable to

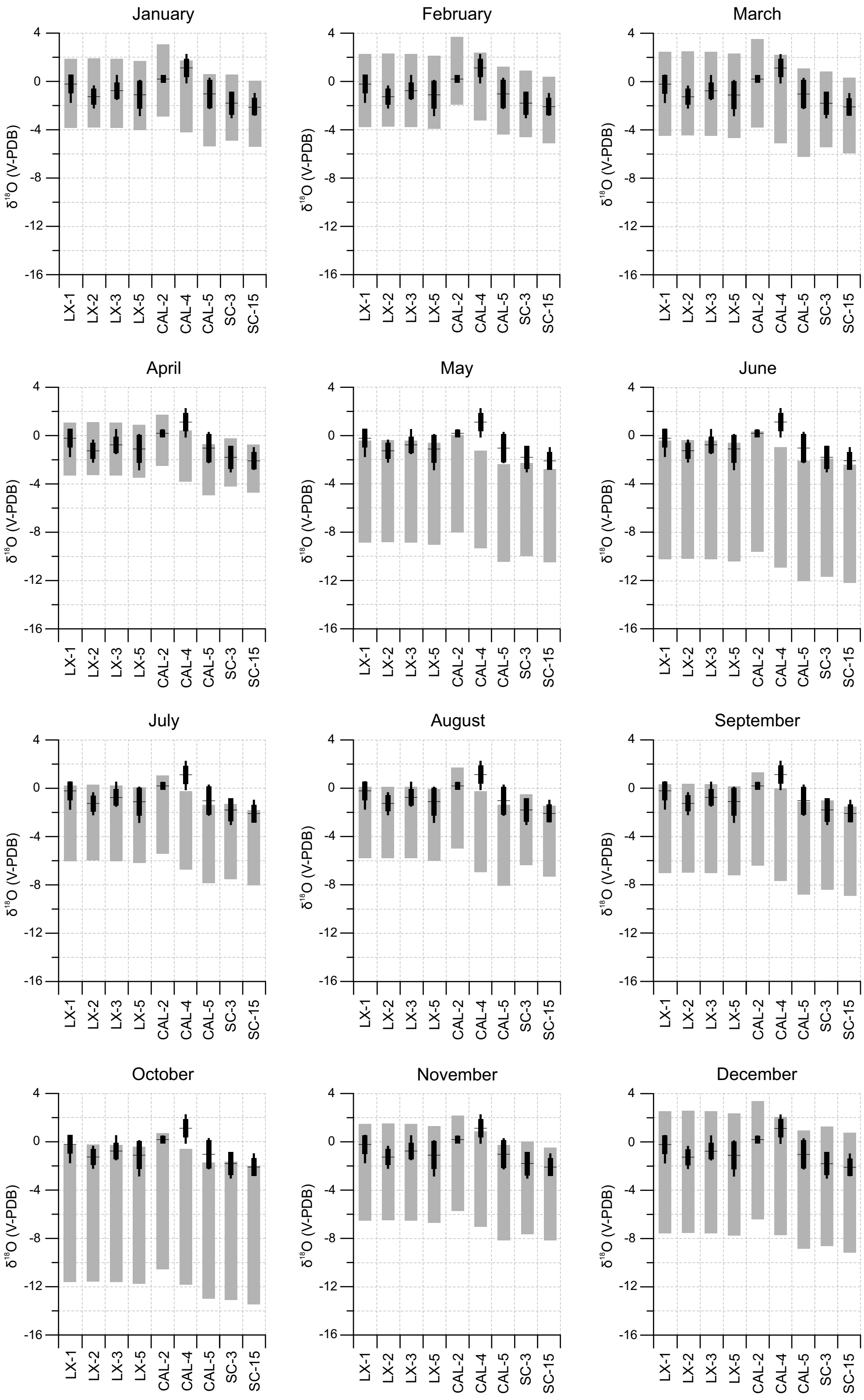

Figure 8. The $\delta^{18}O$ of *C. ilosvayi* compared to the $\delta^{18}O$ range of a calcite in isotopic equilibrium calculated from water temperatures obtained from NWIS stations and precipitation $\delta^{18}O$ from GNIP stations. Horizontal black lines indicate mean values of *C. ilosvayi* $\delta^{18}O$, 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.

C. ilosvayi and vital offsets are also reasonable. Also in this case, a seasonal calcification at the end of the wet season is plausible and calcification during the summer wet season can be excluded 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 temporally restricted calcification periods can reveal information on the seasonal development of temperatures or variations in 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, which were slightly lower, and BiC values, which showed 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 comparison with adults.

However, the isotopic range of *C. ilosvayi* clearly indicates a restricted seasonal calcification period. In cooler climates it is necessary to overcome subzero temperatures during the cold season and this can result in seasonally low abundances (Horne, 1983; Cohen and Morin, 1990). This is not the case in warm regions like Florida or 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). However, this would not explain a restriction of the calcification period to a certain season. Variation in food supply, water conditions, or competition 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 the flooding of dried up areas and higher surface runoff, which has an essential influence on seasonal habitat conditions and the input of organic matter as a food source. This also coincides with findings from the Paraná 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 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 conditions for reproduction are thus more advantageous.

6 Conclusions

In this study we compared site-specific hydrological data with isotopic signatures ($\delta^{18}O$ and $\delta^{13}C$) of the common ostracod species *Cytheridella ilosvayi* from 14 water bodies in South Florida to evaluate habitat-dependent differences caused by seasonal environmental variabilities. The mean $\delta^{18}O$ and $\delta^{13}C$ of *C. ilosvayi* signified the general isotopic characteristics of their host water on a regional basis. 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. In contrast, canal samples reflected low lake water variations caused by the mixing of inflowing water and older lake water. The $\delta^{13}C_{ostr}$ variation separated habitats with a permanent throughflow that reflects mixed $\delta^{13}C_{DIC}$ from the catchment from lentic sites where variation in $\delta^{13}C_{DIC}$ is caused by high-frequency variations in the proportion of photosynthesis to 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. The ranges were the highest in the beginning and end of the wet season and the lowest in April at the end of the dry season. These monthly variations were compared to the isotopic range of *C. ilosvayi* to test a new approach to identify possible calcification times during the year. Using 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 low enough to cause the isotopic signatures observed from *C. ilosvayi*. This seasonality is probably connected to strong seasonal changes in habitat conditions caused by an annual weathering cycle in Florida.

Data availability. All relevant data are presented within the paper or in the Supplement.

The Supplement related to this article is available online at https://doi.org/10.5194/bg-14-4927-2017-supplement.

Author contributions. JM, CW, and WEP carried out the sampling of all water and sediment material and measurements of field data. In addition, JM and CW prepared the ostracod material for isotopic analyses and JM carried them out. AL carried out water analyses. JM prepared the paper with contributions from all coauthors.

Competing interests. The authors declare that they have no conflict of interest.

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