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Otolith elemental fingerprints of juvenile Pacific swordfish *Xiphias gladius*

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The trace element composition of young-of-the-year (YOY) juvenile swordfish *Xiphias gladius* sagittal otoliths were analysed as a preliminary test of the value of otolith elemental fingerprints for determining swordfish nursery ground origins in the central Pacific Ocean. A suite of five elements (Mg, Zn, Sr, Ba and Pb) was assayed with isotope dilution ICP-MS; all elemental concentrations were roughly comparable to otoliths of other marine fishes. Multivariate analyses of elemental fingerprints based on Ba and Sr revealed differences between sample sites, and the magnitude of the differences increased with latitudinal separation. With more comprehensive sampling of nursery grounds, it should be possible to identify origin of nursery ground for adult swordfish by analysing the YOY juvenile portion of the sagittal otolith.

Key words: central Pacific; isotope dilution ICP-MS; juvenile otoliths; otolith chemistry; swordfish nursery areas; *Xiphias gladius*.

INTRODUCTION

The genetic structure of swordfish *Xiphias gladius* L. populations in the Pacific Ocean remains uncertain. Genetic studies utilizing various molecular techniques have yielded conflicting results. Studies reported in Grijalva-Chon *et al.* (1994), Rosel & Block (1996) and Chow *et al.* (1997) revealed no significant population heterogeneity within Pacific swordfish populations while Grijalva-Chon *et al.* (1996) found small but significant differences between populations from the central north Pacific and eastern Pacific near Mexico. More recent genetic evidence (Reeb *et al.*, 2000; R.D. Ward, C.A. Reeb & B.A. Block, pers. comm.) indicates a sideward horseshoe shaped pattern of population structure extending from the western Pacific to the east, with adjacent regions within the North and South Pacific linked by an overlap along the eastern Pacific. The disparity of these findings is probably the result of choice of genetic markers and sample sizes employed. Furthermore, these studies were largely based on samples

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obtained from adults captured on temperate fishing grounds distant from the tropical and sub-tropical spawning and nursery areas. Given their highly migratory capability, even low level genetic exchange is likely to prevent genetic differentiation between nursery areas in Pacific swordfish (Hauser & Ward, 1998). Coupled with the low tag-recapture rates of juvenile and adult swordfish, the search for natural markers of spawning or nursery areas and population structure has broadened to include other approaches.

Variability in the trace element chemistry of fish otoliths has been used as natural geographic markers for fish populations (Campana *et al*., 1995, 2000; Thorrold *et al*., 1998; Rooker *et al*., 2001, 2003). Otolith elemental fingerprinting relies upon two properties of otoliths. Firstly, otoliths grow throughout the life of the fish and, unlike bone, are metabolically inert (Campana & Neilson, 1985). Secondly, the calcium carbonate and trace elements that make up >90% of the otolith are derived mainly from sea water, as modified by ambient temperature (Campana, 1999). The elemental composition of otoliths is thought to reflect the environmental characteristics of the sea water that fishes inhabit, independent of any genetic differences. If the physical and chemical composition of water masses varies spatially, then the central portion of otoliths could presumably record elemental compositions specific to particular nursery areas.

The objective of this study was to provide a preliminary test of the value of otolith elemental fingerprints for differentiating among young-of-the-year (YOY) juvenile (not to include the 1+ year juvenile age class) swordfish from nursery sites at different locations in the central Pacific Ocean. Spatial differences in elemental fingerprints are therefore necessary for future research designed to identify the place of origin of adult swordfish based on the elemental fingerprint of the YOY juvenile portion of the adult otolith. A second objective was to develop the analytical approaches required to accurately assay the tiny otoliths (<2 mm) characteristic of YOY juvenile stage swordfish and other billfishes.

METHODS

The YOY juvenile swordfish ($n = 23$) 62–79 cm eye-to-fork length (L_{EF}), were collected during research vessel operations at the equator (0–1° N; 165° W) in October 1994 and from commercial yellowfin *Thunnus albacares* (Bonnaterre) and bigeye *Thunnus obseus* (Lowe) tuna longline sets off Hawaii (22° N; 165° W) in September to October 1997. Capture location data obtained later in the study indicated that four of the original Hawaii specimens used in this study were actually caught elsewhere; two specimens from south of Hawaii (17° N; 160° W) and two north of Hawaii (31° N; 162° W) (Table I and Fig. 1). Otoliths from adult swordfish, used for bulk analysis in development of the assay method, were collected from 42 adult females (160–199 cm L_{EF}) captured in the swordfish longline fishery north of Hawaii during 1996–1997. The complete sagittal otolith pair was removed from each adult and juvenile fish in the laboratory shortly after collection and stored dry in plastic vials prior to elemental analysis.

In preparation for the elemental analysis, sagittal otoliths from juveniles were placed in a drop of ultrapure (Super-Q) water and cleansed of adhering tissue. Otoliths were then ultrasonically cleaned in Super-Q water for 3 min, rinsed, sonified, rinsed a second time and then air-dried under a Class 100 laminar flow hood. Contamination associated with handling methods is always a concern in these types of studies (Thresher, 1999). The suite of elements and decontamination protocols used here, however, have been shown to be effective in dealing with concerns regarding systematic bias (Campana, 1999). After

TABLE I. Collection data and elemental concentrations of YOY juvenile swordfish otoliths. Data for fish length (L_{EF}), paired otolith mass (M_{OT}) and elemental concentrations are mean ± S.E.

						Elemental concentration (μg g^{-1})				
Site	Date	Location	L_{EF} (cm)	n	M_{OT} (mg)	Mg	Zn	Sr	Ba	Pb
1	October 1994	0–1° N, 165° W	72·6 ± 1·5	12	0·44 ± 0·05	36·3 ± 2·6	9·3 ± 2·6	1967 ± 96	1·9 ± 0·2	1·2 ± 0·5
2	October 1997	22° N, 163° W	69·0 ± 1·8	7	0·36 ± 0·04	33·1 ± 4·7	10·6 ± 2·9	1836 ± 173	3·1 ± 0·4	1·1 ± 0·5
3	September 1997	17° N, 160° W	64·4 ± 2·7	2	0·35 ± 0·05	43·5 ± 15·5	28·3 ± 25·3	1125 ± 25	2·7 ± 1·1	0·4 ± 0·3
4	October 1997	31° N, 162° W	69·9 ± 4·5	2	0·40 ± 0·20	33·5 ± 1·5	8·5 ± 2·0	1650 ± 250	4·2 ± 1·7	1·2 ± 0·2
Grand mean			70·5 ± 1·1	23	0·40 ± 0·03	35·7 ± 2·2	11·3 ± 2·2	1826 ± 88	2·54 ± 0·3	1·1 ± 0·3

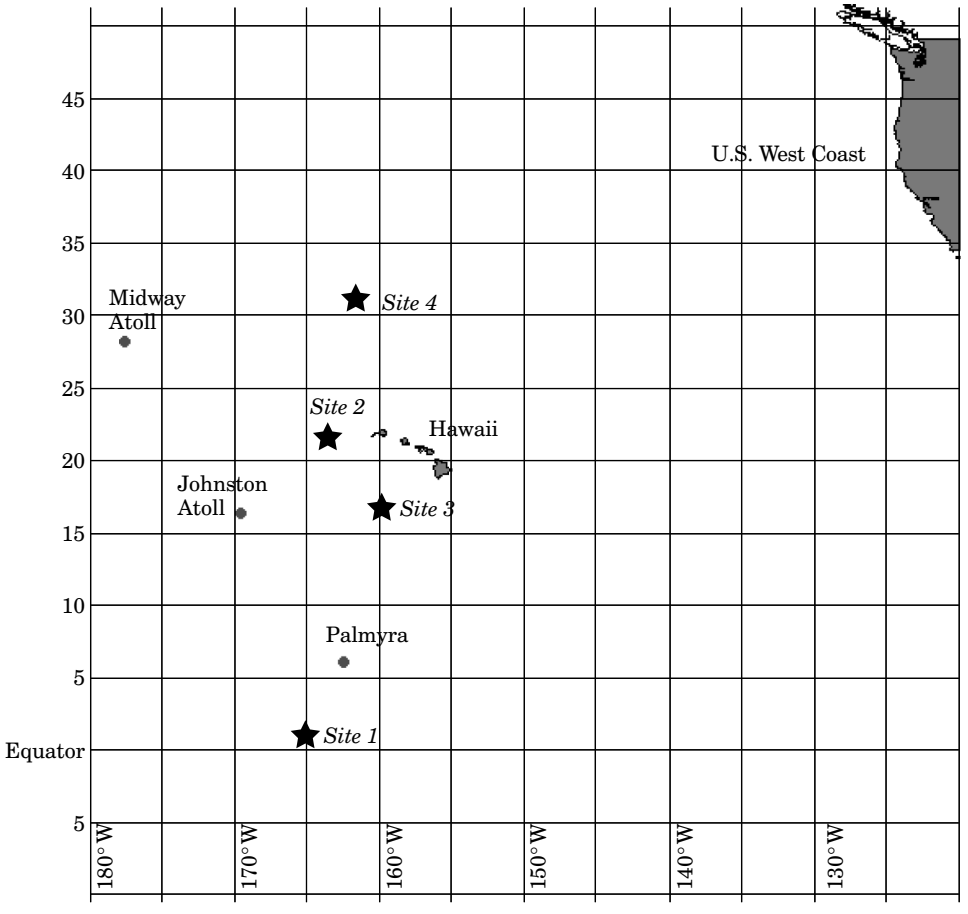

FIG. 1. Locations of the four latitudinal sampling sites within the central equatorial and North Pacific Ocean.

drying for at least 24 h, otoliths were weighed (M_{OT}) to the nearest 5 μg and stored in high-density, acid-washed polyethylene vials. Because juvenile swordfish otoliths are small (<1 mg), left and right otoliths were pooled for subsequent elemental analyses. Blank vials (with no otoliths present) were similarly prepared for blank corrections and to calculate limits of detection.

Adult swordfish otoliths were pooled for semiquantitative assay with inductively coupled plasma mass spectrometry (ICP-MS). This served to identify the suite of elements and probable detection levels anticipated in subsequent analyses of juvenile otoliths. Nine elements (Li, Mg, Al, Mn, Zn, Sr, Cd, Ba and Pb) were detectable by ICP-MS in swordfish otoliths, of which five (Mg, Zn, Sr, Ba and Pb) had higher detection levels more suitable for quantification. Isotope dilution (ID) was the preferred method of quantification because of superior accuracy and precision in otolith assays (Campana *et al.*, 1995).

Juvenile otoliths were dissolved in 200–700 μl of a 10% redistilled nitric acid solution containing the enriched isotopes of the five elements targeted for isotope dilution. Otolith solutions were assayed with a Perkin-Elmer SCIEX ELAN 5000 ICP-MS equipped with a high-efficiency pneumatic nebulizer at the National Research Council Laboratory, Ottawa, Canada. The enriched isotopes used in the spiking procedure were ^{25}Mg, ^{67}Zn,

^{87}Sr, ^{137}Ba and ^{207}Pb, while ^{24}Mg, ^{66}Zn, ^{88}Sr, ^{138}Ba and ^{208}Pb were used for quantification. The assay sequence was systematically randomized across samples to ensure that instrument drift did not artefactually inflate the assay results of one sample site over another.

Sample solutions were introduced into the ICP-MS with a high-efficiency pneumatic nebulizer, rather than the standard concentric nebulizer because of the small size of the juvenile swordfish otoliths. Limits of detection (LOD, 3σ of mean blank values from multiple analyses of five blank vials, based on a 1 mg otolith in a 0·3 mL final volume) for each of the elements were as follows: [Mg] 2·7 μg g^{-1}, [Zn] 1·5 μg g^{-1}, [Sr] 0·09 μg g^{-1}, [Ba] 0·036 μg g^{-1} and [Pb] 0·09 μg g^{-1}.

To evaluate the significance of observed variations in elemental concentrations and fish size on nursery site, univariate (one-way ANOVA) and multivariate (MANOVA) tests were applied. The ANOVA and MANOVA tests were performed using a general linear models (GLM) procedure due to unequal observations between sampling sites. Linear regressions were employed to examine for significant trends in individual elemental concentrations over size and latitude. A stepwise discriminant analysis (SDA) was performed to determine which elements were significant contributors toward the differentiation of sampling sites. Those significant elements were then entered into a canonical discriminant analysis (CDA) and a jackknife cross-validation procedure was run to determine the accuracy of these sampling site classifications based on elemental fingerprints. All statistical analyses were performed at the $P = 0{\cdot}05$ level of significance using SAS® version 8 for Windows® and Statgraphics plus version 3.3 for Windows®. Confidence ellipses about the canonical scores for the equator and Hawaii samples were determined using Statistica® version 4.5 for Windows®.

RESULTS

Semi-quantitative assays of adult swordfish otoliths varied slightly from the more accurate assays of the juveniles, although it was not possible to determine if the differences were real or artefactual. Adult otoliths contained 0·4 μg g^{-1} [Li], 180 μg g^{-1} [Mg], 4 μg g^{-1} [Al], 0·4 μg g^{-1} [Mn], 5 μg g^{-1} [Zn], 2500 μg g^{-1} [Sr], 0·4 μg g^{-1} [Cd], 12 μg g^{-1} [Ba] and 0·3 μg g^{-1} [Pb]. Of the five elements measured in juvenile otoliths, Zn and Pb concentrations were slightly higher, Sr slightly lower, and Mg and Ba five-fold lower than adults (Table I). Only one juvenile otolith was rejected (site 3) due to abnormally high values for most elements.

The individual elemental concentrations for Ba and Sr differed significantly among sites (one-way ANOVA, $P < 0{\cdot}05$). The two measures of fish size (L_{EF} and otolith mass) were not significantly different among sites (one-way ANOVA, $P > 0{\cdot}10$). The multivariate elemental fingerprint did vary among sites (MANOVA, Pillai's Trace = 1·23, $F_{15,51}$, $P < 0{\cdot}05$). A univariate comparison among the equator and Hawaii sites indicates significant elemental differences only for Ba (one-way ANOVA, $P < 0{\cdot}01$). The multivariate elemental fingerprint also differed significantly among the equator and Hawaii sites (MANOVA, Pillai's Trace = 0·63, $F_{5,13}$, $P < 0{\cdot}05$). These results were not due to differences in swordfish size among sites, since there was no significant trend (deviations from slope of zero; P values = 0·21–0·98) in any of the elements with respect to size.

The large-scale geographic differences in elemental fingerprints were apparent using both simple and multivariate techniques. A simple plot of Sr *v.* Ba composition for each individual indicated that the sample sites differed from each other [Fig. 2(a)]. The SDA using all five elements found Ba and Sr were the significant discriminant variables (Ba, $F = 4{\cdot}04$, $P < 0{\cdot}05$; Sr, $F = 8{\cdot}47$,

$P < 0{\cdot}01$). A CDA was performed using only Ba and Sr as the dependent variables. A plot of the canonical scores for each fish, centroid means, and 95% confidence ellipses for the equator and Hawaii samples [Fig. 2(b)] showed greatest dispersion along the first canonical axis (92% of the variation) and this reflected differences in Ba. An increase in Ba concentration with latitude was significant ($P = 0{\cdot}002$ for slope) while Sr displayed a non-significant ($P = 0{\cdot}17$) inverse relationship (Fig. 3). The jackknife cross-validation procedure resulted in a correct classification rate of 83 and 57% for equator and Hawaii juveniles, respectively, for an overall error rate of 30% between these two sites. All four juveniles from south (site 3) and north (site 4) of Hawaii were incorrectly classified. Significant differences between the equator and Hawaii sites were detected despite the small sample sizes and low statistical power. Power analysis indicates that a per site sample size of $n = 37$ and $n = 50$ would be required to provide sufficient power to differentiate between the two and four sample means, respectively, at a desired power of 80% and an assumed S.D. $= 1{\cdot}5$.

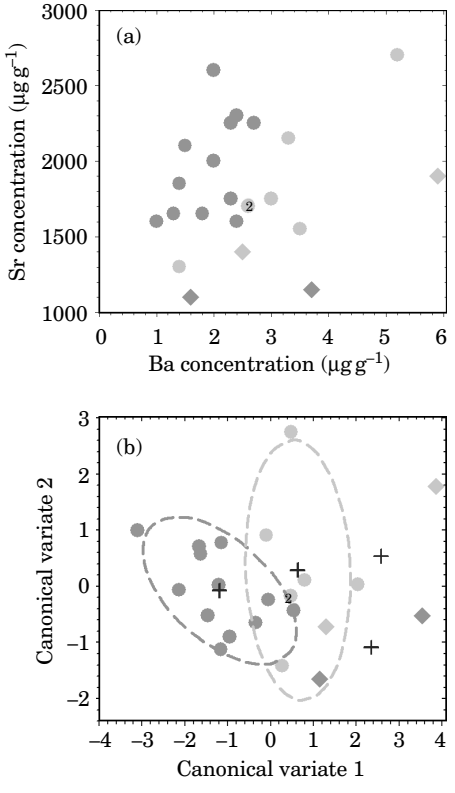

FIG. 2. Elemental fingerprints of YOY juvenile swordfish otoliths in the North Pacific. (a) Plot of Sr *v*. Ba concentration of otoliths and (b) plot of first two canonical variates from canonical discriminant analysis. Samples were from the equator, site 1 (●), Hawaii, site 2 (●), south of Hawaii, site 3 (◆) and north of Hawaii, site 4 (◆). The centroid mean (+) of each site is indicated. The equator and Hawaii groups are demarked by 95% confidence ellipses. Numbers within symbols indicate multiple data points.

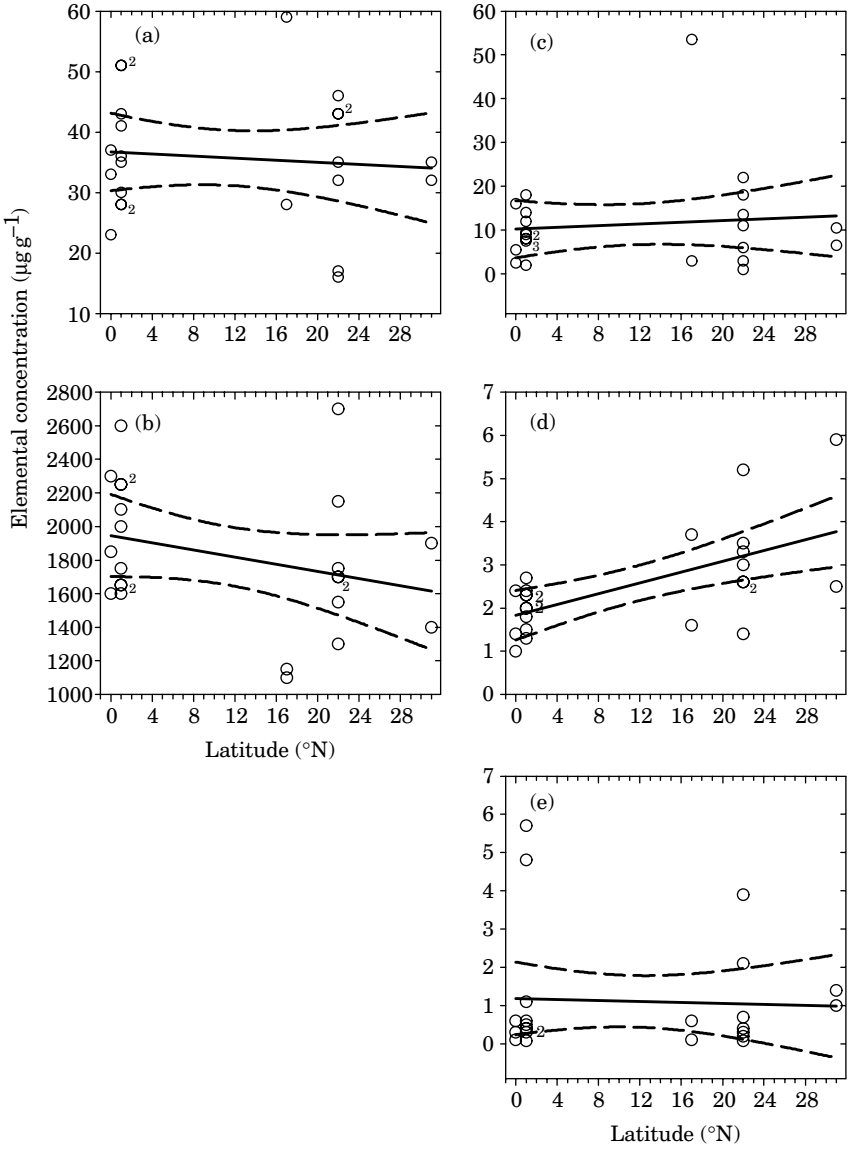

FIG. 3. Otolith elemental concentrations (a) Mg, (b) Sr, (c) Zn, (d) Ba and (e) Pb in relation to latitude of capture. Linear regression and associated 95% confidence limits are shown. Numbers adjacent to symbols indicate multiple data points. The curves were fitted by: (a) $y = 36{\cdot}70 - 0{\cdot}08503\ x$, (b) $y = 1946 - 10{\cdot}69x$, (c) $y = 10{\cdot}22 + 0{\cdot}09618x$, (d) $y = 1{\cdot}835 + 0{\cdot}06253x$ and (e) $y = 1{\cdot}191 - 0{\cdot}006648x$.

DISCUSSION

The elemental composition of swordfish otoliths was comparable to that of other marine fish otoliths (Campana, 1999). In common with other species, most of the elements measured were present at trace levels (<100 ppm); only Sr was

present at a higher concentration (Campana, 1999). The otolith elemental composition of swordfish was no more similar to that of another large pelagic teleost, the bluefin tuna *Thunnus thynnus* (L.), than to any other marine fishes (Rooker *et al.*, 2001; Secor *et al.*, 2002). The evolutionary or ecological significance and ramifications of this result is unclear. The bluefin tuna were collected in areas and years different from the swordfish, so a direct comparison was not possible.

Large pelagic fishes, especially swordfish and other billfishes, have otoliths that are very small (<3 mm) relative to body size (Wilson & Dean, 1983), making them challenging targets for elemental assay. Use of a high-efficiency nebulizer, suited to very small otoliths, proved to be as important in this study as it has been in other studies using otoliths <1 mg (Thorrold *et al.*, 1998). Similarly, use of isotope dilution ICP-MS allowed more accurate assays than would otherwise be possible (Campana *et al.*, 1995; Secor *et al.*, 2002). The suite of elements that could be measured in this study, excluding those elements such as K and Na which are physiologically regulated, was limited (five or six elements) but typical of most otolith analysis studies (Campana, 1999). The fact that Pb could be measured in swordfish, and that its concentration was considerably above the LOD, was a somewhat unusual observation due to its typically lower trace concentrations in oceanic sea water. The same laboratory which carried out the swordfish assays, however, was responsible for assays in bluefin tuna (Secor *et al.*, 2002) and shad *Alosa sapidissima* (Wilson) (Thorrold *et al.*, 1998), neither of which contained significant quantities of Pb. Hence the appreciable Pb content of swordfish otoliths was real although the source remains unknown.

The YOY juveniles examined in this study represent a wide latitudinal sample that was otherwise collected over a narrow longitudinal (160 to 165° W) and temporal (September to October) scale. Yearly effects could not be completely controlled because equatorial samples were less available than those samples from higher latitudes and thus will require an adaptive sampling strategy. The effect of size differences within and between sample sites was considered nominal in this study since juvenile billfishes undergo some of the fastest growth rates recorded for teleosts (Prince *et al.*, 1991; Megalofonou *et al.*, 1995). The size range of YOY juvenile specimens (62–79 cm L_{EF}) represented a maximum age difference of 3 months based on presumed daily otolith increment counts of similar sized individuals off Hawaii (R.L. Humphreys Jr, unpubl. data). The effects of ontogenetic variation or elemental selectivity with respect to otolith elemental composition in young swordfish could not be ascertained in this study.

In the central equatorial and central north Pacific, YOY juveniles have been recorded from the equator to *c.* 37° N latitude, with higher catches at 5–7° N and 15–30° N latitudes and less frequently at 0–4° N, 8–14° N and at latitudes >30° N (R.L. Humphreys Jr., unpubl. data). These records are based on the seasonally infrequent by-catch of YOY juveniles made in the yellowfin and bigeye tuna longline fishery that operates in the equatorial to warm temperate central Pacific. The use of earlier juvenile and larval stages are less feasible for otolith elemental study due to their rarity of capture, their significantly smaller sagittal otoliths and reliance on research cruises to obtain specimens. Evidence for the retention of YOY juveniles to nursery areas is circumstantial. In Hawaii, the

southerly movement of imminent spawners in proximity to the Hawaiian Islands occurs during March to June (DeMartini *et al.*, 2000). The occurrence of larvae off the Island of Hawaii during this same period and appearance of YOY juveniles as by-catch in the offshore yellowfin and bigeye tuna longline fishery during the following August to December period (R.L. Humphreys Jr., unpubl. data) suggest minimal latitudinal dispersal during the YOY juvenile stage. The potential for passive latitudinal dispersal away from natal areas due to current and winds, however, has not yet been investigated. In terms of active movement, tagged juveniles in the western Atlantic typically showed little latitudinal difference between tag and recapture positions (Stone, 2000). On this basis, capture location of YOY juveniles in this study was used as an approximate location of the previous nursery area.

The significance of the geographic variation in the swordfish elemental fingerprint was more one of potential rather than direct application. Differences such as these are mandatory prerequisites for the ability to differentiate between spatially distant nursery sites (Campana, 1999), and show promise for future investigation. An explanation of the observed latitudinal increase in Ba concentration and concomitant decline in Sr remains unclear. Otolith Ba : Ca and Sr : Ca ratios have been shown to increase in marine otoliths as their ambient concentrations in sea water increase (Bath *et al.*, 2000). The Ba enrichment in sea water is associated with increased nutrient levels, such as in upwelling areas, while Sr levels may be an indicator of ambient temperature (Bath *et al.*, 2000). Differences among geographic sites in this study were presumably related to surface water mass characteristics and associated physiological differences; however, the source of the elemental fingerprint differences remains unknown. The observed elemental compositions thus represent only those sites and years that were sampled. Because the elemental composition of the otolith is metabolically stable, though, the YOY juvenile portion of the otolith serves as a natural tag of the nursery area independent of cause (Campana *et al.*, 2000). With a larger sample size, this study could have been used to identify and track the swordfish cohort that used the sampled areas as nursery grounds.

Trace element signatures in otoliths have shown promise in the present study as natural tags of nursery area in swordfish. With more comprehensive sampling of putative nursery grounds, it should be possible to track movement patterns of adult swordfish by sectioning adult otoliths and using laser ablation ICP-MS to probe the YOY juvenile portion of the otolith (Campana *et al.*, 1994; Thorrold *et al.*, 1997). The ability to assign individual fish accurately to their natal or nursery area (Pawson & Jennings, 1996) has considerable implications for the global fisheries management of highly migratory swordfish throughout the Pacific (DeMartini, 1999).

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