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**Temporal and spatial variation in strontium in a tropical river:
implications for otolith chemistry analyses of fish migration**

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Abstract

Analysis of otolith strontium isotope ratios $^{87}\text{Sr}/^{86}\text{Sr}$ is an increasingly utilised approach for studying fish migration. We analysed surface and groundwater from the Daly River catchment in the wet-dry tropics of Northern Australia over two years. Analyses of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were also conducted for freshwater Sooty grunter (*Hephaestus fuliginosus*) and the putatively diadromous Ord River mullet (*Liza ordensis*). Spatial variation in freshwater $^{87}\text{Sr}/^{86}\text{Sr}$ was high (range: 0.71612-0.78059) and there was strong seasonality in water $^{87}\text{Sr}/^{86}\text{Sr}$, with highest values in the wet season. Temporal variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios is attributed to seasonal patterns in surface run-off from geological formations with radiogenic compositions versus input from groundwater aquifers interacting with less radiogenic formations. Temporal variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios precluded robust inference on movement within freshwater for both species, although movement across salinity gradients by Ord River mullet was clearly identified. We conclude that temporally and spatially replicated water Sr data should be a general requisite for studies that analyse otolith Sr ($^{87}\text{Sr}/^{86}\text{Sr}$, Sr/Ca, Sr/Ba) to make inferences about fish movement and migration.

42 **Introduction**

43 Movement and migration by fishes is an important process in many riverine ecosystems,
44 creating ecological connections between spatially distant habitats and functioning as a
45 conduit for the transport of assimilated energy and nutrients across catchments and among
46 terrestrial, freshwater, estuarine and marine ecosystems (Flecker et al. 2010). Although the
47 migrations of riverine fishes are commonly classified into a few broad categories (e.g.
48 anadromy, catadromy, amphidromy, potamodromy), riverine fishes exhibit a wide variety of
49 migration strategies, often with high levels of individual behavioural flexibility (McDowall
50 1988). Understanding the intricacies of fish migration - and how they influence ecosystem
51 function and productivity - is a key challenge for riverine ecologists, and is fundamental to
52 the effective management and conservation of riverine fish populations (Koehn and Crook
53 2013).

54 An increasingly utilised technique for improving our knowledge of fish movement and
55 migration is otolith chemistry analysis. As layers of calcium carbonate accrete on the outer
56 surface of otoliths, dissolved ions from the ambient water are incorporated into the chemical
57 structure, forming a stable chronological record that, at least in part, reflects the ambient
58 water chemistry (Campana 1999). Sr and Ba are the two most commonly used ions for otolith
59 chemistry studies because they are relatively abundant in aquatic environments, are
60 permanently embedded within the otolith crystalline structure via replacement of Ca^{2+} , and
61 their concentrations and bioavailability vary among catchments and across salinity gradients
62 (Walther and Limburg 2012; Doubleday et al. 2014). The most common approach in fish
63 migration studies is to measure variations in the concentrations of otolith Sr and/or Ba
64 relative to Ca to identify movements between chemically distinct water bodies (Gillanders
65 2005). For example, regions of an otolith with relatively low Sr/Ca and high Ba/Ca usually

66 represent periods of freshwater residence, whilst the reverse pattern occurs during marine
67 residence (McMahon et al. 2013).

68 Analysis of otolith isotope ratios, particularly $^{87}\text{Sr}/^{86}\text{Sr}$, has also become a common approach
69 in fish migration studies over recent years. Variations in $^{87}\text{Sr}/^{86}\text{Sr}$ are largely due to
70 accumulation of radiogenic ^{87}Sr from the slow beta-decay of ^{87}Rb (half-life 49.6×10^9 years),
71 and therefore correlate broadly with the Rb/Sr ratio and/or geological age of the Sr source
72 (Faure and Mensing 2005). One of the main advantages of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios over Sr/Ca and
73 Ba/Ca is that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of otoliths directly reflect the source materials, and it is
74 therefore possible to directly relate water and otolith $^{87}\text{Sr}/^{86}\text{Sr}$ (Phillis *et al.* 2011; Brennan et
75 al. 2015 a). There are two main approaches for using otolith $^{87}\text{Sr}/^{86}\text{Sr}$ to study fish migration.
76 The first is to trace the movements of fish among locations within freshwater river networks.
77 This is possible because catchments with different underlying geologies tend to have
78 distinctive water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, allowing movement between locations to be detected as
79 variations in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ (see Barnett-Johnson et al. 2008; Brennan et al. 2015b). The
80 second main use of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios is to trace the movements of fish across salinity gradients.
81 Contemporary seawater has a uniform global $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70918 ± 0.00006
82 (McArthur and Howarth, 2004), whereas $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in fresh water are highly variable
83 and dependent upon catchment geology. Provided the freshwater system under consideration
84 has $^{87}\text{Sr}/^{86}\text{Sr}$ ratios that are dissimilar to the global marine value, it is possible to use
85 variations in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to trace movements across salinity gradients (Kennedy et
86 al. 2002; Hughes et al. 2014).

87 A key premise of both of these approaches is that freshwater $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are temporally
88 stable within a location, thus allowing variation in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to be interpreted as
89 movement by fish among locations (Elsdon et al. 2008). A small number of studies conducted
90 in temperate streams have reported stable and predictable water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios over seasonal

and annual time scales (e.g., Ingram and Weber 1999; Kennedy et al. 2000; Brennan et al. 2015a). However, significant temporal variation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within rivers and streams has also been reported in some instances (Bastin and Faure 1970; Crook et al. 2013; Douglas et al. 2013). To date, few otolith chemistry studies have explicitly examined temporal variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, despite its potential to confound the interpretation of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio data (Elsdon et al. 2008; but see Brennan et al. 2015a). There is a clear need for better understanding of the processes that drive temporal and spatial variation in freshwater $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and to assess the consequences of such variation for inferring fish movement based on otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio analyses (Elsdon et al. 2008; Walther and Thorrold 2009; Brennan et al. 2014).

In this study, we examine temporal and spatial variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Daly River system in the wet-dry tropical region of Northern Australia. We use this information to explore potential implications for the interpretation of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio data using the freshwater Sooty grunter (*Hephaestus fuliginosus*) and the putatively diadromous Ord River mullet (*Liza ordensis*) as case studies. The broader consequences of our findings for the interpretation of otolith chemistry data are discussed with regards to the drivers of temporal variation in surface water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, particularly catchment geochemistry and seasonal patterns of surface run-off and groundwater discharge.

Materials and methods

Study site

The study was conducted in the Daly River catchment in the wet-dry tropical region of the Northern Territory, Australia (Fig. 1). The Daly River is one of the largest rivers in northern Australia, draining an area of 52,500 km² and extending 500 km from the headwaters to the estuary mouth, where it discharges into the Timor Sea (mean annual discharge 6900 GL; Jolly 2002). Tidal influence on water levels and flows extends upstream as far as Daly River

116 Crossing ~105 km upstream of the estuary mouth, whilst salinity may be affected as far
117 upstream as Woolianna (~90 km upstream of estuary mouth) during the dry season. Rainfall
118 in the region comes primarily from the north-west monsoon, as well as from intense rain
119 depressions that form from decaying tropical cyclones. Rainfall is extremely seasonal, with
120 95% falling in the wet season between November and May.

121 Surface water flow in the Daly River main channel (known as the Katherine River upstream
122 of the Flora River junction [Fig. 1]) is strongly dominated by wet season events, with dry
123 season flows contributing only a small fraction of total annual flows (King et al. 2015). As
124 water levels fall in the dry season, the river progressively breaks into a series of long pools
125 separated by runs and riffles. Dry season flows are dominated by groundwater discharge and
126 progressively increase downstream as baseflows are added from different sources (Lawrie
127 2014). Three tributaries of the Daly River (Edith River, Fergusson River, Cullen River) were
128 also sampled in the study (Fig. 1a). The Fergusson River flows in a south-westerly direction
129 and enters the Daly River ~380 km by river upstream of the estuary mouth. The Edith and
130 Cullen rivers enter the Fergusson River 35 km and 60 km upstream of the Fergusson-Daly
131 junction respectively.

132 For much of its course, the Daly River is underlain by sedimentary carbonate-shale-sandstone
133 sequences of the Cambro-Ordovician Daly Basin (Kruse and Munson, 2013). The
134 stratigraphy of this basin comprises, from oldest to youngest, the Tindall Limestone, the
135 Jinduckin Formation, the Oolloo Dolostone and the Florina Formation (Fig. 1b), with a total
136 thickness of ~1 km. These sequences have been folded into a broad basin structure, elongated
137 in a south-east to north-west direction (Fig. 1b) and are locally overlain by Cretaceous
138 sandstone remnants of the Carpentaria Basin. The Tindall and Oolloo are dominantly
139 limestone and dolomite and host major karstic aquifers which are responsible for the bulk of
140 the baseflow in the Katherine and Daly Rivers (Lawrie 2014). Groundwater discharges into

the rivers where they intersect the aquifers. The Jinduckin and Florina formations are composed of siltstone and sandstone with minor limestone layers and only host minor aquifers. Downstream and upstream of the Daly Basin, the Daly/Katherine River system is underlain by ancient crustal basement of the Neoarchean-Proterozoic Pine Creek Orogen (Ahmad and Hollis, 2013). The Pine Creek Orogen is the oldest exposed part of the North Australian Craton and - in the study area - consists of ca. 1860 Ma clastic, carbonate and carbonaceous metasedimentary and volcanic sequences, intruded by ca. 1830 Ma granitic plutons. The largest of these, the >2800 km² Cullen and ~1000 km² Grace Creek Granites, are major components in the headwaters of the Cullen and Fergusson River, and the Katherine/Daly River, respectively. Aquifers in some of these rocks provide minor discharge to the rivers. Baseflow to the Edith, Fergusson and Cullen rivers is derived from Cretaceous sandstone higher in the catchments. The rivers flow over a variety of Proterozoic rocks with potentially highly diverse present-day ⁸⁷Sr/⁸⁶Sr ratios before reaching the sample sites. The largest and most consistent differences, however, are expected between the (i) highly radiogenic but variable run-off from exposed basement and the (ii) much less radiogenic (buffered by marine Sr in limestone and dolostones) and isotopically less variable run-off and groundwater recharge from the Daly Basin.

Study species

The Sooty grunter is a non-diadromous freshwater grunter (Family Terapontidae) distributed across tropical northern Australia from the Daly River eastwards to the Burdekin River in Queensland, as well as southern New Guinea (Pusey et al. 2004). Growing to an adult size of up to 5 kg (more commonly 1 kg), they are a popular angling species and an important food and cultural resource for Indigenous people (Jackson et al. 2012; 2014). Previous studies based on direct observation (Bishop et al. 1995) and mark-recapture (Hogan 1994) have suggested that Sooty grunter may undertake spawning migrations within freshwater.

However, the extent and direction of such movements appears variable among locations and dependent on the availability of inundated floodplains and other habitat features (Pusey et al. 2004). The Ord River mullet (Family Mugilidae) reaches ~2 kg and is a euryhaline species that inhabits marine, estuarine and freshwater habitats across northern Australia. They are not commonly targeted by recreational or commercial fishers, but are utilised as a food resource by Indigenous people (Jackson et al. 2012). There is little empirical information on the movements of Ord River mullet, although it has been suggested that they may be diadromous (Linke et al. 2012).

Water collection and analysis

Surface water samples for $^{87}\text{Sr}/^{86}\text{Sr}$ ratio analysis were collected between July 2012 and November 2014 from the Daly River, tributaries (Edith River, Cullen River, Fergusson River) and across the salinity gradient of the estuary during the 'dry' and 'wet' seasons (defined over the study years as June - November and December - April respectively) (Fig. 1). In the freshwater reaches of the main channel and tributaries, up to six temporal replicate samples were collected per site (Table 1). Sampling of surface water across the estuarine salinity gradient was conducted in November 2013 (dry season) and March 2013 (wet season). Groundwater samples from the Oolloo aquifer were obtained in November 2014 from four locations by pumping water from established boreholes maintained by the Northern Territory Department of Land Resource Management.

The salinity of each water sample was measured in the field using a Quanta[®] water quality meter (Hydrolab Corporation, Loveland, Colorado). This unit has a reported accuracy of +/- 1% and precision of 0.01‰. Most samples were filtered in the field (a few were filtered in the laboratory in Melbourne) using 0.2µm Acrodisc[®] syringe filters (Pall Corporation, Ann Arbor, USA), stored in acid-washed 50 ml polyethylene bottles, refrigerated at 4°C and transferred to the University of Melbourne for analysis. Based on preliminary estimates of Sr

191 concentrations (from electrical conductivity data), volumes of 1-40 ml of water were weighed
192 into clean polystyrene beakers, equilibrated with an appropriate amount of high-purity ^{84}Sr
193 isotopic 'spike' ($^{84}\text{Sr}/^{86}\text{Sr}$ in the spike is 1530) and dried in a HEPA-filtered fume cupboard.
194 Spiked Sr was extracted using a single pass over 0.15 ml (4 x 12 mm) beds of Eichrom[®] Sr
195 resin (50-100 μm). Following Pin et al. (1994), matrix elements were washed off the resin
196 with 2M and 7M nitric acid, allowing collection of a clean Sr fraction in 0.05M nitric acid. Sr
197 yields through the column exceed 90% and column blanks are <50 pg Sr. The Sr blank
198 contributed by filtering was simulated in the laboratory using distilled water and found to be
199 100 pg Sr or less. Based on the signals obtained in the ICP-MS, at least 110 ng of Sr were
200 present in each sample split (usually >400 ng), implying sample to blank ratios of 1100 or
201 higher; blank corrections were therefore insignificant.

202 Sr isotope analyses were carried out on a Nu Plasma multi-collector ICPMS (Nu
203 Instruments[®], Wrexham, UK), with sample introduction in 2% nitric acid via a Glass
204 Expansion[®] PFA nebuliser (0.05 ml/min uptake) and a first generation Cetac[®] ARIDUS
205 desolvator (see Maas et al. 2005). Instrumental mass bias was corrected by normalizing to
206 $^{88}\text{Sr}/^{86}\text{Sr} = 8.37521$ and results are reported relative to a value of 0.71023 for the SRM987 Sr
207 isotope standard. Within-run, or internal, precision (2 standard error [SE]) based on at least
208 30 ten-second integrations is typically better than ± 0.00003 . The reproducibility, or external
209 precision (2 standard deviation [SD]), of the results is ± 0.00004 . This is supported by a
210 triplicate analysis of sample DR-W-071112 which yielded 0.71826, 0.71826 and 0.71825.
211 $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in seven analyses of modern seawater Sr (extracted from a live Enewetak
212 Atoll coral, EN-1) ranged from 0.70913 to 0.70917 (average 0.70916 ± 0.00003 , 2sd, $n=7$),
213 consistent with the accepted ratio of 0.70916 (adjusted to SRM987 = 0.71023, McArthur and
214 Howarth 2004).

Sr concentrations obtained by isotope dilution have a high inherent precision (1%) but may only be minimum estimates if Sr contents in the non-acidified water were unstable between collection and analysis. This effect was minimised by low-temperature storage and rapid transfer to the Sr isotope laboratory. A repeat analysis of one sample after mild acidification in the laboratory (to 0.03M nitric acid) did not increase the actual dissolved Sr concentration. The listed concentrations are thus considered to be very close to, or identical with, actual Sr concentrations.

Mixing models of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (see Phillis *et al.* 2011) over the salinity gradient were calculated for the Daly River in the 2012 dry season and 2013 wet season. End-members used in the models were the least saline freshwater sample from the Daly River main channel in each season (Table 1) and a coastal marine water sample (salinity: 35.8‰, $^{87}\text{Sr}/^{86}\text{Sr}$ ratio: 0.71918, Sr: 3.6 mg/L) collected on 11th August 2010 at the Adelaide River mouth, approx. 130 km to the east of the Daly River mouth.

Sampling of river water for total Sr (mg/L), denoted [Sr] hereafter, was also conducted at a single site (Banyan Farm; Fig. 1) at approximately weekly intervals (n=43) between 7 November 2013 and 25 November 2014. Samples were kept frozen prior to [Sr] analysis. The samples for analysis were acidified with concentrated HNO_3 and then placed in an oven overnight at 60°C. A scan of elemental composition (APHA 2005, method 3125 B), including [Sr], was conducted on an Agilent 7500 series ICPMS operated by Charles Darwin University. The level of detection for [Sr] was 0.1 µg/L (ppb) and results are accurate to within 10%.

Fish collection

Sooty grunter were collected by hook and line fishing between July 2013 and April 2014 from the Daly River (n=5; 204-270 mm standard length [SL], 338-511 g), Edith River (n = 10; 265-385 mm SL, 327-1146 g) and Fergusson River (n = 21; 160-328 mm SL, 138-760 g).

240 Ord River mullet ($n=36$; 175-395 mm SL, 106-1768 g) were collected by boat electrofishing
241 from the main channel of the Daly River in June and July 2012. Upon capture, fish were
242 immediately euthanised by overdose in Aquí-S (175 mg L^{-1}) and measured (SL, $\pm 1 \text{ mm}$)
243 and weighed ($\pm 1 \text{ g}$). The sagittal otoliths were removed in the field and placed into labelled
244 paper envelopes for storage prior to preparation for analysis.

245 **Otolith preparation and Sr isotope analysis**

246 Otoliths were embedded in two-part epoxy resin (EpoFix[®], Struers, Denmark) and
247 transversely sectioned to a thickness of $\sim 300 \mu\text{m}$ through the primordium using a slow speed
248 saw. The sections were polished using lapping film ($9 \mu\text{m}$), rinsed with deionised water, air
249 dried and mounted on glass slides using epoxy resin. Laser ablation-ICPMS was used to
250 measure Sr isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) in the otoliths, following the methods outlined in
251 Woodhead et al. (2005). The analytical system consisted of the MC-ICPMS system described
252 above, coupled to a HeEx laser ablation system (Laurin Technic, Canberra, Australia, and
253 the Australian National University) constructed around a Compex 110 excimer laser (Lambda
254 Physik, Gottingen, Germany) operating at a wavelength of 193 nm.

255 Otolith mounts were placed in the sample cell and the ablation path for each sample was
256 digitally plotted using GeoStar v6.14 software (Resonetics, USA) and a $400\times$ objective
257 coupled to a video imaging system. Ablation transects were run from the otolith core to the
258 proximal edge using a $70 \mu\text{m}$ laser spot and fluence of $\sim 3 \text{ J/cm}^2$. A pre-ablation was
259 conducted prior to the analysis run to remove any surface contaminants with the laser pulsed
260 at 10 Hz and scanned at $20 \mu\text{m/sec}$. The analysis run was then conducted with the laser
261 pulsed at 6 Hz and scanned at $5 \mu\text{m/sec}$. Ablation was performed under pure He atmosphere
262 to minimise the re-deposition of ablated material, and the sample was then rapidly entrained
263 into the Ar carrier gas flow. Corrections for Kr and ^{87}Rb interferences were made following
264 Woodhead et al. (2005) and mass bias was corrected by reference to an $^{86}\text{Sr}/^{88}\text{Sr}$ ratio of

0.11940. The data were processed using Iolite Version 2.13 (see Paton et al. 2011) that operates within IGOR Pro Version 6.2.2.2 (WaveMetrics, Inc., Oregon) with corrections for potential Ca argide/dimer and Rb interferences and instrumental mass bias. All results were normalised to an $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio of 0.70916 for a modern marine carbonate standard run concurrently and known from solution ICPMS analyses to have a modern seawater composition (MacArthur and Howarth 2004). Individual integrations on the mass spectrometer represent 0.2 second time slices. During data processing a one-second moving average was employed to smooth the data. The ranges of water $^{86}\text{Sr}/^{86}\text{Sr}$ ratios recorded for each river (Daly main channel, Edith, Cullen and Fergusson) were overlaid upon the otolith $^{86}\text{Sr}/^{86}\text{Sr}$ ratio transects of each fish to allow visual assessment of otolith $^{86}\text{Sr}/^{86}\text{Sr}$ ratio variation in the context of the surrounding environment. A representative selection of the transect data is presented in the Results and the entire dataset is attached as supplementary material (S1, S2). Annual growth increment formation in the otoliths of Sooty grunter and Ord River mullet has not been validated, so it was not possible to directly relate otolith $^{86}\text{Sr}/^{86}\text{Sr}$ ratios to the age of the fish in the current study.

Results

Water chemistry - freshwater

All water samples collected from freshwater had higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratios than seawater (Table 1), reflecting the old (Proterozoic-Cambrian) bedrock underlying the catchment. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Edith, Fergusson and Cullen rivers (range: 0.72904-0.78059), in particular, were very high compared to the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio global average runoff value of 0.7119 (Palmer and Edmond 1989). Sites on the main channel of the Daly-Katherine River (range: 0.71612-0.74390) had generally lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratios than the three tributaries (Fig. 2). Both the maximum and minimum $^{87}\text{Sr}/^{86}\text{Sr}$ ratio surface water values for all main channel samples (i.e., 0.71612 and 0.74390) were recorded at the same site (Galloping Jacks). $^{87}\text{Sr}/^{86}\text{Sr}$ ratios

290 in groundwater from the Oolloo aquifer (bore samples) were lower (range: 0.71514-0.72090)
291 than surface water in the tributaries, but similar to $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in main channel water
292 during the dry season (Table 1).

293 Strong seasonal variation in surface water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios was observed in the main channel,
294 with no overlap between dry season (range 0.71612-0.72065) and wet season (range:
295 0.72489-0.74390) values across sites and seasons (Fig. 2; Fig. 3). Isotopic variation among
296 sites within the main channel was relatively low in the 2012 dry season (mean: 0.71887,
297 standard deviation [SD]: ± 0.0011), 2012/13 wet season (0.73078 ± 0.0008) and the 2013 dry
298 season (0.71853 ± 0.0011), but there was more variability among main channel sites in the
299 2013/14 wet season (0.73256 ± 0.0082).

300 The Edith River showed extreme seasonal variation, from dry season ratios of 0.72904-
301 0.74855 to much higher wet season ratios of 0.7668-0.7805. By contrast, no consistent inter-
302 seasonal variations were observed in the Cullen River (dry: 0.74968-0.75022, wet: 0.74888-
303 0.75133) and at Fergusson River site FR1 (dry: 0.77297-0.77793, wet: 0.77090-0.77618, Fig.
304 2). However, a single sample taken in July 2013 from location FR2 on the Fergusson River
305 had a much lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (0.7554) than water samples from FR1, indicating strong
306 spatial variation within this tributary.

307 Weekly water sampling at Banyan Farm revealed strong temporal variation in [Sr], with Sr
308 concentrations decreasing during high flows in the wet season and increasing during low flow
309 periods (Fig. 4). There is a strong negative correlation between natural log-transformed river
310 discharge (cumecs) and [Sr] (Pearson product-moment correlation coefficient = -0.926,
311 $P < 0.01$). [Sr] also varied strongly between sampling occasions at the other sites (Table 1), but
312 relationships between $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and [Sr] differed among sites. There was a negative
313 correlation in the main channel sites (Pearson coefficient = -0.852, $P < 0.01$), whereas $^{87}\text{Sr}/^{86}\text{Sr}$
314 ratios and [Sr] were positively correlated in the Edith River (Pearson coefficient = 0.934,

P<0.01). There were no significant correlations between $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and [Sr] in the Fergusson River (Pearson coefficient = 0.587, $P>0.05$) or the Cullen River (Pearson coefficient = -0.226, $P>0.05$), although sample sizes were low at these sites.

Water chemistry - estuarine

As expected, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios decreased substantially, and [Sr] increased, in the estuarine section of the Daly River, due to mixing between seawater and fresh water with contrasting $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and [Sr] characteristics (Table 1, Fig. 5). Modelled $^{87}\text{Sr}/^{86}\text{Sr}$ ratios showed very little change between salinity 36‰ (0.70918) and 5‰ (0.71066), but rose sharply below 5‰ (Fig. 5). Empirical $^{87}\text{Sr}/^{86}\text{Sr}$ ratio values measured at intermediate salinities within the estuary generally aligned closely with predicted values, although a single sample of salinity 0.3‰ collected during the wet season in March 2013 had considerably lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratio than predicted. Linear regression analysis including all of the estuarine samples demonstrated a strong positive relationship between $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and the reciprocal of [Sr] ($^{87}\text{Sr}/^{86}\text{Sr}$ ratio = $0.0003[1/\text{Sr}] + 0.7091$, $r^2 = 0.93$). The variance explained by the regression model was even higher when the outlying March 2013 (wet season) sample was removed ($^{87}\text{Sr}/^{86}\text{Sr} = 0.0003[1/\text{Sr}] + 0.7093$, $r^2 = 0.99$). These results support the mixing models' inherent assumption of conservative mixing between seawater and Daly River freshwater endmembers, although the existence of an outlying value may indicate inputs of freshwater from additional sources into the estuary (i.e., downstream tributaries, run-off from inundated floodplains, groundwater inflow) at particular times and/or locations. Longitudinal patterns of $^{87}\text{Sr}/^{86}\text{Sr}$ in the estuary differed markedly between seasons, with higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratios recorded closer to the estuary mouth in the wet season than the dry season. This reflects the greater input of freshwater into the estuary during the wet season.

Otolith chemistry

Within freshwater - Sooty grunter

340 The otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio transects for Sooty grunter were highly variable both among sites
341 and individuals within sites (Fig. 6; S1). Most transects showed evidence of semi-regular
342 variation of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, suggesting that seasonal variation in water chemistry may have
343 contributed to the observed variation in otolith $^{87}\text{Sr}/^{86}\text{Sr}$. However, the amplitude of these
344 variations was variable even among individuals within the same site (e.g., Fig. 7b versus 7d).
345 The otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio transects of five fish were fully contained within the range of water
346 $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measured at their collection site (Fig. 7a, b, d, f, h). However, regions of the
347 otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio transects of the remaining fish were outside the range of water $^{87}\text{Sr}/^{86}\text{Sr}$
348 ratios at their collection site, suggesting that movement to other regions may have occurred.
349 In several cases, otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from fish collected in the Fergusson River (site F2)
350 greatly exceeded any water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios recorded during the study (Fig. 7i; S1).
351 Presumably, these fish had travelled to more radiogenic regions of the Fergusson River
352 catchment than the collection site during their lives. The strong differences in water $^{87}\text{Sr}/^{86}\text{Sr}$
353 ratios between the two water sampling sites on the Fergusson River demonstrate the potential
354 for high spatial variability in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within tributaries of the Daly River, and
355 this variability appears to be reflected in the otoliths of Sooty grunter.

356 *Across the salinity gradient - Ord River mullet*

357 Transect analyses of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios confirmed the suggested diadromous life history
358 of Ord River mullet. All 40 individuals analysed had near-core otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios close to
359 the marine value of 0.70916, reflecting residence in estuarine or marine water during the
360 early life history, followed by a transition into freshwater (see examples in Fig. 7, S2). The
361 subtle response of water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to estuarine mixing above 5‰ salinity (Fig. 6) does
362 not allow a definitive discrimination between a predominant residence in seawater or within
363 the estuary during the early life history. After transition to freshwater, otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios
364 varied considerably in a similar manner to Sooty grunter. Semi-regular cycling in $^{87}\text{Sr}/^{86}\text{Sr}$

ratios was apparent in most individuals and appears likely to be driven by annual variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within the main channel of the Daly River (where the fish were collected). However, three fish had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios marginally outside the range of water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios recorded in the main channel, possibly suggesting that they may have undertaken forays into tributaries (Fig. 8b, e) or the estuary (Fig. 8f). Alternatively, this may suggest that our water sampling did not capture the full range of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios that occurred within the main channel over the lifetimes of these fish.

Discussion

The results of this study demonstrate substantial temporal variation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within sites on the Daly-Katherine River and associated tributaries. This finding has important implications for the interpretation of otolith chemistry data, as it demonstrates that variation in otolith core-to-edge $^{87}\text{Sr}/^{86}\text{Sr}$ ratio profiles may be concurrently influenced by within-site variation in water chemistry and fish movement among chemically distinct habitats.

Variability of water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios

There was strong spatial variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios among the surface water sampling sites on each sampling occasion. However, considerable overlap among sites occurred across seasons, which was primarily due to the high temporal variability in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within the Edith River and the main channel of the Daly-Katherine River (particularly at the Galloping Jacks site). Water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Cullen and Fergusson rivers were relatively stable over time and did not overlap. With the exception of a single dry season sample from the Edith River, there was no overlap between the tributaries and the main channel of the Daly-Katherine River.

Changes in the relative inputs of surface run-off versus groundwater inflow across seasons (wet versus dry) appear to be the primary driver of the observed temporal variation in riverine

⁸⁷Sr/⁸⁶Sr ratios. Groundwater and surface runoff Sr concentrations and isotopic ratios are determined by the age and composition of rock and soil with which they have had contact (Clark and Fritz, 1997). Since ⁸⁷Sr is the product of radioactive decay of its parent isotope ⁸⁷Rb, old rocks with high Rb content will tend to have more radiogenic (i.e. higher ⁸⁷Sr/⁸⁶Sr) Sr isotope ratios. The geology of the headwater regions of the Daly River (i.e., outside the Daly Basin) consists of Proterozoic sedimentary rocks, granite and volcanics, with areas of Cretaceous sandstone (Fig 1b; Tickell 2011). These rocks impart a highly radiogenic signature on surface runoff and groundwater in the region, which accounts for the relative high ⁸⁷Sr/⁸⁶Sr ratios in the tributaries. During the wet season, the radiogenic surface runoff enters the Daly River main channel via tributaries, resulting in elevated riverine ⁸⁷Sr/⁸⁶Sr ratios. In the dry season, surface runoff into the main channel is negligible and discharge into the river becomes dominated by groundwater, primarily from the Tindall and Oolloo aquifers (Lawrie 2015). As the Tindall and Oolloo formations are mainly comprised of limestones and other marine calcareous rocks, with low Rb content, they impart a less radiogenic signature on the groundwater, resulting in lowered riverine ⁸⁷Sr/⁸⁶Sr ratios during the dry season. This seasonal variation in the relative proportion of discharge into the main channel from groundwater and surface runoff thereby creates an oscillating pattern in riverine ⁸⁷Sr/⁸⁶Sr ratios with annual periodicity.

A further source of complexity in ⁸⁷Sr/⁸⁶Sr dynamics in the Daly River catchment is spatial variation in groundwater ⁸⁷Sr/⁸⁶Sr ratios. Although strong variation in ⁸⁷Sr/⁸⁶Sr ratios among aquifers with different geochemical characteristics is common (Frost and Toner 2004), considerable variation in ⁸⁷Sr/⁸⁶Sr ratios has also been shown to occur within aquifers. Indeed, analysis of small-scale spatial variation in ⁸⁷Sr/⁸⁶Sr ratios of groundwater is an increasingly utilised approach for tracing sub-surface flow pathways within aquifers (e.g., Brenot et al. 2008). In a study of the Oolloo aquifer, Tickell (2011) described three distinct

regions based on sampling of groundwater $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and other chemical properties. The first region, located close to the Daly River in the lower catchment, had groundwater $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of ~ 0.706 . In the second groundwater domain, located more distal to the Daly River channel, water $^{87}\text{Sr}/^{86}\text{Sr}$ was higher (~ 0.715), indicating water-rock interactions with more siliceous sediments, such as the overlying Cretaceous aquifer. Groundwater in the third region near Oolloo Crossing was more radiogenic (~ 0.718 – 0.725) than the first two groups, reflecting upward leakage from the underlying Jinduckin Formation. This was the region from which groundwater samples were taken in the current study. These observations, along with those of the current study, demonstrate the spatial complexity of surface and groundwater $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within the Daly River catchment. Whilst our water sampling was conducted over a very large spatial extent, representation of the catchment remained incomplete and otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios outside of the range of sampled water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were recorded. This emphasises the need for temporally replicated sampling across all potential habitats utilised by the fish under investigation to characterise catchment-scale $^{87}\text{Sr}/^{86}\text{Sr}$ dynamics.

Interpretation of otolith chemistry data

Strong seasonal and spatial variation in fresh water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and $[\text{Sr}]$ has the potential to affect the utility of otolith chemistry data for making inferences about fish movement and migration (see Elsdon et al. 2008). In the current study, overlap in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios between the Edith and Fergusson rivers over space and time made it impossible to confidently distinguish between fish movement among tributaries and temporal variation in water chemistry based on otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio transects. In a minority of cases, the magnitude of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio variation was strongly suggestive of large-scale movement between tributaries and the main channel. For example, significant portions of the transects of two fish collected from the Fergusson River (Fig 7e, g) reflected the chemistry of

the main channel of the Daly River, with values resembling the Fergusson River occurring only immediately prior to capture (i.e. near the otolith margin). However, the relative influences of temporal and spatial variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios could not be differentiated in most cases, even where large (>0.010) variations in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were observed, thus limiting our ability to make detailed inferences on fish movement within fresh water based on the otolith chemistry data.

This finding raises significant issues for the interpretation of otolith chemistry data generally, as many previous otolith chemistry studies do not report on water chemistry or contain only very limited spatial and temporal replication (see Elsdon et al. 2008). For example, Walther et al. (2011) used otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and Sr/Ba ratio transect data without supporting water chemistry data to make inferences about the movements of barramundi *Lates calcarifer* in northern Australia (coincidentally including the Ooloo Crossing site on the Daly River). Walther et al. (2011) observed strong variation in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and Sr/Ba along core-to-edge profiles of barramundi and applied a global zoning algorithm to divide otolith profiles into relatively homogenous zones separated by distinct ‘zone breaks’. These were then used to infer movement by barramundi among chemically distinct ‘habitats’ over the life history.

Re-examination of the data of Walther et al. (2011) in the context of the water $^{87}\text{Sr}/^{86}\text{Sr}$ ratio data presented here provides an informative perspective on this approach to interpreting otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratio data (Fig. 8). Similar to our results for the diadromous Ord River mullet, Walther et al. (2011) effectively identified the transition from saline to fresh water in barramundi from the Daly River, with a zone break corresponding to the transition from estuarine/marine to fresh water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios occurring for all fish during the early life history (Fig. 8). This finding agrees well with current understanding of barramundi life history (Russell and Garrett 1985; McCulloch et al. 2005). Walther et al. (2011) also

identified at least one zone break in the freshwater phase of all six Daly River barramundi examined, with $^{87}\text{Sr}/^{86}\text{Sr}$ ratios among most 'habitats' differing by ~ 0.003 - 0.010 (Fig. 8).

Only one of the seven zone breaks identified in the fresh water phases of the otolith profiles exceeded 0.010 (~ 0.045 , Fig. 8f).

There appears little doubt that fish movement explains at least some of the observed variation in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of fish in the Daly River in the current study and the study of Walther et al. (2011). This is particularly the case for the very large shifts observed in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ profiles of some fish. However, our analysis shows that variations in otolith $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the magnitude used by Walther et al. (2011) to statistically derive zone breaks in the freshwater phase of barramundi would be expected to occur as a result of seasonal variation in water chemistry, even if the fish had remained entirely stationary. Water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were >0.010 higher in the wet season than the dry season along the length of the Daly-Katherine River (Fig. 3) and individual sampling sites varied by up to 0.028 among seasons (Table 1). Based on these observations, we conclude that the application of global zoning algorithms and similar statistical techniques to derive estimates of fish movement should be treated cautiously unless there is complimentary information on the temporal variability of water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to support their validity. The strong seasonal variation in $[\text{Sr}]$ (Fig. 4) suggests that similar concerns may also apply for the interpretation of trace element ratios (e.g. Sr/Ca , Sr/Ba).

Unless the potentially confounding effects of temporal variability in water chemistry can be discounted based on empirical evidence, our findings suggest that robust inference regarding fish movement requires an understanding of the association between sampled otolith material and the water chemistry of the study system at the time the material was deposited. In systems with seasonally oscillating water chemistry, this could potentially be achieved by careful alignment of otolith chemistry profiles against validated annual increments with

known deposition times (not available in the current study). Seasonal otolith chemistry data derived this way could then be examined with regards to spatial patterns of water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios across the study system within seasons. Bataille et al. (2014) provide an example of the approaches available for modelling riverine $^{87}\text{Sr}/^{86}\text{Sr}$ ratios at the catchment scale. However, even in situations with highly predictable seasonality in water chemistry, disentangling the influence of temporal water chemistry variation versus fish movement is a complex task requiring detailed consideration of the spatio-temporal dynamics of the study system, as well as otolith crystallisation rates and the otolith sampling methodology itself (e.g., laser spot sizes and the growth period integrated within each measurement). To date, such detailed linking of otolith chemistry to ambient environmental conditions has seldom been attempted at large spatial scales (but see Brennan et al. 2015b).

When considering the generality of our findings, it should be recognised that fresh water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Daly River catchment are high (up to 0.7806) compared with the global mean of 0.7119 (Palmer and Edmund 1989). The seasonality of rainfall in northern Australia's wet-dry tropics is also much more extreme than temperate areas. Nonetheless, the Daly River example serves to amplify the effects of an issue that is likely to apply to the majority of rivers, lakes, wetlands and estuaries that experience temporal variation in the relative importance of surface run-off versus groundwater input (Sophocleous 2002). Thus, while temporal variation may be more subtle in systems with lower water $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and less extreme seasonal variability in rainfall, the implications of our findings have broad significance for the interpretation of otolith chemistry data. Based on our observations, we conclude that presentation of temporally and spatially replicated water Sr data should be a general requisite for studies that use analyses of otolith Sr ($^{87}\text{Sr}/^{86}\text{Sr}$, Sr/Ca, Sr/Ba ratios) to make inferences about fish movement and migration.

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Table 1: Water chemistry of samples collected from the main channel of the Daly-Katherine River, tributaries of the main channel and the Oolloo aquifer during the study. Site codes for map in Fig. 1 are shown in brackets. “na” = data not available.

Main channel	Sample date	Season	EC (ms/cm)	Sal (‰)	Sr (mg/L)	⁸⁷ Sr/ ⁸⁶ Sr
Galloping Jacks (GJ)	2/07/2012	Dry	0.326	0.16	0.034	0.71908
	1/10/2012	Dry	0.396	0.19	0.056	0.71612
	12/03/2013	Wet	0.092	0.05	0.011	0.73187
	8/07/2013	Dry	0.488	0.24	0.054	0.71795
	27/09/2013	Dry	0.567	0.28	0.061	0.71684
	12/03/2014	Wet	0.082	0.05	0.009	0.74390
Claravale Crossing (CC)	29/09/2012	Dry	0.573	0.28	0.057	0.71853
	13/03/2013	Wet	0.143	0.07	0.021	0.73087
	11/03/2014	Wet	0.154	0.07	0.028	0.72830
Oolloo Crossing (OC)	29/06/2012	Dry	0.560	0.23	0.067	0.71958
	28/09/2012	Dry	0.615	0.27	0.051	0.71844
	6/07/2013	Dry	0.608	0.30	0.079	0.71900
	24/09/2013	Dry	0.625	0.31	0.076	0.71833
	18/03/2014	Wet	0.252	0.12	0.041	0.72489
Mt Nancar (MN)	27/06/2012	Dry	0.552	0.27	0.049	0.72065
Beeboom Crossing (BC)	4/07/2012	Dry	0.576	0.27	0.063	0.71968
	2/10/2012	Dry	0.594	0.25	0.063	0.71851
Daly Crossing (DC)	26/09/2012	Dry	0.585	0.27	0.062	0.71937
	13/03/2013	Wet	0.173	0.09	0.021	0.72992
	25/02/2014	Wet	0.143	0.07	0.022	0.73314
Woolianna (WO)	28/06/2012	Dry	0.528	0.21	0.044	0.71984
	7/11/2012	Dry	0.610	0.30	0.067	0.71826
	5/03/2013	Wet	0.145	0.07	0.016	0.73046
	2/07/2013	Dry	0.563	0.27	0.073	0.72005
	30/09/2013	Dry	0.558	0.27	0.073	0.71901
Bamboo Creek (BA)	26/09/2012	Dry	0.600	0.26	0.063	0.71928
Estuary (E1)	7/11/2012	Dry	0.828	0.41	0.149	0.71214
	5/03/2013	Wet	0.193	0.09	0.044	0.71703
Estuary (E2)	7/11/2012	Dry	6.100	3.38	0.641	0.70976
	5/03/2013	Wet	0.219	0.11	0.056	0.71479
Estuary (E3)	7/11/2012	Dry	10.140	5.70	1.061	0.70962
Estuary (E4)	7/11/2012	Dry	16.000	9.41	1.792	0.70946
Estuary (E5)	7/11/2012	Dry	27.700	17.10	3.261	0.70932
	5/03/2013	Wet	0.602	0.30	0.103	0.71050
Estuary (E6)	5/03/2013	Wet	9.8	5.65	0.995	0.70931
Tributaries						
Cullen River (CR)	3/07/2012	Dry	0.200	0.01	0.033	0.75022
	30/09/2012	Dry	0.200	0.01	0.159	0.74968
	12/03/2013	Wet	0.04	0.03	0.011	0.75133
	9/07/2013	Dry	0.086	0.05	0.033	0.75002
	12/03/2014	Wet	0.037	0.03	0.014	0.74888

Edith River (ER)	3/07/2012	Dry	na	na	0.002	0.72904
	2/10/2012	Dry	na	na	0.005	0.74738
	12/03/2013	Wet	0.063	0.04	0.007	0.78059
	9/7/2013	Dry	0.019	0.02	0.003	0.74644
	15/08/2013	Dry	0.024	0.02	0.004	0.74855
	12/03/2014	Wet	0.041	0.03	0.006	0.76685
Fergusson River (FR1)	3/07/2012	Dry	0.026	0.02	0.005	0.77297
	30/09/2012	Dry	0.047	0.00	0.013	0.77447
	12/03/2013	Wet	0.028	0.02	0.005	0.77618
	9/7/2013	Dry	0.019	0.02	0.012	0.77793
	15/08/2013	Dry	0.051	0.03	0.020	0.77736
	12/03/2014	Wet	0.027	0.02	0.006	0.77090
Fergusson River (FR2)	16/4/2014	Wet/dry	0.014	0.07	0.037	0.75549
Groundwater						
Oolloo aquifer (RN36815)	27/11/2014	Dry	0.601	0.25	0.058	0.71516
(RN36813)	27/11/2014	Dry	0.611	0.25	0.067	0.71514
(RN34364)	27/11/2014	Dry	0.695	0.30	0.046	0.72090
(RN34369)	28/11/2014	Dry	0.612	0.27	0.051	0.71798

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Figure legends

Figure 1: (a) Map of study region showing sampling sites (open circles) and bore water sampling sites (red circles). Site codes: Galloping Jacks (GJ), Cullen River (CR), Fergusson River (FR1, FR2), Edith River (ER), Claravale Crossing (CC), Oolloo Crossing (OC), Daly Crossing (DC), Woolianna (WO), Banyan Farm (BF), estuary sites 1-6 (E1, E6; sites E2-E5 not labelled for clarity). (b) Block diagram showing Daly Basin and underlying structure of major aquifers. Florina Formation (green), Oolloo Dolostone (light blue), Junduckin Formation (pink), Tindall Limestone (aquamarine). Australia's coastline data was obtained from Geoscience Australia (Geoscience Australia, 2004). Surface hydrology and catchment boundary data was supplied by the Department of Land Resource Management, Copyright - Northern Territory of Australia.

Figure 2: Seasonal (dry versus wet) variation in surface freshwater $^{87}\text{Sr}/^{86}\text{Sr}$ in the main channel of the Daly/Katherine River and three major tributaries. For clarity, values within season are offset and lines are drawn between the seasonal medians or single values for each site. Details of the samples are presented in Table 1. Site "FR2" which was only sampled on one occasion is not included.

Figure 3: Longitudinal profiles of $^{87}\text{Sr}/^{86}\text{Sr}$ along the main channel of the Daly-Katherine River from the most upstream site (Galloping Jacks) to the estuary mouth in the dry season 2012 and the wet season 2013.

Figure 4: Total Sr (black symbols) versus daily river discharge (unbroken line). Water samples were collected approximately weekly from Banyan Farm on the Daly River main channel between November 2013 and November 2014.

Figure 5: Mixing models (lines) of water $^{87}\text{Sr}/^{86}\text{Sr}$ across salinity gradient in the Daly River in the dry season of 2012 (a) and the wet season of 2012/13 (b). Symbols represent empirical

721 samples collected from intermediate salinities within the estuary in November 2012 and
 722 March 2013 (Table 1).

723 **Figure 6:** Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Sooty grunter collected from the
 724 Daly River (a-d), Edith River (e-g) and Fergusson River (h-l). The dashed line labelled “SW”
 725 represents seawater $^{87}\text{Sr}/^{86}\text{Sr}$; shaded areas show the ranges of water $^{87}\text{Sr}/^{86}\text{Sr}$ recorded at
 726 each of the regular water sampling sites (Daly River main channel = “MC”, Edith River =
 727 “ER”, Cullen River = “CR”, Fergusson River = “FR1”); the unbroken straight line represents
 728 the $^{87}\text{Sr}/^{86}\text{Sr}$ value of the single sample taken from a second site within the Fergusson River
 729 (“FR2”).

730 **Figure 7:** Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Ord River mullet collected from the
 731 Daly River main channel. The dashed line labelled “SW” represents seawater $^{87}\text{Sr}/^{86}\text{Sr}$;
 732 shaded areas show the ranges of water $^{87}\text{Sr}/^{86}\text{Sr}$ recorded at each of the regular water
 733 sampling sites (Daly River main channel = “MC”, Edith River = “ER”, Cullen River = “CR”,
 734 Fergusson River = “FR1”); the unbroken straight line represents the $^{87}\text{Sr}/^{86}\text{Sr}$ value of the
 735 single sample taken from a second site within the Fergusson River (“FR2”).

736 **Figure 8:** Core-to-edge $^{87}\text{Sr}/^{86}\text{Sr}$ otolith data from barramundi collected at Ooloo Crossing
 737 (redrawn from Walther *et al.* 2011, Figure S1) overlaid on water chemistry data collected in
 738 the current study. Black lines show the otolith $^{87}\text{Sr}/^{86}\text{Sr}$ transect data and grey lines show
 739 modelled habitat occupation and shifts using a global zoning algorithm (Walther *et al.* 2011).
 740 The dashed line labelled “SW” represents seawater $^{87}\text{Sr}/^{86}\text{Sr}$; shaded areas show the ranges of
 741 water $^{87}\text{Sr}/^{86}\text{Sr}$ recorded at each of the regular water sampling sites (Daly River main channel
 742 = “MC”, Edith River = “ER”, Cullen River = “CR”, Fergusson River = “FR1”); the unbroken
 743 straight line represents the $^{87}\text{Sr}/^{86}\text{Sr}$ value of the single sample taken from a second site
 744 within the Fergusson River (“FR2”).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure S1: Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Sooty grunter collected from the Daly River, Edith River and Fergusson River .

Figure S1 (cont): Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Sooty grunter collected from the Daly River, Edith River and Fergusson River .

Figure S1 (cont): Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Sooty grunter collected from the Daly River, Edith River and Fergusson River .

Figure S2: Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Ord River Mullet collected from the Daly River.

Figure S2 (cont): Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Ord River Mullet collected from the Daly River.

Figure S2 (cont): Core-to-edge transects of otolith $^{87}\text{Sr}/^{86}\text{Sr}$ in Ord River Mullet collected from the Daly River.