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Otolith chemistry of triplefn *Forsterygion nigripenne* **indicates estuarine residency**

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Abstract

Understanding the role diferent habitats play in the life histories of fshes is important for the development of holistic aquatic ecosystem management plans. We used otolith trace element analysis to reconstruct the life history of estuarine triplefn *Forsterygion nigripenne* and infer its habitat use. Analysis of otoliths using laser ablation inductively coupled plasma mass spectrometry showed an elemental profle without prominent changes in elemental concentration. The otolith elemental profles were not indicative of movement between waters with substantial Sr:Ca and Ba:Ca diferences. The Sr:Ca and Ba:Ca profles were indicative of within—and near—estuary movements encompassing short time periods. Overall, the otolith microchemical profile was likely indicative of a life history closely associated with estuaries. However, it is difficult to rule out brief excursions to the marine environment given that may not have been of sufficient duration to be recorded in the elemental composition of the otolith. This suggests that *F. nigripenne* is the only species in southern New Zealand known to be closely associated with estuarine habitat for its entire life cycle.

Keywords Life history · Estuary · Otolith · Chemistry · Tripterygiidae

Introduction

Understanding the ways in which fsh use diferent habitats is vital for efective aquatic ecosystem management, and the mitigation of threats facing these habitats through wellinformed conservation practices (Peterson et al. [2000](#page-9-0); Whitfeld [2002](#page-10-0); Courrat et al. [2009;](#page-9-1) Delpech et al. [2010\)](#page-9-2). Estuaries in particular play an essential role in supporting many fish species, contributing in different ways to a variety of life history strategies (Whitfield [1999;](#page-10-1) Potter et al. [2001;](#page-9-3) Elliott et al. [2007;](#page-9-4) Hoeksema et al. [2009\)](#page-9-5). Estuaries can serve as

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nursery habitats for fsh from both freshwater and saltwater environments because of their high productivity and capacity to act as refuges from predators and adverse environmental conditions (Paterson and Whitfeld [2000](#page-9-6); Beck et al. [2001](#page-8-0)). Whilst many fsh species only make transitory use of estuaries as migration routes, some physiologically tolerant species complete their entire life cycle within estuaries (Elliott et al. [2007](#page-9-4)).

A range of techniques can be used to study how fsh utilize estuaries, including tissue stable isotope analysis, otolith microchemistry, and acoustic/radio telemetry (Gillanders and Kingsford [2000;](#page-9-7) Childs et al. [2008;](#page-8-1) Fry and Chumchal [2011](#page-9-8)). As each of these techniques collect diferent types of information, the most appropriate approach is dependent on the objectives of the study. However, the information that tissue stable isotope analysis provides is limited to relatively short time periods due to tissue turnover rates (Buchheister and Latour [2010\)](#page-8-2). The use of electronic tagging is restricted to post-juvenile and adult life stages of larger-bodied species, as the relatively large tags make it difficult or impossible to use the technique on early life-stages and small individuals (Miles et al. [2013;](#page-9-9) Dawson et al. [2015](#page-9-10)), which is problematic given that many larval and juvenile fsh either rear in estuaries or briefy migrate through them. Otoliths, however, record ambient environmental conditions continuously over the lifetime of an individual fsh, potentially enabling an understanding of migration patterns and habitat use of individuals of all sizes and across all life stages (Campana [1999](#page-8-3); Elsdon et al. [2008](#page-9-11)).

The use of otolith trace element analysis to elucidate the life history of a fsh depends on the predictability of the relationship that exists between the otolith chemical composition of the species in question and the ambient water chemistry (Elsdon et al. [2008\)](#page-9-11). It is generally assumed that ratios of trace elements to calcium (Ca), particularly strontium (Sr) and barium (Ba), are deposited chronologically into the otolith material consistently relative to ambient concentrations in the surrounding water (Izzo et al. [2018](#page-9-12)). Therefore, otolith Sr:Ca and Ba:Ca have been used as tracers of the past habitat use by estuarine fsh (Gillanders and Kingsford [2000](#page-9-7); Gillanders [2002a](#page-9-13); Dorval et al. [2005b](#page-9-14); Schilling et al. [2018](#page-9-15)). The predictive relationships between trace elements (i.e., Sr and Ba) and salinity where high Sr/low Ba associated with seawater, and low Sr/high Ba associated with freshwater habitats are often used to reconstruct fsh life histories (Elsdon et al. [2008](#page-9-11)).

Estuarine triplefn *Forsterygion nigripenne* is a species endemic to New Zealand that occurs in estuaries and coastal areas, including the Chatham Islands (Francis et al. [2005](#page-9-16); Wellenreuther et al. [2007;](#page-10-2) Feary et al. [2009](#page-9-17); Kohn and Clements [2011](#page-9-18)). *F. nigripenne* spawns in nests built on shallow and sheltered habitats wherein mud and sand are the dominant substrates (Wellenreuther and Clements [2007\)](#page-10-3). This species is reported to exhibit 69 days of mean pelagic larval duration (PLD) before settlement to the adult habitat (Kohn and Clements [2011](#page-9-18)), a relatively long PLD which provides the potential to promote the dispersal of larvae between estuaries through the marine environment. However, gene fow studies indicate that *F. nigripenne* has limited dispersal potential and low migration rates (Hickey et al. [2009](#page-9-19); Kohn and Clements [2011](#page-9-18)), suggesting the possibility that they remain within estuaries throughout their life cycle. Nevertheless, studies on estuaries located on the South Island of New Zealand have suggested that the life history strategy of *F. nigripenne* comprises a marine larval/juvenile phase (Jellyman et al. [1997](#page-9-20); Sutherland and Closs [2001\)](#page-9-21).

A year-round monthly sampling programme on one of the permanently open estuaries in Otago, southern New Zealand (Waikouaiti Estuary) indicated that *F. nigripenne* occurs in large numbers in the estuary throughout the year, from settlement-age larvae with a translucent body and welldeveloped fns, to post-spawning adults (Taddese and Closs [2020\)](#page-9-22). Conversely, in a recent study, *F. nigripenne* was found to be absent in intermittently closed and open southeastern New Zealand lakes or lagoons (ICOLLs) (Taddese et al. [2018\)](#page-9-23). Thus, whilst it is known that *F. nigripenne* utilize estuaries for much of their life cycle, the precise details of its use across all life history stages are not understood. Hence, this study aimed to use otolith microchemistry to reconstruct the life history of *F. nigripenne* to better understand its habitat use over its full life history. Parameters estimated from otolith microstructural analyses were used to compliment understanding of the habitat use patterns of *F. nigripenne*.

A previous laboratory experiment indicated that otolith trace element analysis is a reliable technique to understand movements of *F. nigripenne* between waters of different Sr:Ca and Ba:Ca compositions. The results of the experiment showed incorporation of Sr:Ca and Ba:Ca into *F. nigripenne* otolith is linearly and positively related to their respective values in the ambient water (Taddese et al. [2019\)](#page-9-24). As otolith elemental signatures collected near the core and the edge refect early life history and habitat use at the time of catch, respectively, (Jessop et al. [2012\)](#page-9-25), we hypothesize that relatively higher Sr:Ca and lower Ba:Ca, indicative of pelagic larval life in the marine environment, will be observed in areas of *F. nigripenne* otoliths closer to the core. Lower Sr:Ca and higher Ba:Ca relative to the core region will be recorded on the outer edge of *F. nigripenne* otolith indicating increased exposure to freshwater inputs, suggesting either the use of estuarine, or near-estuarine coastal habitat subject to the infuence of freshwater inputs.

Materials and methods

Fish sampling

Juveniles of estuarine triplefn were collected using a seine net ($L \times H = 14 \times 1$ m; 10-mm mesh size) from the middle and lower sections of two permanently open estuaries located on the coast of Otago (South Island, New Zealand; Fig. [1\)](#page-2-0): Waikouaiti estuary and Tokomairiro estuary. The areas of the Waikouaiti and Tokomairiro estuaries are estimated to be 1.41 km^2 and 17.5 km^2 , respectively. Average salinity at the lower section of the Waikouaiti estuary ranges from 19 (winter) to 28 (summer) (Taddese and Closs [2020](#page-9-22)). On the other hand, salinity in the lower section of the Tokomairiro estuary fuctuates between 19 and 6, during summer and winter months respectively (Taddese 2020 Unpubl.). Fish [TL mm, 41.8 ± 12 (mean \pm SD)] from the Waikouaiti (*N*=25) and Tokomairiro (*N*=27) were collected in August and November 2016, respectively. Fish were sampled from both the middle (Waikouaiti) and lower (Tokomairiro) reaches of estuaries. Sampling locations were chosen based on prior assessment of the two estuaries (see Taddese et al. [2018\)](#page-9-23). Individuals were euthanized with an overdose of AQUI-S 20E fsh anesthetic (AQUI-S New Zealand Ltd, Lower Hutt, New Zealand) and preserved in 70% ethanol for subsequent otolith extraction and analyses.

Fig. 1 Map of New Zealand and location of sampling sites (Waikouaiti estuary—latitude -45.640917, longitude 170.661694; Tokomairiro estuary—latitude -46.21949, longitude 170.04484)

Otolith extraction, preparation, and analysis

Sagittal otoliths were extracted, cleaned with a nylon brush, air-dried, and stored dry in individual microcentrifuge tubes. Tweezers with plastic tips were used to handle otoliths during extraction and mounting. The tweezers were rinsed with ultrapure water and dried with lint-free wipes before and after handling otoliths from diferent sites. After extraction, both otoliths of each fsh were mounted with the proximal surface upward on a microscope slide using Crystalbond

509 thermoplastic adhesive (SPI Supplies, West Chester, PA), and then polished with 9 μ m and 3 μ m 3MTM diamond lapping flms that are made of polyester until the core was exposed. Once polished, one sagittal otolith from each fsh was transferred and mounted on another microscope slide using double-sided adhesive tape in preparation for laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS) analysis.

The second sagittal otolith was used to determine age using light microscopy (Olympus® Corporation, Tokyo, Japan) to count daily increments. Images of otoliths were taken by an Olympus SC100 camera mounted on a light microscope and examined using ImageJ (version 1.50, National Institutes of Health, USA) image analysis software. Increments were also counted directly using a microscope when they were difficult to count using image analysis. *F*. *nigripenne* otolith microstructure profle is known to show three distinct regions comprising (1) narrow light-colored increments close to the nucleus that become wider and darker in the middle, and (2) a conspicuous dark increment, followed by (3) narrower and lighter increments near the outer edge (Kohn and Clements [2011](#page-9-18)). The conspicuous dark increment that lies next to the intermediate increments is considered to be a settlement mark (Fig. [2;](#page-3-0) Kohn and Clements [2011](#page-9-18)). Hatch, settlement date, and PLD were backcalculated using the settlement mark as a reference from 47 otoliths (*N*=23, Waikouaiti; *N*=24, Tokomairiro). As fve otoliths (two from Waikouaiti and three from Tokomairiro) were damaged during preparation, a total of 47 otoliths were

Fig. 2 Image of a polished *Forsterygion nigripenne* otolith. Gray arrow with black border, wider growth increments before the settlement mark; Black arrow, settlement mark; White arrow, narrower growth increments after the settlement mark. The white broken arrow drawn from the primordium to the posterior end indicates the laser ablation path

used in microstructural analyses from both estuaries. Each otolith was aged twice with two non-sequential randomized, independent reads by the same reader, and the average percent error was calculated. Since no sample ages exceeded 5% error, all 47 samples were included in the results.

Data for eight elements $(^{7}Li, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{55}Mn,$ ${}^{31}P, {}^{39}K, {}^{88}Sr, {}^{137}Ba$ and ${}^{138}Ba$), as well as ${}^{43}Ca$ were quantifed using an Agilent 7900 ICP-MS coupled to an Australia Scientifc Instruments (ASI) RESOlution M-50 laser ablation system powered by a Coherent 193 nm ArF excimer laser. The laser ablated along a predetermined linear transect from the primordium to the otolith margin with a spot size of 50 µm and scan speed of 5 µm/s. Laser repetition rate was set at 7 Hz and on-sample fluence was 2.5 J/cm². A 20 s gas blank was collected prior to each sample, and certifed reference materials (the NIST 610 and NIST 612 glass standards, and the calcium carbonate pellet USGS MACS-3) were ablated every 7–8 samples to allow for correction for any background element counts as well as instrument drift and bias. Each otolith was pre-ablated three times to avoid potential surface contamination from sample preparation.

Data processing

Data were processed using the Trace Elements IS data reduction scheme in Iolite software (version 3.6) run on Igor Pro 6.37 to convert the raw count rate data to element concentrations after normalization to Ca and calibration with NIST 610. Standard reference materials (NIST 612 and MACS-3) were used to assess the linearity, accuracy, and precision of the measurements (see Online resource, Supplementary Material 1). The results for most elements were within $\langle 3\% \rangle$ and $\langle 10\% \rangle$ of published values of NIST 612 and MACS-3, respectively (Pearce et al. [1997;](#page-9-26) Jochum et al. [2012,](#page-9-27) USGS [2012\)](#page-10-4). All the measurements were above the instrument limit of detection (see Online resource, Supplementary Material 2).

Comparison of mean element to Ca (Me:Ca) values before and after the settlement mark enabled comparison of larval and adult habitat signatures. However, the use of the settlement mark as a borderline between otolith chemical signatures from early and adult life stages can be obscured by the lag efect of elemental uptake in otoliths (see Elsdon and Gillanders 2005). Hence, mean \pm SD of Me:Ca from the frst and last 50 µm of otolith transects were taken to approximate the values representing the pre and post-settlement periods consistently. Data were smoothed using a three-point moving median.

Data analysis

After conversion to element concentrations and normalization to Ca, the multi-element data were subjected to multivariate statistical analyses using the PRIMER-e multivariate statistical package (version 7; Quest Research Limited, Auckland, New Zealand). Principal coordinates analysis (PCO) was performed using the Euclidean resemblance matrix constructed from a square-root transformed data to explore sample groupings. A cluster analysis was performed to cross-check sample groupings on the PCO plot. A similarity profle test (SIMPROF) was used to identify statistically signifcant sample groupings by overlaying statistically different SIMPROF clusters on the PCO plot. Although they showed similar patterns with thier respective isotopes, ^{25}Mg and 137Ba were excluded from the PCO to avoid redundancy. The pre- and post-settlement otolith Sr:Ca and Ba:Ca signatures were compared using the Mann–Whitney *U* test. Violations of parametric test assumptions necessitated the use of the Mann–Whitney *U* test.

Results

Hatch and settlement months

The mean \pm SD PLD of *F. nigripenne* was 48.6 ± 11 and 40 ± 6.8 days for fish collected from Waikouaiti and Toko-mairiro, respectively (Fig. [3](#page-4-0)a, b). Most fish from the Waikouaiti settled in their habitats in June and July (Fig. [3](#page-4-0)c, d), whereas the settlement months for most samples from Tokomairiro were September and October. Hatch periods of fsh from the Waikouaiti were evenly distributed across April to June (Fig. [3](#page-4-0)e, f). In Tokomairiro, most fsh hatched in July

and August. The estimated age was 97.4 ± 22.9 (mean \pm SD) and 98.7 ± 31.8 (mean \pm SD) days for samples collected from the Waikouaiti and Tokomairiro estuaries, respectively.

Otolith chemistry

Overall, the otolith chemistry data of *F. nigripenne* showed Sr:Ca and Ba:Ca profles without prominent changes in elemental concentrations across the core to edge transect (Figs. [4,](#page-5-0) [5](#page-6-0)). However, in some otoliths from the Waikouaiti estuary, smaller Ba:Ca peaks covering short time periods were evident nearer the otolith edge (Fig. [4](#page-5-0)a, d). In contrast, in some otoliths from the Tokomariro estuary the Sr:Ca and Ba:Ca ratios co-fuctuated along the transect (Fig. [5](#page-6-0)a–e).

The PCO analysis revealed that the frst two principal coordinates (PCO1 and PCO2) contributed 65.9% the explained variance in the data. Specifcally, PCO1 and PCO2 contributed 46.9% and 19% of the explained variance in the data. Otolith Me:Ca values were grouped between estuaries and pre- and post-settlement mark otolith regions (Fig. [6](#page-6-1)). The pre-settlement otolith Me:Ca values of fsh from the Waikouaiti estuary were signifcantly diferent from the Tokomairiro values. The post-settlement otolith Me:Ca values from both estuaries overlapped to a large extent on the PCO plot. However, some of the post-settlement otolith Me: Ca values from the Waikouaiti estuary were significantly diferent from the Tokomairiro values.

Sr:Ca and Ba:Ca values from the outer edge region were higher than the values from the core region of otoliths. The pre-settlement period Sr:Ca ratios ranged from 2.1 mmol/

Fig. 3 Settlement age in days (**a**, **b**), month of settlement (**c**, **d**) and hatch month (**e**, **f**) were inferred from otolith microstructural analysis for *Forsterygion nigripenne* sampled from the Waikouaiti (*N*=23) and Tokomairiro (*N*=24) estuaries

Fig. 4 Representative plots of otolith Sr:Ca and Ba:Ca profles of *Forsterygion nigripenne* sampled from the Waikouaiti estuary. The vertical broken line in each plot indicates the settlement mark. Plots were selected from a pool of 25 traces

mol to 3.2 mmol/mol for fsh collected from the Waikouaiti (Fig. [7](#page-7-0)a), whereas the post-settlement Sr:Ca values were between 2.9 mmol/mol to 4.7 mmol/mol (Fig. [7](#page-7-0)a). Ranges of pre-and post-settlement Ba:Ca were between 0.3 µmol/mol to 1.5 µmol/mol and between 0.5 µmol/mol to 4.7 µmol/mol, respectively for samples collected from the Waikouaiti (Fig. [7](#page-7-0)b). Similarly, the pre-and post-settlement Sr:Ca values of fsh sampled from the Tokomairiro varied between 2.4 mmol/mol to 3.6 mmol/mol and 2.9 mmol/mol to 4.6 mmol/mol, respectively (Fig. [7a](#page-7-0)). Ba:Ca ranged from 0.3 µmol/mol to 2.1 µmol/mol and from 0.8 µmol/mol to 2.1 µmol/mol for samples collected from the Tokomairiro (Fig. [7](#page-7-0)b). The results of the Mann–Whitney U test supported the diferences between the pre- and post-settlement mark otolith Sr:Ca and Ba:Ca values (Table [1\)](#page-7-1).

Discussion

Other life history parameters inferred from otolith microstructure (i.e., time of hatch and settlement month) were indicative of continuous recruitment of juveniles within estuaries over an extended period. This pattern aligns well with the consistent presence of transparent juveniles with fully developed fns at the middle section of the Waikouaiti (Taddese [2019\)](#page-9-29) and other estuaries (Jellyman et al. [1997](#page-9-20); Sutherland and Closs [2001](#page-9-21)) between January and June. Fish sampling time diference between the Waikouaiti and Tokomairiro caused slight variation in settlement and hatch months among samples from both sites. Fish were collected in August and November from the Waikouaiti and Tokomairiro estuaries, respectively. This was refected in settlement months, whereby most fsh from the Waikouaiti settled in June and July, and those from the Tokomairiro settled in September and October. The average PLD observed in this study (44.1 \pm 9.9 days, mean \pm SD) was also slightly lower than the previously reported PLD in the North Island, New Zealand (69 days, Kohn and Clements [2011](#page-9-18)). Given the association of cold temperature with longer larval life (Green and Fisher [2004\)](#page-9-30), PLD was expected to be longer in the South Island where temperature is relatively lower. Therefore, the lower PLD of *F. nigripenne* observed in the present study could be a result of other biotic and abiotic factors (e.g., food availability) that affect larval traits. It is

Fig. 5 Representative plots of otolith Sr:Ca and Ba:Ca pro fles. of *Forsterygion nigripenne* sampled from the Tokomairiro estuary. The vertical broken line in each plot indicates the settle ment mark. Plots were selected from a pool of 27 traces

Fig. 6 Principal coordinates (PCO) analysis plot of Me:Ca values quantifed from pre- and post-settlement mark parts of otoliths collected from two estuaries [i.e., Waikouaiti (Wk) and Tokomairiro (Toko)]. The plot is based on a resem blance matrix constructed from a square-root transformed data. Each circle represents a signifcantly diferent group as identifed by the similarity profle (SIMPROF) test (see the supplementary material)

Fig. 7 Box plots show the pre-settlement and post-settlement otolith Sr:Ca (**a**) and Ba:Ca (**b**) of *Forsterygion nigripenne* collected from the Waikouaiti (*N*=25) and Tokomairiro (*N*=27) estuaries. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range. *LH* life history, *WK* Waikouaiti estuary, *TK* Tokomairiro estuary, *Pre* pre-settlement mark, *post* post-settlement mark, *SrCa* Sr:Ca (mmol/mol), *BaCa*, Ba:Ca (µmol/mol)

known that higher food abundance can increase in larval growth rate (Claramunt and Wahl [2000](#page-8-4)). It is likely that larvae that grow faster will be able to settle in the adult habitat relatively faster than those which grow slowly (e.g., Kaemingk et al. [2019\)](#page-9-31).

Table 1 Results of the Mann–Whitney *U* test for diferences in otolith elemental signatures between the pre- and post-settlement periods of fish sampled from the Waikouaiti ($N=25$) and Tokomairiro ($N=27$) estuaries

Our fndings showed that the changes in magnitudes of otolith element to Ca (Me:Ca) across transects of *F. nigripenne* otoliths were not substantial enough to suggest movement across waters of markedly diferent Sr:Ca and Ba:Ca values. The most plausible explanation for this steady otolith elemental profle between diferent life stages is either continuous estuarine residency, with perhaps some movement into coastal habitat closely associated with, and strongly infuenced by, estuarine inputs. In a previous experiment, otolith elemental profles of juveniles of this species reared in water spiked with diferent Sr and Ba concentrations showed signifcant diferences in otolith Sr:Ca and Ba:Ca values (Taddese et al. [2019\)](#page-9-24). Therefore, given that otolith elemental uptake in *F. nigripenne* is directly related to the concentrations in the ambient water, the general otolith Me:Ca profle observed in this study was indicative of a life history closely associated with estuaries. The absence of marked variations in Sr:Ca and Ba:Ca along the otolith transects suggests that *F. nigripenne* is likely associated with estuaries over its entire life history, and is perhaps the only species with such a life history strategy in southern New Zealand.

If the larval life stage of *F. nigripenne* involved movement to the wider marine environment for long periods, the presettlement Me:Ca profle would have shown higher Sr:Ca and lower Ba:Ca values than the post-settlement values, such as those seen in species undertaking an amphidromous migration, passing from the marine environment through the estuary and into freshwater (see example profles in Hicks et al. [2017\)](#page-9-32). Alternatively, Me:Ca signatures close to the core would have shown higher Ba:Ca and lower Sr:Ca ratios had the early life history of *F. nigripenne* consisted of a

signifcant stay in the upper freshwater dominated sections of estuaries where Sr concentration is markedly lower.

The pre-settlement otolith Me:Ca variation between the two estuaries seen in the PCO plot could indicate subtle diferences in larval rearing habitat. Whereas the overlap among most of the post-settlement mark otolith Me:Ca values could mean that fsh stay in the estuaries after completing their early life. The estuary-specifc diference in postsettlement mark otolith Me:Ca values may suggest that fish from the Waikouaiti estuary undertake short-term excursions to the near shore areas.

A steady Sr:Ca ratio and minor Ba:Ca peaks were observed in samples from the Waikouaiti estuary. The minor Ba:Ca peaks observed in Waikouaiti samples are presumably the result of Ba concentration variability in the ambient estuarine water, most likely associated with seasonal and periodic increased freshwater infow. Low salinity measurements were recorded in the Waikouaiti estuary during spring and winter months due to increased freshwater inputs (Taddese and Closs [2020\)](#page-9-22). Temporal Ba:Ca variations correlated to freshwater fow were reported for juvenile spotted seatrout *Cynoscion nebulosus* otoliths in Chesapeake Bay (Dorval et al. [2005b\)](#page-9-14). Furthermore, the steady Sr:Ca profle with small Ba:Ca peaks could indicate continuance of saltwater inputs with relatively constant Sr composition in this very open and well-mixed estuary.

A co-fuctuating pattern of Sr:Ca and Ba:Ca was seen in fish from the Tokomairiro estuary. This could reflect shortlived movements between diferent sections of the estuary. Small-scale within estuary variations in water trace element concentration can occur (Dorval et al. [2005a;](#page-9-33) Williams et al. [2018\)](#page-10-5) and infuence otolith composition (Gillanders and Kingsford [2000](#page-9-7); Gillanders [2002a,](#page-9-34) [b](#page-9-13); Sanchez-Jerez et al. [2002](#page-9-35); Dorval et al. [2007](#page-9-36)). We presume that, given the facilitation of Ba incorporation by Sr in *F. nigripenne* otoliths (Taddese et al. [2019](#page-9-24)), the somewhat co-variability of Sr:Ca with Ba:Ca observed in the present study suggests brief movements within the estuary from/to the section(s) where Sr is possibly higher (i.e., the lower reach). A snap-shot measurement of Sr and Ba from the Waikouaiti estuary indicated that Sr is higher in the deep middle sections (Taddese [2019](#page-9-29); Taddese et al. [2019\)](#page-9-24). It is possible that *F. nigripenne* moves between the estuary and the nearby coastal marine environment strongly infuenced by the estuarine inputs. However, the infuence of estuarine inputs, in terms of Sr and Ba, is unlikely to extend far into the marine environment given that estuaries studied are all relatively small in size.

A slight increase in Me:Ca ratios from the portion of the otoliths close to the core to the section representing the time of catch was observed in this study, suggesting that *F. nigripenne* probably spends its larval life in relatively deeper water columns (i.e., the main river channel) of estuaries where the Sr concentration is relatively lower (Taddese [2019;](#page-9-29) Taddese

et al. [2019](#page-9-24)). An alternative explanation for this pattern could be ontogenetic changes related to dietary shifts between different life stages (Buckel et al. [2004](#page-8-5); Engstedt et al. [2012](#page-9-37)). However, diet is known to have minimal infuence on otolith chemistry relative to ambient water conditions (Milton and Chenery [2001](#page-9-38); Walther and Thorrold [2006\)](#page-10-6).

To our knowledge, the present study is the frst to examine the life history of an estuarine dependent fsh species in southern New Zealand estuaries using otolith chemical analysis. Our results highlight the valuable functions estuaries play in supporting the life history of an endemic fsh species. The interpretations of otolith Me:Ca patterns in this study were based on a previous laboratory experiment conducted to validate the relationship between ambient water and otolith chemistry of the study species (Taddese et al. [2019](#page-9-24)). Taddese et al. ([2019\)](#page-9-24) confrmed the extent to which ambient water chemistry infuences elemental accretion in *F. nigripenne* otoliths. The fndings of the experiment established that Sr and Ba incorporated into *F. nigripenne* otoliths at a rate that refects the ambient concentrations. Despite the complexity of estuarine water chemistry, the continuous presence of both Sr and Ba in *F. nigripenne* otoliths indicates the species is estuary-dependent over its entire life history. Relating a time-series estuarine water Me:Ca data to otolith values in future studies will further help in understanding the life history of *F. nigripenne*.

Supplementary Information The online version contains supplementary material available at<https://doi.org/10.1007/s12562-021-01501-0>.

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