Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Journal of Fish Biology (2017) **90**, 311–325
doi:10.1111/jfb.13187, available online at wileyonlinelibrary.com

Otolith morphology varies between populations, sexes and male alternative reproductive tactics in a vocal toadfish *Porichthys notatus*

A. P. H. Bose*, J. B. Adragna and S. Balshine

Aquatic Behavioural Ecology Laboratory, Department of Psychology, Neuroscience, & Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON, L8S 4L8 Canada

(Received 14 April 2016, Accepted 9 September 2016)

In this study, the morphology of sagittal otoliths of the plainfin midshipman fish *Porichthys notatus* was compared between populations, sexes and male alternative reproductive phenotypes (known as ‘type I males or guarders’ and ‘type II males or sneakers’). Sagitta size increased with *P. notatus* size and changes in shape were also detected with increasing body size. *Porichthys notatus* sagittae begin as simple rounded structures, but then elongate as they grow and take on a more triangular and complex shape with several prominent notches and indentations along the dorsal and caudal edges. Moreover, the sagittae of the two geographically and genetically distinct populations of *P. notatus* (northern and southern) differed in shape. *Porichthys notatus* from the north possessed taller sagittae with deeper caudal indentations compared to *P. notatus* from the south. Sagitta shape also differed between females and males of the conventional guarder tactic. Furthermore, guarder males had smaller sagittae for their body size than did sneaker males or females. These differences in sagittal otolith morphology are discussed in relation to ecological and life history differences between the sexes and male tactics of this species. This is the first study to investigate teleost otolith morphology from the perspective of alternative reproductive tactics.

Key words: Batrachoididae; Fourier analyses; plainfin midshipman; sacculus; shape analysis.

INTRODUCTION

The structures involved in teleost audition have held a long-standing interest to researchers from a wide range of disciplines spanning from fisheries biology to neuroscience. Of particular interest are the otoliths of the inner ear. These are three pairs of hard, dense, calcareous structures that play an important role in both audition and balance (Popper & Fay, 1993; Popper & Lu, 2000; Popper *et al.*, 2005). The three pairs of otoliths are termed the sagittae (singular sagitta), lapilli (singular lapillus) and asteriscii (singular asteriscus), housed within the sacculus, lagena and utriculus end organs, respectively. These pairs of otoliths can differ markedly from one another in morphology, *i.e.* shape and size. Of these otoliths, the sagitta is most notable as it displays exceptional morphological diversity between species but remarkably less

---

*Author to whom correspondence should be addressed. Tel.: +1 905 525 9140/26037; email: boseap@mcmaster.ca variation within a species (Schellart & Popper, 1992; Campana, 2004). This has made otolith size and shape analyses, particularly of sagittae, useful tools for discriminating among fish species and even higher order taxonomical groupings (L'Abée-Lund & Jensen, 1993; Paxton, 2000; Tuset *et al.*, 2003, 2015). Within a species, morphological variation of sagittae can be influenced not only by age, but also by genetic and environmental factors (Cardinale *et al.*, 2004; Vignon & Morat, 2010), and can vary with an individual's somatic growth rate, feeding history (Strelcheck *et al.*, 2003; Gagliano & McCormick, 2004) or even habitat (Lombarte & Lleonart, 1993). Thus, intraspecific variation in sagittal otolith morphology is also able to capture differences between fish populations, stocks and year-classes [*e.g.* Atlantic herring, *Clupea harengus* L. 1758, Bird *et al.*, 1986; Atlantic mackerel, *Scomber scombrus* L. 1758, Castonguay *et al.*, 1991; Atlantic cod *Gadus morhua* L. 1758, Campana & Casselman, 1993; haddock, *Melanogrammus aeglefinus* (L. 1758), Begg & Brown, 2000].

Despite decades of otolith research, some aspects of otolith morphology still remain untested. For example, no study has yet investigated within- and between-sex variation in otolith morphology in a species with alternative reproductive tactics. In such species, a particular sex, usually male (Neff & Svensson, 2013), is characterized by a discontinuous distribution of phenotypic traits and so individuals can be categorized into two or more distinct reproductive phenotypes. Each of these phenotypes will have evolved to maximize their chances of reproduction through alternative means (Taborsky *et al.*, 2008). Male alternative reproductive tactics are widespread across animal species (Taborsky *et al.*, 2008), especially fishes (Knapp & Neff, 2008) and are exhibited in several commercially important groups, such as salmonids (Jones, 1959; Thomaz *et al.*, 1997). For example, in one species of Pacific salmon, Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792), there are two separate types of males; hooknose males with large body sizes, develop pronounced secondary sexual characteristics and mature several years after migrating to sea, while other males, jacks, are much smaller in size, do not develop secondary sexual characteristics and mature precociously (Berejikian *et al.*, 2010). Studying otolith morphology in fish species with alternative tactics provides two benefits. First, it provides the opportunity to use study systems that are characterized by divergence in many traits (*e.g.* relating to physiology, behaviour and life history) to test for phenotypic variation in a highly species-specific and seemingly constrained trait, sagittal otolith morphology. Second, it tests the importance of accounting for alternative tactics when using otoliths to estimate population structure, a potentially highly relevant consideration for some commercially valuable species.

In this study, the size and shape variation of sagittal otoliths was studied in a fish that expresses two distinct male alternative reproductive tactics, the plainfin midshipman *Porichthys notatus* Girard 1854. The two male phenotypes of this vocal batrachoidid have been termed guarder and sneaker. At the beginning of the breeding season, males of the guarder or conventional, tactic (also known as type I males), migrate from deep waters to the rocky intertidal zones, where they excavate nesting cavities beneath large rocks (Arora, 1948). From here, they produce low frequency (*c.* 100 Hz) tonal advertisement calls by rapidly contracting the sonic muscles attached to their swimbladder walls (Ibara *et al.*, 1983; Brantley & Bass, 1994; McIver *et al.*, 2014). Females locate guarder males *via* phonotaxis and lay eggs on the roof of the selected male's nest (Brantley & Bass, 1994; McKibben & Bass, 1998). Guarder males then provide sole parental care for the offspring for up to three consecutive months (Cogliati *et al.*, 2013). Adequate nesting sites in the intertidal zone are limited (DeMartini, 1988), and so guarder males compete intensely with each other for nest ownership and will vocalize during agonistic encounters (Brantley & Bass, 1994; McIver *et al.*, 2014). Males of the sneaker, or alternative, tactic (also known as type II males) are much smaller in body size, do not build nests, nor do they court females, provide parental care, or engage in physical competition with other males (Brantley & Bass, 1994). Rather, sneaker males eavesdrop on guarder male vocalizations and then rely upon stealth and satellite spawning to steal paternity from spawning guarder males. In addition to behavioural differences, the alternative tactics in male *P. notatus* are strikingly different with respect to their body size and gonadal investment (Bass & Andersen, 1991), vocal capabilities and neuroanatomy (Bass & Marchaterre, 1989*a*, *b*; Brantley & Bass, 1994; Bass *et al.*, 1996), as well as endocrine hormone profiles (Brantley *et al.*, 1993; Arterbery *et al.*, 2010). An earlier study on *P. notatus* used sagittal otoliths to age juveniles and adults and showed that sneaker males mature at an earlier age than do guarder males (Bass *et al.*, 1996). Given the divergence in traits between the male tactics, it was predicted that sagitta morphology would also differ.

Variation in sagittal otolith morphology was also investigated in relation to the different geographic populations of *P. notatus*. *Porichthys notatus* is widely distributed along the western coastlines of North America from Alaska to Mexico with a notable absence along the Oregon coast, U.S.A. (Miller & Lea, 1972; Walker & Rosenblatt, 1988). Several studies by Cogliati *et al.* (2013, 2014*a*) have shown that the northern population (stretching from Alaska to Washington) appears to be a single population that is genetically distinct from the southern population (stretching from northern California to Mexico). *Porichthys notatus* from the southern population tend to be smaller in overall body size (Cogliati *et al.*, 2014*a*), and are apparently more bioluminescent because of differences in the luciferin content of their diets (Warner & Case, 1980; Thompson & Tsuji, 1989). Given the latitudinal, genetic and ecological separation between the populations, it was predicted that sagitta morphology might also differ.

MATERIALS AND METHODS

INTERTIDAL COLLECTION OF SPECIMENS

Porichthys notatus is not considered threatened or endangered (Collette *et al.*, 2010). Adult reproductive *P. notatus* were collected from intertidal nests, located by carefully overturning large rocks at low tide. Collections occurred during the summers of 2010 ($n = 29$), 2011 ($n = 44$), 2013 ($n = 93$), 2014 ($n = 87$) and 2015 ($n = 48$) from intertidal beaches of the northern population (British Columbia, Canada: 49° 01′ N; 123° 83′ W, 48° 63′ N; 123° 53′ W and 49° 04′ N; 122° 88′ W and Washington, U.S.A.: 47° 71′ N; 122° 89′ W), and also during the summers of 2010 ($n = 7$) and 2011 ($n = 14$) from intertidal beaches of the southern population (California, U.S.A.: 38° 15′ N; 122° 90′ W). All *P. notatus* found within nests are reproductive and have fully developed gonads. Non-reproductive *P. notatus* presumably do not migrate to the intertidal zones. The collected *P. notatus* were all used in a number of other short-term field studies (Cogliati *et al.*, 2013, 2014*a*, *b*, 2015; Bose *et al.*, 2014, 2016), none of which involved long-term housing, changes in diet or manipulations of physiology that could have affected somatic or otolith growth. Each *P. notatus* was sexed and its tactic identified by external morphology (Bass, 1996; Bass & McKibben, 2003) before being measured for standard length (L_S) to the nearest 0·1 cm and body mass to the nearest 0·1 g. All *P. notatus* were euthanized with an overdose of benzocaine (>250 mg l^{-1} for 3 min or until complete loss of responsiveness to tactile stimuli), dissected and the sagittae were removed, wiped clean and stored in dry Eppendorf tubes.

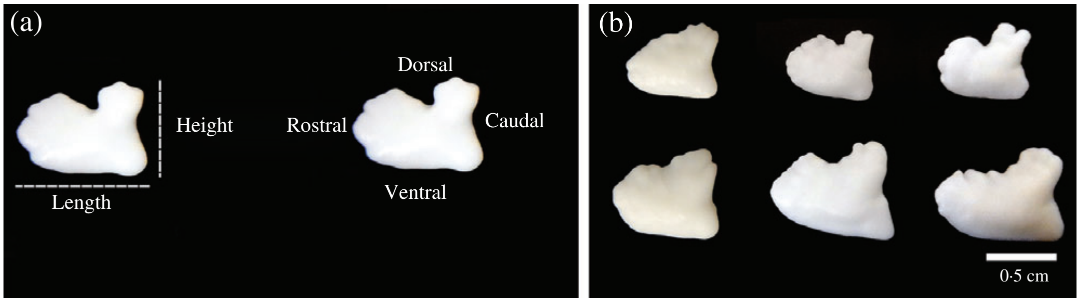

FIG. 1. (a) The major length and height axes as well as axes of orientation (rostral-caudal, dorsal-ventral) for the sagittal otoliths of *Porichthys notatus*. (b) An example of the size and shape variation observed among sagittae of guarder male *P. notatus* sampled from the northern population (note, sulcus side facing down). Scale is the same in (a) and (b).

QUANTIFICATION OF OTOLITH SIZE

Several morphometric measurements were obtained from each sagitta. The length [rostral-caudal major axis, Fig. 1(a)] and height [dorsal-ventral major axis, Fig. 1(a)] of each sagittal otolith was measured with a digital micrometer (to the nearest 0·001 mm, Shimana, SHAYMR171; www.shimana.ca), the mass was measured with a microbalance (to the nearest 0·0001 g, Mettler Toledo, AB204-S/FACT; www.mt.com) and the surface area was calculated using the software package SHAPE ver. 1.3 (to the nearest 0·0001 cm^2, Iwata & Ukai, 2002) from digital photographs (12 megapixels) taken of each otolith at a distance of 15 cm. In many fishes, the masses of the left and right paired otoliths are not equal, and this mass asymmetry is likely to vary in magnitude and direction both between individuals and over the course of an individual's lifetime (Lychakov & Rebane, 2005). In this study, only measurements from left sagittae were used for the otolith size comparisons. From each left sagitta, all four morphometric measurements (length, height, mass and surface area) were standardized and then reduced down to a single principal component (PC), termed sizePC, using a principal component analysis (PCA) using the software R (www.R-project.org).

Otolith mass asymmetry was also calculated for each individual by taking the difference in mass between the left and right sagittae and dividing this difference by the average mass of both sagittae (following Lychakov & Rebane, 2005). A linear mixed effects model was used to test whether there was a left or right bias in sagittal otolith mass asymmetry. *Porichthys notatus* type (*i.e.* female, sneaker male and guarder male) and L_S were included as fixed effects. L_S was first mean-centred in order to make the model intercept interpretable. Sampling year and specific field site of collection were treated as random effects (nlme package; Pinheiro *et al.*, 2015).

QUANTIFICATION OF OTOLITH SHAPE

Shape was quantified from sagittae using an elliptic Fourier principal component analysis provided by the shape analysis software package SHAPE. Elliptical Fourier functions are able to accurately describe information about the outlines of enclosed shapes in a quantifiable manner (Kuhl & Giardina, 1982). The analysis in SHAPE describes the contours of enclosed shapes, *i.e.* otoliths, as the sum of multiple ellipses, or harmonics, each of which is described by trigonometric equations involving a combination of sine and cosine waves (Kuhl & Giardina, 1982). Each harmonic is defined by four coefficients, or elliptic Fourier descriptors (EFDs), that correspond to its sine and cosine parameters. SHAPE normalizes these coefficients based on the ellipse of the first harmonic (following Kuhl & Giardina, 1982), resulting in coefficients that are invariant to size and orientation of the shape of interest. It then uses a principal component analysis based on a variance–covariance matrix to summarize and reduce the large number of coefficients into a manageable number of principal components.

To quantify otolith shape, sagittae were photographed individually against a black background [Fig. 1(b)]. Left otoliths were considered for this shape analysis, though if only the right otolith was available for a given specimen the photograph was still taken and then flipped horizontally (this occurred for 6%, or 19 of the 322 available specimens). The contours of each sagitta were extracted and chain-encoded from the binarized images within the software SHAPE. This technique has been similarly used in previous studies of otolith shape (Tracey *et al.*, 2006). The EFDs were derived using the first 20 harmonics, and normalized with respect to the first harmonic ellipse. The accuracy obtained by using the first 20 harmonics was assessed qualitatively by comparing actual sagitta contours with reconstructions based on the EFDs using the NEFview programme in SHAPE. The shape variation described by the EFDs was then summarized into several composite variables, termed shapePCs, using a PCA in SHAPE. A broken stick model (Jackson, 1993) in conjunction with a scree plot was then used to determine how many shapePCs to interpret. The scree plot displays the per cent variance explained by each shapePC and the broken stick model indicates the per cent variance explained by each PC if the components were determined by chance alone.

COMPARISON OF OTOLITH SIZE AND SHAPE BETWEEN POPULATIONS

In 2010 and 2011, no females or sneaker males were collected from the southern population. Thus, only the sagittae of guarder males were compared between the northern and southern populations in terms of their size ($n_{\text{North}} = 46$, $n_{\text{South}} = 20$) and shape ($n_{\text{North}} = 50$, $n_{\text{South}} = 21$). Sample sizes differ slightly here because the size analysis only considered left sagittae, while the shape analysis also included several right sagittae when the left one was not available. A PCA in R was used to reduce the sagittal otolith morphometric measurements (*i.e.* length, height, mass and surface area) of these specimens down to a single sizePC component, which accounted for 95·2% of the total otolith size variation. An elliptic Fourier PCA in SHAPE was used to summarize the shape variation into five shapePCs, termed shapePC1-5. The five shapePCs respectively explained 33·1, 27·3, 15·8, 5·7 and 3·7% of the total variation in sagitta shape data (respective eigenvalues are $4{\cdot}32\text{e}^{-3}$, $3{\cdot}56\text{e}^{-3}$, $2{\cdot}07\text{e}^{-3}$, $7{\cdot}46\text{e}^{-4}$ and $4{\cdot}86\text{e}^{-4}$). Visualizations of the effects that each shapePC has on otolith shape were constructed in SHAPE following Furuta *et al.* (1995).

In order to compare sagitta size between the guarder males of the northern and southern population, a linear mixed effects model (LMM) was run using sizePC as the independent variable, and *P. notatus* L_{S} (cm) and population (northern *v.* southern) as predictor variables. Sampling year and specific field site of collection were treated as random effects. To compare sagitta shape between the guarder males of the northern and southern population, one LMM was run for each shapePC (five in total). Otolith surface area (cm^2) and population (northern *v.* southern) were included as predictor variables, while sampling year and specific field site of collection were included as random effects. Interaction effects were removed if they did not significantly improve the model fit based on Akaike information criterion. Because each random effects model is considered to be an independent comparison of shape, a Bonferroni correction was applied to maintain a family-wise error rate of 0·05. Thus, a new α of value 0·01 was implemented as the critical threshold for significance for these comparisons.

COMPARISON OF OTOLITH SIZE AND SHAPE BETWEEN SEXES AND TACTICS

Next, the sagittae from each *P. notatus* type, *i.e.* female, sneaker male and guarder male, were compared for size differences ($n_{\text{Females}} = 43$, $n_{\text{Sneaker males}} = 17$, $n_{\text{Guarder males}} = 170$) and shape differences ($n_{\text{Females}} = 46$, $n_{\text{Sneaker males}} = 21$, $n_{\text{Guarder males}} = 177$). Again, sample sizes differ slightly here between size and shape analyses because the size analysis only considered left sagittae while the shape analysis also included several right sagittae when the left was not available. As females and sneaker males were not collected from the southern population, these analyses only consider *P. notatus* that were collected from the northern population. Again, a PCA in R was used to reduce the sagittal otolith morphometrics (*i.e.* length, height, mass and surface area) from these specimens down to a single sizePC, which captured 94·8% of the total size variation. An elliptic Fourier PCA in SHAPE was used to summarize the sagitta shape variation into four shapePCs, termed shapePC1-4, which respectively explained 46·3, 23·8, 10·7 and 4·2% of the total variation in shape data (respective eigenvalues are $9{\cdot}40e^{-3}$, $4{\cdot}84e^{-3}$, $2{\cdot}18e^{-3}$ and $8{\cdot}55e^{-4}$). Again, visualizations of the effects that each shapePC has on sagitta contour were constructed using SHAPE following Furuta *et al.* (1995).

In order to compare sagitta size between the three *P. notatus* types (*i.e.* female, sneaker male and guarder male) a LMM was run using sizePC as the independent variable. *Porichthys notatus* L_S (cm) and *P. notatus* type were fixed effects, while sampling year and specific field site of collection were random effects. To compare the variation in sagitta shape between the three *P. notatus* types, one LMM was run for each shapePC (four in total). Otolith surface area (cm^2) and *P. notatus* type were used as fixed effects, while sampling year and the specific field sites of collection were modelled as random effects. When needed, variance heterogeneity (*e.g.* increasing variance in sagitta size with increasing *P. notatus* L_S) was modelled using a variance function (Zuur *et al.*, 2009). Likelihood ratio tests (LRTs) were used to test for significant main effects or interaction effects of *P. notatus* type and L_S. If significant, this was followed up with *post hoc* pair-wise comparisons (Tukey contrasts, multcomp package; Hothorn *et al.*, 2008) between each of the three *P. notatus* types. Again, as each analysis of a shapePC is considered to be an independent comparison of shape, a Bonferroni correction was applied to maintain a family-wise error rate of 0·05. Thus, a new α of 0·0125 was implemented as the critical threshold for significance for these comparisons.

ETHICAL NOTE

All animal collections and handling were in accordance with the Canadian Department of Fisheries and Oceans rules. All procedures were approved by the McMaster University Animal Research Ethics Board (AUP 13-12-52), as well as the University of Washington Institutional Animal Care and Use Committee (AUP 4079-06) and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

RESULTS

Larger *P. notatus* also had larger sagittal otoliths. This was apparent in the comparison of sagitta size between populations [LMM: $t = 17{\cdot}4$, d.f. = 55, $P < 0{\cdot}001$; Fig. 2(a)] as well as the comparison of sagitta size between the sexes and tactics [LRT: $\chi^2 = 469{\cdot}8$, d.f. = 1, $P < 0{\cdot}001$; Fig. 3(a)]. There was no significant right-left asymmetry in sagittal otolith mass (LMM, intercept, $t = 0{\cdot}82$, d.f. = 2·78, $P > 0{\cdot}05$) nor did asymmetry increase or decrease with *P. notatus* L_S (LMM, $t = 0{\cdot}51$, d.f. = 38·4, $P > 0{\cdot}05$) or vary across *P. notatus* types (LMM, all pair-wise $P > 0{\cdot}05$).

The size of guarder male sagittae did not differ between the northern and southern populations [sizePC, LMM: $t = -1{\cdot}26$, d.f. = 8, $P > 0{\cdot}05$; Fig. 2(a)], however, shape did vary significantly. For the between-population comparison, sagitta shape data were reduced down to five significant principal components, or shapePCs [Fig. 2(b)]. Each shapePC describes a different aspect of sagittal otolith contour shape, which can be visualized in Fig. 2(c). Guarder males from the northern population had taller sagittae along the dorsal-ventral major axis [shapePC1, LMM: $t = -3{\cdot}69$, d.f. = 8, $P < 0{\cdot}01$; Fig. 2(d)] with a more pronounced indentation at the caudal end [shapePC4, LMM: $t = -2{\cdot}94$, d.f. = 59, $P < 0{\cdot}01$; Fig. 2(d)] than did the guarder males from the southern population. No other shape differences were observed between guarder males of the two populations (all $P > 0{\cdot}05$).

Guarder males had smaller sagittal otoliths (after controlling for body size) than did either sneaker males (sizePC, Tukey contrast: $z = -2{\cdot}99$, $P < 0{\cdot}05$) or females [Tukey

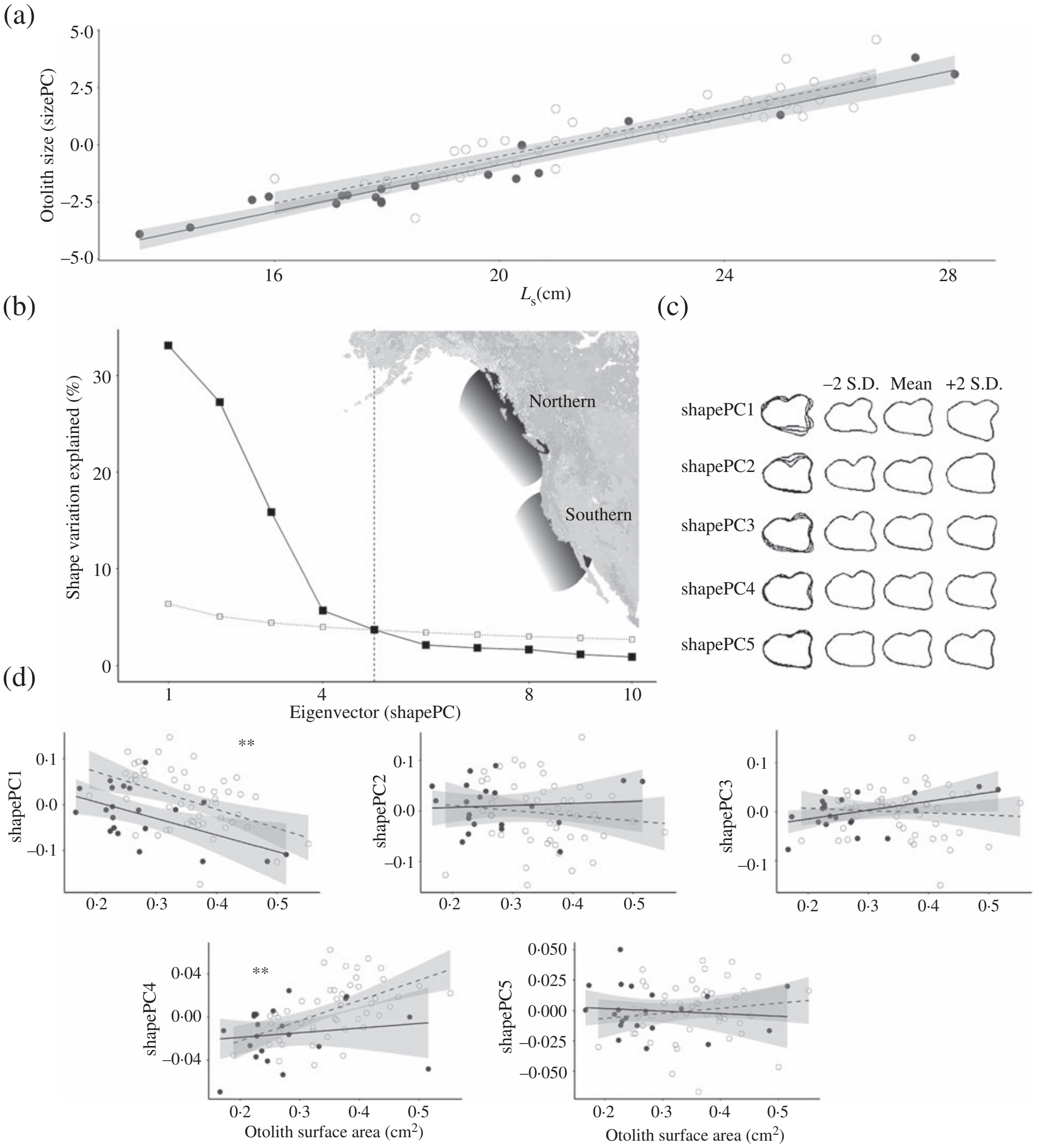

FIG. 2. (a) Sagitta size comparison between the northern (--○--) and southern (—●—) populations of *Porichthys notatus*. (b) A scree plot indicating how many shape principal components (shape PCs, —■—) explain more variance in sagittal otolith shape than can be expected by chance alone (as calculated by the broken stick model, —□—), when comparing the two *P. notatus* populations as shown in the map insert. (c) Contour reconstructions to aid in visualizing the shape variation captured by each of the five shapePCs. Note, the contours beneath mean are all identical, but their shapes change as the scores of each shapePC are increased or decreased (±2 S.D. shown). The leftmost column overlays the three contours to the right. (d) Sagitta shape comparisons between the northern (--○--) and southern (—●—) populations of *P. notatus*, using each of the five shapePCs identified above. $^{**}P < 0·01$.

contrast: $z = −5·88$, $P < 0·001$; Fig. 3(a)]. Sneaker males and females possessed similarly sized sagittae for their body size (Tukey contrast: $z = −1·48$, $P > 0·05$). For the among *P. notatus* type comparison, sagitta shape data were reduced down to four significant principal components, shapePCs [Fig. 3(b)]. Each shapePC used in this analysis can be visualized in Fig. 3(c). Controlling for otolith size, females had narrower

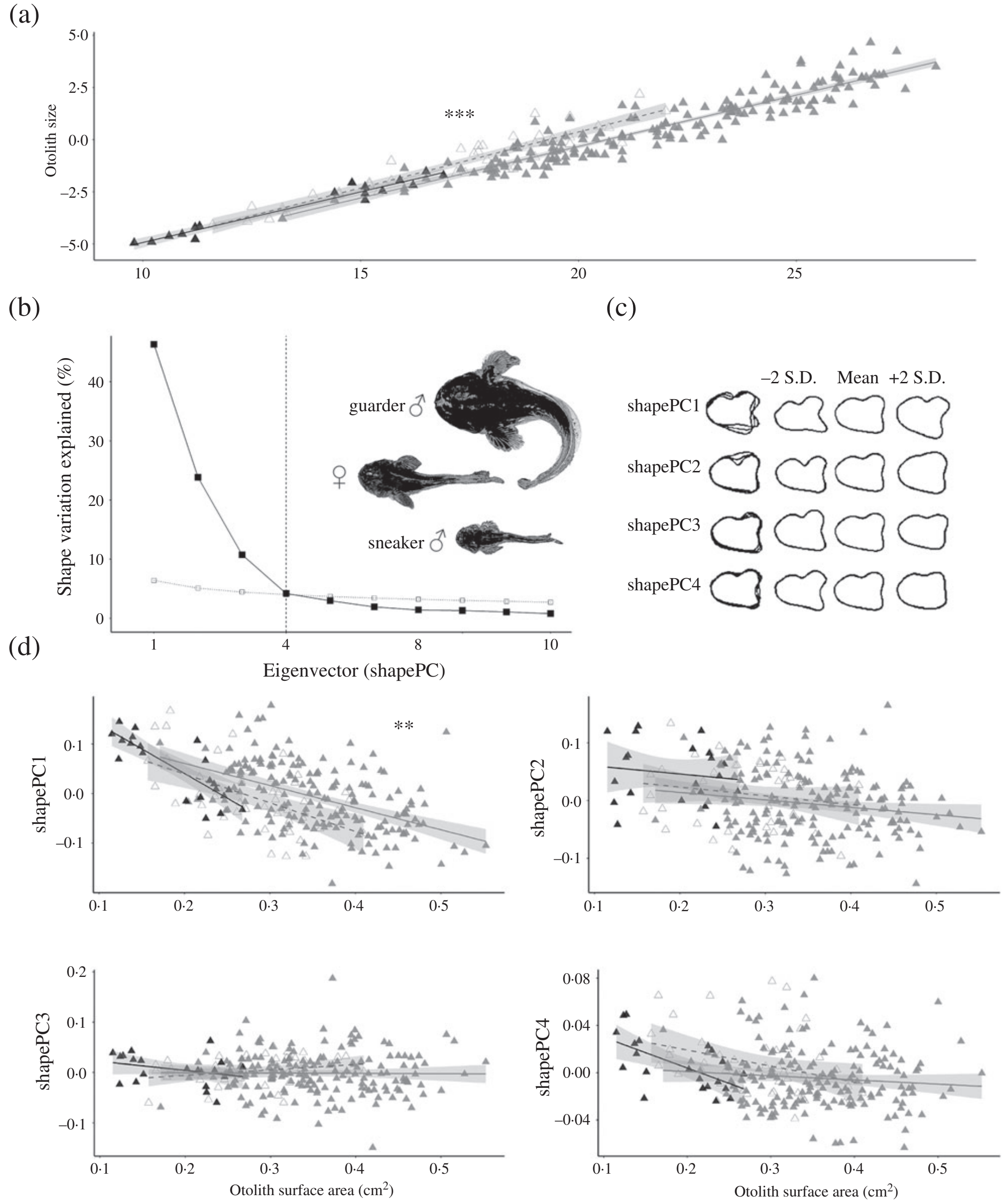

FIG. 3. (a) Sagitta size comparison between the three *Porichthys notatus* types, females (- -△- -), guarder males (—▲—) and sneaker males (—▲—). (b) A scree plot indicating how many shape principal components (shapePCs, —■—) explain more variance in sagittal otolith shape than can be expected by chance alone (as calculated by the broken stick model, —□—), when comparing the *P. notatus* types. (c) Contour reconstructions to aid in visualizing the shape variation captured by each of the four shapePCs. As in Fig. 2(c), the contours beneath mean are all identical, but their shapes change as the scores of each shapePC are increased or decreased (±2 S.D. shown). Again the leftmost column overlays the three contours to the right. (d) Sagitta shape comparisons between the three *P. notatus* types, females (- -△- -), guarder males (—▲—) and sneaker males (—▲—) using each of the four shapePCs identified above. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

sagittae along the dorsal-ventral major axis than did guarder males [shapePC1: $z = 3{\cdot}51$, $P < 0{\cdot}05$; Fig. 3(d)]. No other sagittal shape differences could be detected between the *P. notatus* types (all $P > 0{\cdot}05$).

Lastly, changes in sagittal otolith shape were detected as otolith size increased. These shape changes were apparent in both the comparison between populations and the comparison between *P. notatus* types. Sagittae narrowed along the dorsal-ventral major axis with increasing sagitta size [population comparison shapePC1, LMM: $t = -4{\cdot}51$, d.f. = 60, $P < 0{\cdot}001$; Fig. 2(d); *P. notatus* type comparison shapePC1, LRT: $\chi^2 = 76{\cdot}6$, d.f. = 1, $P < 0{\cdot}001$; Fig. 3(d)].

DISCUSSION

The size of the sagittal otoliths in *P. notatus* was tightly and linearly related to L_S, consistent with findings from other studies (Harvey *et al.*, 2000; Campana, 2004). Asymmetry in sagittal mass was not consistently biased towards the right or left sagitta, nor was it related to the size of the individual, also consistent with previous investigations of fish otoliths (Lychakov & Rebane, 2005). The shape of the otoliths displayed a general ontogenetic shift; beginning as a small simple semi-elliptical structure, and developing into a more complex, elongated and triangular structure often with several prominent notches, or indents, along the dorsal and caudal regions. Across fishes, it is extremely common to observe sagittal otolith shape change over ontogeny and with fish growth; otoliths typically grow from simple forms into more intricate and species-specific shapes (Lombarte & Castellón, 1991; Campana, 2004). It is notable, however, that some large *P. notatus* individuals still had relatively simple sagitta contours, while some smaller *P. notatus* had relatively complicated ones.

OTOLITH SHAPE BUT NOT SIZE DIFFERS BETWEEN GEOGRAPHIC POPULATIONS

Sagittal otolith size (once body size of *P. notatus* was controlled for) did not differ between individuals from the northern *v.* southern populations. Guarder males from the north, however, possessed taller sagittae with more pronounced caudal indents compared to guarder males from the south. Differences in sagitta shape between fish populations and stocks have also been found by numerous other studies. For example, Campana & Casselman (1993) observed differences in the shape of sagittae between stocks of *G. morhua* and found that a large amount of the shape variation could be explained by different growth rates between the populations. Reproductive *P. notatus* in the southern population are on average smaller than *P. notatus* from the northern population (Cogliati *et al.*, 2014*a*). It would be enlightening for future studies to compare somatic growth rates and environmental correlates between the two populations. For example, changes in diet and feeding history, most notably starvation, have been shown to affect otolith shape (Gagliano & McCormick, 2004; Vignon & Morat, 2010). Some evidence does exist for differences in diet between the northern and southern *P. notatus* populations. *Porichthys notatus* in the south have access to bioluminescent prey items, a dietary source of luciferin, while *P. notatus* in the north apparently do not (Thompson & Tsuji, 1989). Interestingly, Cogliati *et al.* (2015) found that guarder males from the southern population had higher parasite loads compared to guarder males from the north. This suggests that the *P. notatus* in each population experience differing ecological conditions. Future studies may now wish to investigate how somatic growth rates differ between the two populations and the mechanisms by which this influences otolith growth, chemistry and morphology.

OTOLITH SIZE AND SHAPE DIFFERS BETWEEN SEX AND TACTIC

Reproductively active male *P. notatus* can differ dramatically in body size; the average guarder male is twice as long and eight times as heavy as the average sneaker male (Bass, 1996; Fitzpatrick *et al.*, 2016). A small amount of size overlap, however, does occur between the two types of males (L_S range, sneaker male: 9·8–16·9 cm; guarder male: 13·2–28·2 cm), while female body size (L_S range, female: 11·6–22·0 cm) overlaps with males of either tactic (Bass, 1996). Interestingly, after controlling for body size, both sneaker males and females had larger sagittae relative to their body size than did guarder males. This result is consistent with the common observation that slower growing fishes tend to possess heavier otoliths than faster growing fishes of the same body size. This pattern has been documented in numerous fish species including striped bass *Morone saxatilis* (Walbaum 1792) (Secor & Dean, 1989), guppies *Poecilia reticulata* Peters 1859 (Reznick *et al.*, 1989) and gag *Mycteroperca microlepis* (Goode & Bean 1879) (Strelcheck *et al.*, 2003). In *P. notatus*, the two male tactics differ markedly in their age at maturation, with sneaker males investing heavily into gonadal growth and maturing precociously, and guarder males investing more into somatic growth and maturing later (Bass, 1996). Like sneaker males, females must also invest heavily into reproductive growth at the expense of somatic growth. Since sneaker males and females invest less into somatic growth than do guarder males, relative somatic growth rates may be able to explain these inter-tactic and inter-sex differences in sagitta size, though this hypothesis still requires explicit testing. Bass (1996) and Forlano *et al.* (2015) have noted that for many somatic, neurological and hormonal traits, sneaker males tend to resemble females more closely than guarder males, and it appears that this relationship is upheld with respect to sagitta size as well.

The physiological costs associated with surviving the breeding season are probably vastly different between *P. notatus* guarder males, sneaker males and females. *Porichthys notatus* guarder males breed and remain in their nests to provide parental care for up to three consecutive months (Cogliati *et al.*, 2013). Over this time period, guarder males must also physically compete with one another for nest ownership and access to females, while refraining from foraging and limiting their food intake (with the exception of some egg cannibalism, Bose *et al.*, 2014; Cogliati *et al.*, 2015). Guarder males, therefore, often suffer a significant deterioration in body condition over the breeding season (Sisneros *et al.*, 2009; Bose *et al.*, 2014, 2015). Unlike guarder males, sneaker males and females are not confined to a nest and are presumably free to actively forage over the course of the breeding season. Since periods of acute stress have been associated with disruptions to the deposition of daily otolithic growth increments [coho salmon, *Oncorhynchus kisutch* (Walbaum 1792), Campana, 1983], it would be interesting to know whether otolith growth in *P. notatus* is significantly affected by the stresses and costs associated with finding and preparing a nest, breeding, providing parental care and fasting.

While no consistent differences could be found between the shapes of sagittae from sneaker males and guarder males, females had narrower sagittae than guarder males. Sex differences in otolith shape have been reported in some studies or species [*e.g. G. morhua*, Campana & Casselman, 1993; *C. harengus*, red mullet *Mullus barbatus* L. 1758, common dab *Limanda limanda* (L. 1758), Mille *et al.*, 2015], though many other studies have failed to detected any sex differences [*e.g. S. scombrus*, Castonguay *et al.*, 1991; lake trout *Salvelinus namaycush* (Walbaum 1792), Simoneau *et al.*, 2000; *G. morhua*, Cardinale *et al.*, 2004; whiting *Merlangius merlangus* (L. 1758), megrim *Lepidorhombus whiffiagonis* (Walbaum 1792), Mille *et al.*, 2015]. In general, when male−female differences in otolith shape are reported, the sex effect tends to be much smaller in magnitude, and hence harder to detect, than the differences associated with age and body size (Campana & Casselman, 1993; Campana, 2004). A common explanation for sex differences in otolith shape is that the sexes probably differ in terms of their somatic growth rates, physiology and metabolisms (Campana & Casselman, 1993; Begg & Brown, 2000; Cardinale *et al.*, 2004; Mille *et al.*, 2015). Future studies should investigate the ecological, physiological and life history differences between the sexes in *P. notatus* that might influence the otolith shape differences observed.

Alternative reproductive tactics are both common and widespread in teleosts (Knapp & Neff, 2008), yet their presence in many species is often overlooked. In some species, alternative tactics may be ignored in part due to the difficulties associated with differentiating tactics based on morphological and physiological traits alone (*i.e.* without observing any behavioural traits, Kotiaho & Tomkins, 2001; Taborsky *et al.*, 2008). Yet accounting for the presence of same-sex alternative morphs within a species can be of considerable importance, especially when alternative morphs are at risk of being misclassified. Sneaker males are often ignored or misclassified as non-reproductive males, juveniles or even females because of their frequent phenotypic similarities to these groups (Bass, 1990; Taborsky *et al.*, 2008). Although with sufficient training, *P. notatus* reproductive types and sexes can be reliably discriminated *via* external morphology, the results of this study show that sneaker males in *P. notatus* have the potential to be misclassified as females based on sagitta morphology alone, especially otolith size. Thus, it is recommended that any future efforts using otolith morphology in order to discriminate age-classes or assess population composition of a species with alternative tactics, including *P. notatus*, should acknowledge the potential differences between sexes and alternative reproductive morphs.

We would like to thank K. Cogliati, A. Hassan, J. Marentette, C. Chui, J. Reynolds, A. Mistakidis, H. Howe, H. Kou, E. Balke and N. Luymes for assistance with field collections. N. Luymes also assisted with shape data collection. We would also like to thank A. Bass and E. McCallum for helpful comments on the manuscript. Research was supported by the Natural Sciences and Engineering Research Council of Canada (A.P.H.B., S.B.), the PADI Foundation (A.P.H.B.), the McMaster University Graduate Students Association (A.P.H.B.), McMaster University's Department of Psychology, Neuroscience & Behaviour (A.P.H.B.), Ontario Innovation Trust (S.B.), Canadian Foundation for Innovation (S.B.) and the Canada Research Chairs program (S.B.).

References

Arora, H. L. (1948). Observations on the habits and early life history of the batrachoid fish, *Porichthys notatus* Girard. *Copeia* **1948,** 89−93. doi: 10.2307/1438409

Arterbery, A. S., Deitcher, D. L. & Bass, A. H. (2010). Corticosteroid receptor expression in a teleost fish that displays alternative male reproductive tactics. *General and Comparative Endocrinology* **165,** 83–90. doi: 10.1016/j.ygcen.2009.06.004

Bass, A. H. (1990). Sounds from the intertidal zone: vocalizing fish. *Bioscience* **40,** 249–258. doi: 10.2307/1311261

Bass, A. H. (1996). Shaping brain sexuality. *American Scientist* **84,** 352–363.

Bass, A. H. & Andersen, K. (1991). Intra- and intersexual dimorphisms in the sound generating motor system in a vocalizing fish: motor axon number and size. *Brain, Behavior and Evolution* **37,** 204–214.

Bass, A. H. & Marchaterre, M. A. (1989*a*). Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphism in the ultrastructure of myofibrils. *Journal of Comparative Neurology* **286,** 141–153. doi: 10.1002/cne.902860202

Bass, A. H. & Marchaterre, M. A. (1989*b*). Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphisms and general synaptology of sonic motor nucleus. *Journal of Comparative Neurology* **286,** 154–169. doi: 10.1002/cne.902860203

Bass, A. H. & McKibben, J. R. (2003). Neural mechanisms and behaviors for acoustic communication in teleost fish. *Progress in Neurobiology* **69,** 1–26.

Bass, A. H., Horvath, B. J. & Brothers, E. (1996). Nonsequential developmental trajectories lead to dimorphic vocal circuitry for males with alternative reproductive tactics. *Journal of Neurobiology* **30,** 493–504. doi: 10.1002/(SICI)1097-4695(199608)30:4<493::AID-NEU5>3.0.CO;2-Z

Begg, G. A. & Brown, R. W. (2000). Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Transactions of the American Fisheries Society* **129,** 935–945. doi: 10.1577/1548-8659(2000)129<0935:SIOHMA>2.3.CO;2

Berejikian, B. A., Van Doornik, D. M., Endicott, R. C., Hoffnagle, T. L., Tezak, E. P., Moore, M. E. & Atkins, J. (2010). Mating success of alternative male phenotypes and evidence for frequency-dependent selection in Chinook salmon, *Oncorhynchus tshawytscha*. *Canadian Journal of Fisheries and Aquatic Sciences* **67,** 1933–1941. doi: 10.1139/F10-112

Bird, J. L., Eppler, D. T. & Checkley, D. M. Jr. (1986). Comparisons of herring otoliths using Fourier series shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **43,** 1228–1234. doi: 10.1139/f86-152

Bose, A. P., Cogliati, K. M., Howe, H. S. & Balshine, S. (2014). Factors influencing cannibalism in the plainfin midshipman fish. *Animal Behaviour* **96,** 159–166. doi: 10.1016/j.anbehav.2014.08.008

Bose, A. P., McClelland, G. B. & Balshine, S. (2015). Cannibalism, competition, and costly care in the plainfin midshipman fish, *Porichthys notatus*. *Behavioral Ecology* **27,** 628–636. doi: 10.1093/beheco/arv203

Bose, A. P., Kou, H. H. & Balshine, S. (2016). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish. *Behavioral Ecology* **27,** 1507–1514. doi: 10.1093/beheco/arw075

Brantley, R. K. & Bass, A. H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). *Ethology* **96,** 213–232. doi: 10.1111/j.1439-0310.1994.tb01011.x

Brantley, R. K., Wingfield, J. C. & Bass, A. H. (1993). Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Hormones and Behavior* **27,** 332–347. doi: 10.1006/hbeh.1993.1025

Campana, S. E. (1983). Calcium deposition and otolith check formation during periods of stress in coho salmon, *Oncorhynchus kisutch*. *Comparative Biochemistry and Physiology. Part A, Physiology* **75,** 215–220. doi: 10.1016/0300-9629(83)90073-7

Campana, S. E. & Casselman, J. M. (1993). Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 1062–1083. doi: 10.1139/f93-123

Cardinale, M., Doering-Arjes, P., Kastowsky, M. & Mosegaard, H. (2004). Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **61,** 158–167. doi: 10.1139/f03-151

Castonguay, M., Simard, P. & Gagnon, P. (1991). Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. *Canadian Journal of Fisheries and Aquatic Sciences* **48,** 296–302. doi: 10.1139/f91-041

Cogliati, K. M., Neff, B. D. & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics. *Behavioral Ecology and Sociobiology* **67,** 399–408. doi: 10.1007/s00265-012-1460-y

Cogliati, K. M., Mistakidis, A. F., Marentette, J. R., Lau, A., Bolker, B. M., Neff, B. D. & Balshine, S. (2014*a*). Comparing population level sexual selection in a species with alternative reproductive tactics. *Behavioral Ecology* **25,** 1524–1533. doi: 10.1093/beheco/aru147

Cogliati, K. M., Balshine, S. & Neff, B. D. (2014*b*). Competition and cuckoldry: estimating fitness of alternative reproductive tactics in plainfin midshipman. *Behaviour* **151,** 1209–1227. doi: 10.1163/1568539X-00003180

Cogliati, K. M., Danukarjanto, C., Pereira, A. C., Lau, M. J., Hassan, A., Mistakidis, A. F., Bolker, B. M., Neff, B. D. & Balshine, S. (2015). Diet and cannibalism in plainfin midshipman *Porichthys notatus*. *Journal of Fish Biology* **86,** 1396–1415. doi: 10.1111/jfb.12649

DeMartini, E. E. (1988). Spawning success of the male plainfin midshipman. I. Influences of male body size and area of spawning site. *Journal of Experimental Marine Biology and Ecology* **121,** 177–192. doi: 10.1016/0022-0981(88)90254-7

Fitzpatrick, J. L., Earn, D. J., Bucking, C., Craig, P. M., Nadella, S., Wood, C. M. & Balshine, S. (2016). Postcopulatory consequences of female mate choice in a fish with alternative reproductive tactics. *Behavioral Ecology* **27,** 312–320. doi: 10.1093/beheco/arv159

Forlano, P. M., Sisneros, J. A., Rohmann, K. N. & Bass, A. H. (2015). Neuroendocrine control of seasonal plasticity in the auditory and vocal systems of fish. *Frontiers in Neuroendocrinology* **37,** 129–145. doi: 10.1016/j.yfrne.2014.08.002

Furuta, N., Ninomiya, S., Takahashi, S., Ohmori, H. & Ukai, Y. (1995). Quantitative evaluation of soybean (*Glycine max* L., Merr.) leaflet shape by principal component scores based on elliptic Fourier descriptor. *Breeding Science* **45,** 315–320. doi: 10.1270/jsbbs1951.45.315

Gagliano, M. & McCormick, M. I. (2004). Feeding history influences otolith shape in tropical fish. *Marine Ecology Progress Series* **278,** 291–296.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal* **50,** 346–363. doi: 10.1002/bimj.200810425

Ibara, R. M., Penny, L. T., Ebeling, A. W., van Dykhuizen, G. & Cailliet, G. (1983). The mating call of the plainfin midshipman fish, *Porichthys notatus*. In *Predators and Prey in Fishes* (Noakes, D. L. G., Lindquist, D. G., Helfman, G. S. & Ward, J. A., eds), pp. 205–212. Dordrecht: Springer.

Iwata, H. & Ukai, Y. (2002). SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity* **93,** 384–385. doi: 10.1093/jhered/93.5.384

Jackson, D. A. (1993). Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* **74,** 2204–2214. doi: 10.2307/1939574

Jones, J. W. (1959). *The Salmon*. New York, NY: Harper & Brothers.

Knapp, R. & Neff, B. D. (2008). Alternative reproductive tactics in fishes. In *Fish Behaviour* (Magnhagen, C., Braithwaite, V. A., Forsgren, E. & Kapoor, B. G., eds), pp. 411–433. Enfield, NH: Science Publishers Inc..

Kotiaho, J. S. & Tomkins, J. L. (2001). The discrimination of alternative male morphologies. *Behavioral Ecology* **12,** 553–557. doi: 10.1093/beheco/12.5.553

Kuhl, F. P. & Giardina, C. R. (1982). Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* **18,** 236–258. doi: 10.1016/0146-664X(82)90034-X

L'Abée-Lund, J. H. & Jensen, A. J. (1993). Otoliths as natural tags in the systematics of salmonids. *Environmental Biology of Fishes* **36,** 389–393. doi: 10.1007/BF00012418

Lombarte, A. & Castellón, A. (1991). Interspecific and intraspecific otolith variability in the genus *Merluccius* as determined by image analysis. *Canadian Journal of Zoology* **69,** 2442–2449. doi: 10.1139/z91-343

Lombarte, A. & Lleonart, J. (1993). Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes* **37,** 297–306. doi: 10.1007/BF00004637

Lychakov, D. V. & Rebane, Y. T. (2005). Fish otolith mass asymmetry: morphometry and influence on acoustic functionality. *Hearing Research* **201,** 55–69. doi: 10.1016/j.heares.2004.08.017

McIver, E., Marchaterre, M. A., Rice, A. N. & Bass, A. H. (2014). Novel underwater soundscape: acoustic repertoire of plainfin midshipman fish. *Journal of Experimental Biology* **217,** 2377–2389. doi: 10.1242/jeb.102772

McKibben, J. R. & Bass, A. H. (1998). Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. *Journal of the Acoustical Society of America* **104,** 3520–3533. doi: 10.1121/1.423938

Mille, T., Mahe, K., Villanueva, M. C., De Pontual, H. & Ernande, B. (2015). Sagittal otolith morphogenesis asymmetry in marine fishes. *Journal of Fish Biology* **87,** 646–663. doi: 10.1111/jfb.12746

Miller, D. J. & Lea, R. N. (1972). Guide to the coastal marine fishes of California. *California Department of Fish and Game Bulletin* **157,** 72–73.

Neff, B. D. & Svensson, E. I. (2013). Polyandry and alternative mating tactics. *Philosophical Transactions of the Royal Society B* **368,** 20120045. doi: 10.1098/rstb.2012.0045

Paxton, J. R. (2000). Fish otoliths: do sizes correlate with taxonomic group, habitat and/or luminescence? *Philosophical Transactions of the Royal Society B* **355,** 1299–1303. doi: 10.1098/rstb.2000.0688

Popper, A. N. & Fay, R. R. (1993). Sound detection and processing by fish: critical review and major research questions (part 1 of 2). *Brain, Behavior and Evolution* **41,** 14–25. doi: 10.1159/000113821

Popper, A. N. & Lu, Z. (2000). Structure–function relationships in fish otolith organs. *Fisheries Research* **46,** 15–25. doi: 10.1016/S0165-7836(00)00129-6

Popper, A. N., Ramcharitar, J. & Campana, S. E. (2005). Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine and Freshwater Research* **56,** 497–504. doi: 10.1071/MF04267

Reznick, D., Lindbeck, E. & Bryga, H. (1989). Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 108–112. doi: 10.1139/f89-014

Schellart, N. A. M. & Popper, A. N. (1992). Evolution of the auditory system in fish. In *The Evolutionary Biology of Hearing* (Webster, D. B., Fay, R. R. & Popper, A. N., eds), pp. 295–322. New York, NY: Springer New York.

Secor, D. H. & Dean, J. M. (1989). Somatic growth effects on the otolith-fish size relationship in young pond-reared striped bass, *Morone saxatilis*. *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 113–121. doi: 10.1139/f89-015

Simoneau, M., Casselman, J. M. & Fortin, R. (2000). Determining the effect of negative allometry (length/height relationship) on variation in otolith shape in lake trout (*Salvelinus namaycush*), using Fourier-series analysis. *Canadian Journal of Zoology* **78,** 1597–1603. doi: 10.1139/z00-093

Sisneros, J. A., Alderks, P. W., Leon, K. & Sniffen, B. (2009). Morphometric changes associated with the reproductive cycle and behaviour of the intertidal-nesting, male plainfin midshipman *Porichthys notatus*. *Journal of Fish Biology* **74,** 18–36. doi: 10.1111/j.1095-8649.2008.02104.x

Strelcheck, A. J., Fitzhugh, G. R., Coleman, F. C. & Koenig, C. C. (2003). Otolith–fish size relationship in juvenile gag (*Mycteroperca microlepis*) of the eastern Gulf of Mexico: a comparison of growth rates between laboratory and field populations. *Fisheries Research* **60,** 255–265. doi: 10.1016/S0165-7836(02)00171-6

Taborsky, M., Oliveira, R. F. & Brockmann, H. J. (2008). The evolution of alternative reproductive tactics: concepts and questions. In *Alternative Reproductive Tactics: An Integrative Approach* (Taborsky, M., Oliveira, R. F. & Brockmann, H. J., eds), pp. 1–21. Cambridge: Cambridge University Press.

Thomaz, D. M. P. F., Beall, E. & Burke, T. (1997). Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceedings of the Royal Society of London B* **264,** 219–226. doi: 10.1098/rspb.1997.0031

Thompson, E. M. & Tsuji, F. I. (1989). Two populations of the marine fish *Porichthys notatus*, one lacking in luciferin essential for bioluminescence. *Marine Biology* **102,** 161–165. doi: 10.1007/BF00428276

Tracey, S. R., Lyle, J. M. & Duhamel, G. (2006). Application of elliptical Fourier analysis of otolith form as a tool for stock identification. *Fisheries Research* **77,** 138–147. doi: 10.1016/j.fishres.2005.10.013

Tuset, V. M., Lombarte, A., Gonzalez, J. A., Pertusa, J. F. & Lorente, M. (2003). Comparative morphology of the sagittal otolith in *Serranus* spp. *Journal of Fish Biology* **63,** 1491–1504. doi: 10.1111/j.1095-8649.2003.00262.x

Tuset, V. M., Imondi, R., Aguado, G., Otero-Ferrer, J. L., Santschi, L., Lombarte, A. & Love, M. (2015). Otolith patterns of rockfishes from the northeastern pacific. *Journal of Morphology* **276,** 458–469. doi: 10.1002/jmor.20353

Vignon, M. & Morat, F. (2010). Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Marine Ecology Progress Series* **411,** 231–241. doi: 10.3354/meps08651

Walker, H. J. Jr. & Rosenblatt, R. H. (1988). Pacific toadfishes of the genus *Porichthys* (Batrachoididae) with descriptions of three new species. *Copeia* **1988,** 887–904. doi: 10.2307/1445712

Warner, J. A. & Case, J. F. (1980). The zoogeography and dietary induction of bioluminescence in the midshipman fish, *Porichthys notatus*. *The Biological Bulletin* **159,** 231–246. doi: 10.2307/1541021

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer New York.

Electronic References

Campana, S. E. (2004). *Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean Canadian Special Publication of Fisheries and Aquatic Sciences No. 133*. NRC Research Press. Available at http://publications.gc.ca/collections/collection_2016/mpo-dfo/Fs41-31-133-eng.pdf/

Collette, B., Acero, A., Betancur, R., Cotto, A. & Rojas, P. (2010). *Porichthys notatus*. The IUCN Red List of Threatened Species. Version 2014.3. Available at www.iucnredlist.org

Harvey, J. T., Loughlin, T. R., Perez, M. A. & Oxman, D. S. (2000). Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean. Seattle, WA, NOAA/National Marine Fisheries Service (NOAA Technical Report NMFS, 150). Available at https://core.ac.uk/download/files/331/11018240.pdf/

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2015). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-121. Available at http://CRAN.R-project.org/package=nlme/