Journal of Fish Biology (2006) **68,** 1909–1917
doi:10.1111/j.1095-8649.2006.01079.x, available online at http://www.blackwell-synergy.com

The differentiation of *Stegastes partitus* populations using lapillar and sagittal otolith chemistry

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(Received 17 May 2005, Accepted 19 January 2006)

The comparison of elemental concentrations of sagittal and lapillar otoliths from the same individuals of *Stegastes partitus* indicated significant differences for several elements. Sagittal otoliths were superior at differentiating individuals, yet the differentiation of individuals was further improved when the elemental concentrations of both otolith types were used in the same analysis.

Key words: discrimination; lapillus; otolith chemistry; sagitta.

The majority of teleosts have three types of otoliths (sagitta, lapillus and asteriscus, listed in decreasing size in most adult fishes), which through their continual growth incorporate trace elements from the environment (Campana, 1999; Thorrold *et al.*, 2002; Elsdon & Gillanders, 2003). Unfortunately, because otolith chemical studies have overwhelmingly favoured the use of sagittal otoliths to investigate the spatial arrangement of populations and the movement of individuals, little information is available regarding the chemical nature of lapillar otoliths (the asteriscus is not discussed because its crystal structure differs from that of the sagitta and lapillus). The only chemical studies comparing sagittal and lapillar otoliths were for temperate (Atlantic cod *Gadus morhua* L., Campana & Gagne, 1995) and subtropical species (flathead mullet *Mugil cephalus* L., Meyer-Rochow *et al.*, 1992; Gulf menhaden *Brevoortia patronus* Goode, Chesney *et al.*, 1998). Of these studies, Campana & Gagne (1995) and Meyer-Rochow *et al.* (1992) showed significant differences between otolith types, but only the former study compared the ability of sagitta and lapillus to discriminate populations.

In this study, comparisons were made between elemental concentrations from sagittal and lapillar otoliths of the bicolour damselfish *Stegastes partitus* (Poey), a common Caribbean reef fish (maximum standard length, L_S, of 6 cm) that was collected from several sites around Turneffe Atoll, Belize. *Stegastes*

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partitus is an ideal study species because it displays strong site fidelity from settlement to adulthood (Nemeth, 1998; Valles *et al.*, 2001) and thus their otolith elemental concentrations reflect a relatively fixed area (home range <2 m^2; pers. obs.). The specific goals of this study were to determine: a) if elemental concentrations vary between otolith types (sagitta and lapillus) of *S. partitus*, b) if both otolith types are equivalent at discriminating individuals collected from different sites and times, and c) whether combining the elemental concentrations of otolith types results in improved discrimination.

Juvenile *S. partitus* (n = 80) were selected from collections that were made in June (n = 42) and July (n = 38) 2002, at seven sites around Turneffe Atoll, Belize (Fig. 1). Individuals ranged in size from 1·1 to 3·4 cm L_S with a mean ± S.D. of 1·9 ± 1·0 cm (median of 1·8 cm). From each fish one sagitta and lapillus were removed, mounted in Crystal Bond®, and polished in a transverse plane using a range of lapping film (30–3 μm). In a class 100 clean room, multiple otolith sections (up to 35) were fixed to a microscope slide, cleaned in a milliQ water-bath and sonicated for 2·5 min, triple rinsed with 95% ethanol, and then triple rinsed with milliQ water. Each slide was then allowed to dry overnight in a laminar flow, HEPA-filtered fume hood.

Otoliths were chemically analysed at the Great Lakes Institute of Environmental Research, University of Windsor, Canada, using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (see Table I). A

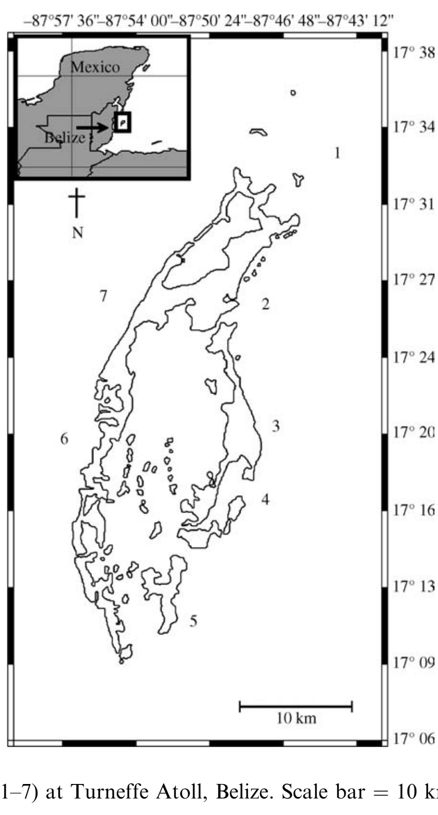

FIG. 1. Map of study sites (1–7) at Turneffe Atoll, Belize. Scale bar = 10 km.

TABLE I. Instrument operating conditions

Specification		Setting
Laser ablation system	Continuum Surelite I	
	Laser type	Nd:YAG
	Wave length	266 nm
	Frequency	20 Hz
	Flash lamp power	1·15 kV
	Beam diameter	45 μm
	Depth of ablation	15 μm
	Raster type	Line
ICP-MS	Thermo Elemental X7 ICP-MS	
	Plasma gas	Argon
Data acquisition	Background signal acquisition	60 s
	Otolith signal acquisition (maximum)	40 s
Standards	NIST	610
	Internal standard	Calcium

NIST, National Institute of Standards and Technology.

transect inwards from the otolith edge (*i.e.* perpendicular to growth increments) was targeted using an automated microscope stage moving at a speed of *c.* 5 μm s^{-1}. In total, 12 isotopes (Li_7, Mg_{25}, Mn_{55}, Cu_{65}, Zn_{66}, Rb_{85}, Sr_{86}, Cd_{111}, Sn_{120}, Ba_{138}, Ce_{140} and Pb_{208}) were analysed by LA-ICP-MS and chemical concentrations (parts per million) were calculated using Lamtrace software (van Achterbergh *et al.*, 2001). In order to standardize for the amount of material ablated in both sagitta and lapillus (thus ensuring that levels of detection did not vary between otolith types), the signal selected for use in Lamtrace was limited to 20 μm of the transect (*i.e. c.* 4 s of ablation) immediately inwards from edge of either otolith type. Because of differences in growth rates this ablation length corresponded to *c.* 2–3 days of sagittal growth (based on estimates of settlement-stage fish by Wilson & Meekan, 2002) and 6–8 days of lapillar growth (pers. obs.). Although signal selection for lapillus probably accounted for a longer interval of time in the life of a fish relative to that for sagitta, it is unlikely that the additional lapillus material will cause concentration differences between otolith types. Instead, the site-attached nature of *S. partitus* means that this extra material will simply reflect the chemical concentration of the same environment (*i.e.* site of collection) for a longer period.

After elemental concentrations were calculated, Li, Mg, Mn, Zn, Sr, Sn, Ba, Ce and Pb were retained for statistical analysis because they met specific levels of measurement precision and accuracy (Chittaro *et al.*, 2005). Of the 80 individuals that were chemically analysed, 14 were removed based on outlier analysis (a value was removed if it was three times the interquartile distance; Fowler *et al.*, 1995). Using these nine elements a Wilcoxon matched-pairs test was used to investigate whether elemental concentrations differed between sagittal and lapillar otoliths from the same fish (a non-parametric test was used since paired differences between lapillus and sagitta for concentrations of several elements did not meet the assumption of normality for paired *t*-tests). This analysis indicated significant differences between otolith types such that concentrations of Li, Mn, Sn, Ba and Ce were two to nine times greater in lapillar than in sagittal otoliths, while concentrations of Pb were 19 times greater in sagittal otoliths (Fig. 2 and Table II). These differences in elemental concentrations between sagittal and lapillar otoliths from *S. partitus* suggest that there is differential regulation of ions passing through each otolith vestibule (*i.e.* sacculae and utriculus), into the endolymph, and finally into the otolith. Unfortunately, this is speculative since few studies exist that compare trace elemental concentrations of sagittal and lapillar otoliths (Meyer-Rochow *et al.*, 1992;

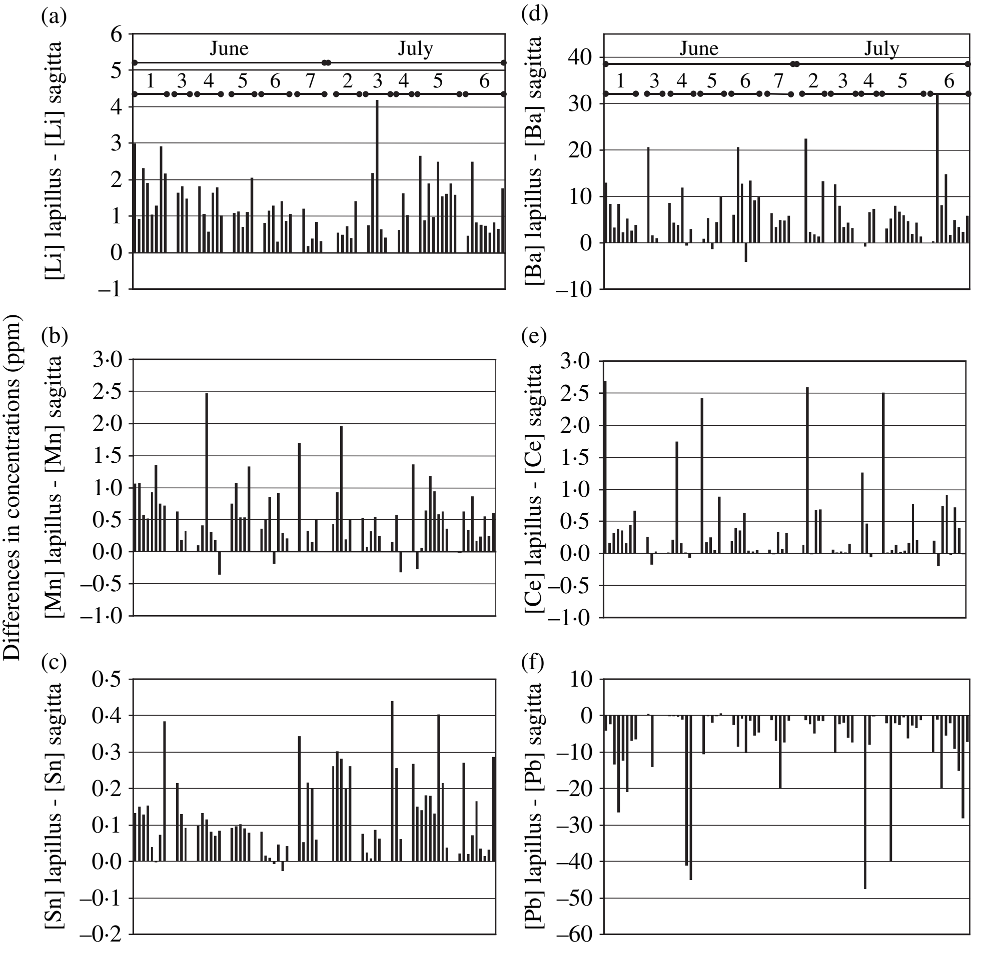

FIG. 2. Differences in concentrations in terms of (a) Li, (b) Mn, (c) Sn, (d) Ba, (e) Ce and (f) Pb between lapillar and sagittal otoliths for each fish (each bar represents one individual). All differences were significantly different, $P < 0{\cdot}001$ ($n = 65$) (Wilcoxon matched-pairs tests, which assessed whether there were significant differences in elemental concentrations between otolith types). Sites (1–7, see Fig. 1) and time of collection (June and July) are indicated.

TABLE II. Isotopes included in statistical analyses. Means ± S.D. provided for detection limit by sample ablation, concentrations per otolith type and the ratio of lapillus concentration to sagitta concentration. Values are based on 16 different sets of otolith ablations

Isotope	Mean ± S.D. detection limit by sample (ppm)	Mean ± S.D. lapillus concentration (ppm)	Mean ± S.D. sagitta concentration (ppm)	Ratio of lapillus to sagitta concentration
Li (7)	0·08 ± 0·08	1·49 ± 0·86	0·20 ± 0·16	7·45
Mg (24)	2·03 ± 1·53	8·41 ± 4·90	8·13 ± 4·74	1·03
Mn (55)	0·17 ± 0·18	0·86 ± 0·45	0·30 ± 0·19	2·87
Zn (66)	0·27 ± 1·64	1·86 ± 2·10	1·36 ± 1·54	1·37
Sr (86)	0·63 ± 0·46	3129 ± 652	3347 ± 1085	0·93
Sn (120)	0·06 ± 0·03	0·21 ± 0·10	0·08 ± 0·04	2·63
Ba (138)	0·01 ± 0·05	10·93 ± 7·40	4·54 ± 3·57	2·41
Ce (140)	0·003 ± 0·002	0·45 ± 0·65	0·05 ± 0·05	9·00
Pb (208)	0·03 ± 0·01	0·45 ± 0·80	8·65 ± 11·1	0·05

Campana & Gagne, 1995; Chesney *et al.*, 1998), or rates of deposition for each otolith type.

Following the Wilcoxon matched-pairs test, a linear discriminant function analysis (DFA) was used to determine whether elemental concentrations from sagitta or lapillus were more useful at discriminating fish from different sites and times. Specifically, separate DFAs were conducted for each otolith type such that the grouping variable corresponded to site and time sets (*i.e.* one of seven Turneffe Atoll sites, and one of two times, June or July 2002). For each DFA, a classification matrix and partial Wilks' lambda statistic was determined; the former indicates the per cent of fish that were correctly identified to the site and time from which they were collected, while the latter indicates the unique contribution of an element to the discriminatory power of the model (StatSoft, Inc., 2001). To interpret which element best accounted for the separation within a discriminant root, factor correlations >0·33 (representing *c.* 10% of the variance; Tabachnick & Fidell, 1996) were examined (elements were $\log_{10}$ transformed to improve normality for the DFA).

The differentiation of individuals to the sites and times from which they were collected was successful regardless of otolith type, but superior for sagittal otolith chemistry (Table III). Specifically, the DFA using sagittal otolith chemistry correctly classified 77% of the individuals (*i.e.* 51 out of 66 individuals) compared to 69% (*i.e.* 46 out of 66 individuals) when lapillar otolith chemistry was used (Table III). Interestingly, despite significant differences between otolith types in terms of Li, Sn and Pb (see Fig. 2), this same suite of elements best explained the discrimination of individuals when sagittal or lapillar otoliths were analysed (*i.e.* partial Wilks' Lambda was smallest for Sn, followed by Li and Pb for both DFA models, and these elements best accounted for the separation within the first two discriminant roots, *i.e.* had a factor correlations

TABLE III. Results of the discriminant function analyses (DFA) to differentiate groups (*i.e.* sites and times of fish collections) using elemental concentrations of sagitta, lapillus and sagitta, and lapillus. Specifically, information is provided regarding Wilks' Lambda (the variance not explained by the discriminant functions), χ^2 statistic (the measure of statistical significance) and the proportion of the variance represented by significant roots (per cent). Elements with factor correlations >0·3 are indicated (only for root 1 or 2)

DFA (mean per cent correct)	Wilks' lambda	d.f.	*F*	*P*	χ^2	d.f.	*P*	Per cent	Root modules	Li	Sn	Pb
Sagitta (77)	0·02	90, 322	2·7	<0·001	206	90	<0·001	82	1		0·83	
									2	−0·61		−0·49
Lapillus (69)	0·04	90, 322	2·2	<0·001	173	90	<0·001	78	1	−0·37	0·31	−0·40
									2	0·56		−0·39
Sagitta and lapillus (94)	0·001	180, 346	2·5	<0·001	362	180	<0·001	87	1		−0·58S	
									2	−0·30S	−0·34S	

S, concentrations from sagittal otoliths.

>0·33; Table III). Campana & Gagne (1995) also reported that sagittal otolith chemistry correctly classified more individuals than lapillar otoliths (94% compared to 83%), yet a slightly different suite of elements was found to be important for either otolith type.

Because it was determined that each otolith type reflected the environment differently (*i.e.* significant differences in elemental concentrations were observed between sagittal and lapillar otoliths; Fig. 2) and each otolith type had sufficient spatial and temporal variability in elemental concentrations (*i.e.* each DFA indicated that a high proportion of individuals were correctly classified; Table III), the discrimination of individuals was expected to further improve when sagittal and lapillar otolith chemistries were combined. In fact, reviews on the use of stable isotopes in ecology by Hobson (1999) and Rubenstein & Hobson (2004) highlighted that the use of multiple tissues would improve the ability to track organisms (as well as differentiate populations) since each tissue has different turnover rates making them unique recorders of the environment.

When the concentrations of all nine elements of both otolith types were used in a single analysis (*i.e.* DFA whereby concentrations of elements from lapillus and sagittal otoliths were all separate variables in the same dataset; therefore there were 18 variables) an increase in discriminatory ability was observed, such that the correct classification of individuals improved to 94% (*i.e.* 62 out of 66 individuals, whereby the greatest discriminatory power was provided by concentrations of Sn from sagittal otoliths, while concentrations of Li and Sn from sagittal otoliths best accounted for the separation within the first two discriminant roots; Table III). This improvement in discriminatory power is likely to be an important development, particularly as the spatial and temporal scales of studies get smaller, yet expectations of identifying population structure remain high. For example, a number of studies, such as Gillanders *et al.* (2001), Patterson *et al.* (1999, 2004) and Chittaro *et al.* (2005), have reported poor discriminatory ability at a variety of spatial scales (100s of m to 10s of km, 50–100 km, 2·5–20 km and 0·8–20 km, respectively) using sagitta chemistry alone. If the improvement in discriminatory ability is not specific to *S. partitus* then the use of sagittal and lapillar otolith chemistry would probably provide additional differentiation needed in studies like those mentioned above.

Overall this study indicated that there was considerable variability in elemental concentrations between sagittal and lapillar otoliths and that the ability to discriminate collections of individuals was superior for the former otolith type. In addition, this study highlighted that the combination of sagittal and lapillar otolith concentrations provided useful information that allowed greater separation of fish collected from different sites and times. Although more studies are needed to test the utility of using both otolith types (*e.g.* the cost associated with the analysis of both otolith types *v.* its benefits, in addition to whether similar results are likely for other species) the inclusion of both types will probably provide the improved level of chemical resolution necessary for studies at finer spatial and temporal scales, or in cases where chemical differences among sites of interest are relatively small.

We would like to thank L. D. Corkum, J. Lovett-Doust, C. Mora, P. Usseglio, J. D. Hogan, J. Kritzer and two anonymous referees for their constructive comments. This study was supported by an NSERC CRO (grant no. 227965) awarded to P. F. Sale and an Ontario Graduate Scholarship awarded to P.M.C.

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