

Text S1. Justification of randomly selecting otoliths

Paired otoliths in flounder from Order Pleuronectiformes and bilaterally symmetrical flatfishes have been observed as morphologically and chemically dissimilar (Loher et al. 2008, Farmer et al. 2013, Kajajian et al. 2014, Jackman et al. 2016). The left (blind-side) otolith of adult individuals was used for analysis in the presented study as it has been shown to have a higher classification accuracy in flounder due to a clearer and more consistent structure (de Pontual et al. 2000, Farmer et al. 2013, Kajajian et al. 2014). The right otolith was significantly larger than the left otolith for adult sand flounder (paired sample t-test; $t = 11.86$, $p < 0.0001$), which supported the standardisation of only using the left otolith of adults. For juvenile flatfish, there is less evidence to suggest that their paired otoliths are morphologically and chemically dissimilar (Forrester & Swearer 2002, Loher et al. 2008). Thus, a random sagittae was selected for the juvenile analysis, which was supported by no significant difference of the multi-elemental concentrations between the left and right otoliths for juvenile sand flounder (PERMANOVA, Pseudo- $F_{1,89} = 1.03$, $P_{(perm)} = 0.40$) and no significant difference in mass between paired otoliths (paired sample t-test; $t = 1.11$, $p = 0.29$).

Table S1. Details of machine precision estimates ($\mu \text{ g}^{-1} \pm 2 \text{ S.D.}$) for each trace element in (a) MACS-3 and (b) NIST SRM 612 reference materials are given from LA-ICP-MS using horizontal transects (adult ablation), vertical spots (juvenile ablation) and reference values. Reference values for MACS-3 are GeoReM preferred values based on Jochum et al. 2019. Reference values for NIST SRM 612 are GeoReM preferred values based on Jochum et al. 2011. All values were rounded to the nearest whole number, with the exception of values that would have been rounded down to zero.

a) MACS-3

Element	Adult ablation	Juvenile ablation	Reference
Lithium	69 ± 4	66 ± 3	63 ± 4
Sodium	5942 ± 323	5991 ± 320	5850 ± 940
Magnesium	1710 ± 72	1758 ± 97	1720 ± 200
Phosphorus	97 ± 7	87 ± 10	103 ± 16
Potassium	6 ± 2	6 ± 6	1 ± -
Manganese	590 ± 13	571 ± 24	512 ± 50
Copper	126 ± 4	110 ± 5	116 ± 8
Zinc	85 ± 10	85 ± 8	124 ± 42
Rubidium	0.02 ± 0.02	0.02 ± 0.02	0.04 ± -
Strontium	6701 ± 227	6626 ± 320	6640 ± 340
Barium	59 ± 2	56 ± 3	60 ± 3
Lead	68 ± 4	57 ± 4	60 ± 6

b) NIST SRM 612

Element	Adult ablation	Juvenile ablation	Reference
Lithium	42 ± 1	40 ± 1	40 ± 1
Sodium	101177 ± 1278	101178 ± 1121	101640 ± 2278
Magnesium	61 ± 1.0	60 ± 1	68 ± 5
Phosphorus	30 ± 1	30 ± 4	47 ± 7
Potassium	62 ± 1	60 ± 1	62 ± 2
Manganese	40 ± 1	39 ± 1	39 ± 1
Copper	38 ± 1	38 ± 0.4	38 ± 2
Zinc	38 ± 1	38 ± 1	39 ± 2
Rubidium	31 ± 0.3	31 ± 0.4	31 ± 0.4
Strontium	79 ± 1	78 ± 1	78 ± 0.2
Barium	39 ± 0.5	38 ± 1	39 ± 1
Lead	38 ± 1	36 ± 1	38 ± 0.2

Table S2. Details of isotopes and mean limits of detection (LOD) for adult and juvenile sand flounder otolith samples outputted from the algorithms within IOLITE version 3.71 (Paton et al. 2011) run on Igor Pro 7.

Element	Isotope	LOD μ g-1
Lithium	⁷ Li	0.02
Sodium	²³ Na	0.51
Magnesium	²⁵ Mg	0.09
Phosphorus	³¹ P	1.75
Potassium	³⁹ K	0.30
Manganese	⁵⁵ Mn	0.12
Copper	⁶³ Cu	0.01
Zinc	⁶⁶ Zn	0.04
Rubidium	⁸⁵ Rb	0.01
Strontium	⁸⁸ Sr	0.01
Barium	¹³⁸ Ba	0.0005
Lead	²⁰⁸ Pb	0.003

Table S3. Average 2 standard deviations, provided as an indicator of internal precision, of the elemental concentrations within the post-settlement region time-series of adult (n = 81) and juvenile sand flounder otoliths (n = 90) given in μ g⁻¹.

Element	Adult samples	Juvenile samples
Lithium	0.5	0.5
Sodium	948.0	964.4
Magnesium	16.0	20.7
Phosphorus	26.80	88.20
Potassium	219.2	201.0
Manganese	1.9	3.0
Copper	0.4	0.2
Zinc	1.8	0.7
Rubidium	0.1	0.1
Strontium	559.9	708.5
Barium	2.0	1.1
Lead	0.1	0.0

Table S4. Canonical analysis of principal component (CAP) classification matrix constructed using otolith multi-elemental signatures (Li, Na, Mg, Mn, K, Zn, Rb, Sr, Ba) from juvenile (age 0+) sand flounder in east Otago, New Zealand to classify juveniles to individual estuaries (fine-scale resolution). Rows list the original collection site and columns lists the predicted site of collection based on the CAP with leave-one-out classification.

Station collected	Station assigned based on isotopic signature									No. correct by CAP	No. analysed	% correct
	Andersons Bay Inlet	Hoopers Inlet	Inner Waitati	Kaikorai Lagoon	Otago Harbour	Outer Waitati	Papanui Inlet	Purakaunui Inlet	Waikouaiti River estuary			
Andersons Bay Inlet	5	0	0	0	0	5	0	0	0	5	10	50
Hoopers Inlet	0	4	0	0	0	0	4	2	0	4	10	40
Inner Waitati	0	0	7	1	0	0	0	1	1	7	10	70
Kaikorai Lagoon	0	0	1	9	0	0	0	0	0	9	10	90
Otago Harbour	0	1	0	0	5	0	0	3	1	5	10	50
Outer Waitati	4	1	0	0	0	4	1	0	0	4	10	40
Papanui Inlet	0	1	0	0	0	1	8	0	0	8	10	80
Purakaunui Inlet	0	2	0	0	3	0	0	5	0	5	10	50
Waikouaiti River estuary	1	0	0	0	3	0	0	2	4	4	10	40
<i>Overall</i>	10	9	8	10	11	10	13	13	6	51	90	56.7%

% chance per site = 11.1%

Permutation test trace statistic = 1.83, p: 0.0001

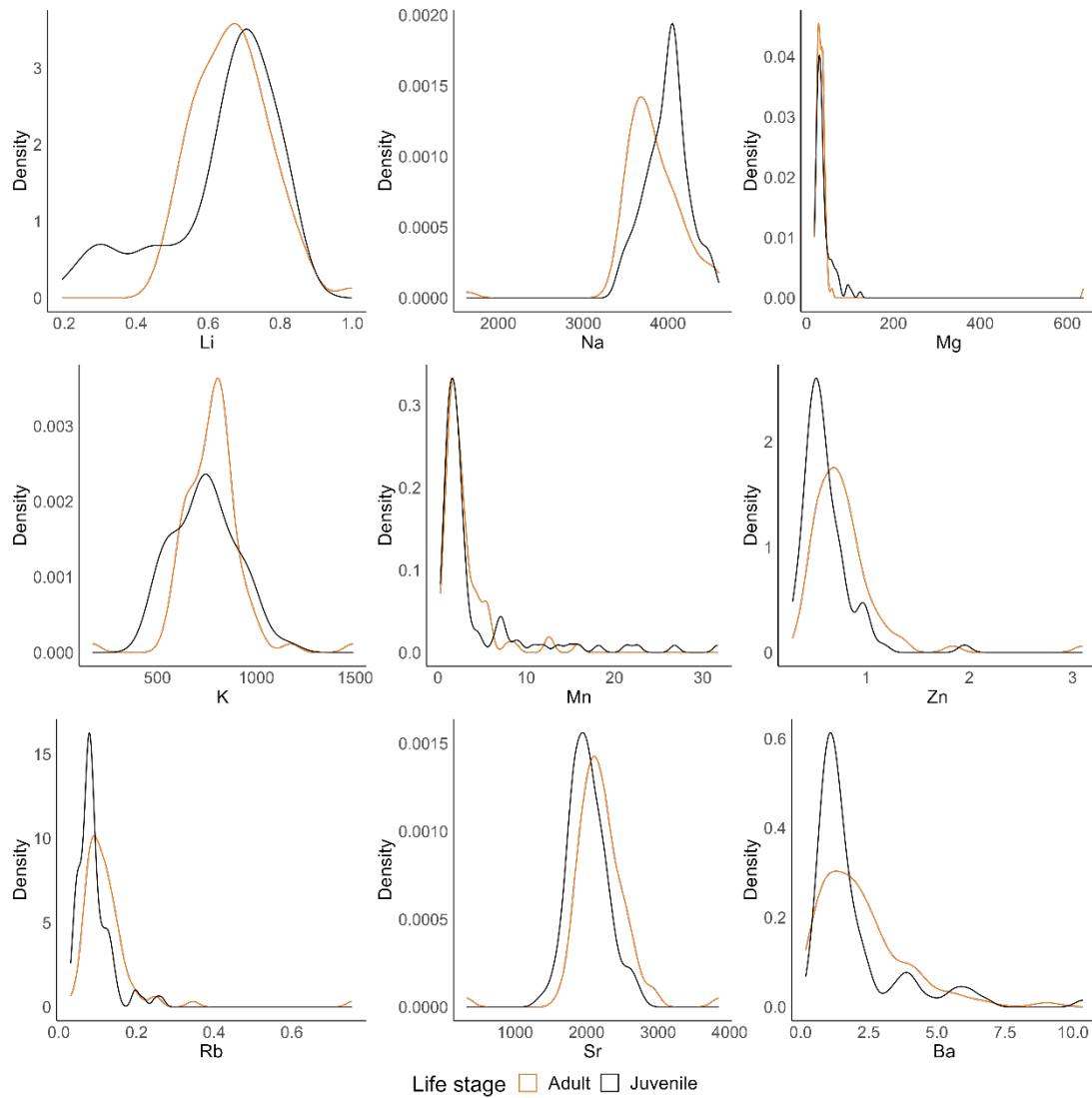


Figure S1. Distribution of means of nine elements ($\mu\text{ g}^{-1}$) measured within the post-settlement region of juvenile (age 0+, n = 90) and adult (age 1+, n = 81) sand flounder collected from the east Otago coastline in New Zealand.

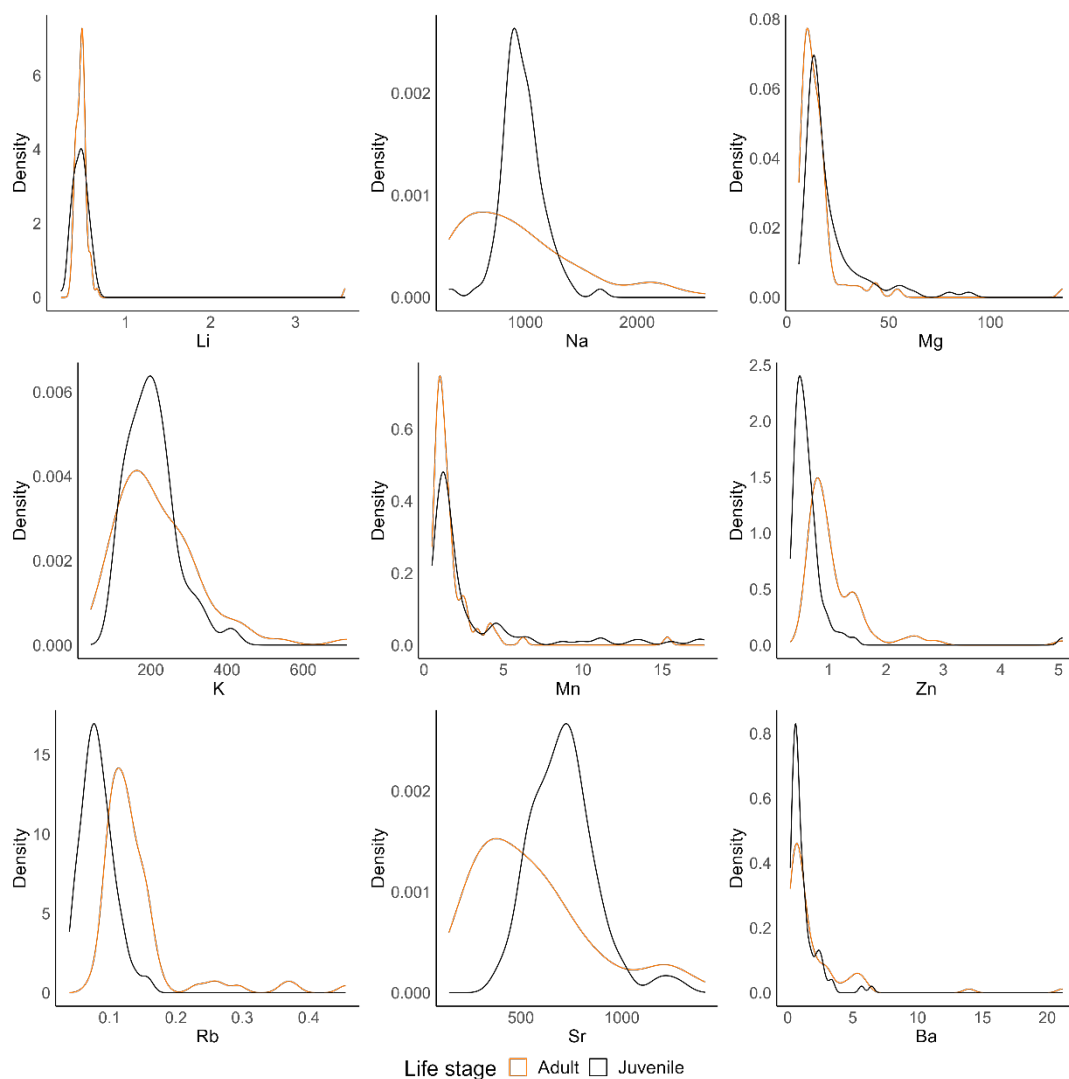


Figure S2. Distribution of the average 2 standard deviations of nine elements ($\mu \text{ g}^{-1}$) measured within the post-settlement region of juvenile (age 0+, n = 90) and adult (age 1+, n = 81) sand flounder collected from the east Otago coastline in New Zealand.

References

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