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# Growth and otolith morphology vary with alternative reproductive tactics and contaminant exposure in the round goby *Neogobius melanostomus*

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Round goby Neogobius melanostomus sagittal (saccular) otolith morphology was compared between males of the two alternative reproductive tactics (termed guarder and sneaker males) and between males captured from sites of high or low contamination. Otolith size increased with fish size and also displayed an ontogenetic shift in shape, becoming relatively taller as otoliths grew in size. Despite a considerable overlap in age between males adopting the two reproductive tactics, size-at-age measurements revealed that guarder males are significantly larger than sneakers at any given age and that they invest more into somatic growth than sneaker males. Controlling for body size, sneaker males possessed heavier sagittal otoliths than guarder males. Subtle otolith shape differences were also found between the two male tactics and between sites of high and low contaminant exposure. Sneaker males had relatively shorter otoliths with more pronounced notching than guarder males. Fish captured at sites of high contamination had otoliths showing slower growth rates in relation to body size and their shapes had more pronounced caudal points and ventral protrusions when compared with fish captured at sites of low contamination. The results are discussed in relation to life-history tradeoffs between the male tactics in terms of reproductive and somatic investment as well as the putative metabolic costs of exposure to contaminants. Overall, this study reveals that male alternative reproductive tactics and environmental contaminants can have small, yet measurable, effects on otolith morphology and these factors should be accounted for in future research when possible.

### KEYWORDS

Great Lakes, Hamilton Harbour, invasive species, *Neogobius melanostomus*, size at age, somatic growth

# 1 | INTRODUCTION

Teleost otoliths have provided a powerful lens through which to better understand fish biology and ecology (Popper *et al.*, 2005). By studying the structure and chemical composition of otoliths, researchers have extracted a wealth of valuable information about fish age (Campana & Thorrold, 2001), metabolism and somatic growth rates (Armstrong *et al.*, 2004; Colloca *et al.*, 2003; Hoff & Fuiman, 1993), the timing of major life-history events (Neilson *et al.*, 1985; Rehberg-Haas *et al.*, 2012) and even the chemical composition of the environment (Elsdon & Gillanders, 2003, 2004). In particular, the saccular (sagittal) otolith, one of three paired otoliths found within the inner ear, displays high interspecific variation in shape and size and relatively less intraspecific variation, making it useful for discriminating between fish species (Campana, 2004; Schellart & Popper, 1992; Tuset *et al.*, 2003). For example, sagittal otolith morphology (*i.e.*, size and shape) has been used to identify various prey species and their size classes in the diets of predators based on recovered undigested otoliths from faecal samples (Radhakrishnan *et al.*, 2010; Suter & Morel, 1996). Though otolith shape generally varies less within-

urnal of **FISH**BIOLOGY

species compared with between-species, intraspecific variation in sagittal otolith shape has also been used to discriminate between fish stocks, populations, sexes and age-classes in many species (*e.g.*, Atlantic cod *Gadus morhua* L. 1758; Campana & Casselman, 1993; Cardinale *et al.*, 2004; haddock *Melanogrammus aeglefinus* (L. 1758); Begg & Brown, 2000; red snapper *Lutjanus campechanus* (Poey 1860); Beyer & Szedlmayer, 2010; plainfin midshipman *Porichthys notatus* Girard 1854; Bose *et al.*, 2016). However, in order to confidently use otolith morphology to discriminate between species, populations, sexes, age classes, *etc.*, it is important to understand the numerous factors that can affect otolith size and shape (Mille *et al.*, 2015).

Otolith morphology is influenced by both genetic and environmental factors (Vignon & Morat, 2010). Numerous factors such as diet (Strelcheck *et al.*, 2003), rearing temperature (Hoff & Fuiman, 1993) and habitat depth (Lombarte & Lleonart, 1993; Tuset *et al.*, 2003, 2015) have been shown to correlate with otolith size and shape within species, probably due to their influence on metabolism and growth. In this study, two additional factors were investigated to assess their potential effects on sagittal otolith morphology: male alternative reproductive tactics and environmental pollutants. How these factors affect otolith morphology has received minimal research attention to date. This is rather surprising because many commercially-important fishes, such as salmonids, express distinct male alternative reproductive tactics (Berejikian *et al.*, 2011; Gombar *et al.*, 2017; Jones, 1959) and anthropogenic pollution is of increasing concern for aquatic ecosystems (Bernhardt *et al.*, 2017; Strayer & Dudgeon, 2010).

Alternative reproductive tactics (ART) occur in species when individuals of one sex express two or more distinct reproductive morphs, with each morph attempting to gain reproduction through alternative means (Gross, 1996; Neff & Svensson, 2013; Taborsky et al., 2008). In most species with ARTs, males display a discontinuous distribution of phenotypic traits, which results in two or more reproductive morphs persisting together in a population that are morphologically, physiologically, or behaviourally distinct from one another. One morph is typically classified as the conventional morph (often called the guarder, parental or bourgeois morph) and is characterized by being large, aggressive, territorial and acquires reproduction by courting and physically competing for females, while the alternative morph (often called the sneaker or parasitic morph) is typically small, non-aggressive and secures reproduction by stealth, cuckoldry, or forced copulations (Knapp & Neff, 2008; Taborsky et al., 2008). Otolith morphology is known to differ between males adopting either of two alternative reproductive tactics in P. notatus (Bose et al., 2016). Alternative reproductive tactics could therefore be an important source of intraspecific variation in otolith morphology that warrants further investigation.

ARTs can arise through various pathways. Tactics may be flexibly expressed (such that individuals can switch between tactics throughout their lifespan depending on current physiological, ecological, or social condition, *e.g.*, guppy *Poecilia reticulata* Peters 1859 (Godin, 1995)) or tactics can become established at an early age and be fixed for life (*e.g.*, bluegill sunfish *Lepomis macrochirus* Rafinesque 1819 (Gross, 1984, 1991); *P. notatus* (Bass *et al.*, 1996)). Tactics may also be expressed sequentially across a lifetime, such that individuals adopt one tactic early in life and then switch to another tactic later in life (*e.g.*, Mediterranean wrasse *Symphodus ocellatus* (L. 1758); Alonzo *et al.*, 2000). Because otolith morphology does not alternate or change rapidly on the same time scales as behaviour, flexible tactics are not expected to be associated with specific otolith morphologies. However, otolith morphology is known to change ontogenetically (Campana, 2004); consequently, males adopting sequential tactics may differ in otolith morphology simply due to their inherent age differences. Lastly, in species with fixed ARTs, suites of traits can coevolve rather independently between the tactics if intra-locus tactical conflict is low or resolved (Morris *et al.*, 2013; Pike *et al.*, 2017). Thus, otolith morphology could also differ between males expressing fixed ARTs irrespective of their age (Bose *et al.*, 2016).

Many environmental stressors, such as pollutants, affect metabolism and therefore have the potential to alter teleost otolith growth and morphology. For example, exposure to textile factory effluent resulted in African catfish *Clarias gariepinus* (Burchell 1822), having smaller sagittal otoliths for their body size than non-exposed fish (Adeogun & Chukwuka, 2011). Other environmental stressors, such as parasite load, can also affect resource allocation and growth and influence sagittal otolith shape (*e.g.*, European eel *Anguilla anguilla* (L. 1758); Sahyoun *et al.*, 2007). Because otolith morphology plays a large role in many aspects of fisheries biology and many fishes live and reproduce in environments that are affected by environmental stressors, more research is needed to evaluate the relationship between environmental contamination and otolith size and shape.

In this study, the effects of male ARTs and contaminant exposure on sagittal otolith size and shape were investigated in the round goby Neogobius melanostomus (Pallas 1814). Neogobius melanostomus is a convenient study species for four reasons. First, N. melanostomus male ARTs have been described morphologically and physiologically and are easily categorized into two distinct morphs, a conventional guarder tactic and a parasitic sneaker tactic (Bleeker et al., 2017; Marentette et al., 2009). Although males may not be capable of rapidly switching between tactics, it is currently not known if the two tactics represent sequential or fixed strategies. Second, numerous previous studies of N. melanostomus have revealed considerable variation in body size within age classes (Duan et al., 2016; French & Black, 2009; Gümüs & Kurt, 2009; Huo et al., 2014; MacInnis & Corkum, 2000; Sokołowska & Fey, 2011). Unfortunately, none of these previous studies accounted for the presence of male alternative reproductive tactics. Third, N. melanostomus can be readily collected from both highly polluted and less polluted sites. In this study, fish were collected from sites in Hamilton Harbour, Ontario, Canada, an area of heterogeneous sediment contamination based on historical industrial steel production, urban run-off and combined sewer overflows (Hamilton Harbour Remedial Action Plan, 1992, 2002; Zeman, 2009). The movement and migration patterns of N. melanostomus still require in-depth study, though several early mark-recapture studies have suggested that guarder males are philopatric during their spring- summer breeding season and possess small home ranges of c.  $5 \text{ m}^2$ (Marentette et al., 2011; Ray & Corkum, 2001). Philopatry combined with a benthic lifestyle means that N. melanostomus comes in direct contact with (contaminated) sediments and can remain in polluted areas for long periods of time (Marentette et al., 2010). Fourth, as an invasive species in the Laurentian Great Lakes and in western Europe, considerable research efforts have been made to uncover how

journal of **FISH**BIOLOGY

*N. melanostomus* might influence native ecosystems and food webs and what can be done to mitigate their effect (*e.g.*, Johnson *et al.*, 2010; Kornis *et al.*, 2012; Taraborelli *et al.*, 2010). Thus, understanding what factors influence *N. melanostomus* populations and their growth and age more generally is highly pertinent to current research on this invasive fish.

In this study, three analyses were conducted. One, to gain insights into whether ARTs in *N. melanostomus* are fixed or sequential, fish size-at-age data were examined for overlap in age between the two tactics. Two, to determine if males adopting different ARTs vary in sagittal otolith morphology, otolith size and shape were quantified and compared. Three, to uncover if contamination levels are reflected in otolith morphology, otolith size and shape were quantified and compared between fish collected from sites of high or low contamination.

# 2 | MATERIALS AND METHODS

### 2.1 | Field collections

Reproductive adult male N. melanostomus were collected between May and November 2016 (n = 341) from seven field sites in and near Hamilton Harbour, Canada: LaSalle Park, Grindstone Creek, Desjardins Canal, Fisherman's Pier, Pier 27, Sherman Inlet and Fifty Point Marina (43° N; 79° W). Two of these locations, Sherman Inlet and Pier 27, were sites of considerable industrial contamination from historical steel processing, urban run-off and combined sewer overflows (Hamilton Harbour Remedial Action Plan, 1992, 2002) and were thus classified as high contamination sites. Sediments at these sites contain high concentrations of metals (e.g., cadmium, zinc, lead), polyaromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) that exceed provincial guidelines for "probable effect levels" (PEL; Milani et al., 2017; Neff et al., 2016). Specimens collected from these areas have higher tissue burdens of metals, are smaller in body size, show evidence of endocrine disruption via internal and external male intersex, pelvic fin erosion and behavioural perturbations compared with N. melanostomus collected from less contaminated sites (Marentette et al., 2010, 2012; McCallum et al., 2014). The remaining five sites, LaSalle Park, Grindstone Creek, Desjardins Canal, Fisherman's Pier and Fifty Point Marina, are relatively less affected and were therefore classified as low-contamination sites (Marentette et al., 2010;

McCallum *et al.*, 2014). Specimens were caught using minnow traps baited with *c*. 25 g of frozen corn and placed in the near-shore habitat, following Young *et al.* (2010) and McCallum *et al.* (2014). The traps were recovered 3–24 h later. All fish were euthanized with an overdose of 0.025% benzocaine (Sigma Aldrich; www.sigmaaldrich.com) immediately upon capture, measured for total body mass (*M*, 0.001 g) and standard length ( $L_s$ , 0.01 cm). The reproductive tactic of each male was assigned based on external morphology and later confirmed during dissection by examining the testes, seminal vesicles and calculating gonadosomatic index ( $I_G$ ; males with  $I_G > 1\%$  are considered reproductive: Marentette & Corkum, 2008, Marentette *et al.*, 2009; Zeyl *et al.*, 2014). During dissection, the two sagittal otoliths were extracted, wiped clean and stored dry (Figure 1). Any otoliths that were chipped or broken during the handling process were not used.

# 2.2 | Aging N. melanostomus sagittal otoliths

Neogobius melanostomus males of both reproductive tactics were captured from clean and contaminated sites between May and November in 2007 and 2008 using the methods described above. These males were aged from whole sagittal otoliths by an experienced otolith reader with no prior knowledge of the N. melanostomus tactics or site of origin (15 guarders and 16 sneakers in 2007, 19 guarders and 30 sneakers in 2008). Fish were selected randomly from each of five body size categories based on total length ( $L_T$ ; delimited by the 20th, 40th, 60th, 80th and 100th percentiles). This selection ensured proportionate representation across all body sizes. Otoliths were cleaned, immersed in water and viewed under transmitted light at ×80 magnification with a Leica GZ6 stereomicroscope (www.leica.com). Annuli of the otoliths were enumerated from the core to the edge and the type and amount of growth at the edge noted. A single age estimate was obtained for each fish. A linear model (LM) was fit including  $L_s$  as the response variable and age estimate, male reproductive tactic, site contamination level and year as predictor variables. All interaction terms were investigated and dropped from the models if non-significant.

### 2.3 | Quantifying sagittal otolith size

The samples collected in 2016 comprised 630 otoliths (289 pairs of otoliths, 52 unpaired otoliths) from 341 males (154 guarders and 74 sneakers from clean sites; 53 guarders and 60 sneakers from



**FIGURE 1** Examples of round goby *Neogobius melanostomus* sagittal otoliths displaying size and shape variation. The otolith surface that contacts the auditory sensory epithelium, *i.e.*, the sulcus acousticus, is facing down

contaminated sites). Each otolith was weighed in duplicate using a digital microbalance (to the nearest 0.0001 g, Mettler Toledo, AB204-S/ FACT; www.mt.com) and then photographed against a black background under a dissection microscope (Nikon SMZ1500 scope; www. nikon.com) at ×10 magnification (Q Imaging Retiga 2000R Fast 1394 CCD camera; www.gimaging.com) next to a  $0.35 \times 0.35$  cm square to provide scale. A summary of the fish and otoliths collected from each field site is given in Table 1. A linear mixed-effects model (LMM) using the lme4 package in R (www.r-project.org; Bates et al. 2015) was used to test for differences in otolith size between reproductive tactics and between sites of high and low contamination. Log-transformed otolith mass  $(M_{\Omega})$  was the response variable, with male alternative reproductive tactic, contamination level and their interaction as predictor variables and log L<sub>s</sub> was included as a covariate. Field site was included as a random intercept, as was fish ID, because most fish in the analysis contributed two otoliths.

# 2.4 | Quantifying sagittal otolith shape

The digital photographs of sagittal otoliths were used to calculate the surface area of each otolith (to the nearest 0.0001 cm<sup>2</sup>) with the shape analysis software package SHAPE 1.3 (Iwata & Ukai, 2002). All images of left otoliths were also flipped horizontally to be in the same orientation as right otoliths before their shape was quantified using the elliptic Fourier PCA provided by SHAPE. SHAPE uses elliptic Fourier shape functions, which can accurately capture shape information about enclosed contours (e.g., otoliths) in great detail (Kuhl & Giardina, 1982). The analysis describes contours as the sum of multiple ellipses, or harmonics. Given an increasing number of harmonics, the shape of any fully enclosed contour may be described with ever-improving accuracy (Kuhl & Giardina, 1982). In this study, a total of 15 harmonics were used to describe the shape of each N. melanostomus otolith. This number of harmonics was obtained by using a Fourier power spectrum to determine how many harmonics would capture at least 99.9999% of the otolith shape (following Crampton, 1995). A PCA DURNAL OF **FISH**BIOLOGY

based on a variance-covariance matrix was then implemented to reduce the large number of coefficients into a more manageable number of principal components, each of which describes a different aspect of otolith shape (for further details on methodology see Crampton, 1995 and Iwata & Ukai, 2002). Four principal components were found to be significant as determined by the broken-stick model (Jackson, 1993) and a scree plot. While the scree plot displays how much variation is explained by each principal component, the broken stick model indicates the variation that would be explained by each component by chance alone.

The PCA summarized the sagittal otolith shape variation from the elliptic Fourier shape analysis into four principal components (Figure 2 (a)) explaining 32.9, 22.7, 10.9 and 7.3% of the total variation in shape data (respective Eigen values are 1.91e–3, 1.32e–3, 6.30e–4 and 4.21e–4). Otolith shape variation (generated in SHAPE following Furuta *et al.*, 1995) described by each PC is shown in Figure 2(b).

Otolith shape was compared between male alternative reproductive tactics and sites of high and low contamination by fitting a linear mixed-effects model for each significant principal component and applying a conservative Bonferroni correction to maintain the familywise error rate at 0.05. Principal component scores were modelled as response variables, while male alternative reproductive tactic (i.e., guarder v. sneaker), site type (i.e., high v. low contamination), otolith surface area and all three of their pairwise interactions were included as predictors. Field site and fish ID were both included as random intercepts. Non-significant interaction terms were dropped from the models. Yeo-Johnson power transformations were applied to the response variables to optimize the normality of each model's residuals (Yeo & Johnson, 2000). Heteroscedasticity in the data (i.e., variance in shape tends to increase as otoliths get larger) was accounted for by adding variance functions to the models that were selected to minimize model AIC. All statistical analyses in this study were conducted in R 3.3.1, (www.R-project.org).

**TABLE 1** Summary of Neogobius melanostomus samples collected from field sites in Hamilton Harbour, Canada. Shaded regions indicate sites of high contamination and unshaded regions indicate sites of low contamination

|                    | Male spawning tactic | n    |          | L <sub>s</sub> (cm)               |          | M <sub>O</sub> (mg)              |         |
|--------------------|----------------------|------|----------|-----------------------------------|----------|----------------------------------|---------|
|                    |                      | Fish | Otoliths | Mean $\pm$ S.D.                   | Range    | Mean $\pm$ S.D.                  | Range   |
| LaSalle Park       | Guarder              | 49   | 89       | $\textbf{8.41} \pm \textbf{1.43}$ | 5.9-11.9 | 4.0 ±1.63                        | 1.4-8.1 |
|                    | Sneaker              | 35   | 65       | $\textbf{5.93} \pm \textbf{1.03}$ | 4.1-9.5  | $2.0 \pm 0.80$                   | 1.0-5.4 |
| Grindstone Creek   | Guarder              | 5    | 10       | $8.00 \pm 0.72$                   | 7.0-8.9  | $3.0 \pm 0.37$                   | 2.4-3.5 |
|                    | Sneaker              | 2    | 4        | $6.25 \pm 0.06$                   | 6.2-6.3  | $\textbf{2.3} \pm \textbf{0.13}$ | 2.1-2.4 |
| Desjardins Canal   | Guarder              | 14   | 26       | $\textbf{7.53} \pm \textbf{1.14}$ | 6.1-10.5 | $\textbf{3.2} \pm \textbf{1.19}$ | 2.3-6.7 |
|                    | Sneaker              | 5    | 7        | $\textbf{6.11} \pm \textbf{1.42}$ | 4.5-8.1  | $\textbf{2.0} \pm \textbf{0.64}$ | 1.1-2.8 |
| Fisherman's Pier   | Guarder              | 34   | 59       | $\textbf{9.03} \pm \textbf{1.11}$ | 6.6-11.1 | $\textbf{4.4} \pm \textbf{1.02}$ | 2.3-7.0 |
|                    | Sneaker              | 12   | 23       | $\textbf{6.26} \pm \textbf{1.10}$ | 5.0-9.1  | $\textbf{2.4} \pm \textbf{0.86}$ | 1.3-4.7 |
| Fifty Point Marina | Guarder              | 52   | 97       | $\textbf{9.05} \pm \textbf{1.30}$ | 6.8-11.6 | $\textbf{4.8} \pm \textbf{1.48}$ | 2.6-7.9 |
|                    | Sneaker              | 20   | 38       | $\textbf{6.91} \pm \textbf{0.86}$ | 5.7-8.5  | $\textbf{3.2} \pm \textbf{0.93}$ | 2.0-4.8 |
| Pier 27            | Guarder              | 28   | 53       | $\textbf{7.21} \pm \textbf{1.09}$ | 5.6-9.2  | $\textbf{3.1}\pm\textbf{0.94}$   | 1.9-5.1 |
|                    | Sneaker              | 27   | 50       | $\textbf{5.22} \pm \textbf{1.05}$ | 3.8-8.4  | $\textbf{1.8}\pm\textbf{0.66}$   | 1.0-3.4 |
| Sherman Inlet      | Guarder              | 25   | 48       | $\textbf{6.82} \pm \textbf{0.98}$ | 5.0-8.4  | $\textbf{2.4} \pm \textbf{0.69}$ | 1.6-4.2 |
|                    | Sneaker              | 33   | 61       | $\textbf{5.35} \pm \textbf{0.81}$ | 3.6-7.5  | $\textbf{1.6} \pm \textbf{0.53}$ | 0.7-3.0 |

 $L_{\rm S}$  = standard length;  $M_{\rm O}$  = ototlith mass; n = Sample size.



**FIGURE 2** (a) Scree plot illustrating how many principal components (----) describe more variation in sagittal otolith shape than by chance alone (i.e., the broken stick model, ----). The vertical dashed line represents the crossover between the data and the broken stick model. (b) Otolith contour reconstructions. Contours under the Mean column represent the average otolith shape in *Neogobius melanostomus*. Contours on either side of the mean column illustrate the effect that each PC has on otolith shape. Contours under the Overlay column allow for easier visualization of the shape variation captured by each PC. Arrows indicate areas of greatest difference

# 3 | RESULTS

Guarder males were on average larger (mean  $L_{\rm s} \pm$  s.D. = 8.2  $\pm$  1.5 cm) than sneaker males (5.8  $\pm$  1.1 cm) despite considerable overlap in age (guarder-male age range = 1–4 years, mean  $\pm$  std. dev = 1.9  $\pm$  0.7 years; sneaker-male age range = <1–3 years, mean  $\pm$  s.D. = 1.2  $\pm$  0.5 years). The males used in this analysis were selected to cover the full size range of each tactic and body sizes overlapped considerably

between the two male tactics (guarder-male  $L_s$  range = 5.0–11.9 cm, sneaker-male  $L_s$  range = 3.6–9.5 cm). Guarder males tended to display faster somatic growth than sneaker males (indicated by a steeper relationship between log $L_s$  and log(age)) though this difference did not quite reach statistical significance (LM: interaction term, t = -1.90, d.f. = 64, P = 0.063; Figure 3). Note that the two sneaker males found to be < 1 year old were estimated to be approximately 0.5 years old.



**FIGURE 3** Standard length ( $L_s$ )-age comparison (mean  $\pm$  S.D.) between sneaker-male (- $\bigcirc$ -) and guarder-male (- $\bigcirc$ -) *Neogobius melanostomus* in (a) low-contamination and (b) high-contamination environments

Otolith mass increased with fish length (Figure 4) and this relationship was significantly steeper in fish from the sites of low contamination compared with fish from sites of high contamination (LMM: interaction term, t = 2.51, d.f. = 334.2, P < 0.05). Sneaker males also possessed heavier otoliths for their body size than guarder males (Figure 4), although this difference was only apparent in sites of low contamination (LMM: interaction term, t = 2.51, d.f. = 332.2, P < 0.05). This tactic difference persists even when the model is rerun using only fish that fall in the body size overlap range between sneaker and guarder males ( $L_S$  range = 5.02–9.54 cm, LMM: body size x contamination level interaction term, t = 1.76, d.f. = 261.6, P > 0.05; tactic × contamination level interaction term, t = 2.45, d. f. = 260.4, P < 0.05).

The shape of *N. melanostomus* sagittal otoliths changed with body size. As fish grew, otoliths became relatively taller along the ventraldorsal axis (PC1, LMM, slopes differed between male tactics, guardermale slope, t = 14.01, d.f. = 287, P < 0.001; sneaker-male slope, t = 5.31, d.f. = 287, P < 0.001, Figure 5(a)). Whereas, dorsal notches (PC2, LMM: main effect slope, t = -3.14, d.f. = 288, P < 0.01, Figure 5(b)), caudal points (PC3, LMM: main effect slope, t = -3.41, d.f. = 288, P < 0.001, Figure 5(c)) and ventral protrusions (PC4, LMM, slopes differed between site types, low contamination slope, t = -4.55, d.f. = 287, P < 0.001; high contamination slope, t = -7.16, d.f. = 287, P < 0.001, Figure 5(d)) all became less pronounced as fish grew in size. Guarder-male otoliths became taller at a faster rate than sneaker-male otoliths (PC1, LMM: interaction term, t = 3.89, d.f. = 287, P < 0.001, Figure 5(a)). Sneaker-male otoliths also had a more pronounced notch along their dorsal edge than did the otoliths of guarder males (PC2, LMM: main effect, t = 2.82, d.f. = 338, P < 0.01, Figure 5(b)). Fish from high contamination sites had otoliths with more pronounced caudal points than fish from lowcontamination sites (PC3, LMM: main effect, t = -4.25, d.f. = 338, P < 0.001, Figure 5(c)). Furthermore, the ventral otolith protrusion became less pronounced at a faster rate in fish from high contamination sites than in fish from low contamination sites (PC4, LMM:



**FIGURE 4** Standard length ( $L_S$ )-sagittal otolith mass ( $M_O$ ) comparison showing that *Neogobius melanostomus* sneaker males (---) have heavier sagittal otoliths at length than guarder males (---) when collected from low contamination sites. Photographs reprinted with permission from Marentette *et al.* (2009)

IOURNAL OF **FISH**BIOLOGY

interaction term, t = -4.99, d.f. = 287, P < 0.001, Figure 5(d)). Note that with the exception of PC1, these results are robust when the models are re-run using only otoliths that fall within the size overlap between sneaker and guarder-male otoliths (otolith surface area range = 2.94–7.70 mm<sup>2</sup>) (PC1, interaction term, t = 1.19, d.f. = 228, P > 0.05; PC2, main effect, t = 3.86, d.f. = 273, P < 0.001; PC3, main effect, t = -3.93, d.f. = 273, P < 0.001; PC4, interaction term, t = 2.63, d.f. = 228, P < 0.01).

# 4 | DISCUSSION

The study of otolith size and shape offers fisheries biologists and fish ecologists a tool to investigate growth patterns across individuals, assess population structure and manage fish stocks. By gaining a more comprehensive understanding of the various factors that affect otolith size and shape, the usefulness of otolith morphology analyses will be further enhanced. For example, in many fish species the size of the sagittal otolith is known to relate strongly to fish size and this relationship can often be well-described with a simple linear regression (Harvey et al., 2000; examples for Neogobius melanostomus, Duan et al., 2016; Huo et al., 2014). Pooling all N. melanostomus from this study, the relationship between otolith mass (y; mg) and fish  $L_s$  (x; cm) is described by logy = 1.89logx - 2.68 and accounts for 90% of the variability in the data. Thus, while sagittal otolith mass can be a strong predictor of fish size, 10% still remains unexplained. The current study investigated two largely unexplored factors that can explain additional variation in sagittal otolith morphology, male ARTs and environmental contamination.

# 4.1 | Somatic growth differs between male reproductive morphs

Guarder males had larger body sizes and tended to have faster somatic growth rates (P = 0.06) than sneaker males despite a considerable overlap in age. Both male tactics also overlapped considerably in body size, which is in contrast to the findings of Bleeker et al. (2017) who found little to no overlap in the body sizes of the two male tactics in several Dutch N. melanostomus populations. The present data (overlapping ages and body sizes but different somatic growth rates) lend support to the idea that male ARTs in N. melanostomus represent two separate developmental pathways, becoming fixed within the first year of life. Whether these tactics are the result of a genetic polymorphism or the result of a genetically based developmental switch point (Oliveira et al., 2008; Shuster & Wade, 2003) still remains to be determined. It is possible that N. melanostomus sneaker males still follow a sequential pathway, beginning their lives as sneakers and eventually adopting the guardermale tactic later in life after surpassing a particular threshold. But, clearly this switch point is not based on surpassing a single population-wide body-size threshold. Rather, the switch point may differ from individual to individual or even be socially determined. More work is needed to uncover the proximate mechanisms that underlie tactic expression in N. melanostomus.



FIGURE 5 (a, b) Otolith shape compared between the two male alternative reproductive tactics of Neogobius melanostomus, sneaker males (----) and guarder males (-----). (c, d) Otolith shape compared between males from sites of high (-----) and low (-----) environmental contamination. Plots show partial residuals from linear mixed-effects models (generated using 'visreg' package in R, Breheny & Burchett 2017). Black arrows point towards the aspects of otolith shape that are primarily affected by each principal component

#### 4.2 | Sagittal otolith size varies with male reproductive tactic and environmental contamination

Although sagittal otolith mass increased with fish size, sneaker males possessed heavier otoliths after accounting for body size than did guarder males. A similar finding was documented by Bose et al. (2016) in Porichthys notatus Girard 1854, a marine toadfish that expresses two developmentally distinct fixed male ARTs that are also termed guarder and sneaker males. One explanation for why sneaker males might possess relatively heavier otoliths centres around tactic-specific somatic growth rates; a common finding among fishes is that slower growing individuals typically possess heavier otoliths compared with faster growing individuals after accounting for body size (e.g., striped bass Morone saxatilis (Walbaum 1792) (Secor & Dean, 1989); P. reticulata (Reznick et al., 1989); gag Mycteroperca microlepis (Goode & Bean 1879) (Strelcheck et al., 2003); salema Sarpa salpa L. 1758 (Abaad et al. 2016)). Individuals that invest more into somatic growth are expected to have smaller, lighter otoliths for their body size than individuals that invest less into growth (Secor & Dean, 1989). In many fish species with male ARTs, guarder males invest highly into somatic growth while sneaker males invest highly into reproductive tissues at the expense of somatic growth; Marentette et al. (2009) found that N. melanostomus guarder males were on

average 3.6 times heavier than sneaker males, but sneaker males possessed relative testes sizes that were on average 2.6 times greater than guarder males. Further studies would be valuable to examine what biological implications relative otolith size differences might have on hearing or vestibular performance between the tactics.

Environmental contamination also appeared to affect the relationship between otolith mass and fish size in N. melanostomus in this study. First, the difference in relative otolith mass between sneaker and guarder males was only apparent in sites of low contamination; no difference in relative otolith mass could be detected between males adopting the different tactics in sites of high contamination. Second, all male N. melanostomus (of both tactics) captured at sites of high contamination had slower otolith growth, given their body sizes compared with males from sites of low contamination. Many environmental contaminants and stressors are known to affect metabolism through mitochondrial toxicity (Meyer et al., 2013; Jayasundara, 2017). Environmental pollutants may also increase ATP demand for detoxification and cellular homeostasis, potentially altering otolith growth rates relative to somatic growth. In this study, N. melanostomus from sites of high contamination would have been chronically exposed to PAHs, PCBs and metals such as lead, zinc and cadmium in their environments (Milani et al., 2017; Neff et al., 2016; RAP, 1992; Zeman, 2009). Previous studies also suggest that

URNAL OF **FISH** BIOLOGY

*N. melanostomus* from these high contamination sites have decreased activity levels (Marentette *et al.*, 2012), forage less, consume fewer food items (McCallum *et al.*, 2017) and are smaller in average body size (McCallum *et al.*, 2014) than fish from low contamination sites. Thus, in order to understand the growth of *N. melanostomus* and their otoliths in sites of high and low contamination, future work will be required to evaluate the metabolic consequences of contaminants, activity and feeding and how they all interplay with the very different life history strategies adopted by each alternative male tactic.

# 4.3 | Sagittal otolith shape varies with male reproductive tactic and environmental contamination

Neogobius melanostomus otoliths became proportionally taller as they grew larger. However, the otoliths not only displayed a general ontogenetic shift in shape as the fish grew, but otolith shape followed subtly different trajectories depending on male tactic and the degree of environmental contamination. Guarder-male otoliths grew taller at a faster rate (described by PC1) and had less pronounced notches along their dorsal edges (described by PC2) than did the otoliths of sneaker males. Otoliths sampled from sites of high contamination had more pronounced caudal points (described by PC3) and less prominent ventral protrusions (described by PC4), compared with those from sites of low contamination. Ontogenetic changes in otolith shape are commonly observed across fish species, where shape tends to become progressively more species-specific as otoliths grow (Campana, 2004; Lombarte & Castellón, 1991). What biological implications these shape differences have on hearing or vestibular performance remains to be evaluated. Nevertheless, the shape differences described here in N. melanostomus are similar to those found in a previous study on P. notatus, wherein sagittal otolith shape also differed between male tactics and also differed between geographically-separated populations (Bose et al., 2016). Otolith shape is known to differ between age-classes, sexes and populations in numerous species (e.g., Atlantic mackerel Scomber scombrus L. 1758 (Castonguay et al., 1991); Gadus morhua (Campana & Casselman, 1993); silver hake Merluccius bilinearis (Mitchill 1814) (Bolles & Begg, 2000)).

Reports of otolith growth or structural alterations due to contaminant exposure are rare. Burbot Lota lota (L. 1758) living in lakes polluted with PCBs and metals have shown crenulations or lace-like growths on sagittal otoliths not present in other populations (Pulliainen & Korhonen, 1994). Alterations to otolith shape could impair hearing in N. melanostomus, which rely, in part, on sound to detect predators and communicate with conspecifics (Rollo & Higgs, 2008). Also, fish with otolith malformations have been shown to have altered behavioural responses to feeding stimuli, as well as increased cortisol levels relative to fish with normal otoliths (e.g., red drum Sciaenops ocellatus (L. 1766) (Browning et al., 2012)). It would be valuable to conduct controlled laboratory studies rearing juveniles under exposure to various contaminants as such studies would uncover which environmental pollutants most drastically influence otolith morphology and what biological consequences emerge.

In summary, the use of otolith morphology as a means to discriminate fish based on factors such as age, body size, sex and population requires a strong understanding of all the factors that can influence otolith size and shape (Mille et al., 2015). The present study makes use of N. melanostomus to show that the relationships between sagittal otolith morphology and fish size can depend in-part on male reproductive tactic and the level of environmental contamination. It should be stressed, however, that the effect sizes uncovered here are small. Otolith shape displayed a high amount of variation that overlapped between male tactics, degrees of environmental contamination and across body sizes. As such, using otolith morphology alone to predict male tactic and contaminant exposure history would be difficult. Nevertheless, future studies on N. melanostomus could benefit by accounting for variation between male ARTs as well as anthropogenic pollutants (or other environmental stressors). For example, accounting for this variation is important when attempting to use otolith morphology to infer specific details about individual fish, such as their body size. Furthermore, it has been common practice for studies to estimate N. melanostomus size at age by using back-calculation techniques (Duan et al., 2016; French & Black, 2009; Gümüs & Kurt, 2009; Huo et al., 2014; MacInnis & Corkum, 2000; Sokołowska & Fey, 2011), the present study suggests that the efficiency of these techniques could be improved by considering guarder males separately from sneaker males. While alternative tactics are widespread among fishes and anthropogenic effects on aquatic ecosystems are a growing concern, their effects on otolith morphology have only rarely been studied. Future studies using additional species and exposure regimes would therefore be valuable to further characterize the effects of ARTs and contaminants on otolith morphology.

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### Author contributions

The study was conceived by A.P.H.B. and S.B. Data were collected by all co-authors. A.P.H.B. conducted the formal statistical analyses. A.P.H.B. and E.S.M. jointly wrote the first draft of the manuscript and all co-authors contributed to editing and revisions.

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BOSE ET AL.

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