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Using otolith organic matter to detect diet shifts in *Bardiella chrysoura*, during a period of environmental changes

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ABSTRACT: Accurate knowledge on fish trophic ecology and its modifications is crucial for understanding the impact of global change on ecosystems. In this context, we investigated the value of the $\delta^{13}C$ and $\delta^{15}N$ of otolith soluble organic matter (SOM) for identifying temporal diet shifts in American silver perch *Bairdiella chrysoura* over a 30-yr period characterized by strong changes in its population size and habitats within the Terminos Lagoon (Mexico). We first compared the otolith SOM isotopic signatures from present-day adults to those of muscle and the main local prey. Our results suggest that otolith SOM can be confidently extracted and analyzed for both present and past otoliths of this species. The mean otolith SOM signatures obtained (−15.92 ± 1.35‰ for $\delta^{13}C$ and 9.38 ± 0.93‰ for $\delta^{15}N$) were consistent with those of the diet as 85% of the individual signatures were included within the prey isotopic niche area. Moreover, this study supports a trophic enrichment factor between diet and otolith ($TEF_{diet\text{-}otolith}$) close to 0 for $\delta^{15}N$, while for $\delta^{13}C$, the $TEF_{otolith\text{-}muscle}$ of +0.02‰ warrants further investigation. Then, we compared past and contemporary otolith SOM signatures to investigate temporal diet shifts in *B. chrysoura*. This showed that $\delta^{13}C$ and $\delta^{15}N$ differed significantly between the past and present period even if the temporal shift remained relatively small (respectively +1.17‰ and −0.55‰). The present study substantiates the use of otolith SOM $\delta^{13}C$ and $\delta^{15}N$ as a proxy of fish present and past trophic position, opening the possibility for major progress in studies of temporal changes in food web ecology.

KEY WORDS: Trophic ecology · Stable isotope analysis · Coastal ecosystem · *Bairdiella chrysoura* · Terminos Lagoon

INTRODUCTION

There is now strong evidence that the environmental changes induced by natural or anthropogenic pressures cause major alterations in species assemblages (Walther et al. 2002, Myers & Worm 2005). In marine habitats, this can lead to strong regime shifts, with important ecological and economic consequences (Chavez et al. 2003). However, little is currently known about the effects of mid- or long-term environmental changes on trophic interactions between sympatric species, especially with regards to macroorganisms (Winder & Schindler 2004, Herbst 2006). As these interactions strongly drive species demogra-

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phy (Carpenter et al. 1985), evaluating their sensitivity to temporal shifts in environmental conditions is currently one of the greatest challenges for forecasting the impacts of global change on the biosphere.

So far, this kind of investigation has been principally limited by the difficulty in obtaining reliable estimates of lifetime trophic positions for multiple species, even in the same ecosystem (Edwards & Richardson 2004). This is particularly true in aquatic habitats where the observation of animals in their natural environment requires major technical, human and financial input (Chapman & Rice 1971, Savino & Stein 1989, Cutler & Swann 1999). Historically, 2 main methods were used to describe trophic position and diet in aquatic animals: stomach content analysis and stable isotope analysis (SIA) of soft tissues. Although they have proved very useful for identifying trophic interactions and food web structures in varied marine and freshwater ecosystems (e.g. Vander Zanden et al. 1999, Pinnegar & Polunin 1999), their worth for understanding species' responses to temporal environmental changes is limited by the fact that they only provide limited temporal information, from days to months, on the actual lifetime diet of the animals (Hyslop 1980, Davis et al. 2012).

SIA on calcified tissues with low metabolic turnover rates (e.g. scales, bones, otoliths) might overcome this problem since these tissues grow over the entire life of the animal, thereby providing temporally integrated information on its diet (Schoeninger & DeNiro 1984, Szpak 2011). The first technique tested for this was developed on fish scales (Wainright et al. 1993, Trueman & Moore 2007). However, the validity of this technique has been called into question as scales are likely to be shed and regenerate very quickly, leading to ecological misinterpretations. By contrast, otoliths (ear stones) grow continuously inside the inner ear of most teleost fishes, without later resorption or reworking of the concentric layers sequentially laid down on their periphery (Campana & Neilson 1985). Because these inert calcified structures are easily preserved over long time periods, decadal to centennial otolith collections are currently available for many species and ecosystems worldwide. These show great promise for studying long-term shifts in fish lifetime trophic positions and interactions (Rowell et al. 2010).

Over the last 3 decades, several studies have investigated the possibility of using SIA on fish otoliths as an indicator of their diet (e.g. McMahon et al. 2011). Most concluded that otoliths *in toto* cannot be used with confidence since their structure is largely dominated by inorganic carbonates whose signature mainly reflects the surrounding environment rather than the organic matter ingested by the fish (Solomon et al. 2006). In addition, the very low organic matter (OM) content of these calcified structures makes both the extraction of otolith OM and the analysis of its $\delta^{13}C$ and $\delta^{15}N$ very challenging (Degens et al. 1969), which until now has limited the use of otoliths for investigating historical change in aquatic trophodynamics.

However, a new protocol for otolith OM extraction was recently proposed that enables separation of otolith soluble proteins from the insoluble ones (Grønkjær et al. 2013). By doing this, it was demonstrated that the $\delta^{13}C$ and $\delta^{15}N$ signatures of otolith soluble organic matter (SOM) from tank-raised Atlantic cod *Gadus morhua* can be reliable proxies of those of the prey ingested, the trophic enrichment (i.e. the difference in signature between the otolith SOM of the fish and the flesh of its prey) being very close to zero for both elements (Grønkjær et al. 2013). If this innovative method is validated for other species and in the wild, SIA on otolith SOM could not only simplify and improve current investigations of fish lifetime diet and their spatial variations, but also allow assessment of fish past trophic positions from archived otoliths, thereby providing a valuable new tool to evaluate the impact of global change on coastal species and food webs.

To test this, we focused on a sub-tropical sciaenid, the American silver perch *Bairdiella chrysoura* (Lacepède, 1802) and its population within the Terminos Lagoon (Mexico), a coastal estuarine system that has experienced drastic environmental changes since 1980 (Ramos-Miranda et al. 2005b, Sirot et al. 2015a). The modifications of abiotic conditions and the loss of favourable habitats in the lagoon over this period have caused marked decreases in fish abundance (–41%) and biomass (–58%) in this estuarine ecosystem, and the resulting changes in fish taxonomic and functional diversity (Villéger et al. 2010, Sirot et al. 2015a) have had significant consequences on the structure and functioning of local food webs (Sosa-López et al. 2005). *B. chrysoura* is one of the fish species that has suffered most from the environmental changes. This species, listed as one of the most abundant and widely distributed of the Gulf of Mexico in the 1980s (Chao & Musick 1977), has experienced a dramatic decline since then, of ~90% in both its abundance and biomass within the lagoon (Sirot et al. 2015a). As this species is of commercial and recreational importance in the Gulf of Mexico (Ayala-Perez 2006) and since it is a key component of coastal ecosystems, being the habitual prey for several large estuarine predators (Luczkovich et al. 2000, Heupel & Hueter 2002, Blewett et al. 2006, Grammer et al. 2009), regular scientific surveys con-

ducted in the Terminos Lagoon since the beginning of local environmental disturbances in the 1980s has been undertaken in order to determine the reasons for this decline. This has involved repeated sampling of *B. chrysoura* individuals (gut samples, otoliths) and of their prey over the last 3 decades, which provides a unique opportunity to explore the value of SIA of fish otolith SOM as a technique for studying temporal diet shifts in fish populations facing modifications of their biotic and abiotic environment.

To this end, we first tested the feasibility of SOM extraction based on the technique of Grønkjær et al. (2013) on past and present-day otoliths of adult *B. chrysoura*. Then, the first objective of this study was to investigate the accuracy of otolith SOM $\delta^{13}C$ and $\delta^{15}N$ values for the estimation of the current diet of the species in Terminos by comparing these otolith SOM $\delta^{13}C$ and $\delta^{15}N$ to (1) the $\delta^{13}C$ and $\delta^{15}N$ signatures available for juvenile and adult *B. chrysoura* muscle tissue in the lagoon, and (2) those expected for its main lifetime local prey. Finally, the second objective of this study was to compare otolith SOM signatures of past (1979–1981) and contemporary (2006–2007) *B. chrysoura* adults from the lagoon and used the comprehensive knowledge gathered on the Terminos Lagoon ecosystem and the evolution of its biocenosis over the last 30 yr, to draw conclusions about temporal changes in the diet of *B. chrysoura* and the value of the SIA of otolith SOM for detecting and quantifying temporal diet shift in this species.

MATERIALS AND METHODS

Studied area and species

The Terminos Lagoon is the third largest lagoon in the world (1660 km^2), and the largest estuarine system along the coast of Mexico. Located in the southwest part of the Gulf of Mexico (Fig. 1), it is separated from the sea by a barrier island, Carmen Island, and communicates with the sea through 2 wide channels, located at either end of this island, the Puerto Real Inlet to the east and the Carmen Inlet to the west. Freshwater inputs to the lagoon originate mainly from 3 rivers located on its southern edge (Rio Palizada in the west and Rio Chumpan and Rio Candelaria in the east). As the water in the lagoon generally circulates from east to west, the southwestern part of the lagoon tends to have brackish water while the salinity of the northwestern part is close to marine levels (Ramos-Miranda et al. 2005a). Tropical climate in the area is characterized by 3 distinct seasons: the classical dry (D) and wet (W) seasons, from February to May and from June to September, respectively, and the nortes (N) season from October to January (Yáñez-Arancibia & Day 1982) characterized by a decrease in temperature and by strong winter storms coming from the North. Based on this variability in abiotic parameters, researchers have subdivided the region into 5 habitats based on their environmental characteristics, i.e. water salinity, bottom type, transparency, and underwater vegetation (see Fig. 1; Yáñez-Arancibia et al. 1988). In the 1980s, the lagoon was of great ecological and economic importance because of its size and its biodiversity: for example, around 120 fish species were reported, some of them being endemic (Yáñez-Arancibia et al. 1988). However, the lagoon has since suffered from diverse anthropogenic pressures, including fishing by artisanal shrimp trawlers, off-shore oil exploitation, increasing human populations along its shoreline and associated pollution (Ramos-Miranda et al. 2005a,b). This has resulted in marked shifts in its abiotic and biotic parameters (Ramos-Miranda et al. 2005b, Villéger et al. 2010) and caused a dramatic decline in the abundance and biodiversity of its fish

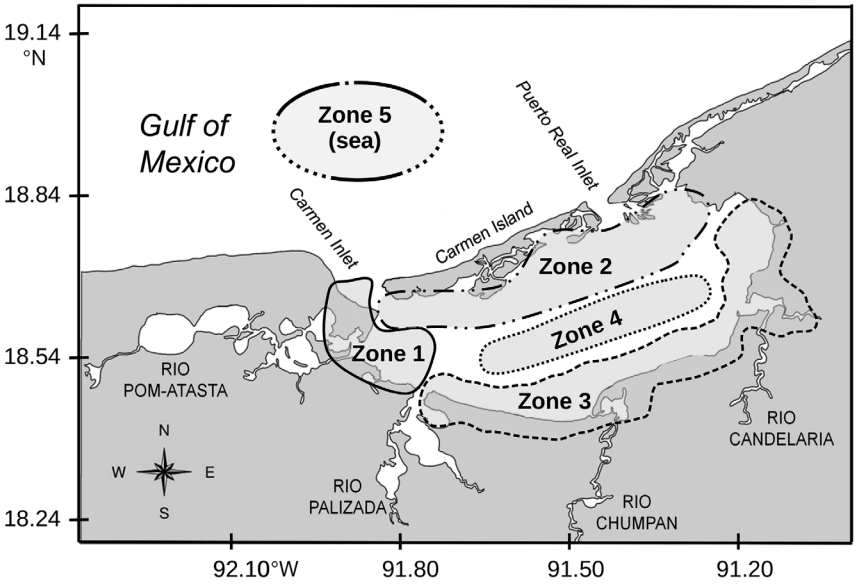

Fig. 1. Sampling zones in the Terminos Lagoon (southern Gulf of Mexico) where specimens of *Bairdiella chrysoura* were collected in past (1979–1981) and present (2006–2007) periods. Sampling zones were defined based on environmental parameters (for details, see Yáñez-Arancibia & Day 1982)

community (Sirot et al. 2015a). This has impacted differently the various trophic compartments of local food webs, resulting, for example, in a drastic decline of the fish species with intermediate trophic levels, while detrivorous species and top predators have increased in abundance (Sosa-López et al. 2005).

The studied species, the American silver perch *Bairdiella chrysoura*, is an estuarine carnivorous sciaenid commonly fished on the west Atlantic coast (from New York to the south of Louisiana) and in the Gulf of Mexico. The maximum size reached by this species is 230 mm standard length (SL) which corresponds to a 6 yr old individual (Grammer et al. 2009). Its length at maturity is 100 mm SL, corresponding to a 5 mo old individual (Chavance 1984). The population decline of this species in Terminos since the 1980s is mainly attributed to the disappearance of its juveniles from the lagoon, associated with a noticeable decrease in growth rate during the first 4 mo of life (Sirot et al. 2015b). This suggests that the main reason for the local decline in the population of *B. chrysoura* may be that the habitats in Terminos are now less suitable for early juvenile growth and survival. Environmental conditions in the lagoon however appear to allow compensatory growth in the individuals that survive this early demographic bottleneck (Sirot et al. 2015b). The key for the conservation of *B. chrysoura* therefore lies in the identification of the mechanisms responsible for this phenomenon. These might include a marked change in the species' diet at the juvenile stage, in response to the environmental modifications that occurred in the lagoon, as the availability of suitable prey on the nursery grounds often control growth and survival in juvenile fish (Mayer & Wahl 1997, Hoxmeier et al. 2004, Graeb et al. 2004). Before this work, very few studies had investigated the diet of *B. chrysoura* (Chavance 1984, Waggy et al. 2007). Juveniles of the species of up to 40 mm SL have been reported to feed mainly on amphipods, copepods and shrimp larvae (Waggy et al. 2007). At this size, they begin to feed on small fishes (early juveniles or adults of small species) and on polychaete worms, while adults feed mainly on shrimps and small fishes (Engraulidae, small Ariidae) (Chavance 1984).

Fish and prey sampling

The *B. chrysoura* individuals used for this work were collected monthly throughout the year during 2 sampling campaigns separated by a ~30 yr interval: in 1979–1981 and in 2006–2007, referred to hereafter as past and present periods respectively. These 2 surveys were both carried out in the 5 zones of the Terminos lagoon, in order to take into account the environmental variability within the system (Fig. 1). For each period and each zone, *B. chrysoura* juveniles (<100 mm SL; Chavance 1984) and adults (≥100 mm SL) were collected using a 5 m bottom otter trawl with 2.5 m mouth opening diameter (4.2 m and 5.4 m respectively for the head and foot rope) and 19 mm mesh size. All the *B. chrysoura* individuals collected (904 in total in 1979–1981 and 79 in 2006–2007) were measured (SL, mm), weighed (wet weight g) and kept refrigerated for later dissection.

In addition, samples of most of the prey and other diet components reported for *B. chrysoura* in the literature (i.e. fishes, shrimps, crabs, small crustaceans, polychaetes and seaweeds or seagrass remains; Chavance 1984) were collected during the 2006–2007 sampling campaign. Large prey (i.e. fishes, shrimps and crabs) were collected together with *B. chrysoura* individuals. Seaweeds and sea grass leaves were also collected from the trawl, where they were often found intertwisted with the benthic organisms. Amphipods were sampled using dedicated plankton nets (mesh size 300 µm) and polychaetes and Tanaidacea were caught by sediment digging. All these potential dietary items were rinsed on arrival at the laboratory, sorted under a stereomicroscope and kept frozen (−20°C) until preparation for SIA.

Comparison of present-day prey, muscle and otolith isotopic signatures

The comparison of *B. chrysoura*'s otolith signatures with those of its main local prey (Fig. 2A) required beforehand the improvement of the current list of *B. chrysoura*'s prey in the lagoon. We analyzed stomach content of the 79 fish captured during the 2006–07 campaign. Among them, only 30 (9 juveniles and 21 adults, 70 to 146 mm SL) had full stomachs. Prey items were sorted under a binocular microscope and identified to the class level, except for fish and crabs that were sorted down to the family level and shrimps, which were determined to the species.

Prey isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) were then assessed by randomly sub-sampling among those captured in all 5 zones (n ≤10 per zone) during the 2006–07 campaign. For large animal prey items, flesh samples (white dorsal muscle for fish, tail muscle for shrimps and claw muscle for crabs) were collected and analyzed separately for at least 3 distinct individuals, while for smaller prey (e.g. small crustaceans and polychaetes) and plants, several individuals were

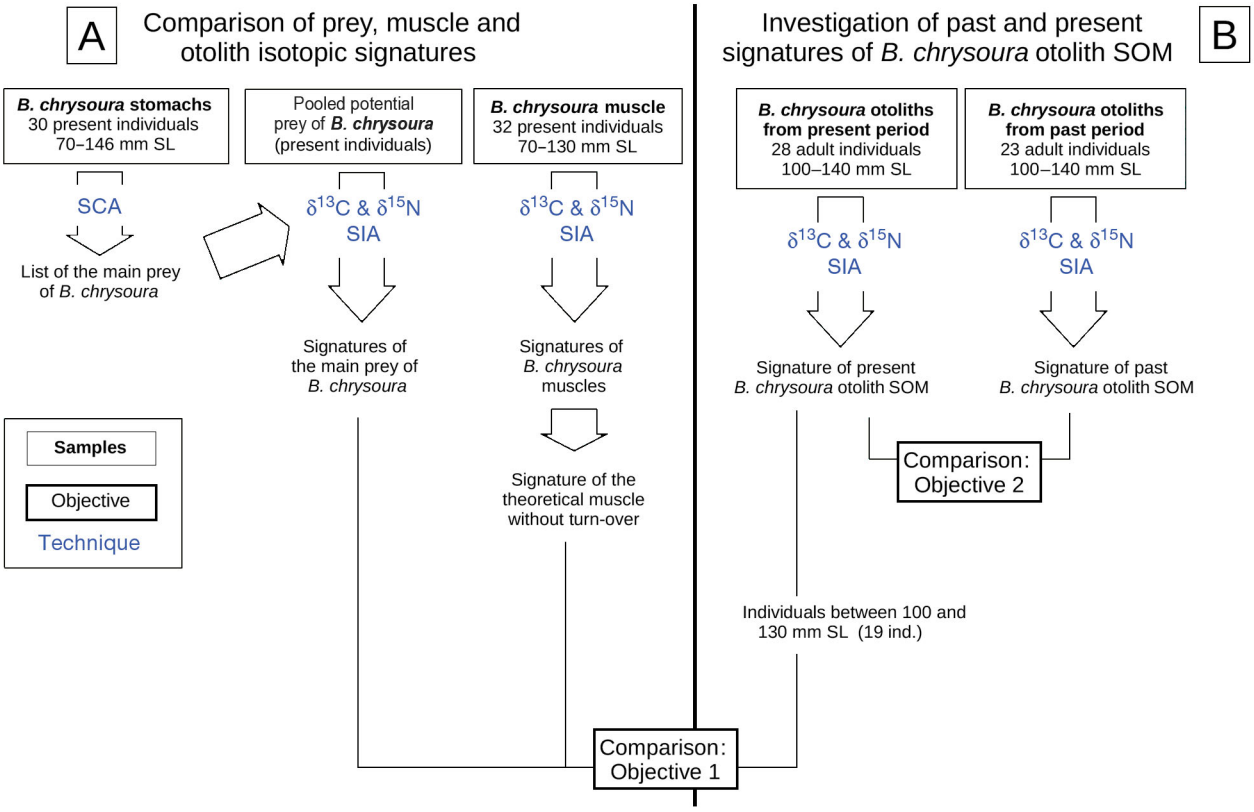

Fig. 2. Methodology applied to assess the use of otolith soluble organic matter (SOM) to detect diet shifts in *Bairdiella chrysoura*, showing the steps involved in determining (A) the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of the average present-day (2006–2007) diet of *B. chrysoura* individuals (70–130 mm SL), and (B) the $\delta^{13}C$ and $\delta^{15}N$ signatures of *B. chrysoura* otolith SOM for the past (1979–1981) and present periods. Samples required and objectives of this study are shown in boxes with thin and bold outlines respectively; techniques are shown in blue. SCA: stomach content analysis; SIA: stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$

pooled if needed, in order to obtain at least 3 different isotopic measures per prey or plant item. The soft tissue samples obtained were all freeze-dried, ground into fine powder and kept separately in a desiccator until analysis. For fish muscle, the lipid correction was performed mathematically according to Post et al. (2007) in order to standardize $\delta^{15}N$ signatures; the other prey items had sufficiently low lipid contents to avoid this additional step. Isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) were performed on powder samples of 0.5 mg in each case, using the continuous flow isotope-ratio mass spectrometer available at the Laboratory of Mass Spectrometry in the Centro Interdisciplinario de Ciencias Marinas at the Instituto Politécnico Nacional in La Paz, Mexico. SIA results are expressed as parts per thousand (‰) from a standard reference material using the notation:

$$\delta(X) = [(R_{sample} / R_{reference}) - 1] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N, R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and $\delta(X)$ is the measure of heavy to light isotope in the sample (precision <0.3‰). Measurements were expressed relative to atmospheric N_2 for nitrogen and relative to Vienna Pee Dee Belemnite for carbon.

Regarding the comparison of muscle and otolith stable isotopic signatures (Fig. 2A), since these 2 materials do not show the same turnover rate (weeks to months for muscles and absence of turnover in physiological conditions for otoliths), direct paired comparison of signatures between muscles and otoliths would have made no sense ecologically due to ontogenic changes of diet. To avoid this problem, we reconstructed the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of a theoretical muscle that experiences no turnover. To this end, we considered the signature of this theoretical muscle as the sum of the muscle signatures of each stage of life weighted by the somatic growth occurring during those stages. Specifically, we (1) analyzed the isotopic signatures of muscle from individuals smaller than 130 mm SL (n = 32, 10 juveniles and 22 adults, 70–130 mm SL), following the procedure described for SIA of prey fish muscle in the previous paragraph, then (2) calculated the

average signature for each 10 mm SL category (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m575p137_supp.pdf) and (3) reconstructed the signature by following (see Table S2 for more details on the choice of the parameter):

$$\delta(X)‰ = \sum_{\text{SizeCat}} (L_{\text{SizeCat+1}} - L_{\text{SizeCat}}) / L_{\max} \times \delta(X)_{\text{SizeCat+1}} \quad (2)$$

where $L_{\text{SizeCat+1}}$ and L_{SizeCat} are, respectively, the size of individuals of a 10 mm SL size category (SizeCat) and the next biggest size category (SizeCat+1), $L_{\max}$ is 130 mm SL and $\delta(X)_{\text{SizeCat+1}}$ is the muscle signature of the 'SizeCat+1' individual. This signature was then compared to those of otoliths (Fig. 2A).

Based on the additional information about *B. chrysoura*'s diet and the calculated prey and muscle isotopic signatures, we determined the most likely contribution to the global average diet (% in weight) for each prey, through a Bayesian mixing model procedure, using the 'siar' package (https://cran.r-project.org/web/packages/siar/index.html). Since tropic enrichment factors (TEF) in $\delta^{13}C$ and $\delta^{15}N$ have not yet been assessed specifically for *B. chrysoura*, TEF values for muscle tissue derived from multiple fish species (1.3 ± 0.7‰ for $\delta^{13}C$ and 2.3 ± 0.8‰ for $\delta^{15}N$; Caut et al. 2009) were implemented in the model.

Temporal shift in *B. chrysoura* otolith SOM signatures

As otoliths grow throughout fish life, SIA on whole adult otoliths should allow identification of potential modifications in *B. chrysoura* diet over time, irrespective of the life stage (larval, juvenile or adult, without however being able to identify the life stage affected by the modification). Therefore, past and contemporary signatures of *B. chrysoura* otolith SOM were assessed using 52 young adults of similar sizes (100 to 140 mm SL), randomly sub-sampled from those captured during the 2 sampling campaigns (n = 23 for 1979–81 and n = 29 for 2006–07; Fig. 2B, Table 1).

Otoliths, right and left sagittae, were extracted, rinsed with milliQ water, dried with Kimtech wipes and stored in dry micro-tubes. They were then prepared following Grønkjær et al. (2013): they were cleaned of any adhering organic material by plunging them into a bath of 0.2 mol l^{-1} of NaOH for 1 h at room temperature. The remaining adherent tissues were removed by sonication (1 min) and by manual cleaning. Once rinsed with MilliQ water and dried at 30°C for 48 h, otoliths were ground into a fine powder using a ball mill grinder. To remove inorganic carbonates, the resulting otolith powder was immersed in 2 ml of 0.1 mol l^{-1} HCl, and kept at 5°C for 24 h before adding 4.4 ml of 0.5 mol l^{-1} HCl until demineralization was complete. To separate the SOM from the rest of the organic matter, demineralized samples were centrifuged at 14 400 rpm (12 000 × *g*) for 10 min and filtered with an Amicon Ultra 10 kDa. The otolith SOM was then cleaned by adding 3.5 ml MilliQ water twice and centrifuging the samples at 4000 rpm (2500 × *g*) until the volume was 50 to 100 µl. After ultrafiltration, otolith SOM of each sample was transferred into pre-weighted tin capsules, freeze dried and stored in a desiccator until analysis.

Table 1. Summary of samples used to investigate the use of otolith soluble organic matter (SOM) to detect diet shifts in *Bairdiella chrysoura* in the Terminos Lagoon (southern Gulf of Mexico): (A) number of individuals per period and per zone (see Fig. 1 for location of sampling zones); (B) number of individuals per period and per season

Variable	Number of individuals caught Past (1979–1981)	Present (2006–2007)
(A) By lagoon zone		
1	0	12
2	6	0
3	9	10
4	7	0
5 (Sea)	1	6
(B) By season		
Dry	12	12
Nortes	5	4
Wet	6	12
Total	23	28

The isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of otolith SOM was carried out by continuous flow isotope ratio mass spectrometry (IRMS) at the AMS ^{14}C Dating Center, Department of Physics and Astronomy, Aarhus University, Denmark. A minimum of 0.1 mg of otolith SOM is needed for reliable measures of $\delta^{13}C$ and $\delta^{15}N$ by IRMS (Grønkjær et al. 2013). For Atlantic cod *Gadus morhua*, this corresponds to at least 100 mg of total otolith powder. However, SOM content in the otolith varies among species (Asano & Mugiya 1993, Hüssy et al. 2004). We therefore decided to analyze all samples with more than 65 mg of otolith powder for *B. chryoura*, making the assumption that the otolith of this species contains at least as much SOM as in cod. For large adults, this amount of SOM could be collected from a single otolith (in this study the left), but for smaller individuals (n = 15) both otoliths (right and left sagittae) had to be pooled (after being cleaned and weighted) to reach this threshold.

Statistical analysis

The potential change of otolith SOM quality over time were investigated by comparing (1) C/N ratios of the SOM between past and present-day otoliths (Student's *t*-test; *t-test* function in R) and (2) the relationships between the amount of SOM material obtained and the initial weight of otolith, i.e. before grinding (analysis of covariance, ANCOVA; *anova* function in *R*). To test whether growth was similar for past and present-day otoliths, we also compared the relationships between initial otolith weight and fish size obtained for the 2 periods (ANCOVA).

The SIA of present-day *B. chrysoura* muscles was computed from individuals between 70 and 130 mm SL (n = 32). For comparison of otolith SOM, muscle and diet signatures we only used otolith SOM signatures ($\delta^{13}C$ and $\delta^{15}N$) obtained for present-day individuals smaller than 130 mm SL (n = 19, SL = 100–130 mm) in order to ensure that comparisons were made among individuals in the same period of life.

The comparison between past and present otolith SOM signatures was carried out using adult otoliths (n = 52, SL = 100–140 mm) in 2 consecutive steps, investigating successively (1) the concomitant change in both $\delta^{13}C$ and $\delta^{15}N$ and (2) $\delta^{13}C$ and $\delta^{15}N$ individually. First, to test for concomitant modification of the $\delta^{13}C$ and $\delta^{15}N$ signatures over time, we compared barycenters of past and present scatterplots using a permutational multivariate analysis of variance (PERMANOVA) performed with the *adonis* function in the *Vegan* package (Anderson 2005). Then, we tested whether the dispersion of the points around their barycenters was similar for the 2 periods using the functions *betadisper* and *permutest* in *Vegan* (this is equivalent to Levene's test in multivariate conditions).

We then investigated the change in $\delta^{13}C$ and $\delta^{15}N$ signatures individually over the time using ANOVA. Variables analyzed included Period, Fish length, Zones and Season, in order to check the possible bias due to the sampling design. To this end, we first used the function *anova* to fit the following linear models (always checking for the normal distribution of residuals):

$$\delta^{n}X \leftarrow \mathrm{lm}(\mathrm{Period} + \mathrm{Season} + \mathrm{Zone} + \mathrm{Fish\ length}) \quad (3)$$

where $\delta^{n}X$ is the stable isotope signature (either $\delta^{13}C$ or $\delta^{15}N$)

Then, we compared the fits of each possible model including these 4 factors based on the Akaike information criterion adjusted for small sample sizes (AICc) using the function *dredge* in the *MuMin* package (https://cran.r-project.org/web/packages/MuMIn/index.html). Once the best model(s) had been selected, we determined the effect each factor had an explaining the variability of stable isotope signatures since 1980 using ANOVA.

All statistical analyses were performed using the R software (R Development Core Team 2016). For all the tests that require permutations, number of permutations was set at 1000 and we took $\alpha = 0.05$ as indicating statistical significance.

RESULTS

Implementation of the SOM extraction method on *B. chrysoura* otoliths

Out of the 52 otolith samples of *B. chrysoura* prepared in this study, one was damaged during the initial OM extraction procedure. The 51 remaining otolith samples, with initial weights of 68 to 155 mg, all provided exploitable SOM $\delta^{13}C$ and $\delta^{15}N$ signatures. These 51 samples included both past (n = 23) and present-day (n = 28) otoliths, all from fish of 100 to 140 mm SL. Their final otolith SOM signatures ranged between −19.40‰ and −12.95‰ (−16.44 ± 1.57‰, mean ± SD) for $\delta^{13}C$ and between 7.22‰ and 11.52 ‰ (9.81 ± 1.02‰) for $\delta^{15}N$.

The relationships between otolith weight and fish size obtained for 1979–81 and 2006–07 individuals did not differ significantly (ANCOVA, df = 1, $F = 0.31$, p = 0.57), suggesting that otolith deposition rate for *B. chrysoura* in Terminos did not change significantly over time (Fig. 3A). The amount of SOM extracted was highly correlated ($R^2 = 0.67$) with otolith weight, irrespective of the period (ANCOVA, df = 1, $F = 116.7$, $p = 1.9 \times 10^{-14}$) (Fig. 3B). However, the amount of SOM extracted per milligram of otolith was significantly lower (ANOVA, df = 1, $F = 11.98$, p = 0.001) in past samples ($3.8 \times 10^{-3} \pm 0.001 \times 10^{-3}$ mg mg^{-1}) than in present-day ones ($4.8 \times 10^{-3} \pm 0.001 \times 10^{-3}$ mg mg^{-1}) (Fig. 3B) but no interaction between period and otolith weight was apparent (df = 1, $F = 0.13$, p = 0.71)

C/N ratios for otolith SOM were also higher on average ($p = 5 \times 10^{-3}$) in past otoliths (4.88 ± 0.22) than in present-day ones (4.65 ± 0.22) (Fig. 3C).

Comparison of present-day prey, muscle and otolith isotopic signatures

Stomach content analysis (from 30 individuals between 70 and 146 mm SL) enabled expansion of the existing list of prey species of *B. chrysoura* in the

Table 2. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$, ‰) of prey and other food items consumed by *Bairdiella chrysoura* and, (below) for comparison, average values for muscle tissue of *B. chrysoura* caught in the Terminos Lagoon (n = 29 individuals, 70–130 mm SL) during the present period (2006–2007)

Food source		No. of replicates	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{15}N$ Mean	$\delta^{15}N$ SD
Seaweed		11	−19.69	2.36	6.36	0.83
Seagrass		2	−11.33	1.62	4.23	0.52
Polychaetes	Small	3	−15.80	0.84	5.73	1.03
	Large	3	−18.11	0.88	8.52	0.44
Tanaidacea		3	−19.22	1.74	6.27	1.27
Amphipoda		3	−17.11	1.02	5.09	0.96
Shrimps		3	−16.67	1.07	7.72	0.31
Portunidae		7	−14.39	2.02	7.46	0.54
Fish	Engraulidae	3	−16.64	0.24	11.04	0.27
	Other species (smaller sp.)	3	−18.45	2.27	8.86	0.82
B. chrysoura muscle		29	−18.13	1.58	11.8	1.27

area. Fishes (mainly Engraulidae but also smaller individuals of Syngnathidae, Gobiidae, Paralichthyidae and Cynoglossidae), shrimps (Penaeidae), crabs (Portunidae), polychaetes and Tanaidacea were commonly found, as well as, in much smaller amounts, amphipods, and remains of seaweeds and seagrasses.

Isotopic signatures varied greatly among these prey items (Table 2, Fig. 4), with respective averages (±SD) ranging from −19.69 ± 2.36‰ (seaweeds) to −11.33 ± 1.62‰ (seagrasses) for $\delta^{13}C$ and from 4.23 ± 0.52‰ (seagrasses) to 11.04 ± 0.27‰ (Engraulidae) for $\delta^{15}N$ (Table 2). Analysis using the 'siar' package of *B. chrysoura* muscle signatures indicated that the current diet of the late juveniles and early adults of the species (70–130 mm SL) mainly consisted of polychaetes (38%), fish (21%), shrimps (18%), Tanaidacea (13%) and Portunidae (7%), with seagrasses, seaweeds and amphipods each contributing less than 1% (Fig. 4, Table S3).

The present-day otolith SOM signatures used for this part of the study were derived from 19 adults (100–130 mm SL) and ranged from −19.40‰ to −13.35‰ (−15.92 ± 1.35‰, mean ± SD) for $\delta^{13}C$ and from 7.22‰ to 10.81‰ (9.38 ± 0.93‰) for $\delta^{15}N$ (Fig. 4). A comparison between these signatures and those of the potential prey of *B. chrysoura* showed a large overlap between the 2 stable isotope niche areas. Moreover, for the majority of individuals, otolith signatures were included in the stable isotopic niche area of *B. chrysoura* prey (only 3 otoliths, i.e. 15%, are not included in the grey polygon of Fig. 4B). Importantly, the averaged signature of present otolith SOM was surrounded by the 3 main prey of the species (i.e. polychaetes, fish and especially Engraulidae and shrimps).

Muscle signatures varied between −21.98‰ and −15.93‰ for $\delta^{13}C$ and between 9.30‰ and 12.75‰ for $\delta^{15}N$ for adults (n = 22, 100–130 mm SL) and from −19.51‰ to −12.21‰ for $\delta^{13}C$ and from 7.64‰ to 14.39‰ for $\delta^{15}N$ for juveniles (n = 10, 70–100 mm SL). Thus, the isotopic values were found to differ significantly according to the life stage for $\delta^{13}C$ ($p = 0.01$), with higher values in the juveniles (−16.89 ± 2.11‰) than in the adults (mean −18.87 ± 1.22‰), but to

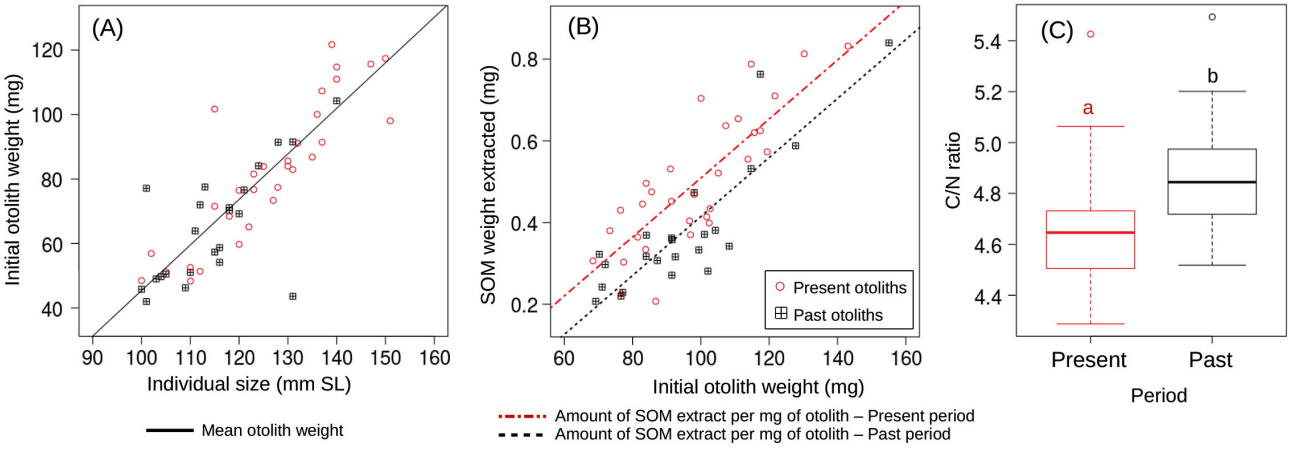

Fig. 3. Comparative data on *Bairdiella chrysoura* from Terminos Lagoon (southern Gulf of Mexico) for past (black, 1979–1981) and present (red, 2006–2007) periods: (A) initial otolith weight (mg) vs. size of individuals, measured as standard length (SL, mm); (B) weight of soluble organic matter (SOM) extracted (mg) vs. initial otolith weight (mg); (C) otolith SOM C/N ratio. Regression lines are shown in (A) and (B); the single line in (A) indicates that the otolith deposition rate did not change significantly over time. (C) Box-and-whisker plots show the range, median and upper and lower quartiles of the C/N ratio for the past and present periods; dots are outliers; letters 'a' and 'b' indicate significant differences between periods

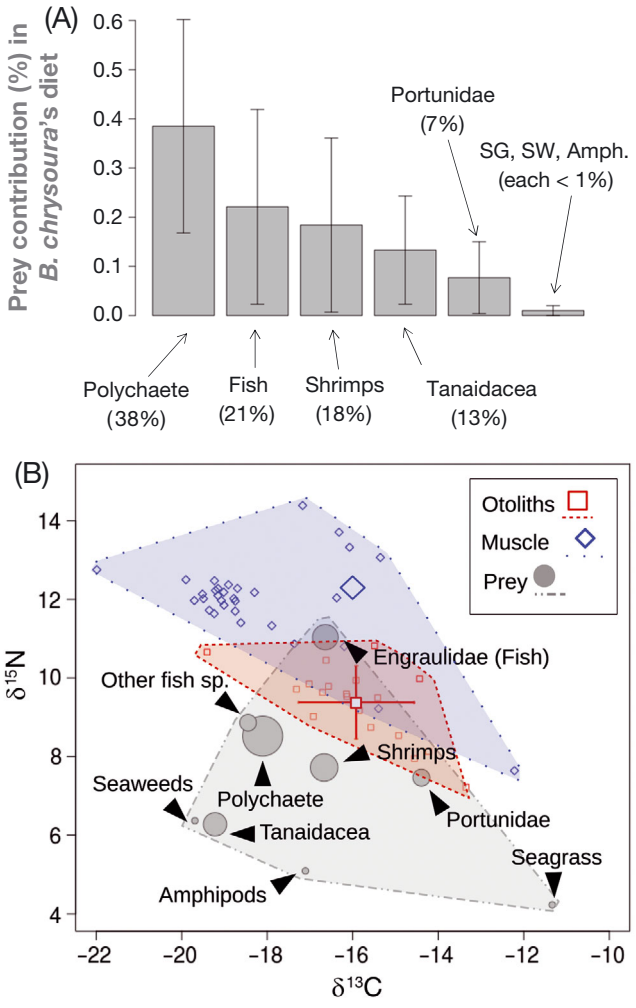

Fig. 4. Comparison of isotopic signatures of diet, otolith soluble organic matter (SOM), and muscle tissue of *Bairdiella chrysoura*. (A) Contribution (average ± SD) of prey and other components of the diet of *B. chrysoura*, based on 'siar' performed on *B. chrysoura* muscle tissue (n = 29, 70–130 mm SL). For more details see Table S3 in the Supplement. SW: seaweed; SG: seagrasses; Amph: amphipods. (B) Isotopic signatures of prey (grey circles), otolith SOM (n = 19, red squares), muscle (n = 29, small blue diamonds) and theoretical stomach contents of present-day *B. chrysoura* (only 1 calculated value, large blue diamond). In each case, the shaded polygon outlines the range of data points. For data on prey, the area corresponds to the contribution of the prey item to the diet. For data on otolith SOM and theoretical stomach contents, small symbols show results for individual samples, while large symbols indicate means and, in the case of otolith SOM, bar lines show SD

be similar (p = 0.65) for $\delta^{15}N$ (11.86 ± 1.27‰). These signatures also overlapped with those of present otolith SOM and interestingly showed a similar pattern for the 2 stable isotope niche areas. However, the values differed significantly (t = 4.87, df = 46.79, p = 1.30×10^{-5} for $\delta^{13}C$ and t = –7.98, df = 46.63, p = 2.85×10^{-10} for $\delta^{15}N$) with the theoretical muscle isotopic signature estimated as –15.94‰ for $\delta^{13}C$ and 12.10‰ for $\delta^{15}N$.

SOM isotopic signatures for present and past otoliths of *B. chrysoura*

The otolith SOM signatures obtained for the 23 adults collected in 1979–81 (–19.02‰ to –12.95‰ for $\delta^{13}C$ and 8.15‰ to 11.52‰ for $\delta^{15}N$) were of the same magnitude as those found for the 28 adults from 2006–07 (between –19.40 and –13.08‰ for $\delta^{13}C$ and between 7.22‰ and 11.08‰ for $\delta^{15}N$). Otolith SOM signatures from present and past individuals thus showed a large overlap (Fig. 5). The dispersions of the signatures around their barycenter were similar between the 2 periods (dispersion test, F = 0.07, df = 1, p = 0.81). However, average isotopic signatures differed statistically between them (PERMANOVA, F = 6.94, p = 0.011).

When $\delta^{13}C$ was individually investigated, the $\delta^{13}C$ values of otolith protein showed an average increase of +1.17‰ between the 2 periods (from –17.08 ± 1.54‰ in 1979–81 to –15.91 ± 1.41‰ in 2006–07.

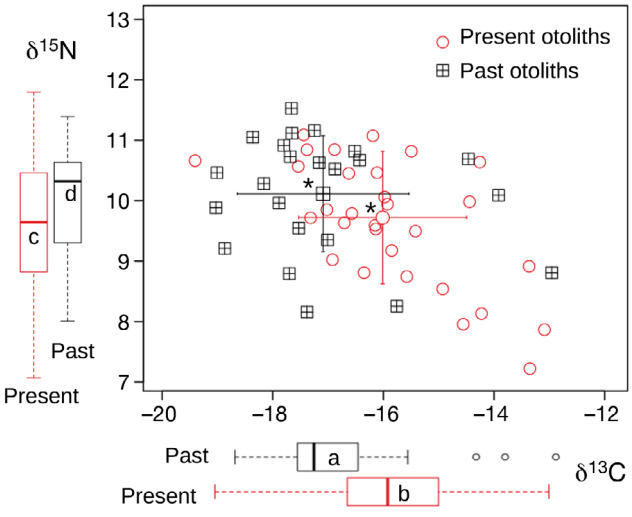

Fig. 5. Comparison of isotopic signatures from *Bairdiella chrysoura* otolith SOM (n = 55, 100–140 mm SL) between past (black, 1979–1981) and present (red, 2006–2007) periods. On the scatter plot, symbols intersected by vertical and horizontal bars show the mean and the standard deviation for each period; asterisks indicate a significant difference between periods based on results of PERMANOVA. Box-and-whisker plots show the range of the signatures for the 2 periods investigated (x-axis: $\delta^{13}C$; y-axis: $\delta^{15}N$), the median and the first and last quartiles of the signature. Circles represent extreme values; letters inside the boxes indicate significant differences between signatures

Table 3. Evaluation of models tested to explain variation in isotopic signatures in otoliths of *Bairdiella chrysoura* collected from the Terminos Lagoon, southern Gulf of Mexico (see Fig. 1 for zones), in past (1979–1981) and present (2006–2007) periods, based on AICc values for (A) $\delta^{13}C$ and (B) for $\delta^{15}N$. The best fit models are shown in **bold** type

Fish length	Period	Season	Zone	df	AICc	ΔAIC	AIC weight
(A) $\delta^{13}C$							
·	**+**	·	·	**3.00**	**188.80**	**0.00**	**0.39**
+	**+**	·	·	**4.00**	**189.98**	**1.18**	**0.22**
·	**+**	**+**	·	**5.00**	**190.75**	**1.95**	**0.15**
+	·		·	3.00	192.73	3.93	0.06
·	+	·	+	7.00	192.86	4.06	0.05
+	+	+	·	6.00	192.95	4.15	0.05
·	·	·	·	2.00	194.29	5.49	0.03
+	+	·	+	8.00	195.68	6.87	0.01
·	+	+	+	9.00	195.99	7.19	0.01
·	·	+	·	4.00	196.17	7.37	0.01
+	·	+	·	5.00	196.26	7.46	0.01
·	·	+	+	8.00	197.35	8.55	0.01
·	·	·	+	6.00	198.05	9.25	0.00
+	+	+	+	10.00	198.56	9.75	0.00
+	·	·	+	7.00	200.01	11.20	0.00
+	·	+	+	9.00	200.26	11.46	0.00
(B) $\delta^{15}N$							
+	**+**	·	·	**4.00**	**143.59**	**0.00**	**0.50**
+	+	·	+	8.00	145.90	2.31	0.16
+	+	+	·	6.00	146.89	3.30	0.10
+	·	·	+	7.00	148.91	5.32	0.03
·	·	·	+	6.00	148.93	5.34	0.03
·	+	·	·	3.00	148.95	5.37	0.03
·	+	·	+	7.00	149.10	5.51	0.03
+	·	·	·	3.00	149.78	6.19	0.02
+	+	+	+	10.00	150.13	6.54	0.02
·	+	+	·	5.00	150.41	6.82	0.02
·	·	·	·	2.00	150.48	6.89	0.02
·	·	+	·	4.00	151.49	7.90	0.01
+	·	+	+	9.00	151.67	8.08	0.01
·	·	+	+	8.00	151.70	8.11	0.01
+	·	+	·	5.00	151.74	8.15	0.01
·	+	+	+	9.00	151.98	8.39	0.01

According to the AICc, the best models to explain the variability in otolith $\delta^{13}C$ signatures were those taking into account (1) the period, (2) the period and the fish length and (3) the period and the season (Table 3A). In 100% of these models, the period was a significant factor to explain the variability of the $\delta^{13}C$ values ($p < 0.02$; Table 4). Moreover, fish length seemed to have a significant effect even if this effect remained marginal (this factor was significant in only 20% of the models). Finally, we confirmed that the season had no effect on the $\delta^{13}C$ value of the otolith. We therefore concluded that the $\delta^{13}C$ value significantly increased (+1.17‰) since 1980 and that a hypothesized unbalanced sampling design (i.e. incorporating neither seasonal nor spatial bias) was not responsible for this change.

When $\delta^{15}N$ was individually investigated, the $\delta^{15}N$ values of otolith protein experienced a small average decrease of −0.55‰ between the 2 periods (from 10.11 ± 0.95‰ in 1979–81 to 9.56 ± 1.02‰ in 2006–07). According to the AICc, the best model to explain the variability in otolith $\delta^{15}N$ values was that taking into account both the period and the fish length (Table 3B). In this model, period was a significant factor to explain the variability of the $\delta^{15}N$ values ($p < 0.007$; Table 4). Regarding fish length, this factor seemed significant to explain $\delta^{15}N$ even if it was only significant in 50% of the models. Thanks to these new results, we concluded that $\delta^{15}N$ values decreased since 1980 (−0.55‰) and that a hypothesized unbalanced sampling design (i.e. incorporating neither seasonal nor spatial bias) was not responsible for this change.

Table 4. Effects of period, zone and season on $\delta^{13}C$ and $\delta^{15}N$ signatures of otolith protein of *Bairdiella chrysoura*, based on results of ANOVA. See Table 3 for the list of models considered in the analysis. Significant effects ($p < 0.05$) are shown in **bold**

Source of variation		$\delta^{13}C$			$\delta^{15}N$		
		df	F	p	df	F	p
Period		**1**	**8.039**	**0.006**			
Period + Length	Period	**1**	**8.05**	**0.006**	**1**	**4.29**	**0.04**
	Length	**–**	1.12	0.29	**–**	**7.84**	**0.007**
Length + Period	Length	**–**	**4.12**	**0.047**	**–**	3.38	0.07
	Period	**1**	**5.05**	**0.02**	**1**	**8.76**	**0.004**
Period + Season	Period	**1**	**8.15**	**0.006**			
	Season	2	1.36	0.26			
Season + Period	Season	2	1.51	0.23			
	Period	**1**	**7.85**	**0.0073**			

DISCUSSION

First *in situ* validation of the technique of Grønkjær at al. (2013)

The demonstration by Grønkjær et al. (2013) that otolith SOM $\delta^{13}C$ and $\delta^{15}N$ in fish can mirror the isotopic signatures of their prey had been conducted under controlled conditions, using Atlantic cod *Gadus morhua* individuals grown in tanks and fed only one type of prey (blue mussel *Mytilus edulis*, lesser sandeel *Ammodytes marinus*, or whiting *Merlangius merlangus*) with isotopic signature not only known but also constant. To our

knowledge, our study is the second to investigate the value of these 2 proxies combined (for $\delta^{15}N$ only, see Vandermyde & Whitledge 2008, Rowell et al. 2010) and the first to do so on wild fish, free to feed on various prey in their natural environment. Our results, in particular the high success rate (98%) in extracting and analyzing otolith SOM for *B. chrysoura*, shows that the technique of Grønkjær et al. (2013) can be applied to various species, even with contrasting ecologies. Moreover, we managed to get reliable isotopic signatures with only 65 mg of *B. chrysoura* otolith powder (compared to a minimum of 100 mg for cod otoliths; Grønkjær et al. 2013). This suggests that otolith SOM content in this sciaenid is at least as high as in cod, which is encouraging regarding the applicability of this technique to other fish species.

This study also confirms the value of otolith SOM $\delta^{13}C$ and $\delta^{15}N$ signatures as indicators of fish trophic positions within food webs, our results showing a strong similarity between the isotopic signatures of *B. chrysoura* otolith SOM and those of its prey. Indeed, the SOM signatures of *B. chrysoura* otoliths were largely (85%) included in the surface delineated by the signatures of the prey it ingested at the juvenile and adult stages.

Regarding the comparison between muscle and otolith SOM signatures, this study showed that these signatures differed by −0.02‰ for $\delta^{13}C$ and by +2.7‰ for $\delta^{15}N$. For $\delta^{15}N$ (i.e. comparison of the theoretical muscle signature and the average of the otolith signatures), this difference of signature is very close to that obtained in Grønkjær et al. (2013) (between +3‰ and +3.9‰ depending on prey type) and contradicts Rowell et al. (2010) and Vandermyde & Whitledge (2008), who found differences of +0.76‰ and +1.1‰, respectively. This result seems to confirm the conclusions of Grønkjær et al. (2013), i.e. $TEF_{diet\text{-}otolith}$ for $\delta^{15}N$ is close to zero. By contrast, Grønkjær et al. (2013) found a difference of +1 and +1.5‰ in $\delta^{13}C$ between muscle and otolith SOM (compared to 0.02‰ in this study). There are many possible explanations for these different values. The first reason could implicate a TEF between diet and otolith SOM signatures that is higher for *B. chrysoura* than for cod. Grønkjær et al. (2013) found a maximum $TEF_{diet\text{-}otolith}$ around 0.23 ± 0.05‰ while we obtained an estimated $TEF_{diet\text{-}otolith}$ between +1‰ and +1.3‰ if we assumed that $TEF_{diet\text{-}muscle}$ = 1.3‰ (Caut et al. 2009). Another reason could be that analyzed otoliths and muscles did not come from the same individuals. This potential difference of $TEF_{diet\text{-}otolith}$ between this study and Grønkjær et al. (2013) could therefore come from inter-individual variability of diet leading to ingestion of prey with different $\delta^{13}C$ signatures. A final explanation could be that the present fish muscle signatures were obtained from individuals larger than 70 mm SL, and therefore that the theoretical muscle signature calculated in this study missed the signatures corresponding to the prey ingested during earlier fish life (from birth to 70 mm SL), which is in contrast to the otoliths, whose signatures reflect a lifetime integrated diet. The omission of the isotopic signatures of early life stages (muscle and prey from the larvae and juveniles below 70 mm SL) within our calculation could, at least in a part, explain this difference of the $TEF_{diet\text{-}otolith}$ between the 2 studies. This is further supported by a negative relationship between muscle $\delta^{13}C$ and size, i.e. smaller individuals have higher muscle $\delta^{13}C$ values.

Despite all these potential biases however, otolith SOM signatures were very close to those of the main prey listed for wild *B. chrysoura*, which confirms the strong potential of otolith SOM $\delta^{13}C$ and $\delta^{15}N$ for accurately reconstructing fish diet. Regarding the determination of TEF between otolith SOM and diet signatures, our results should be used with more caution and will require further investigation to assess more precisely the $TEF_{diet\text{-}otolith}$ for $\delta^{13}C$ and $\delta^{15}N$ in this species.

Interpreting temporal changes in *B. chrysoura* otolith signatures

The comparison between past (1979–1981) and present (2006–2007) otolith SOM signatures suggested an increase of 1.17‰ in $\delta^{13}C$ and a slight decrease in $\delta^{15}N$ of 0.55‰ for the prey ingested by *B. chrysoura*. Interestingly, this slight decrease in $\delta^{15}N$ is supported by recent simulations of the evolution of food webs in the Terminos Lagoon over this time period (Abascal-Monroy et al. 2016). Indeed, using the ECOPATH model (Polovina 1983), these authors showed that the average trophic level of local *Sciaenidae*, the family of *B. chrysoura*, had probably decreased by 0.15 (from 3.34 to 3.19) since 1980, which corresponds to a diminution in $\delta^{15}N$ of 0.36‰ based on the average tropic enrichment factor (+2.3‰) proposed for fish (Caut et al. 2009). This result seems to match fairly well the shift in $\delta^{15}N$ observed in this study (−0.55‰), which corroborates the use of the method for investigating changes in fish diet over time. Although the temporal shift in carbon and nitrogen isotopic values is relatively small, this change could be due to the changes that

occurred in the food web leading to *B. chrysoura* over the same period (Abascal-Monroy et al. 2016). Indeed, although the range of prey ingested by the species has apparently remained the same in the lagoon since 1980 (Chavance 1984), the relative contributions of different components of *B. chrysoura*'s diet have dramatically changed over these 3 decades. In the past, *B. chrysoura*'s diet consisted mainly of fish (60.1%), and to a lesser extent of crabs (26.3%), with very few shrimps and polychaetes (Chavance 1984), while our results indicate a current dominance of polychaetes (38%), fish (21%) and shrimps (18%) (Table S4).

Despite the fact that the temporal change in otolith SOM signatures seems driven by the change in diet, other mechanisms may influence the small but significant difference in $\delta^{13}C$ and $\delta^{15}N$ between past and present periods. Possible mechanisms, acting singly or together, include: (1) a temporal change in habitat use by *B. chrysoura* in the Terminos Lagoon, (2) a modification over time of the average isotopic signatures of the prey, or (3) a post-mortem alteration of the SOM isotopic signature in the past otoliths.

Temporal changes in habitat use by the *B. chrysoura* population living in the Terminos Lagoon are very likely to be part of the equation, especially since the isotopic signatures of several prey eaten by the species during its earliest life stages (e.g. amphipods; Chavance 1984) were shown to vary spatially within the lagoon (Raz Guzmán & de la Lanza 1993). Indeed in the 1990s, the locations where these prey (zooplankton, amphipods, shrimps, crabs) had $\delta^{13}C$ values above −16‰ were very few and restricted to the mouth of the Rio Candelaria and to the seagrass meadows that grew along the south shore of Carmen Island (Raz Guzmán & de la Lanza 1993). Due to sand extraction for human construction projects, the seagrass meadows, which are preferred nurseries for *B. chrysoura* (Rooker et al. 1998), have disappeared from the Terminos Lagoon in recent decades (Villalobos Zapata et al. 2002). Moreover, as the lagoon has also experienced a marked increase of its average water salinity since the 1980s (Sirot et al. 2015a) and since *B. chrysoura* prefers brackish waters (Chavance 1984), the loss of its preferred nursery grounds within Terminos Lagoon in the late 1990s might have resulted in the translocation of its juvenile habitats toward the mouths of the local rivers. This would explain the marked drop in the number of *B. chrysoura* juveniles captured within the lagoon in recent decades (Sirot et al. 2015b). It would also contribute to the temporal shift in *B. chrysoura* otolith SOM signatures if the main nursery habitats of the species are now located near the mouth of rivers where prey $\delta^{13}C$ signatures are less negative, based on reported values for Rio Candelaria (Raz Guzmán & de la Lanza 1993). However, even if the change in *B. chrysoura* habitat is a potential mechanism explaining the modification of $\delta^{13}C$ signatures, our conclusions regarding these new habitats have to be taken with caution and the determination of their exact locations would require a more comprehensive survey of the signature of *B. chrysoura's* potential prey.

An overall modification of the isotopic signatures of the baseline organic matter sources sustaining the food webs in the Terminos Lagoon might also have occurred since the 1980s, in line with the marked increases in salinity and eutrophication levels observed since in the region (Ramos-Miranda et al. 2005b). However, augmentation of the contribution of marine sources to the lagoon food webs fails to explain the increase in otolith SOM $\delta^{13}C$ observed over this time period as prey at sea tend to show lower $\delta^{13}C$ values than in the lagoon (Raz Guzmán & de la Lanza 1993). Similarly, while eutrophication can increase organisms' $\delta^{13}C$ values in aquatic food webs, it generally results in a marked increase in their average $\delta^{15}N$ signatures (McClelland & Valiela 1998, Brenner et al. 1999, Neumann et al. 2002). As $\delta^{15}N$ in otolith SOM did not show any increase, but rather decreased slightly, this does not support the notion of a temporal modification of the baseline signature due to eutrophication.

Finally, otolith degradation during storage is unlikely to have altered SOM signatures, even for the otoliths collected in the 1980s. Indeed, unless they are submitted to high temperatures or pressures (which was not the case here), degradation rate for biological tissues depends largely on the susceptibility of their organic matter to microorganism attacks, which varies from days for soft tissues to centuries or even more for hard structures such as bones (Eglinton & Logan 1991, Collins et al. 2002). When it is combined with mineral structure, organic matter seems to be protected against bacterial alteration (Collins et al. 2000). Consequently, proteins from past hard structures (bones, teeth) are commonly used for reconstructing prehistorical animal diet or even phylogeny (Bocherens et al. 1991, Collins et al. 2000). Although little is known still about the post-mortem evolution of the quality and the isotopic signature of the otolith organic matrix, it has been demonstrated that $\delta^{18}O$ isotopic signatures are well preserved even in fossil otoliths from the Pliocene era (Dufour et al. 2000), which suggest very low to non-

existent alteration of otolith organic matter signature over time. Moreover, as the otoliths of our study did not undergo any process of diagenesis during their storage (in dry microtubes at room temperature for only up to 30 yr), it is reasonable to assume that the difference between past and present otolith SOM signatures is not due to OM degradation during storage.

Our results revealed a slight but significant temporal change in the trophic ecology of population of *B. chrysoura* within the Terminos Lagoon since 1980. It is important to note that whether the temporal shift in otolith signatures observed comes from a change in diet or in habitat, these 2 mechanisms may both lead to a decrease of individual fitness (Le Pape 2005) and probably contributed to population decline of *B. chrysoura* in the area. Indeed, change in diet at juvenile stage could have resulted in a decrease in the nutritional values of the ingested prey. As population demography is strongly related to individual fitness (Violle et al. 2007) and particularly at juvenile stage (Le Pape 2005), this diet modification could have affected *B. chrysoura* abundance or exacerbated an existing decline. In the case of a nursery ground modification, the loss of these key habitats or the degradation of their quality is now well known to decrease individual fitness as these areas provide food and protection against predators for juveniles (Blaber & Blaber 1980, Riley et al. 1981, Ruiz et al. 1993, Gibson 1994).

Advantages, limits and improvements of using otolith SOM signature for investigating changes in fish diet

The application of the method of Grønkjær et al. (2013) for detecting change in fish trophic ecology is based on the analysis of the stable isotopic signatures of the soluble organic matter extract from historical and present otoliths. As for all techniques based on isotopic measures, this method is used to detect change in otolith signature but does not provide information on the mechanism(s) responsible for the observed change. To disentangle the exact cause(s) of the trophic modification, further investigations are generally required usually involving complementary results or techniques (e.g. the isotopic signature of the baseline or of potential prey; Vander Zanden et al. 1999). In our case for instance, more information is required to determine which mechanism(s) led to the temporal change observed in otolith SOM signatures since 1980. In this context and, since a change of juvenile key habitat potentially explains the temporal change in SOM signatures, the combination of SIA with an analysis of otolith elemental microchemistry could allow characterization of key past and present habitats of *B. chrysoura* (Vasconcelos et al. 2008) and consequently validation or refutation of the hypothesis that change of nursery area since 1980 is the cause of the change in isotopic signatures of *B. chrysoura* otoliths since the 1980s.

Another limit of using the isotopic signatures of otolith organic matter is that it is generally more invasive than a technique based on muscle sample biopsies (although most reported isotopic studies on muscle involved fish death; e.g. Hansson et al. 1997, Jennings et al. 2002, Escalas et al. 2015). In most species, this limitation does not constitute a problem, but it could be an important factor to take into account for studying diet of endangered fish (e.g. for tuna; Das et al. 2000).

Finally, as for all isotopic methods based on soft tissues, one major constraint of the approach used in this study is the limited access to historical specimens, i.e. of tissue and otoliths (Jennings et al. 2002). However, in contrast to soft tissues, otoliths can easily be stored for longer periods (Arrington & Winemiller 2002), allowing important collections of historical otoliths to be assembled in laboratories or in natural history museums and made available to researchers. A key objective in the future should therefore be to continue enriching otolith collections.

So far and due to the sensitivity of the mass spectrometer, the technique has been limited by the amount of material required for the isotopic analysis (0.1 mg of otolith SOM, i.e. around 60 mg of otolith powder). However, one really important advantage of the technique of Grønkjær et al. (2013) is the low sampling required. Indeed, in contrast to soft tissues that have high turnover ratio (Weidel et al. 2011), otoliths do not experience any resorption (Campana & Neilson 1985) and should consequently record diet thorough the individual's life. Investigating adult otolith signatures would therefore provide access to the diet of the whole life of the individual, consequently limiting the number of samples required to study historical change in trophic ecology. Moreover, the rapid improvement of mass spectrometer sensitivity and of the extraction technique now allows analysis of as little as 20 mg of otolith powder, thereby opening up new opportunities to characterize the signature of each seasonal/annual otolith increment and consequently to study ontogenic shifts in diet or to determine more precisely the exact life stage affected by a diet change.

CONCLUSIONS

This study demonstrates that the method of Grønkjær et al. (2013) to extract otolith SOM could be successfully applied to present and past otoliths of a tropical estuarine sciaenid. These promising results suggest that this technique could be applicable to very different species with contrasting ecologies. Moreover, our findings indicate that, as is the case for cod, $\delta^{13}C$ and $\delta^{15}N$ from *B. chrysoura* otolith SOM were consistent with the isotopic signature of their lifetime prey. Further investigation is now required to quantify the exact $TEF_{diet\text{-}otolith}$ for this species and more generally for numerous other species.

By applying this state-of-the-art method to historical (1979–81) and contemporary (2006–07) otoliths, we found a small but significant change (increase in $\delta^{13}C$ and the slight decrease in $\delta^{15}N$) in the isotopic signature of *B. chrysoura* lifetime otolith SOM over the last 3 decades. The exact mechanisms leading to these shifts are still not known: although a marked modification of the local diet of *B. chrysoura* juveniles in Terminos seems the more probable mechanism, a change in the location of its nursery grounds within the lagoon could not be excluded.

This study demonstrates the great potential of SIA of otolith SOM for detecting changes in fish diet, providing an additional tool for conservation managers attempting to unravel the ongoing processes leading to demographic changes in fish species facing environmental alterations of their ecosystem and paving the way for new approaches to investigating the effects of global change on marine food webs.

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