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Life-history studies by non-lethal sampling: using microchemical constituents of fin rays as chronological recorders

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Chemical properties of fin rays were investigated in nine fish species to test whether life-history characteristics can be analysed using a non-lethal and minimally invasive methodology. Fish specimens from public aquariums were acquired after fishes died in captivity. Analyses concentrated on exploring the differences between the wild and captive life periods of each fish, which were known from aquarium records. Differences between the two life periods were observed in both the trace-element and stable-isotope compositions of the chemical matrix of the fin ray. Trace-element concentrations in fin rays were compared with those in otoliths using measures of resolved variance and cross-correlation to test the assumption of conserved matrices in the fin ray. Divalent ions and positively charged transition metals (*i.e.* Fe and Co) had strong associations between the two structures, suggesting conservation of material. Stable-isotope values of δ^{13}C and δ^{15}N differed between the wild and captive life periods in most of the fishes, also suggesting conserved matrices. δ^{13}C and δ^{15}N were derived from the organic matrix within the fin ray, which may present a stable-isotope chronology. Future studies can use these chronologies to study diet and movement trends on a temporal scale consistent with the entire lifetime of an individual.

Key words: microchemistry; mineral matrix; otolith chemistry; proteinaceous matrix; trophic chronology.

INTRODUCTION

Microchemical techniques can provide complementary information to existing methods used for fish movement and diet studies. Trace-element analysis (TEA) of calcified structures in fishes has not been used extensively in diet studies, but instead has been used most commonly to study movement in fishes (Elsdon *et al.*, 2008). Using TEA, elemental fingerprints help researchers map the movements of fishes by first tracing elemental concentrations along chronological landmarks within the calcified structures and then comparing the observed trends with known variation in the ambient environment (Dierking *et al.*, 2012). In comparison, stable-isotope analysis (SIA) has been used to both obtain trophic information (Galvan *et al.*, 2010) and to infer movement (Gillanders *et al.*, 2003; Dierking *et al.*, 2012). More specifically, δ^{13}C has been used to identify basal-resource dependence (Hobson, 1999; March & Pringle, 2003; Solomon

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et al., 2011) and δ^{15}N has been used to estimate trophic level (Vanderklift & Ponsard, 2003; Galvan *et al.*, 2010). δ^{13}C and δ^{15}N can also be used to track movements of fishes against background isotopic levels, which are mapped as isoscapes (Graham *et al.*, 2010; Radabaugh & Peebles, 2014).

Stable-isotope ratios in fishes have primarily been measured in muscle tissue. Muscle tissue, however, has a turnover rate of weeks to months and thus SIA of muscle provides short-term perspectives (Nelson *et al.*, 2011; Ankjaero *et al.*, 2012). To date, five types of fish tissue (otoliths, eye lenses, vertebral cartilage, scales and fin rays) have been used to reconstruct longer chronological histories of stable-isotope ratios (Estrada *et al.*, 2006; Elsdon *et al.*, 2008; Wallace *et al.*, 2014; Woodcock & Walther, 2014; Tzadik *et al.*, 2015). While all five structures appear to be effective recorders of stable isotopes, only the analysis of scales and fin rays is non-lethal; however, it can be difficult or impossible to obtain age-specific isotope measurements from fish scales (Hutchinson & Trueman, 2006; Helfman *et al.*, 2009). Fin rays, as in other calcified structures (*e.g.* otoliths, fin spines, scales and cleithra), are incremental structures that can be used for age and growth determination in many fishes (McFarlane & King, 2001; Murie & Parkyn, 2005; Muir *et al.*, 2008; Khan & Khan 2009; Murie *et al.*, 2009; Glass *et al.*, 2011), although limitations exist in fishes with high metabolic rates such as billfishes (istiophorids and xiphiids), which cannot be aged using fin rays due to resorption of annuli (Antoine *et al.*, 1983). Despite this, fin rays offer a potential structure for non-lethal ageing and chemical profile mapping.

In a variety of species, studies have documented conserved trace-element concentrations (especially divalent ions such as Ba and Sr, which have similar ionic radii to Ca) within otoliths and fin rays that correlate with concentrations in ambient water (Clarke *et al.*, 2007; Woodcock *et al.*, 2013). While elements deposit into otoliths and fin rays through different internal pathways, the correlation of certain elements with the ambient environment suggests layers retain their chemical properties over time in both structures, rather than being re-worked (Clarke *et al.*, 2007; Allen *et al.*, 2009; Smith & Whitledge 2010; Jaric *et al.*, 2011; Phelps *et al.*, 2012; Woodcock & Walther, 2014). Inner fin-ray layers become encapsulated by growing outer layers, after which the encapsulated inner layers lose their vascularization, thus inhibiting tissue turnover within the inner layers (Sire & Huysseune, 2003).

Direct comparisons of elemental chronologies (either by TEA or SIA) between otoliths and fin rays have not been made, nor has the conservation of organic material within the fin ray been tested. The present study uses fishes with known histories of wild and captive life periods to investigate whether the annuli of fin rays retain chemical characteristics over time. Specifically, the study tests the assumption of conservation of trace elements within the inorganic matrix of fin rays (primarily $CaPO_4$) by comparing values in fin rays with those in otoliths and conservation of stable-isotope ratios within the organic matrix of fin rays (primarily collagen) to test whether changes occur when ambient water conditions are altered.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fishes were obtained from public aquariums after they had died in captivity. All fishes donated to the study were wild before being captured and raised in captivity. These conditions

TABLE I. Species list of all specimens (n) used in the study and the number of fishes that had otoliths available for trace element analysis (n_{TEA}), as donated by: Mote Marine Laboratory and Aquarium, Sarasota, FL; Guy Harvey Rum Fish Grill restaurant, St Petersburg, FL; Vancouver Aquarium, Vancouver, BC; Rookery Bay Learning Center, Naples, FL; Pier Aquarium, St. Petersburg, FL

Species	Family	n	n_{TEA}	Donor
Centropomus undecimalis	Centropomidae	12	6	Mote Marine Lab
Epinephelus morio	Epinephelidae	5	4	Rum Fish Grill
Sebastes pinniger	Sebastidae	1	1	Vancouver Aquarium
Sebastes caurinus	Sebastidae	4	4	Vancouver Aquarium
Sebastes melanops	Sebastidae	1	1	Vancouver Aquarium
Sebastes flavidus	Sebastidae	3	3	Vancouver Aquarium
Sebastes ruberrimus	Sebastidae	1	1	Vancouver Aquarium
Pogonias cromis	Sciaenidae	1	0	Rookery Bay Learning Centre
Sciaenops ocellatus	Sciaenidae	2	0	Pier Aquarium

allowed for comparison between known wild and captive life periods over longer time frames (*i.e.* years in the present study) than would be feasible *via* laboratory-controlled experimentation (Table S1, Supporting Information). The specimens originated from different families (Table I), thus offering better inference on the generalities of whether the annuli of fin rays retain chemical characteristics over time. Thirty individuals were obtained, of which 20 were used for comparison of otoliths (due to tissue availability) with fin rays by TEA. Fin rays from all 30 individuals were used in comparisons with SIA. Each fish was aged using otoliths and fin rays and an estimate of aquarium residency time was established based on aquarium records, which allowed estimation of the location of the wild-to-captive transition on the calcified structures.

FIN-RAY AND OTOLITH PREPARATION

Fin rays were excised from all individuals to include the distal pterygiophores and then frozen at −20° C or colder. Once removed from the freezer, the fin rays were defrosted in a drying oven for 3 h at 55° C. Plastic forceps were used to peel away as much skin and membrane as possible. Fin rays were soaked in 30% hydrogen peroxide (H_2O_2) for 5 min to loosen any remaining adhering tissue, which was then removed using plastic forceps and paper towels. Once the fin rays were cleaned, they were secured to a petrographic slide using Crystalbond (Aremco; www.aremco.com). Fin rays were sectioned as close to the base of the ray as possible at a 1·5 mm thickness using stacked diamond wafering blades on a Buehler IsoMet low-speed saw (www.buehler.com), producing two or three cross sections. Two readers aged all cross sections under a dissecting microscope before further processing.

Sagittal otoliths were removed using rubber-tipped forceps. Otoliths were rinsed in ultrapure Milli-Q (Millipore; www.emdmillipore.com) water upon removal and soaked in 30% H_2O_2 for 5 min before being mounted and sectioned as above, except with 1·0 mm section thickness. Transverse sections were taken from all otoliths across all families. Cross-sections were repositioned and mounted onto a single slide.

Prepared slides with samples were sonicated in ultrapure Milli-Q (Millipore) water for 5 min using an FS30H sonicator (Fisher Scientific; www.fishersci.com). Samples were placed in a class-100 laminar flow clean hood where they were air-dried for a minimum of 24 h before analysis. All trace-element and stable-isotope analyses were conducted at the University of South Florida, College of Marine Science.

TABLE II. Mean resolved variance (β) and cross correlation (r_{CC}) values for 12 elements that were above the limits of detection* within three families of fishes. Higher values of β and r_{CC} indicate stronger matches between datasets, with a maximum value of 1·0. *%P*, the percentage of individuals in each family where $P < 0{\cdot}05$, based on the Monte Carlo simulations; Σ *%P*, the total percentage of fishes with significant values see Table SII for full list of β, r_{CC}, and *%P* values

Element		Centropomidae ($n = 6$)	*%P*	Sebastidae ($n = 10$)	*%P*	Epinephelidae ($n = 4$)	*%P*	Σ *%P*
Li	β	−668·49	0	−17·92	0	−365·46	0	0
	r_{CC}	−0·05	0	0·04	0	0·16	25	5
Na	β	−3·27	0	−1·87	30	−432·76	25	20
	r_{CC}	−0·11	0	−0·25	20	−0·13	0	10
Mg	β	−4·33E+04	0	−6·75E+04	0	−5·92E+05	0	0
	r_{CC}	0·54	0	−0·23	0	−0·27	0	0
P	β	−1·34E+06	0	−1·59E+06	0	−2·20e+06	0	0
	r_{CC}	−0·31	17	0·26	10	−0·12	25	15
V	β	−22·42	83	−955·38	40	−537·60	50	55
	r_{CC}	−0·04	0	−0·05	0	−0·10	0	0
Mn	β	−152·54	0	−760·35	0	−2292·21	0	0
	r_{CC}	0·24	33	−0·17	0	−0·23	0	10
Fe	β	0·90	100	0·47	100	0·85	100	100
	r_{CC}	0·04	0	0·08	0	0·09	0	0
Co	β	0·72	100	−936·72	100	−1·86	100	100
	r_{CC}	0·00	0	0·01	0	0·03	0	0
Zn	β	−3·50E+04	0	−6·83E+03	10	−7·73E+03	0	5
	r_{CC}	0·42	17	0·23	10	−0·29	0	10
Cu	β	−1·98E+02	83	−9·91E+04	40	−1·06E+07	25	50
	r_{CC}	0·01	0	−0·21	10	0·06	25	10
Sr	β	0·78	100	0·78	100	0·72	100	100
	r_{CC}	0·33	33	−0·03	30	0·39	50	30
Ba	β	0·42	100	−566·29	80	−967·47	75	85
	r_{CC}	0·06	0	−0·10	30	−0·53	50	25

*Elements analysed: Li^{7}, Na^{23}, Mg^{24}, P^{31}, Ca^{43}, Sc^{45}, V^{51}, Cr^{53}, Mn^{55}, Fe^{57}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{64}, Cu^{65}, Ge^{72}, Rb^{85}, Sr^{88}, Y^{89}, Cd^{114}, Sn^{118}, Ba^{137}, Au^{197}, Pb^{208}, Th^{232}, U^{238}.

TRACE-ELEMENT ANALYSIS

Core-to-edge transects were ablated on each structure using a Photon Machines Analyte 193 excimer laser ablation system (Evisa; www.speciation.net) that was connected to an Agilent 7500 ICP-MS (Agilent Technologies; www.agilent.com). The laser system operated at a wavelength of 193 nm and a maximum output of 8 mJ. The ablations of otolith and fin-ray samples were conducted with 86% power, a 5 Hz frequency and a 108 µm spot size. The laser moved across each structure at a speed of 10 µm s^{-1}. Background counts were monitored for 60 s between laser transects to ensure sufficient removal of residue from the previous transect. Measurements were made for 26 unique isotopes. Out of these, 12 were above detection limits for both structures and used in statistical analysis (Table II). Calcium was used as an internal standard for the other 25 analytes being measured due to its stoichiometric abundance within the $CaCO_3$ (primarily aragonite) and $CaPO_4$ (primarily hydroxyapatite) inorganic matrices, where Ca was 40·0% of the molecular mass of $CaCO_3$ (Campana, 1999) and 27·5% of $CaPO_4$. [Ca] of fin rays was verified after acid digestion within polypropylene vials at 180 ° C in 16 M HNO_3 for 2 h. Digested samples were diluted with 2% HNO_3 and quantitatively analysed in the ICP-MS

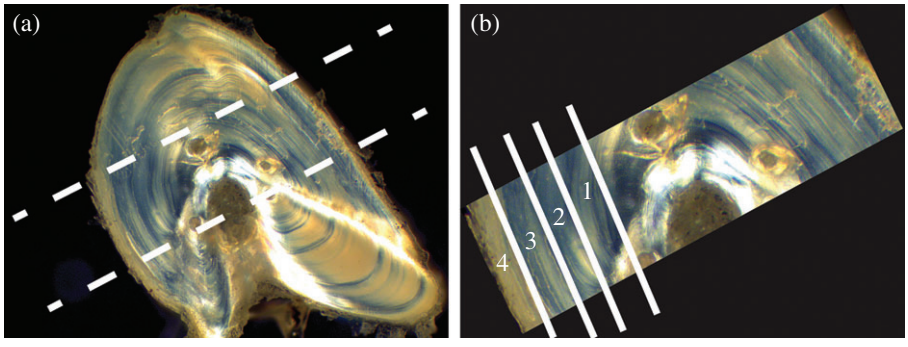

FIG. 1. Separation of annuli from a cross section of a dorsal-fin ray. (a) ---, line along which a rectangular section was taken from the cross section; (b) ——, excision lines separating individual annuli.

to obtain [Ca]. Drift of the ICP-MS during the solution-based analysis was monitored and calibrated using scandium as an internal standard. The calibration line, which establishes a transfer function from original measurements to a scale-normalized quantity, ranged from 5 to 50 mg l^{-1} for Ca.

Agilent Technologies instrument-control software was used for data collection. One external glass and one synthetic calcium carbonate standard with known isotopic compositions (NIST 612 and MACS-4) were used to calibrate the instrument. The MACS standard was analysed prior to and immediately following the analysis of all samples. The U.S. National Institute of Standards and Technology (NIST) standard was analysed both prior to and following all analyses, as well as in between each sample, allowing external drift correction. Concentrations were recorded as counts s^{-1} and then converted to mg l^{-1} using MATLAB R2015a (www.mathworks.com), with functions created in the Fathom Toolbox (Jones, 2014). Concentrations (mg l^{-1}) were used in all subsequent analyses.

STABLE-ISOTOPE ANALYSIS

A second cross section from each fin ray was used to isolate individual annuli for SIA. Each cross section was further cut to create a rectangular slice comprising radial bands that collectively represented the entire lifetime of the fish [Fig. 1(a)]. Slices were cut using a modified feather-blade guillotine. By inserting a second blade and a 0·10 mm spacer, segments were sliced without using mounting adhesive. Each slice was then cut into smaller, perpendicular subsections using the single blade of the guillotine to mechanically separate the annuli [Fig. 1(b)]. Each fin-ray subsection was representative of a different life period. The same procedure was not applied to the otolith samples because the quantification of organic material within incremental sections was beyond the capabilities of modern instrumentation.

Fin-ray sections were then classified as wild period or captive period. Each subsection was then analysed for bulk molar concentrations of carbon and nitrogen (C, N and C:N) and stable isotope ratios (δ^{13}C and δ^{15}N). A 200–1200 µg sample of each cross section was weighed on a precision micro-balance (Mettler-Toledo; www.mt.com), encapsulated in tin and loaded into a zero-blank autosampler (Costech Technologies; www.costech.com). Samples were combusted at 1050 °C in a Carlo-Erba NA2500 Series-II elemental analyser (EA; ThermoScientific) coupled in continuous-flow mode to a Finnigan Delta Plus XL isotope ratio mass spectrometer (IRMS; ThermoScientific). Stable-isotope compositions were expressed as ‰ using delta notation: *e.g.*, $\delta^{15}N = (R_{sample}R^{-1}{}_{standard}) - 1]1000$, where $R = {}^{15}N^{14}N^{-1}$. The C:N measurements were calibrated and δ^{13}C and δ^{15}N were normalized to the AT-Air and Vienna peedee belemnite (VPDB) scales, respectively, using NIST 8573 and NIST 8574 L-glutamic acid standard reference materials. Analytical precision, estimated by replicate measurements of a laboratory working standard (NIST 1577b bovine liver SRM, $n = 30$), was ± 0·25 δ^{13}C, 0·10‰ δ^{15}N and ± 0·43 C:N.

STATISTICAL ANALYSIS

In order to compare trace element concentrations between otoliths and fin rays, a Gaussian filter was applied to the otolith data, which were then standardized to match the smaller dataset associated with the fin ray for each fish. This methodology was created specifically for this project and differs from other techniques that correlate unknown concentrations in calcified structures (*i.e.* otoliths and fin rays) to ambient water concentrations. A measure of resolved variance (Mann *et al.*, 1998) was used as well as cross-correlation values (Legendre & Legendre, 2012) to compare the two structures. The resolved variance statistic measures how effectively the variance of one data series (*i.e.* otolith) is explained by the other (*i.e.* fin ray);

$$\beta = 1 - \left[\sum \left(y_{oto}\text{-}y_{fin}\right)^2\right]\left[\sum \left(y_{oto}\right)^{-2}\right],$$

where y_{oto} is a series of elemental concentrations in the otolith (after Gaussian standardization) of a single fish from time of birth to time of death and y_{fin} is the series of the same element over the same time period in the fin ray. The resolved variance (β) was calculated for each element that was above the limits of detection (Tables II and SII,) in each fish and compared with values of cross-correlation using the same time series. The resolved variance statistic was chosen as the primary metric for comparison because it is a more robust comparison of datasets than traditional correlations, as it measures the correspondence based on the relative departure from the mean, the mean itself and the absolute variances of the two datasets. The use of the resolved variance statistic allowed for the exploration of matches between datasets that were not apparent from the cross-correlation values. Higher values of both β and cross-correlation values indicate stronger matches between datasets, with a maximum value of 1·0. In addition, a two-sample Kolmogorov-Smirnov test was used to test whether either correspondence variable (*i.e.* β or cross-correlation) differed between life periods.

Significance levels for both β and cross-correlation values were estimated by Monte Carlo simulations ($n = 1000$ permutations) that took serial correlation into account. The serial correlation derived from a null model of AR(1) red noise was used (*i.e.* an auto-regressive model with a lag of one). Degrees of freedom were based on the autocorrelation coefficients with lag-one of the two series.

Stable-isotope comparisons were made for both $\delta^{13}C$ and $\delta^{15}N$ values in fin-ray sections between the captive and the wild life periods for each fish by calculating absolute differences. Absolute differences were used instead of signed differences because there was not an *a priori* reason to expect that one life period would have a higher or lower isotopic value than another and thus the magnitude of differences was of concern rather than the sign of differences. In order to assess the significance of this magnitude between the two life periods, a bootstrapping technique was used to estimate the range of average differences (99% C.I.) around the observed mean value. In addition, a Procrustes analysis was used to test how well the data from the two periods matched (Peres-Neto & Jackson, 2001). Specifically, how well did captive and wild-period data agree? Differences between periods should be expected, given associated differences in background levels and feeding history. All statistical analyses were conducted using MATLAB version R2015a (www.mathworks.com/).

RESULTS

TRACE-ELEMENT CHRONOLOGIES

The comparison statistics of core-to-edge transects between otoliths and fin rays of the same fishes varied among elements and individual fishes (Table II). Significant values of β were consistently observed for the concentrations of Fe, Co, Ba and Sr between structures (Fig. 2 and Table SIII). No other element had values that were significant in more than 55% of the samples. The cross-correlation values were not consistently

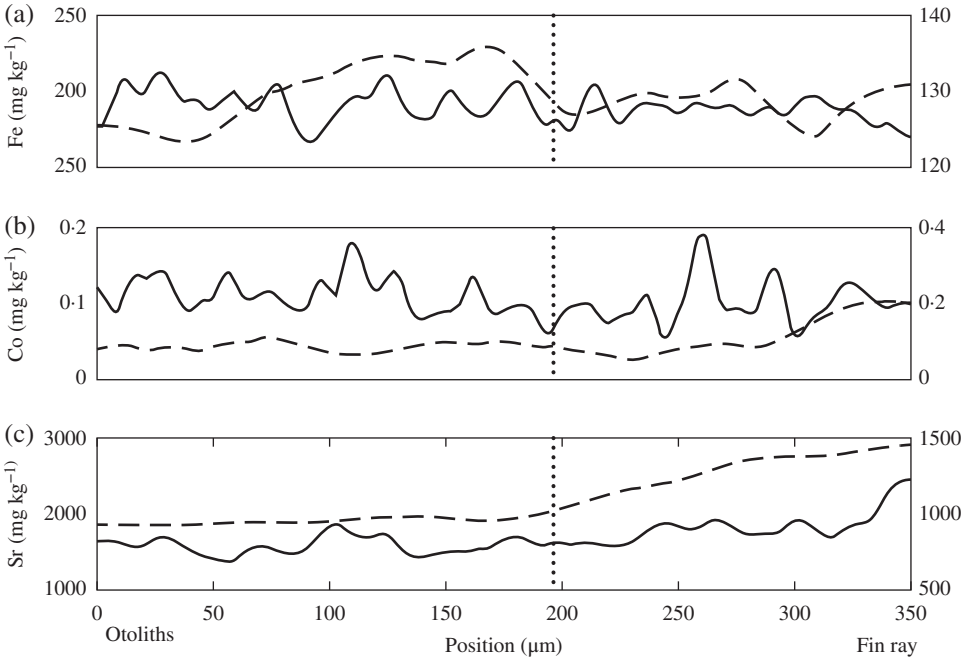

FIG. 2. Concentrations of elements with a 2+ charge, *i.e.* (a) iron (Fe), (b) cobalt (Co) and (c) strontium (Sr) over time in the otolith after smoothing (——) *via* a Gaussian filter and fin-ray concentrations (– – –) of a copper rockfish *Sebastes caurinus.* ....., the documented time of capture (*c*. 200 μm). This sample represents a fish with particularly strong β values for elemental concentrations, but is representative of the general trend observed among all samples. Profiles were run from the core of the structure (on the left, corresponding to birth) to the edge of the fin ray (on the right, corresponding to end of life).

significant for any single element, but had the highest occurrence of significance for Ba and Sr (Table II). Neither measure of correspondence differed between life period for the elemental concentrations of Fe, Co, Ba and Sr ($\overline{ks} = 0{\cdot}35$, $\overline{P} > 0{\cdot}05$), thus further analyses focused on entire lifetime comparisons of each fish instead of by life period.

Differences among families for each element were apparent for some elements, but not others. While β was consistently significant for Fe, Co, Ba and Sr across all families, other elements such as V and Cu were more often significant for centropomids than for other families (Table II). Significant values of cross-correlation in most elements were unevenly distributed among families. For example, while no centropomids had significant values of cross correlation for Ba, half of the epinephelids did (Table II).

STABLE-ISOTOPE CHRONOLOGIES

The values of δ^{13}C in all fishes ranged from −24·65 to −11·33 (mean ± S.E., −18·29 ± 0·44) for the wild periods and −21·63 to −11·24 (mean ± S.E., −16·29 ± 0·29) for the captive periods (Table III). Values of δ^{15}N ranged from 7·98 to 13·47 (mean ± S.E., 10·53 ± 0·19) for the wild periods and 7·86 to 13·62 (mean ± S.E., 10·63 ± 0·19) for captive ones. All families showed δ^{13}C enrichment and a more narrow range of values after being put into captivity (Table III). The mean absolute difference between wild

TABLE III. Mean values (‰) of $\delta^{13}C$ and $\delta^{15}N$ between wild and captive life periods in all fishes. Absolute differences are presented with lower (−C.L.) and upper confidence limits (+C.L.) for the 99% confidence interval as calculated by the bootstrapping technique

				Difference		
		Wild	Captive	−C.L.	Mean	+C.L.
Centropomidae	$\delta^{13}C$	−17·05	−15·18	1·63	2·47	3·41
	$\delta^{15}N$	10·33	10·12	0·46	0·71	0·97
Sebastidae	$\delta^{13}C$	−19·74	−17·51	1·23	2·24	3·63
	$\delta^{15}N$	11·90	12·00	0·25	0·40	0·55
Epinephelidae	$\delta^{13}C$	−19·32	−16·63	1·93	3·01	4·02
	$\delta^{15}N$	9·11	9·92	0·65	1·14	1·66
Sciaenidae	$\delta^{13}C$	−18·15	−16·63	1·88	3·31	4·74
	$\delta^{15}N$	12·08	12·40	0·54	0·69	0·83

and captive periods for individual fishes was 2·55‰ (14%) for $\delta^{13}C$ and 0·71‰ (7%) for $\delta^{15}N$. All values were significant at $P < 0{\cdot}01$.

The Procrustes analysis of all fishes further illustrated the differences between wild and captive periods *via* the comparison of paired samples ($m^2 = 0{\cdot}47$, $P < 0{\cdot}001$) (Fig. 3). When separated by family, the centropomid ($m^2 = 0{\cdot}46$, $P < 0{\cdot}001$) and sebastid samples ($m^2 = 0{\cdot}34$, $P < 0{\cdot}001$) differed strongly between wild and captive periods. While the Procrustes analyses performed on the epinephelids produced a high procrustean statistic ($m^2 = 0{\cdot}77$, $P > 0{\cdot}05$), the two periods were not significantly different. The sciaenids that were analysed were also not significantly different, although only three individuals were tested ($m^2 = 0$, $P > 0{\cdot}05$) (Fig. 4).

DISCUSSION

The data presented in this study were generated to address the hypothesis that trace elements and stable-isotope values were conserved over time in the inorganic and organic matrices of fin rays in fishes. The correspondence of certain divalent cations between otoliths and fin rays in the same fishes is consistent with the assumption of conserved inorganic-matrices (Fig. 2). Similarly, the differences in $\delta^{13}C$ and $\delta^{15}N$ values between wild and captive periods are consistent with organic-matrix conservation. The conservation of matrices in fin rays is one parsimonious explanation for the observed trends in the data, but further testing is necessary to verify the actual mechanism behind these trends. The data presented provide a framework from which to further investigate the uses and applications of trace element and stable-isotope chronologies in the fin rays of fishes.

TRACE-ELEMENT CHRONOLOGIES

The high β values generated from comparisons of divalent cations (alkaline earth and transition metals) between otoliths and fin rays in all fishes that were analysed suggest a high level of correspondence among elemental values between the two structures.

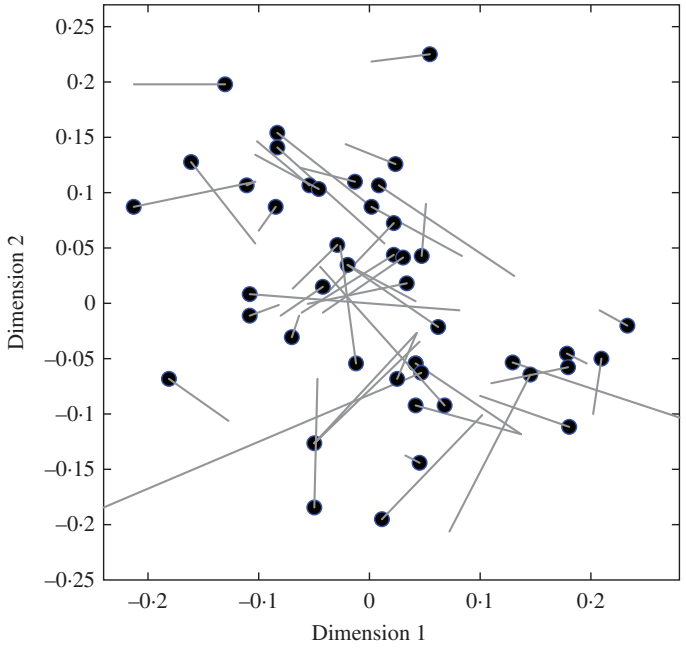

FIG. 3. Procrustean superimposition plot of wild and captive life-period paired samples of fin rays from all fishes. The *m*-statistic (m^2) for this analysis can range from 0 to 1 where smaller values represent more similarity between datasets; here, the $m^2 = 0{\cdot}47$. The dimensions describe the rotated data of the δ^{13}C and δ ^{15}N values prior to and after capture. ●, scaled wild life-period values of the rotated data; ——, residual lengths indicating the amount of difference between the samples.

This metric should not be confused with the correlation term (CC), as it measures a different aspect of the data. While CC is a measure of how well the departures from the mean correspond between two datasets, the β term offers a more robust comparison by accounting for the mean itself, the relative departure from the mean and the variances of the two datasets. Owing to the charge and size of atomic radii, the elements with high levels of correspondence can substitute for the Ca-cation in the inorganic matrices of both calcium carbonate and hydroxyapatite. Interestingly, other elements with 2+ charges (*i.e.* Mg and Mn) did not show strong correspondence between structures, possibly due to different substitution rates in $CaCO_3$ (otoliths) compared with $Ca_5(PO4)_3(OH)$ (fin rays) in the marine environment, as is common with Mg (Martens & Harriss, 1970). Trace elements, acquired from either diet or ambient water, are absorbed into the bloodstream of a fish and are incorporated into mineral matrices at the time of osteogenesis (Mahamid *et al.*, 2010; Woodcock *et al.*, 2012). The different elements tested could be expected to have different incorporation pathways, bioavailabilities or substitution affinities for each of the two calcified structures analysed. It could therefore be assumed that individual trace elements would have different rates of incorporation into each calcified structure within the fish. The correspondence of certain elements, however, is suggestive of similar incorporation processes and may indicate the conservation of material, as both structures retained similar values over time. Overall, the TEA was consistent with the hypothesis of conserved inorganic

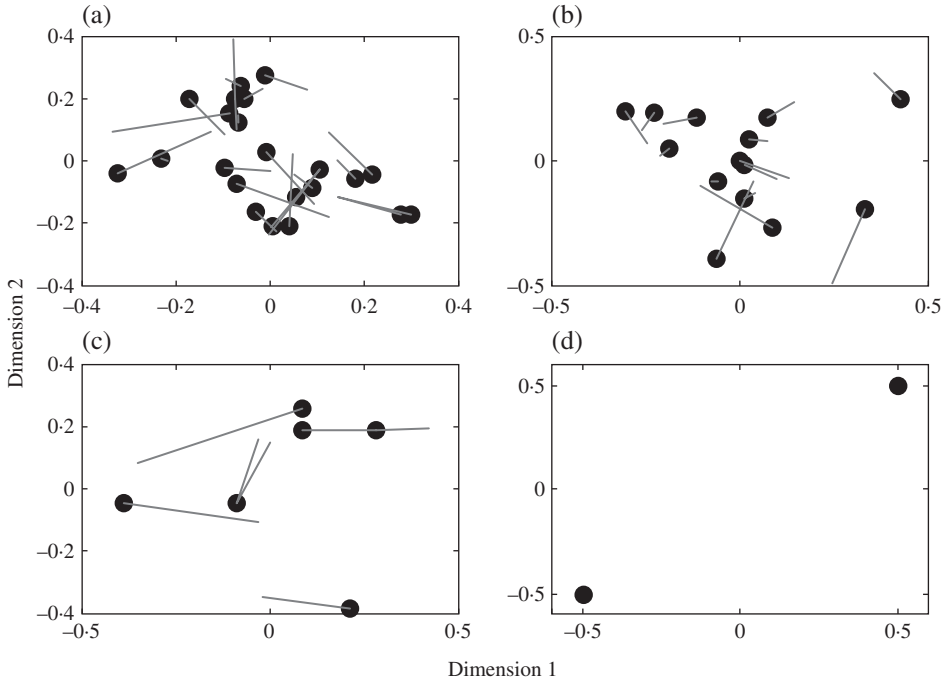

FIG. 4. Procrustean superimposition plot of wild and captive life-period paired samples of fin rays from all fishes, separated by family: (a) Centropomidae, $m^2 = 0{\cdot}46$; (b) Sebastidae, $m^2 = 0{\cdot}34$; (c) Epinephilidae, $m^2 = 0{\cdot}0$; (d) Sciaenidae $m^2 = 0{\cdot}77$. ●, scaled wild life-period values of the rotated data; ——, residual lengths indicating the amount of difference between the samples.

matrices in fin rays, as the concentrations corresponded strongly to those of the otolith, which has been documented to be a conserved matrix (Campana, 1999).

STABLE-ISOTOPE CHRONOLOGIES

Stable-isotope analysis in fin rays required several assumptions with regard to chemical stability. Most notably, the assumption of little to no turnover is critical to the effective use of stable-isotope chronologies for the inference of life-histories in individual fishes. The high level of resolved variance in divalent cation concentrations between otoliths and fin rays was consistent with this assumption, as the inorganic matrix in fin rays is embedded within the organic one (Mahamid *et al.*, 2010). Thus, as one matrix is encapsulated and its properties are conserved over time, the same could be expected for the other matrix. In addition, encapsulated layers are not vascularised, thus little to no turnover would be expected. The differences in $\delta^{13}C$ and $\delta^{15}N$ values between wild and captive periods could have resulted from differences in ambient water, differences in diet, tissue turnover or tissue decay. The reason for the unidirectional enrichment of $\delta^{13}C$ values in the captive period remains unclear, but may have resulted from carbon-based filtration systems in aquarium tanks, such as those that use activated carbon. Differences in water chemistry and diet between the natural environment and an artificial one (*i.e.* public aquariums) are suggestive of the most

parsimonious explanation for the observed differences. Tissue turnover would have probably resulted in equal values across both life periods, while tissue decay would have exhibited consistent directional differences (most likely depletion) in both $\delta^{13}C$ and $\delta^{15}N$ values. Other explanations for the observed differences include differential turnover rates among annuli, incomplete turnover (*i.e.* where turnover only occurs in one part of the ray) and different incorporation rates among annuli.

A last assumption of chronological SIA involved the time period of deposition. In this study, the organic matrix within each annulus was assumed to originate at the corresponding age of the fish. Further studies are necessary to verify this assumption as there may be a depositional lag due to metabolic pathways.

The stable-isotope trends observed in this study were consistent among three families and seven species. It should be noted, however, that differences among families cannot be differentiated from differences among ambient conditions, as samples within families all came from the same aquariums. Centropomids and sebastids had stable-isotope values that were significantly different between wild and captive periods based on the Procrustes analysis (Fig. 4). The epinephelids also showed a strong trend towards differences between life periods (*i.e.* the highest observed *m*-statistic); however, no significant difference was observed, possibly as a result of a low sample size ($n = 5$). The only family that had outlying trends was Sciaenidae, which did not show differences in stable-isotope values between wild and captive periods in the Procrustes analysis (Fig. 4). No sciaenid otoliths were available for analysis, so the TEA trends were undocumented. The anomalous trend among SIA in sciaenids may be due to small sample size ($n = 3$) or a true homogeneity between life periods. A larger sample size should be used to identify the cause of this anomaly.

METHODOLOGICAL LIMITATIONS AND POTENTIAL USES

Microchemical analyses in fin rays have several limitations that are primarily related to the chemical composition of each sample. Values of $\delta^{13}C$ need to be interpreted carefully, as inorganic carbon noise can affect the final output from the mass spectrometer. Even though fin rays are primarily composed of hydroxyapatite, carbonate molecules commonly substitute for phosphate and the resulting carbon noise in the analysis of the organic component in fin rays (and all bones) will obstruct the signal (Peroos *et al.*, 2006). Limitations also exist due to the size of cross sections in fin rays and the annuli therein. Cutting curved annuli with a straight blade is challenging. Owing to mechanical limitations and the small sizes of the samples used in this study, temporal resolution was limited to two, multi-year periods. Further refinement with this method and instrumentation with higher precision (*e.g.* micro-elemental analysis mass spectrometry) could lead to stable-isotope chronologies that are representative of smaller time gaps. Fishes with larger fin rays can be used with existing instrumentation (Tzadik *et al.*, 2015).

In its current form, the methods presented here could be used to track migratory and trophic patterns across ontogeny for individual fishes. If matrices are indeed conserved in fin rays, then life-history attributes for each fish are recorded continuously so that a complete record is available for each individual. These records can be used to test actual life-history trends in individuals as opposed to assumed life periods (that are ultimately researcher defined), such as ontogenetic migrations (Allen *et al.*, 2009). Traditional SIA using muscle tissue is limited in temporal inference due to relatively fast

turnover rates. A conserved matrix of organic material could be used instead to infer trends over time with much smaller sample sizes than would be necessary through the use of muscle tissue. Combining stable-isotope chronologies with existing isoscapes can lead to detailed studies on individual movements by recording how baseline values change over time. Inferring movements from isoscapes is relatively common in terrestrial ecology, but has yet to be used extensively in the marine environment. Chronological recorders of stable isotopes in fishes (*e.g.* fin rays and eye lenses) can be used to bridge the gap between these two fields. The use of fin-ray analysis for management purposes should be considered especially when endangered species are in question, as fin-ray removal is minimally invasive and does not affect growth or survival (Zymonas & McMahon, 2006).

The results presented here are consistent with the hypothesis that chemical matrices in fin rays are conserved over time. These matrices can be used to measure trace-element and stable-isotope values over time that are representative of the inorganic and organic matrices, respectively. These types of analyses appear to present viable alternatives to lethal techniques to study life-history characteristics in fishes where culling activities are inappropriate.

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Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE SI. Ages, time spent in captivity, and date of death (collection date) for all samples used in the study. Age estimates were derived from fin rays, and verified by otoliths when available.

TABLE SII. A list of mean values of the limits of detection (LOD) and the per cent relative standard deviation (%RS.D.). Each value was calculated as a mean value across each sample and each run of the instrument.

TABLE SIII. A complete list of resolved variance statistics (β), cross correlation (r_{CC}) values and associated P-values ($^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$) for each specimen among all elements tested.

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