

Disentangling and quantifying sources of otolith shape variation across multiple scales using a new hierarchical partitioning approach

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ABSTRACT: Otolith shape analysis provides a practical basis for stock separation, useful in fisheries management. A wide range of factors affect otolith morphology and a fundamental challenge in morphometric stock identification is to develop a consensus on the biological interpretation of otolith shape variation. To date, there are few comprehensive reports that have examined various factors simultaneously at multiple scales in natural conditions. In this study, I identified sources of otolith shape variation across spatial and taxonomic scales using a new hierarchical partitioning method embedded in a geometric morphometric framework. Various environmental, taxonomic and endogenic factors which affect otolith shape were quantitatively investigated in 2077 coral reef fishes. Using the new partitioning method, allometry accounted for a considerable degree of otolith shape variation at all scales and contributed more variation to regional differences than did habitats or islands. While large-scale variations are expected to be associated with significant shape variation, the study provides quantitative evidence that both local environmental variables and large-scale patterns contribute equally to total otolith shape variation. Models that aim at discriminating stocks and forecasting stock boundaries implicitly assume that the within-stock morphological variation is negligible in regard to the variation that exists among stocks. The importance of local environmental variables may therefore act as an important confounding effect into those predictive models. More generally, the degree of contribution of some variables differed substantially among the taxonomic scales, as did their relationship among spatial scales. A scale-dependent understanding of factors affecting otolith morphology is critical in understanding the integrity of fish populations and is an effective tool in management of fisheries resources.

KEY WORDS: Morphological disparity · Morphospace · Regression tree · Coral reef fish · Determinants · Taxonomic scales · Geographic scales

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INTRODUCTION

Teleost fishes possess 3 pairs of largely aragonitic mineralizations (otoliths) in their inner ear which are an important and widely-used tool in ichthyological studies (Campana 2005). There are many diverse applications of otoliths in fisheries research. Otoliths can provide accurate estimates of age and growth at both daily and yearly scales and a basis for a record of growth patterns from individual to population level (Campana & Thorrold 2001). Among otolith features, the analysis of the shape appears particularly relevant

in taxonomic, biological and ecological investigations. While the morphology of sagittal otoliths is sufficiently conservative to be regarded as specific (L'Abée-Lund 1988, Stransky & MacLellan 2005, Tuset et al. 2006), their morphological variability is considerable and mainly influenced by sex, age and year class (Castonguay et al. 1991, Lombarte & Castellón 1991, Lombarte & Lleonart 1993, Begg & Brown 2000, Monteiro et al. 2005, Reichenbacher et al. 2009). However, there is also a strong variability related to environmental factors such as depth, water temperature and substrate type (Lombarte & Lleonart 1993, Gagliano &

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McCormick 2004, Mérigot et al. 2007, Hüseyin 2008). As a consequence, otoliths have been used for decades as a popular tool for the discrimination of species, population and stock differences related to geographic locations and to chemical and physical properties of the environment (Campana & Casselman 1993, DeVries et al. 2002, Cardinale et al. 2004, Tracey et al. 2006, Burke et al. 2008).

The utility of otolith shape analysis extends beyond these differentiations, as it also provides insights into events that influence the life history of individual fishes (Campana & Thorrold 2001, Campana 2005). The advantage otolith shape offers is that otoliths are a permanent record and no reabsorption occurs. There has been more interest in recent years in the use of morphological features of the otoliths for even more detailed analyses. For example, it has been suggested that the otolith shape of tropical fish may directly reflect the body condition/health of individuals regardless of fish size and age (Gagliano & McCormick 2004). It has also been suggested that the history of growth and environmental conditions incorporated within the otoliths may be a powerful tool in early life history studies of marine fishes (Thorrold et al. 2007, Lemberget & McCormick 2009). Of particular interest is that, excluding directional asymmetry naturally occurring in flatfishes, the asymmetry between the left and right otolith has been proposed as a useful index of body condition/health (Somarakis et al. 1997, Grønkvær & Sand 2003, Gagliano et al. 2008, Palmer et al. 2010) and may provide an important insight into the history of larval condition (Gagliano & McCormick 2004, Fey & Hare 2008, Gagliano et al. 2008, Lemberget & McCormick 2009). While there is no general consensus that asymmetry correlates positively with stressful environmental conditions (e.g. Díaz-Gil et al. 2015), it has been suggested that otolith microstructure contains a history of stress in larval fish. In this case, the asymmetry between the left and right otolith may thus reveal information about processes important to the replenishment of fish populations (Leary & Allendorf 1989, Lemberget & McCormick 2009).

It is therefore important to identify and quantify the relative importance of the main factors that affect otolith shape variability. The usefulness of morphological characters depends entirely on how well their biological properties are understood. While annuli spacing is biologically interpretable (i.e. related to age and growth), the interpretation of other features, such as otolith shape, is not straightforward since its determinants are not fully understood and a wide range of factors can act simultaneously. Although the

use of otolith shape is common, there are few comprehensive studies that have analysed its variation across multiple scales (e.g. Lombarte & Castellón 1991, Torres et al. 2000, Gauldie & Crampton 2002, Cardinale et al. 2004, Criquet et al. 2009), and, to date, none have quantitatively attempted to disentangle the specific contribution of the different factors affecting otolith shape at multiple scales. In particular, the question whether variations in otolith morphology occur at a large, regional or local scale remains largely unexplored. Little theoretical and practical work has considered how multiple factors may shape the otolith morphology. In other words, do large-scale variations (e.g. among individuals collected from distant localities) predominantly affect the magnitude of morphological differences, compared to regional or local variations? Local intra-population variation in otolith shape has, to the best of my knowledge, never been finely quantified in comparison to the variation at larger scales (but see Lombarte & Castellón 1991, Torres et al. 2000). However, only this comparison can determine the relative importance of local variation in otolith morphology and the legitimacy of ignoring it at a relevant scale. Moreover, given the growing interest in the use of otolith asymmetry, it is necessary to disentangle the relative importance of asymmetry compared to the level of naturally occurring variation in otolith shape at multiple scales. This information is fundamental if otolith shape asymmetry is to be used as an effective tool for investigating the history of stress and condition/health in fish, which could then provide a practical basis for fisheries management and replenishment of fish populations.

In previous studies, otolith shape variation was used to investigate small-scale location and movement patterns (i.e. within a few hundred metres) of the bluestripe snapper *Lutjanus kasmira* on the north coast of Moorea (Society Archipelago, French Polynesia, southcentral Pacific, Vignon et al. 2008, Vignon 2012). At a larger scale, otolith shape variation was also compared between bluestripe snappers collected from 3 different islands throughout the Pacific Ocean (over 1000s of km, Vignon & Morat 2010). Moreover, the non-indigenous bluestripe snapper introduced in the Hawaiian archipelago was used to investigate the genetic influence of otolith shape variation (Vignon & Morat 2010). The authors discriminated between individuals belonging to different lineages but growing under the same environmental conditions and cohabiting within the same shoals in the wild. To date, data from the above-mentioned studies have not been subject to a combined analysis and review. This pro-

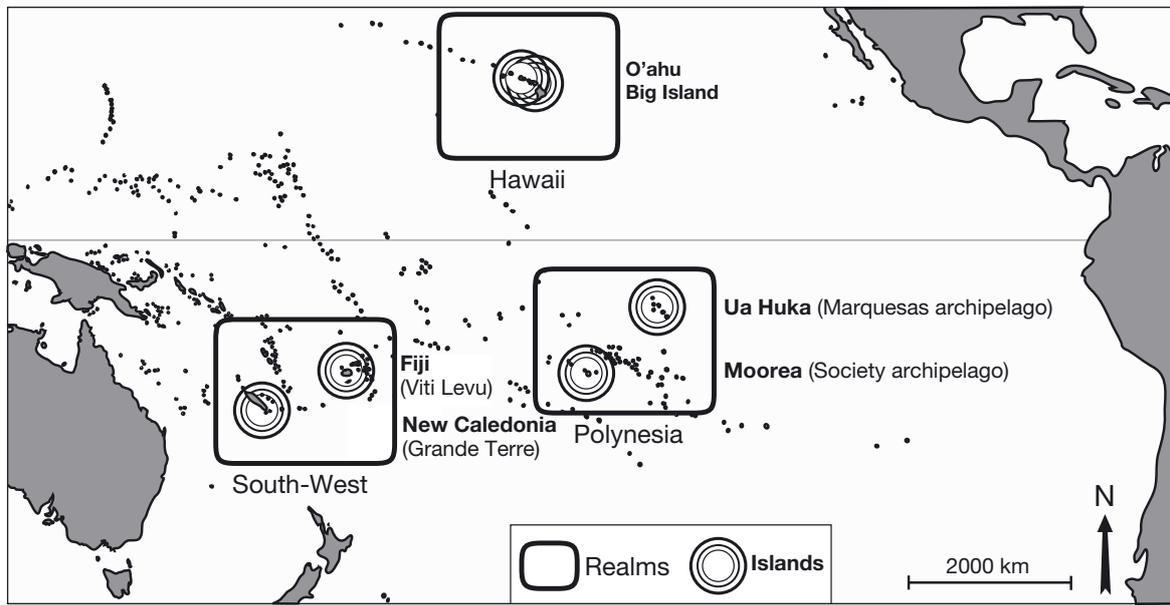


Fig. 1. Sampling locations in the Pacific Ocean. Three realms and 6 islands were investigated

vides an interesting opportunity for simultaneously evaluating the relative importance of factors affecting otolith shape across multiple scales.

The general purpose of this paper was to study the variation in sagittal otolith shape over different taxonomic and spatial scales. More specifically, I investigated to what extent non-exclusive factors affect the shape of otoliths in a geometric morphometrics context. The relative importance of these non-exclusive factors is quantified and I specifically determined whether large-scale factors have a larger effect on otolith shape than local ones and if the relative importance of local factors differs among larger units. I also investigated the degree of asymmetrical shape variation between right and left otoliths compared to the level of naturally occurring variation in otolith shape at multiple scales. For comparative reasons I also included data from related and non-related spe-

cies to assess the within species variation compared to between species variation and to estimate if the main factors affect otolith shape in a specific/generic manner. This study suggests a new method for quantitative hierarchical partitioning of otolith shape variation across multiple scales which could be useful in fisheries for stock discrimination.

MATERIALS AND METHODS

Sites and fish collection

A total of 2077 coral reef fish from 2 families and 8 species were collected between 2005 and 2007 during an extensive epidemiological survey from islands in the Pacific Ocean (Fig. 1, Table 1). All species have a

Table 1. List of fish species. A total of 2077 fish were sampled. n.c.: not collected

Species	Family	Size range (cm)	N	—Moorea—			Ua Huka 2007	Fiji 2007	New Caledonia 2007	—O'ahu—			Big Island	
				2005	2006	2007				2005	2006	2007	2005	2006
<i>Lutjanus fulvus</i>	Lutjanidae	8.0–29.9	365	n.c.	77	138	13	29	15	n.c.	41	52	n.c.	n.c.
<i>Lutjanus gibbus</i>	Lutjanidae	12.4–25.4	24	n.c.	10	14	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
<i>Lutjanus kasmira</i>	Lutjanidae	9.7–31.3	529	n.c.	99	132	72	34	14	n.c.	66	88	8	16
<i>Cephalopholis argus</i>	Serranidae	12.3–35.6	348	94	38	63	n.c.	n.c.	19	25	17	31	48	13
<i>Cephalopholis urodeta</i>	Serranidae	4.6–16.9	276	n.c.	174	75	n.c.	22	5	n.c.	n.c.	n.c.	n.c.	n.c.
<i>Epinephelus hexagonatus</i>	Serranidae	6.7–23.0	122	n.c.	60	62	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
<i>Epinephelus fasciatus</i>	Serranidae	16–33.1	215	n.c.	87	126	n.c.	n.c.	2	n.c.	n.c.	n.c.	n.c.	n.c.
<i>Epinephelus merra</i>	Serranidae	3.6–21.2	198	n.c.	77	78	n.c.	11	32	n.c.	n.c.	n.c.	n.c.	n.c.

reduced home range and limited movement capabilities. Local environmental variables such as habitat, distance from the coast and depth were recorded during capture and are assumed to reflect long-term environmental conditions experienced by individuals and thus the effect of local variables on specific otolith shape of wild fish. Fish were collected using a spear gun, and each individual was measured (standard length, SL, and eviscerated weight, W_e), respectively, to the nearest mm and g. Fulton's condition index ($K = W_e / SL^3$) is believed to be a good indicator of general well-being of a fish assuming isometric growth (Bolger & Connolly 1989). To avoid a species-specific confounding effect (pairwise comparisons indicated significant differences in K between some species), W_e was regressed onto SL^3 and residuals of species-specific regression were used as an unbiased index of biological conditions. Due to residual homoscedasticity among species, values were not standardized. Sex was determined (except for immature juveniles) based on gonadal development.

Otolith shape analysis

The present study focuses on the saccular otolith, the sagitta, which is the largest and most massive of the 3 types of otoliths in most groups of teleost fishes. Each of the 4154 unbroken otoliths was cleaned, placed with the sulcus acusticus oriented towards the observer, and then examined under a stereomicroscope fitted with a digital camera linked to a computer. The high-contrast images were thresholded and binarized from the orthogonal projection of the contour for outline extraction by ImageJ software (<http://rsb.info.nih.gov/ij/>). While shape analyses of otolith 2D contours are generally performed through Fourier analysis, otoliths have recently been successfully studied using geometric morphometrics (Monteiro et al. 2005, Ponton 2006, Palmer et al. 2010, Ramírez-Pérez et al. 2010, Vignon & Morat 2010, Vignon 2012), providing the proper framework in which to quantify otolith shape variability. However, none of these studies has made use of this analytical development to quantitatively establish the degree of morphological dissimilarity among the otoliths across multiple scales. In this context, a geometric morphometric approach using semi-landmarks was used for analyzing variation patterns.

Following Monteiro et al. (2005), the outline of each specimen was stored as pixel coordinates which were reduced to 100 points equally spaced (using contour length as a parameter) along the contour,

starting at the intersection of the major axis with the anterior margin of the otolith. This reference point (biologically corresponding among individuals) is referred to as a landmark and the remaining 99 points as semi-landmarks (see Vignon & Morat 2010). The coordinates of the landmark and semi-landmarks were digitized using the software tpsDig v.2.10 (from the TPS package, Rohlf 2015). Generalized orthogonal least square procrustes superimpositions of landmark and semi-landmark coordinates were then computed with tpsRelw v.1.45, minimizing bending energy with respect to a mean reference form (Green 1996, Bookstein 1997) to determine the criteria for sliding semi-landmarks along outlines.

The primary goal of this study was to identify the main determinants of otolith shape variability at various spatial and taxonomic scales. To do so, 12 factors, both categorical and continuous, were used as potential explicative variables (Table 2). All of the variables mentioned above have already been investigated separately and are potentially relevant predictors that can affect otolith shape over several scales. Because of protogyny, it was difficult to unequivocally attribute values of relative importance to sex and size in groupers, as the interdependence of these factors precluded clear partitioning. Sex was therefore discarded and subsequently only fish length was analyzed. Similarly, because of inherent size heterogeneity among species, lengths were standardized independently for each species (ranging from 0 for the smallest sampled individual to 1). First, a hierarchical partitioning of the morphological disparity (see below) was performed based on all fish species collected together from all islands (global scale). Subsequent analyses were performed at a lower scale with a subset of data. A total of 4 spatial scales were considered: global scale, biogeographic scale (3 realms), regional scale (6 islands) and local scale (12 habitats). Additionally, 4 taxonomic scales were considered: global scale, family scale (2 families), generic scale (3 genera) and specific scale (8 species). A top-down view along the hierarchy of scales allowed evaluating how the relative contributions of variables changed among scales. To quantify the degree of similarity among variable contributions among scales, a Spearman's rank correlation was performed on the mean contribution values of variables in pair-wise combinations of scales. A lack of significance ($p < 0.05$) in a correlation indicated that the relative importance of variables differed among scales. The approach also allowed estimating the grouped contribution of environmental, intrinsic and taxonomic variables (see Table 2).

Table 2. List of variables used in the multivariate regression tree. Cat: categorical; cont: continuous; W_e : eviscerated weight; SL: standard length

Factors	Cat/cont	Factor status
Taxonomic		
Species	cat	8 species (see Table 1 for details)
Family	cat	2 families (see Table 1 for details)
Intrinsic		
Size	cont	SL (standardized between 0–1 per species)
Fulton's index	cont	Fish health (species-specific residuals of W_e regressed onto SL^3)
Genetic	cat	2 origins: Marquesas-Society (for Hawaiian fish only)
Sides	cat	2 categories (right and left side, nested within individuals)
Environmental		
Island	cat	6 islands (2 islands per realm, see Fig. 1 for details)
Habitat	cat	4 habitats: outer/fringing reefs, lagoon, bay (2 per island)
Depth	cont	Individual sampling depth (m)
Coastal distance	cont	Individual sampling distance (m)
Year	cat	3 categories (sampling year: 2005, 2006 and 2007)
Individual		
Individual	cat	Not used directly in the partitioning process. Its relative influence is instead calculated as the unexplained part of the total variance (residuals)

Artificial variation could be due to measurement error (ME). To estimate ME, otolith outlines from 100 randomly selected fish were extracted 3 times based on independent images (replicates) of the same otolith. A Procrustes ANOVA was performed using individuals as a random effect and body sides as a fixed effect. The among-individual main effect represented individual shape variation. The main effect for the sides expressed directional asymmetry in shape, and side individual interaction served as a measure of fluctuating asymmetry. The residual variance component among replicated measurements (3 replicates) provided an estimation of the ME (Klingenberg & McIntyre 1998).

Hierarchical partitioning of morphological disparity

Within the last 10 yr, there has been increasing interest in the use of classification and regression tree (CART) analysis for complex and highly interactive ecological data that may include non-linear relationships between variables (De'ath & Fabricius 2000). This is important as the shape of otoliths may be determined by a large number of interactive factors. Within CART, multivariate regression trees (MRT) are relatively new in ecology (De'ath 2002) and, to date, have not been used for the analysis of morphological data with multiple explanatory variables. In this study, I introduce a novel approach for partitioning morphological shape variation and

quantitatively investigating the main determinants of shape disparity. The combined use of landmark-based geometric morphometrics and MRT may provide a practical framework to address both exploratory and predictive issues. Hierarchical partitioning (as commonly accepted, not in the meaning of Chevan & Sutherland 1991) refers to the partitioning of variation into contributions from 2 or more groups, with the variation of each group being potentially partitioned into contributions from subgroups, and so on. Such an approach generates a tree-like hierarchical structure that decomposes the total variation into independent components. MRT is indeed a tree-building recursive method that constructs a hierarchical tree by repeatedly splitting the set of observations into 2 mutually exclusive subgroups, each of which is as homogeneous as possible. MRT can handle numerical as well as categorical variables. For categorical explanatory variables with k levels, any combinations of levels (among the $2^{k-1}-1$ possible splits) can be used to form a split. For numerical explanatory variables, a split is defined by values less than, and greater than, some chosen value. Thus, only the rank order of numerical variables determines a split, and for u unique values there are $u-1$ possible splits. At each step, from all possible splits of all explanatory variables, the method selects the one that maximizes the between-group variation. This method was used to build hierarchical groups of observations to analyse which factors (see list in Table 2) may affect otolith shape variation and compare their relative contributions (i.e. percentage of

the total variance explained). Splitting continues until an overly large tree is obtained (i.e. numerous terminal groups with very few otoliths). To preclude overfitting, tree size was selected using 10-fold cross-validation and the minimum cross-validated error was chosen using the '1SE' rule (Breiman et al. 1984).

Disparity (D) based on landmark data in the geometric morphometrics framework is a common measure that refers to the morphological diversity of a group. The measurement of disparity follows that established by Foote (1993)

$$D = \frac{\sum_{j=1}^N d_j^2}{N-1} \quad (1)$$

where d_j represents the distance of otolith j from the overall centroid (i.e. the grand mean calculated over the N otoliths being analyzed). This measure is size-sample free and is considered to be robust (Foote 1993). In contrast, the partial disparity (PD)

$$PD = \frac{d_j^2}{N-1}$$

quantifies the contribution that a particular otolith makes to the overall disparity of a larger group. PD is therefore a metric that allows partitioning disparity additively, so that hierarchical disparity (i.e. partitioning of morphological variation into contributions from 2 or more subgroups) can be quantified. Overall disparity can indeed be partitioned into a within subgroup contribution and a between subgroup contribution. To determine the PD of a subgroup, one simply sums the contributions to the disparity of all the otoliths in that subgroup. The between-subgroups contribution equals overall disparity in the initial group minus the sum of the 2 subgroups' PDs. The distance metric we adopted is the full Procrustes distance, calculated from the shape of an individual and the grand mean shape. When applying a typical MRT process to coordinates of points, the multivariate sums of square deviations about the mean (SS-MRT) is used as a measure of impurity, expressed as

$$SS-MRT = \sum_{ij} (x_{ij} - \bar{x}_j)^2 \quad (2)$$

with x_{ij} the coordinates for (semi-)landmark j and otolith i and \bar{x}_j the mean coordinates of (semi-)landmark j (De'ath 2002). This results in minimizing the sum of squared distances between corresponding (semi-)landmarks, thus reducing the variance for all the coordinates over all (semi-)landmarks within each of the 2 subgroups obtained by a split. Of particular interest is that we can compute shape disparity directly by estimating Procrustes distances, or we can

calculate the variances of coordinates obtained by a generalized least squares (GLS) Procrustes superimposition. The 2 approaches yield the same results because the sum of squared coordinates obtained by GLS equals the squared Procrustes distance to the mean (Zelditch et al. 2004). However, for this purpose, distances must be Euclidean so that only Procrustes distances in tangent space were considered. As a consequence, applying MRT directly to either coordinates obtained by GLS or Procrustes distances makes it possible to calculate the disparity for all subgroup pairs for all possible splits (according to the available explanatory variables) and select the split that minimizes the morphological disparity within each of the 2 subgroups. In other words, the MRT procedure repeatedly splits observations into mutually exclusive subgroups, each of which are as homogeneous as possible. This tends to reduce the morphospace occupied by each subgroup in a significant manner. Equivalently, the procedure maximizes the morphological differences between the mean shapes of the 2 groups (i.e. maximize the between-subgroup contribution).

The MRT procedure initially generated a pruned tree based on '10-fold' cross-validation and then calculated the relative influence/importance of each explicative variable. The measure of relative influence/importance of each predictor variable is defined as the weighted sum across all splits in the tree of the between-subgroups contribution (percentage variance explained) when the variable is used as a splitter. Due to computational limitations (all possible splits for the 'individual' variable presented more than 10^{600} combinations), 'individuals' were not used directly in the partitioning process, and their relative influence must therefore be interpreted as the percentage of unexplained inter-individual variance (residuals) when all other components have been quantified. In this context, the relative influences from all variables sums up to 100. The relative contribution/importance, expressed as a percentage, was plotted for each variable and scale. The grouped contributions of the environmental, intrinsic and taxonomic variables were determined by summing the percentage contributions of their constituent variables. These grouped contributions could thus be incorporated into a ternary diagram.

Tree structure is subject to high variability depending on data (i.e. model instability due to small changes in the data). Bagging (bootstrap aggregating) is a nonparametric machine-learning ensemble designed to improve the stability and accuracy of machine-learning algorithms such as MRT (Breiman

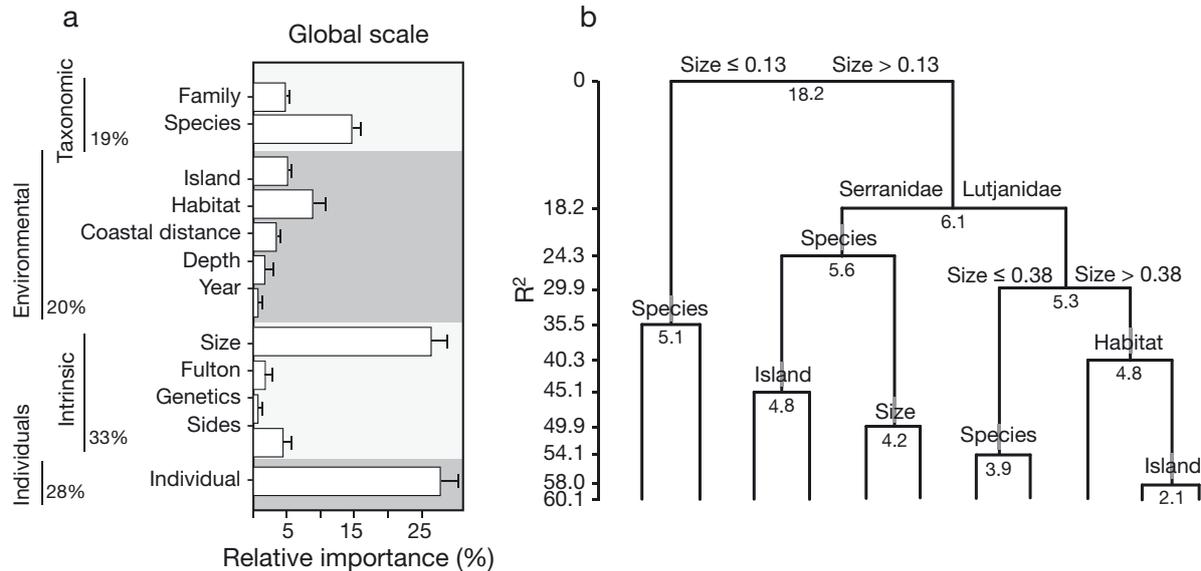


Fig. 2. (a) Relative influence of the main factors on otolith shapes at the global scale. At each scale, the relative influences sums up to 100, with higher numbers indicating stronger influence on otolith shape variation. (b) Composite exploratory tree depicting major interactions among factors at a global scale. The node labels show the factor selected for the split. The fraction of the variance (R^2) accounted for by the clusters formed at each partition level is reported (numbers under the nodes), as well as the cumulative explained variance (scale on the left)

1996). It does so by building a large number of regression trees based on a subset of randomly sampled (bootstrap) data points. This typically renders the model output stochastic, and variance is decreased through model averaging (Prasad et al. 2006, De'ath 2007). The original data were selected according to the scale of interest. The selected otoliths were further resampled for model-building using a bagging fraction of 50%. Each tree is then created as previously explained, minimizing the cross-validated error. To limit the stochasticity in model outcomes caused by the bagging, I created an ensemble of 500 trees at each scale and then averaged the results. In this context, the importance values for all 500 trees are simply averaged to obtain an overall mean (\pm SD) measure of the variable importance. For visual interpretation of both relative importance and interactions among factors, no bagging was allowed and the full dataset was used (global scale). Although outputs from a single model invariably deviated somewhat from the 500-model ensembles, explorations indicated that they were adequate for visualization and successfully depicted the main morphological trends in otolith shape. This tree contains the first 10 binary partitions and eleven terminal groups along a scale representing the proportion of the shape variation explained by each partition (R^2).

The novelty of the proposed hierarchical disparity method (i.e. partitioning of morphological variation into contributions from 2 or more subgroups) relies

on the conjugation of MRT embedded in a geometric morphometric context. Here, I partitioned the morphological disparity of sagittal otoliths across multiple scales in relation to environmental, intrinsic and taxonomic variables to quantify their relative importance. Analyses were performed using the *mvp* package of the R statistical software (Therneau et al. 2004). Codes and a tutorial that includes full-worked examples are included in the Supplement at www.int-res.com/articles/suppl/m534p163_suppl/.

RESULTS

Using disparity as a measure of shape variation and geometric morphometric techniques, I analysed/ investigated the degree of otolith shape variation in relation to various factors at multiple scales. Within the Procrustes ANOVA, measurement sum-of-squares error accounted for only 0.8% of total shape variation. In comparison, asymmetry-related variation (both directional and fluctuating) accounted for about 5% of variation, 6-fold larger than ME. ME is therefore assumed to be insignificant compared to other sources of variation, and hereafter results should not be artifactually biased by image acquisition. At the global scale (Fig. 2a), differences among species and/or families only accounted for 19% of total shape variation, with interspecific differences being more pronounced than between snappers and groupers. Similarly, envi-

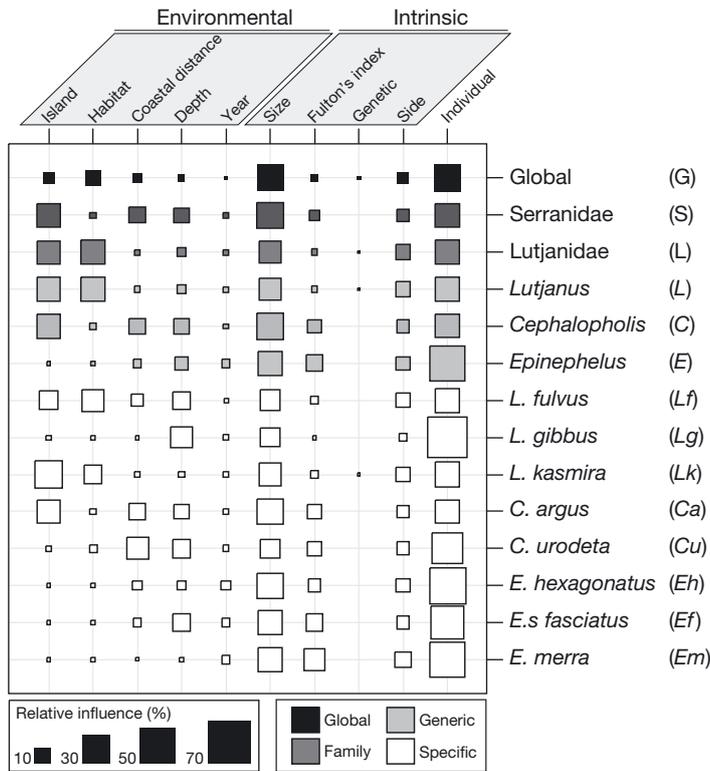


Fig. 3. Mean relative influence measures of environmental, intrinsic and individual factors at all taxonomic scales. At each scale, the relative influences sum up to 100

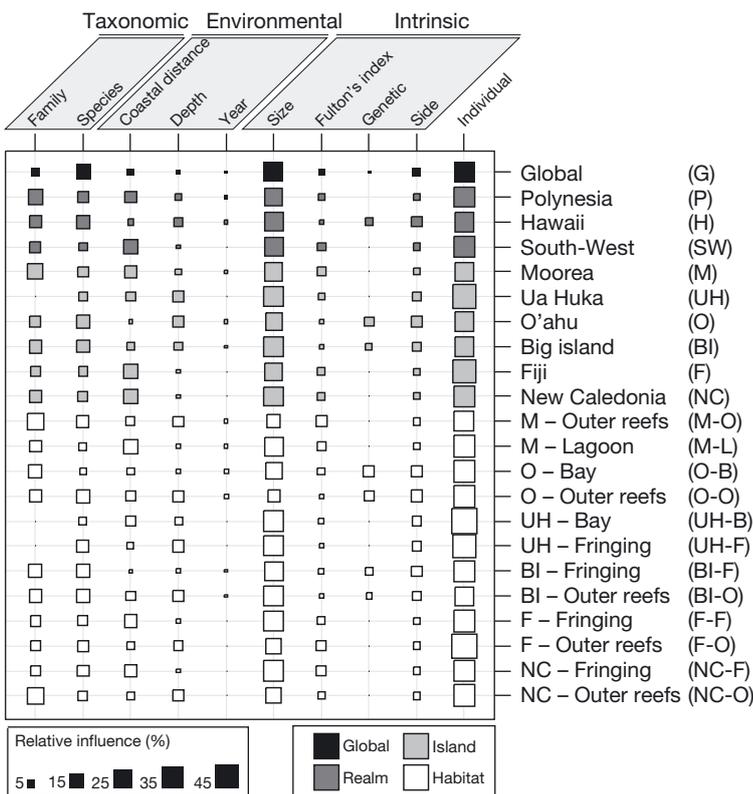


Fig. 4. Mean relative influence measures of taxonomic, environmental, intrinsic and individual factors at all spatial scales. At each scale, the relative influences sums up to 100

ronmental variables accounted for 20 % of shape variation, with local habitat being the most important factor (8.8 %). In contrast, 28 and 33 % of shape variation, respectively, are due to individuals and endogenous (i.e. intrinsic) factors. It is noteworthy that size (used as a proxy of ontogeny) is by far the most substantial intrinsic variable that accounted alone for 26.6 % of total variation, almost as much as the remaining inter-individual variability (28.0 %). The tree illustrates both relative importance and interactions among factors. The pruned tree identified 10 significant partitions using 5 different predictor variables (Fig. 2b). Hierarchical partitioning explained 60.1 % of total shape variation. The first partition was based on whether the standardized size was greater or less than 0.13 and intrinsically explained 18.2 % of total shape variation. The group with smaller sizes correspond to young-of-the-year that have recently passed through the reef crest to settle onto the reef. Otolith shape variation in these fish remains extremely limited, with differences being predominantly specific. Within the group with greater sizes, otoliths have significantly higher levels of shape variation with species, habitat and island being nested with each other depending on families. Size appears as a predominant factor for snappers, with interspecific variations being insignificant and habitat important, irrespective of island. In contrast, otolith shape of groupers is much more related to species, with important differences among islands, irrespective of habitats.

Figs. 3 & 4 provide a more in-depth view of the relative influence of all variables along the hierarchy of taxonomic and spatial scales. The results of this study do not support the hypothesis that large-scale differences in environmental conditions are associated with important shape variation. In contrast, the data clearly emphasize that, at any scale, local environmental variables such as habitat, coastal distance or depth contribute contribute as much to total shape variation as large-scale patterns (i.e. comparisons between isolated islands or non-related families). This is of primary importance because most studies exclusively focus on large-scale differences, omitting equally important local-scale differences that should not be neglected and

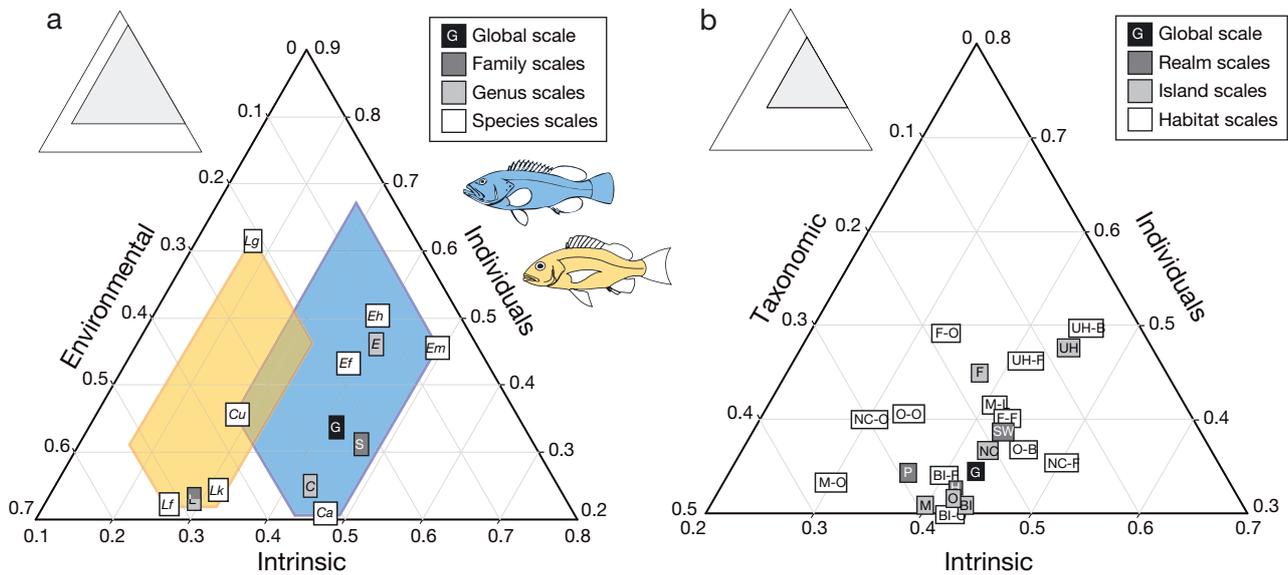


Fig. 5. (a) Ternary plots showing the relative contributions of environmental, intrinsic and individual factors at various taxonomic scales, as described in Fig. 3 (Serranidae within the blue area and Lutjanidae within the yellow area) and (b) taxonomic, intrinsic and individual factors at various spatial scales, as described in Fig. 4. Shaded triangles at the top left of both panels specify the focus region

that can be easily overlooked when precise information on the sampled fish is missing. In addition, that otolith shape from snappers differs markedly with respect to environmental variables (to the detriment of intrinsic factors), while otoliths from groupers rather differ with respect to intrinsic variables (to the detriment of environmental factors) (Fig. 5). On a practical basis, the data emphasize that otoliths can be used to record both large- and small-scale factors.

In most of the spatial and taxonomic scales of our system, size is substantial in determining the shape of coral reef otoliths. The contributions of the remaining variables can, however, drastically change among taxonomic scales, as well as among spatial scales (Fig. 6). Among the 28 possible inter-specific pairwise comparisons, 13 and 18 comparisons were insignificantly correlated, respectively, for $p > 0.05$ and $p > 0.1$, indicating that the variable importance hierarchy often differs among species. Similarly, among the 66 possible inter-habitat comparisons, 22 and 25 comparisons were insignificantly correlated, respectively, for $p > 0.05$ and $p > 0.1$, indicating that the variable importance hierarchy differs among

habitat. Interestingly, only 2 and 5 comparisons were insignificant at the island scale ($p > 0.05$ and $p > 0.1$, 15 possible pairwise comparisons). None of the comparisons between the 3 main realms was insignificant,

Spearman's correlation

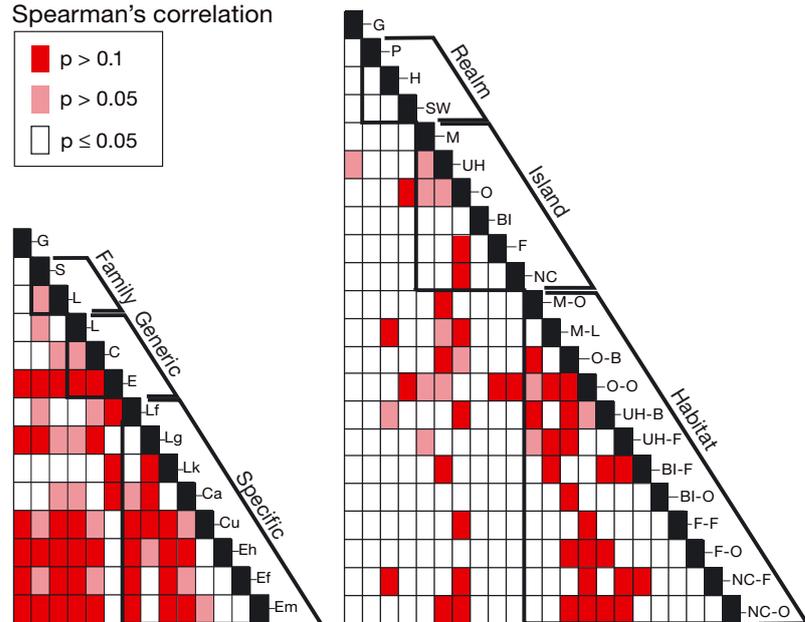
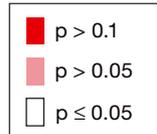


Fig. 6. P-values associated with Spearman's correlation coefficients evaluating the mean contributions of the variables at taxonomic (left) and spatial (right) scales. A lack of statistical significance ($p > 0.05$ and > 0.1) indicates differences in the contribution of variables among scales. Abbreviations of taxonomic and spatial scales as defined in Figs. 3 & 4). Dark black lines delimit intra- and inter-hierarchical comparisons

indicating similar a determinant pattern among the biogeographic regions. This clearly indicates that large scales (i.e. realms and islands) are homogeneous, compared to fine scales that exhibit local highly contrasted determinants. Otolith shape variability at a given scale may develop from interacting lower-level units but may also be imposed by large-scale constraints. Here, aggregating otoliths from multiple sources at a large scale encompasses local determinants and results in more homogeneously determined entities.

DISCUSSION

Otolith shape determinants

Conclusions about the determinants of shape variability patterns in ecological, evolutionary or developmental studies are often derived from correlative multiple regression settings (e.g. Lombarte et al. 2010). However, identification of the predictor variables most probably affecting variation in morphological variables by commonly used regression methods can be problematic, particularly if predictor variables are significantly intercorrelated and exhibit high-order interactions (Mac Nally 2000, Graham 2003). The number of putative explanatory factors may also be substantial, precluding an exhaustive investigation of the whole model's landscape. In this context, numerous studies have focused exclusively on *a priori* hypotheses rather than investigating morphological variation in an exploratory manner. And even when exploratory analyses were conducted, results did not quantitatively investigate the source of shape variation. While results from morphological investigations and predictions are critically affected by the scales addressed and their corresponding scale-dependent processes, the comparison of results originating from different scales is rarely discussed explicitly. This common neglect of scale-related questions contrasts with the strong need for scale-explicit understanding of ecological processes and patterns. Of particular interest is that a top-down view along the hierarchy of scales can help to identify lower-level constraints on higher-level disparity, as well as trans-scale influences. Compared to traditional approaches, the present study shows that hierarchical partitioning methods provide deeper insights into the main determinants of otolith shape variation at multiple scales. The decomposition of sagittal shape variation shows that disparity is highly variable, both at the community and popula-

tion levels. The importance of this disparity has already been documented (e.g. Lombarte & Castellón 1991), but is here quantified for the first time with simultaneous regard to species, size, space, time, genetics (only for snappers introduced in Hawaii), individuals and symmetry. The high level of remaining inter-individual variation across all scales could have been overestimated and is difficult to fully comprehend as it may depend on several uncovered (i.e. not recorded in the field) scale-specific variables. The other factors are reviewed hereafter.

Inter-specific differences

Not surprisingly, although exhibiting considerable variation among conspecific individuals, the shape (outline) of the aragonitic sagittal otoliths is species-specific, with consistent variation among adult species (Campana & Thorrold 2001). Other studies based on simple morphological indices have already pointed out the specific nature of some combinations of morphological characteristics which may be of use in the discrimination between species. Such species identification from otolith shapes is of key interest for ecological marine studies. For example, otoliths recovered from the stomachs or droppings of piscivorous animals (e.g. seals and seabird) are used to determine areas of feeding or to reconstruct species composition of diet (Jobling & Breiby 1986). The basis for these different purposes is either a reference collection of otoliths from the local fish species or published atlases (e.g. Harvey et al. 2000, Campana 2004, Tuset et al. 2008). In addition, the morphology of otoliths sometimes allows taxonomic relationships to be evaluated, with implications for phylogeny, systematics and paleontological studies (Nolf 1985, Assis 2003, 2005, Reichenbacher et al. 2007). This relationship is, however, not apparent for related species derived from rapid adaptive radiation, such as polar nototheniids that exhibited a weak relationship between otolith shape and phylogeny, with most of the variability being related to trophic niche (Lombarte et al. 2010). In the present study, the otoliths from closely related fish species (i.e. congeneric species) are more alike than those of different families. Because of their function as sound transducers, otolith shape variation should influence the frequency sensitivities and directional hearing in fish (Popper et al. 2005). As a result, the within-species shape variation should be conservative to maintain function and might explain the small intraspecific shape variation in relation to interspecific variation.

Surprisingly, data reveal that the within-species shape disparity explains a large amount of variation at a global scale, predominantly due to allometric changes and residual (non-explained) inter-individual differences. This contradicts conventional wisdom that maintains that interspecific shape differences are large compared to within-species variation, even when comparing species in the same genus (e.g. Monteiro et al. 2005).

Spatial variation

In various systems, the shape of the sagittal otolith has been described as varying from region to region in relation to environmental factors. However, the responses of organisms to different sources of habitat heterogeneity may vary, and, consequently, different sources of heterogeneity may interact to shape the morphology of otoliths. In any case, otolith shape often shows clinal variation by distance, or by geographical location and depth (Wilson 1985, Torres et al. 2000, Begg et al. 2005). Regional variation in otolith shape raises the possibility of using this variation as a discrimination tool for stocks, distinguishing different groups of fishes from different locations when comparative genetic data are not available (Castonguay et al. 1991, Campana & Casselman 1993). However, only a few studies have specifically examined links between otolith shape and environmental/biotic variables (Hüssy 2008). The distribution of otolith morphotypes may reflect adaptations to optimize fish survival in the context of different sound environments (Gauldie & Crampton 2002). However, whether the shape differences observed are indicative of functional differences in the hearing of sounds between the different islands remains to be clarified.

On a theoretical basis, the particular abiotic characteristics of the environments occupied by fish can influence otolith growth (Morales-Nin 2000), which in turn affects otolith shape. Consequently, the local factors that influence growth rate also act on the morphology of the whole otolith (Lombarte et al. 2003). However, most studies were carried out on large geographic scales, such as latitudinal gradient and marine ecological regions. In contrast, studies dealing with small-scale variations (i.e. over a few hundred metres) are scarce (Vignon et al. 2008, Vignon 2012). The paucity of studies at small scale may reflect the lack of expectation that local habitat heterogeneity would affect otolith shape over short distances. Variability at large scales is hardly considered in empirical works, where otolith morphology is

considered to adequately reflect the stocks, suggesting thereby that within-stock variation is of negligible importance in regard to inter-stock differences. The results of this study indeed support the hypothesis that large-scale differences in environmental conditions are associated with important shape variation, as measured by absolute increase in disparity when fishes from different localities are pooled together. Of particular interest is that the data also support that conditions at a lower scale (i.e. within-island habitats) affect otolith shape with the same order of magnitude as conditions at a higher scale, mostly due to local change in size and ontogenetic niche shift (see details for ontogenetic trajectories of *Lutjanus kasmira* in Vignon 2012). This duality clearly illustrates that local factors can act as important confounding effects when higher regional patterns are investigated. As a consequence, the results of this study indicate that local processes should be better taken into consideration, as local environmental variables must be accounted for in order to apply some corrections. Omitting such local covariables may result in a spurious discrimination of stocks over a large scale.

Temporal variation

Although in this study only 2 sampling years were available for most species, the results indicate temporal stability of otolith shape within a location in these 2 consecutive years. These findings coincide with those of other authors (e.g. Jónsdóttir et al. 2006). Yet, the use of otolith shape to discriminate between stocks may not always be straightforward because the discrimination might have been caused by age- and year-class effects and would therefore highlight sample differences rather than stock discrimination. Therefore, it has been suggested that for stock discrimination, otolith shape characteristics have to be recalculated each year for each major age group (Castonguay et al. 1991, Begg & Brown 2000).

Allometry

In fishes, a significant part of the morphological differences are associated with size-related changes (which may correlate with habitat use) due to variations in rates or timing of growth and developmental processes (Simoneau et al. 2000, Monteiro et al. 2005, Doering-Arjes et al. 2008). In many cases, while conspecific adults present consistent shapes, small oto-

liths have a more rounded and undifferentiated shape (Curin-Osorio et al. 2012). This causes the shape among juveniles from different species to overlap considerably. As the fish grew, the otolith or portion of otoliths developed, resulting in a more distinct shape. The ontogenetic trajectory of otolith shape has specifically been investigated for *L. kasmira* (Vignon 2012), and otoliths from other snappers mostly change in a similar manner. This shows that the development of otolith size and the general shape of the otolith is an ontogenetic process, but that the finer details of the otolith shape may be modulated both by local and regional conditions.

Genetics

Although there may also be a genetic contribution to otolith shape differences between stocks, environmental factors are considered to be the major determinants of otolith growth. These determinants remain mostly elusive in the system under consideration, except for *L. kasmira* in its introduced range (i.e. Hawaii) where both genetic and environmental influences play a substantial role in determining otolith shape. More specifically, while environment induces an overall change in otolith shape, genetically induced changes locally affect otolith shape. This specific aspect has already been investigated in depth in Vignon & Morat (2010).

Fluctuating asymmetry

Among individual fishes of the same species that have otolith shapes different from each other, the asymmetry between left and right otoliths of each individual is not insignificant (ranging from 2 to 10% of total variance, according to scale). Such high symmetry between paired structures that are completely separated from each other in their growth implies a high degree of genetic determination in otolith shape (Gauldie & Crampton 2002). On a theoretical basis, bilaterally symmetrical traits should be expressed identically on both sides, because constraints act symmetrically on the 2 otoliths and the product of the development should produce nearly symmetric final forms under ideal conditions (Palmer & Strobeck 1986). However, deviations from perfect symmetry are frequently observed. Environmentally or genetically induced deviations from the ideal phenotype provide information about the precision of development, with lower precision reflecting the disruptive

effects of environmental stressors, poor genetic quality, or both (Leary & Allendorf 1989). In this context, in absence of obvious stressful conditions, the present findings illustrate the level of naturally occurring variation in otolith shape. For comparison, fluctuating asymmetry of the otolith shape in the razor fish *Xyrichtys novacula* from 5 populations accounted for 23.2% total shape variability (Palmer et al. 2010). In this study, fluctuating asymmetry was quantified using the individual-side interaction term from a Procrustes ANOVA. While the current analysis quantifies overall asymmetry-related variation, Procrustes ANOVA allows quantifying the specific contribution of fluctuating and directional asymmetry. In this context, direct quantitative comparison of results from different statistical approaches is unfortunately not straightforward and needs further investigation. In any case, such quantitative results are scarce and offer a good ecological perspective on the use of differences between left and right otolith shape to interpret quality and survivability of fishes, especially because this level of variability has been investigated in the broader context of other sources of variability.

Stock identification

The concept of stock is a useful grouping that is considered crucial in management of fisheries resources (Begg et al. 1999, Begg & Waldman 1999). The understanding of the stock structure of a given fish species is crucial for outlining pertinent management regulations in those fisheries where multiple stocks are differentially exploited at the same time (Dankel et al. 2008). In this context, otoliths have long been used to discriminate species, population and stock differences. On a practical basis, while the use of the otolith has been frequently advocated, there are several concerns associated with this approach as there are various intrinsic confounding effects (i.e. sex, age, year class). Thus, the implicit assumption that the within-stock morphological variation is negligible in regard to the variation that exists among stocks cannot be made. In addition, although otoliths may effectively identify phenotypic stocks (i.e. a group of individuals within a species that maintains common characters that are environmentally dependent, related to geographic locations and to chemical and physical qualities of water masses) (Cadrian & Friedland 1999), the determinants of otolith shape variation and underlying processes are not fully understood. Several studies have indicated that otolith shape differences between stocks are likely to be

caused by both environmental and stock (i.e. genetic) influences (Cardinale et al. 2004, Vignon & Morat 2010), although it is unclear to what extent phenotype (environmental conditions affecting plausibly growth rates) and genotype affect otolith shape. Facing the difficulty of disentangling the genetic/environmental relative contributions in natural conditions, it is at least necessary to disentangle the sources and to quantify the amount of environmentally induced shape variation. There has indeed been a growing interest for understanding the relationships between environmental conditions and otolith shape variation. Such an eco-morphological understanding is not only of interest in a stock discrimination context but also because there is more evidence for the specialized adaptation of otolith shape to the hearing requirements of particular fish habitats in terms of optimal frequencies (Gauldie & Crampton 2002). However, there are surprisingly few studies that have tried to relate ecological heterogeneity and morphological disparity (Gauldie & Crampton 2002, Hüsey 2008). This constitutes an obviously important direction for future research.

During the last few decades, there have been several empirical and descriptive attempts to identify the key determinants of otolith shape in a variety of species, and several key variables have been elucidated at various scales. However, despite these efforts, most of the investigations were pursued to test *a priori* hypotheses based on a limited number of potential determinants, and multi-scale quantitative assessments of factors structuring otolith shape have been elusive. Morphological differences are traditionally used in a specific context at a particular scale, but the use of otolith shape as a relevant indicator of stocks must be comprehended in a more general view, across multiple spatial scales. These perspectives necessitate the knowledge of scale-dependent phenomena, because the overall morphology of any otolith is the result of processes operating at both small and large spatial scales during the fish's life. Hence, there is a quantitative need to understand how morphology changes across multiple spatial scales. Nevertheless, the paucity of available quantitative data strongly limits such attempts. In this context, disentangling the extent to which intrinsic, local, regional and realm characteristics contribute to otolith shape variability is also fundamental for better otolith-based decision support in fisheries management. Defining and quantifying such key factors is equally important in determining the effect of scale on otolith shape and ensuring predictability.

CONCLUSIONS

The challenge for morphometric stock identification in future is to develop a consensus on biological interpretations of geometric analyses (Cadrin 2000). The usefulness of morphological characters depends entirely on how well their biological properties are understood. As the biochemistry and physiology of otoliths have become better known, so has their value to the biologist. The quantitative measurement of otolith shape determinants calls for special consideration and further investigation. By focusing on shape variation across multiple hierarchical spatial scales, we could thus identify the scale at which the importance of a given determinant is maximized or minimised. The use of hierarchical partitioning is a major step forward in understanding the rationale for phenotypic stock discrimination. Such understanding of the scaling of otolith shape variation is crucial if otolith shape is to be used in the future as an effective tool to improve our understanding of the integrity of fish populations and in the management of fisheries resources.

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