| 1 | Use of otolith chemistry and acoustic telemetry to |
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| 2 | elucidate migratory contingents in barramundi |
| 3 | Lates calcarifer |
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22 Abstract

Migration is a fundamental aspect of the life history of many fishes and must be well 23 understood for targeted conservation and management. We used acoustic telemetry and 24 otolith ⁸⁷Sr/⁸⁶Sr analysis and annual ageing to study intra-specific variation in the movements 25 26 of barramundi Lates calcarifer in rivers of the Northern Territory, Australia. Acoustic transmitters were surgically implanted into 25 barramundi (420-1010 mm TL, median 510 27 mm TL) collected from freshwater reaches of the South Alligator River and their movements 28 tracked over 2 years. ⁸⁷Sr/⁸⁶Sr transect analysis was also conducted on the otoliths of 67 29 barramundi from the Daly, Mary, South Alligator and Roper rivers. Acoustic telemetry 30 showed that most fish remained in fresh water across wet and dry seasons in both years. 31 Higher rates of movement occurred during the wet season and a minority of primarily larger 32 33 fish moved into the estuary during high flows. Otolith chemistry analyses revealed high diversity in salinity histories among individuals. The telemetry and otolith chemistry data are 34 35 integrated to examine migration as a function of the stage of sexual development, and we propose a revised life history model that identifies three life history contingents. We conclude 36 that anthropogenic disturbance, such as modified river hydrology, has the potential to alter 37 38 the frequency of life history contingents in barramundi populations.

39

40 Introduction

Behaviour associated with mating and reproduction is a key determinant of the evolutionary, 41 ecological and behavioural characteristics of species (De Bruyn et al. 2011) and may vary 42 considerably among individuals within species (Gross 1996). Intra-specific variation in 43 reproductive behaviour has traditionally been studied in the context of male-female 44 45 differences, but there is growing recognition that variation within the sexes is widespread across many taxa, and that evolution often gives rise to a diversity of reproductive 46 phenotypes, rather than a single best male and female phenotype for each species (Gross 47 1996). A well-known example in fishes is the two male breeding phenotypes that commonly 48 49 occur within riverine salmonid populations: an anadromous phenotype that grows to a large size whilst at sea and invests heavily in display and courtship on the spawning grounds; and 50 an early maturing, non-migratory phenotype that is much smaller, invests proportionally 51 52 more in testes for sperm competition, and reproduces by "sneaking" fertilisations with females attempting to mate with larger males (Fleming 1996; Young et al. 2013). 53 54 Migration is a fundamental aspect of mating and reproduction in many fishes and the migratory characteristics of species must be well understood for the development of targeted 55 56 conservation and management strategies and policy (Cooke et al. 2016). Intra-specific variation in migratory behaviour is very common in fishes. For example, many species 57 exhibit facultative migration (also known as "partial migration"; Chapman et al. 2011), where 58 populations are comprised of sympatric migratory and non-migratory individuals. 59 60 Components of populations that exhibit consistently different migratory behaviour are 61 referred to as migratory "contingents" (Secor 1999). Additionally, many species exhibit "skipped spawning", where some individuals within populations fail to develop sexually, and 62 therefore do not migrate, in some years due to poor nutritional status or other factors (Milton 63 64 and Chenery 2005; Rideout and Tomkiewicz 2011).

Developing our understanding of the processes that give rise to divergent migratory strategies 65 within fish populations is an important research area for evolutionary biologists, but is also of 66 practical significance to the management and conservation of aquatic ecosystems. The 67 expression of life history traits, such as migration, can be considered a conditional strategy 68 that relies on individuals making "decisions" (e.g., whether or not to migrate) within the 69 context of their physiological status and external environment (Gross 1996; Kendall et al. 70 71 2015). By altering the external environmental context, anthropogenic disturbances such as changes to river hydrology and human-induced climate change, have the potential to affect 72 73 the outcomes of these decision-making processes and, thereby, influence the frequency of reproductive phenotypes within populations. 74

The barramundi or Asian sea bass *Lates calcarifer* is a large perciform fish (family Latidae) 75 that occurs in coastal and fresh waters throughout the Indo-West Pacific region including 76 77 India, Myanmar, Sri Lanka, Bangladesh, southern China, Taiwan, the Malay Peninsula, Java, Borneo, Sulawesi, the Philippines, Papua New Guinea and northern Australia (Grey 1987). 78 Barramundi are sequential, protandrous hermaphrodites, developing initially as males at 3-5 79 80 years of age and then into females at 4-8 years of age (Davis 1987). The movements and reproductive biology of barramundi in Australia and Papua New Guinea were subject to 81 considerable research from the 1950s to 1980s, including studies of early life history (Moore 82 1980; 1982; Russell and Garrett 1983, 1985; Davis 1984a), spawning and reproductive 83 behaviour (Dunston 1959; Garrett 1987), sexual development (Moore 1979, 1980; Davis 84 85 1982, 1984b), and mark-recapture analyses of growth and movement (Moore and Reynolds 1982; Davis 1986; Davis and Kirkwood 1984; Russell and Garrett 1988). Based on available 86 information, Grey (1987) presented a generalised conceptual model of the life history and 87 88 catadromous migrations of barramundi in Australia (Fig. 1a). Even at this time, however, it was recognised that the biology of barramundi is extremely complex and that much variation 89

90 in life history characteristics occurs among individuals and populations (Grey 1987; Davis91 1987).

Subsequent otolith and scale chemistry analyses of barramundi in Australia and New Guinea 92 revealed high intra-specific variation in salinity histories within populations and found that a 93 94 proportion of individuals remain within saline coastal/estuary habitats throughout life, 95 without ever undertaking catadromous migrations into fresh water (Pender and Griffin 1996; Cappo et al. 2005; McCulloch et al. 2005; Milton and Chenery 2005; Milton et al. 2008; 96 97 Halliday et al. 2012). Milton and Chenery (2005) also found that a proportion of large, freshwater resident fish did not return to the estuary and suggested that such individuals may 98 not participate in spawning during their lives. Notwithstanding the identification of these life 99 history variants, current management of barramundi still relies heavily upon the tenets of 100 Grey's (1987) life history model as a generalised descriptor of the barramundi life cycle (e.g., 101

102 NT Fisheries 2006; WA Fisheries 2016; Fig. 1b).

The aim of the current study was to integrate data collected from an acoustic telemetry study and otolith chemistry analyses to examine individual and among-population variation in life history strategies of barramundi in rivers of the Northern Territory, Australia. These data are used to revisit the tenets of the generalised life history model for barramundi and a revised life history model is presented that takes into account information from the current and previous studies.

109 Methods

110 Study sites

111 The study was conducted in the wet-dry tropical region of the Northern Territory, Australia, 112 between the Daly River, which flows into the Timor Sea, and the Roper River, which flows 113 into the western Gulf of Carpentaria (Fig. 2). Fish for otolith analysis were collected from the 114 Daly, Mary, South Alligator and Roper rivers. These rivers all experience predictable periods

of high flow during the monsoonal wet season. The Daly and Roper rivers are perennial, with 115 stable dry season flows occurring due to input from extensive groundwater aquifers. The 116 upper reaches of the Mary and South Alligator rivers are intermittent, ceasing to flow during 117 the dry season and forming series of hydrologically disconnected main channel billabongs. 118 Barramundi were collected for tagging with acoustic transmitters from main channel 119 billabongs (Yellow Water, Home Billabong, Mardugal Billabong) on the South Alligator 120 121 River in Kakadu National Park. These billabongs are located ~120 km upstream of the estuary mouth and are surrounded by extensive areas of floodplain wetlands that are 122 123 inundated annually during the wet season.

124 Water sampling and analysis

Surface water samples for ⁸⁷Sr/⁸⁶Sr analysis were collected from the freshwater reaches of the 125 four rivers to allow for calculation of mixing models across the salinity gradient for each 126 127 system (see Phillis et al. 2011) (Table 1). Samples were collected from the Daly River in October 2012, the South Alligator River in November 2013 and the Mary and Roper rivers in 128 November 2014. The salinity of each water sample was measured in the field using a 129 Quanta[®] water quality meter (Hydrolab Corporation, Loveland, Colorado) and salinity values 130 are expressed as dimensionless units on the Practical Salinity Scale of 1978 (PSS 78). 131 Samples were filtered in the field using 0.2µm Acrodisc[®] syringe filters (Pall Corporation, 132 Ann Arbour, USA), stored in acid-washed 50 ml polyethylene bottles, refrigerated at 4°C and 133 transferred to the University of Melbourne for analysis. Water samples weighing 10-20 grams 134 were equilibrated with a pure ⁸⁴Sr spike, dried and Sr was extracted using a small column of 135 EICHROM[®] Sr-resin. ⁸⁷Sr/⁸⁶Sr and Sr concentrations were measured using a Nu Plasma 136 multi-collector ICPMS (Nu Instruments®, Wrexham, UK), with sample introduction via a 137 low-uptake PFA nebuliser and Cetac® ARIDUS desolvator. Instrumental mass bias was 138 corrected by internal normalization to ⁸⁸Sr/⁸⁶Sr=8.37521 (exponential law). Sr isotope 139

analyses have typical internal (2se) precisions of ± 0.000020 or less, and are reported relative 140 to SRM987 = 0.710230; the reproducibility, or external precision (2 standard deviation [SD]), 141 of the results was ± 0.00004 (see Crook *et al. in press* for further details of the analytical 142 procedure). Mixing models for each river system were calculated using the riverine sample as 143 the freshwater endmember and a coastal marine water sample collected in August 2010 at the 144 Adelaide River mouth as the marine endmember (salinity: 35.8, ⁸⁷Sr/⁸⁶Sr: 0.70918, Sr: 3.6 145 mg/L). The ⁸⁷Sr/⁸⁶Sr value corresponding to a salinity of 1 from the mixing model for each 146 system was then plotted on the otolith ⁸⁷Sr/⁸⁶Sr graphs (see Results). 147

148 Otolith preparation and Sr isotope analysis

An initial sample of otoliths from 18 large barramundi (640–1220 mm TL) was analysed 149 using archived samples held by NT Fisheries, Department of Primary Industries (Daly, n=6; 150 Mary, n=6, South Alligator, n=3, Roper, n=3). We later augmented these samples with an 151 152 additional 49 otoliths from the Daly River (n=30) and Mary River (n=19) (500–1260 mm TL). Due to space limitations, the results of these additional analyses are presented as 153 Supplementary Material (S1). Samples from the largest available barramundi were used in 154 the analyses to provide information on the salinity histories of individual fish across all 155 phases of the protandrous life history. Fish were sampled between 2008 and 2014 from 156 commercial (gill nets) and recreational fishers (angling), as well as targeted research 157 sampling (boat electrofishing). Each fish was measured to the nearest 5 mm (TL) and the 158 otoliths were removed and placed in paper envelopes or plastic vials. In preparation for 159 analysis, one otolith from each fish was embedded in two-part epoxy resin (EpoFix®, Struers, 160 Denmark) and transversely sectioned to a thickness of ~300 µm through the primordium 161 using a slow speed saw. The sections were polished using lapping film (9 μ m), rinsed with 162 163 deionised water, air dried and mounted on glass slides using epoxy resin. Laser ablation-MC-ICPMS was used to measure Sr isotope ratios (87Sr/86Sr) in the otoliths, following the 164

methods outlined in Woodhead *et al.* (2005). The experimental system consisted of the MCICPMS system described above, coupled to a Australian Scientific Instruments RESOlution
laser ablation system with Laurin Technic (Canberra, Australia) ablation cell constructed
around a Compex 110 excimer laser (Lambda Physik, Gottingen, Germany) operating at a
wavelength of 193 nm.

170 Otolith mounts were placed in the sample cell and the ablation path for each sample was digitally plotted using GeoStar v6.14 software (Resonetics, USA) and a 400× objective 171 coupled to a video imaging system. Ablation transects were run from the otolith core to the 172 proximal edge using a 40 μ m laser spot and fluence of ~3 J/cm². A pre-ablation was 173 conducted prior to the analysis run to remove any surface contaminants with the laser pulsed 174 at 5 Hz and scanned at 20 µm/sec. The analysis run was then conducted with the laser pulsed 175 at 5 Hz and scanned at 10 µm/sec. Ablation was performed under pure He atmosphere to 176 177 minimise the re-deposition of ablated material, and the sample was then rapidly entrained into the Ar carrier gas flow. Corrections for Kr and ⁸⁷Rb interferences were made following 178 Woodhead *et al.* (2005) and mass bias was corrected by reference to an ⁸⁶Sr/⁸⁸Sr ratio of 179 180 0.1194. The data were processed using Iolite Version 3.32 that operates within IGOR Pro Version 6.2.2.2 (WaveMetrics, Inc., Oregon) with corrections for potential Ca argide/dimer 181 and Rb interferences and instrumental mass bias. All results were normalised to an ⁸⁷Sr/⁸⁶Sr 182 isotope ratio of 0.70916 for a modern marine carbonate standard run concurrently and known 183 from solution ICPMS analyses to have a modern seawater composition (MacArthur and 184 185 Howarth 2004). Following otolith chemistry analysis, the otolith sections were examined under a dissecting microscope and annual increments counted to estimate the age of each fish 186 (see Stuart and McKillop 2002). The locations of annual increments were measured along the 187 ⁸⁷Sr/⁸⁶Sr transect for each fish using image analysis software (Image-Pro Plus, Media 188 Cybernetics, Rockville, MD, USA). 189

190 Acoustic telemetry

191 Fish for tagging with acoustic transmitters were collected by boat electrofishing (MBS-

2DHP-PS-SR, ETS, Madison, WI, USA) and their total length (TL) measured to the nearest 5 192 mm. The transmitters (V13-1L, Vemco, Nova Scotia) were surgically implanted into the 193 peritoneal cavities of 25 barramundi (420-1010 mm TL, median 510 mm TL) from 8-16 194 October 2013. Fish were anaesthetised with Aqui-S (0.03 ml L⁻¹) and placed in a V-shaped 195 foam holding cradle lined with wetted absorbent cloth. The transmitters were sterilised with 196 Hibitane disinfectant (100 ml L⁻¹) and rinsed with sterile saline prior to implantation. 5-6 197 198 scales were removed from the ventral surface approx. 10 cm anterior to the anal vent and slightly offset from the midline, and the area swabbed with Betadine. An incision of ~25 mm 199 length was then made in an anterior-posterior orientation into the peritoneal cavity using a 200 201 sterilised scalpel. The transmitters were inserted into the peritoneal cavity through the 202 incision and the incision was then closed with a single layer closure using three interrupted sutures placed into the musculature 3-4 mm beneath the skin (2.0 metric, absorbable 203 monofilament, 26 mm swaged needle). Betadine was then applied to the area. An assistant 204 irrigated the gills using fresh river water throughout the procedure (~4 min). After the 205 procedure, fish were held in fresh river water until normal posture and gill movement had 206 resumed and were then released at the site of collection. An array of 30 acoustic receivers 207 (VR2W, Vemco, Nova Scotia) was deployed from the estuary mouth to the upstream 208 209 billabongs, including two receivers in Yellow Water and one each in Home and Mardugal billabongs (Fig. 1). Two receivers were also deployed at the mouths of the nearby Wildman, 210 West Alligator and East Alligator rivers. Data was downloaded from the receivers at ~3 211 monthly intervals between October 2013 and June 2015. 212

- 213 **Results**
- 214 *Water* ⁸⁷*Sr*/⁸⁶*Sr* mixing models

⁸⁷Sr/⁸⁶Sr in the freshwater samples used to calculate mixing models across the salinity 215 gradient were all higher than seawater (Daly = 0.7161, Mary = 0.7585; South Alligator = 216 0.7450; Roper = 0.7175), reflecting the ancient geology (Proterozoic-Cambrian) and 217 relatively radiogenic bedrock underlying the region (Ahmed and Munson 2013). In the South 218 Alligator and Daly rivers, modelled ⁸⁷Sr/⁸⁶Sr showed very little change from salinity 2–36, 219 but rose sharply below 2 (Fig. 2). The Mary River was similar except that modelled ⁸⁷Sr/⁸⁶Sr 220 began to rise sharply below ~5. The Roper River mixing model showed a more gradual 221 increase in modelled ⁸⁷Sr/⁸⁶Sr across the salinity gradient than the other three rivers due to the 222 223 higher salinity and Sr concentration of the freshwater endmember (Table 1, Fig. 3).

224 Otolith chemistry analysis

Estimates of the ages of the barramundi analysed in the study ranged from 5 to 17 years (Fig. 225 4, S1). The otolith ⁸⁷Sr/⁸⁶Sr analysis revealed high variation in individual salinity histories in 226 barramundi among and within the river systems (Fig. 4, S1). Some of the otolith transects 227 also exhibited distinct annual oscillations in ⁸⁷Sr/⁸⁶Sr at salinities associated with estuarine 228 (e.g., Fig. 4i, r) and freshwater residence (e.g., Fig. 4l, p). In agreement with previous studies 229 (see Milton 2009), all fish showed evidence of residence in saline or brackish water during 230 the very early life history, with near-core 87 Sr/ 86 Sr values corresponding to water salinities >1 231 and most very close to the global marine ⁸⁷Sr/⁸⁶Sr value of 0.70918. 232

Although there was high individual variation in the otolith ⁸⁷Sr/⁸⁶Sr data, several broad types
of movement behaviour were identifiable based on the otolith chemistry analysis. For
example, a contingent of fish (14 of the 67 fish analysed) showed no evidence of extended
residence in fresh water throughout the life history (Fig. 4 c, d, e, h, i, j, r, see also S1). Four
of these had ⁸⁷Sr/⁸⁶Sr ratios close to the marine value across the entire transect, suggesting
that they had remained in relatively saline water (salinity >5) throughout their life (Fig. 4 e, j,
S1). Six fish showed evidence of only brief and irregular freshwater residence (Fig. 4 c, d, r,

S1), whilst four had alternating periods of residence in water of varying salinity

241 approximately corresponding to their annual growth increments (Fig. 4 h, i, S1).

242 Other individuals occupied freshwater habitats for extended periods during the life history.

243 Of these, nine of the 67 fish had transitioned from saline to fresh water in the early life

history and then remained in fresh water for 2–5 years, before moving back downstream into

saline/brackish water (Fig. 4 a, g, k, S1). This pattern is broadly consistent with the

246 generalised life history model for barramundi, where return migration to the estuary coincides

247 with the onset of male maturation (3–4 years of age, Fig. 1).

Another contingent (14/67) of fish, collected from both fresh water and estuarine habitats,

249 had transitioned from saline water to fresh water in the early life history, but remained in

250 fresh water until age 6 or greater (Fig. 4 b, f, l, m, p, q, S1). The distinguishing feature of

these fish was that they had remained in fresh water beyond the usual age of initial male

252 maturation. Of these 14 fish, the six sampled from estuarine habitats had resided in

freshwater until the ages 6-10 before returning to the estuary (Fig. 4 b, f, p, S1), whilst the

eight fish sampled from fresh water were aged 6-11 years of age at the time of collection(Fig. 4 l, m, q, S1).

The sex of the three fish collected from the freshwater reaches of the South Alligator River 256 was confirmed via visual examination in the laboratory. The largest of these fish (Fig. 4 m; 257 1030 mm TL) was a six year old, mature female (Fig. 5) and the two smaller fish (Fig. 4 n, o; 258 640 and 725 mm TL) were both mature males. These two smaller fish were 5 years of age at 259 the time of sampling, so it is not possible to determine whether they would have migrated 260 into the estuary or continued to reside in freshwater for an extended period. Unfortunately, 261 262 information on the sex of the other fish analysed in the study (from archived otolith collections) was not available. 263

The 30 fish not classified into the above contingents were collected from fresh water and ranged from 2-5 years of age at the time of collection (2 years n=8; 3 years n=4; 4 years n=6; years n=12). Given the age of these fish, it is not possible to determine whether they would have undertaken downstream migrations to saline water according to the generalised life history model or delayed migration beyond the age of male maturation.

269 *Acoustic telemetry*

270 Twenty (80%) of the 25 tagged barramundi were detected >2 months post-tagging,

271 confirming longer-term survival and transmitter retention in these fish (Fig. 6). One of these

fish (Fig. 6f) was recaptured by an angler and data relating to its capture reported to the

273 project team. This fish had increased in length from 480 mm TL when tagged on 9th October

274 2013 to 625 mm TL when recaptured on 2nd December 2014 (an increase of 145 mm over

 \sim 275 \sim 14 months). It is important to consider the rapid growth rate of barramundi when

interpreting the acoustic telemetry data, as the sizes of the tagged fish would have increased

277 considerably, and their maturation status may have changed, during the >2-year period over

278 which tracking was conducted.

In the first 6 weeks post-tagging, all fish were restricted to the billabong system in which they
were tagged. During this time, the three billabongs (Yellow Water, Home, Mardugal) were
connected to each other via small channels, but not to the downstream reaches of the South
Alligator River and estuary. Occasional excursions among the three billabongs occurred
during this time, with three barramundi moving >5 km between billabongs (Fig. 6 a, e, q).

The 2013/14 wet season began in early November with rainfall from a monsoonal weather system inundating surrounding floodplains and initiating connecting flows between the

upstream billabongs and the main channel/estuary. Fish movement increased dramatically in

response to the wet season flows, with eight fish moving 6-80 km downstream into the main

channel/estuary of the South Alligator River between November and March (Fig. 6 c, e, m, n, 288 p, q, r, s). A concurrent radio-telemetry study (authors unpubl. data) also showed that tagged 289 barramundi moved large distances (commonly >1 km from permanent water) onto newly 290 inundated floodplains during this event and continued to utilise floodplain habitats for several 291 months during the wet season. As the acoustic listening stations were located only in the 292 billabongs and main river channel downstream, occupation of inundated floodplain habitat 293 294 resulted in a decrease in the number of detections of many fish during the wet season (e.g., Fig. 6 f, h, o). Of the eight fish that moved downstream into the main channel during the 295 296 2013/14 wet season, all except one moved back into the billabongs in which they were originally tagged by March 2014. The one fish that did not return home moved 54 km 297 downstream in early February and had moved 22 km back upstream by late February, after 298 which time detections ceased (Fig. 6 s). 299

300 Movement during the 2014 dry season was restricted to the three billabongs, with only occasional movements among billabongs (Fig. 6 e, f, k, l, t). Thirteen barramundi were still 301 302 being detected at the onset of the 2014/15 wet season. Of these, seven were subsequently detected only in the billabongs, with reduced detection rates suggesting utilisation of 303 surrounding inundated floodplains similar to the previous year (Fig. 6 a, f, h, k, l, o, t). Three 304 305 fish entered the main channel downstream of the billabongs during high river flows in January and returned upstream to the billabongs by early February (Fig. 6 c, g, r). The other 306 three barramundi with active transmitters moved into the lower reaches of the estuary and 307 308 continued to reside in the tidal region between the estuary mouth and \sim 70 km upstream for the remainder of the study. These included the two largest tagged fish (1010 and 860 mm TL) 309 and a fish of 550 mm TL at the time of tagging. None of the fish tagged in the South 310 Alligator River were detected on receivers in the adjacent river mouths during the study. 311

312 Discussion

313 Integrating otolith chemistry and telemetry data

The otolith chemistry and acoustic telemetry data presented here provides independent and 314 complementary information that strengthens the inferences that can be drawn regarding the 315 movements and life history of barramundi in the study rivers. For example, a major 316 conclusion from the otolith chemistry analyses was that the majority of fish that transitioned 317 into fresh water during the early life history remained resident within freshwater for 5-11 318 319 years (as opposed to the 3–4 years described in the existing barramundi life history model; Fig. 1). The acoustic tracking data supports this by demonstrating that the majority of tagged 320 321 fish remained within fresh water over multiple wet seasons. These findings are also in agreement with previous otolith chemistry analyses of barramundi in Papua New Guinea 322 (Milton and Chenery 2005) and Australia (McCulloch et al. 2005), who similarly reported 323 extended freshwater residence by some fish and little evidence of regular movements 324 between fresh water and estuarine/coastal habitats. Whilst our telemetry study revealed a 325 large increase in the rate and extent of movement within fresh water during the wet season, 326 both the otolith chemistry and telemetry data demonstrated that such movements were 327 restricted to freshwater (including inundated floodplains) for most individuals. An important 328 area for future research is to quantify the relationships between barramundi movement and 329 riverine discharge across all stages of the life history and among freshwater and estuarine 330 habitats. 331

An interesting feature of the otolith chemistry data was the occurrence of annual oscillations
in ⁸⁷Sr/⁸⁶Sr within the freshwater phase of some fish (e.g., Fig. 4 l, p, q). A similar pattern
was also reported in Sr/Ca and Ba/Ca in the otoliths of barramundi collected from several
northern Australian rivers, including the Daly and Roper rivers (Halliday *et al.* 2012).
Regular variation in otolith ⁸⁷Sr/⁸⁶Sr might either be attributed to regular movement by fish
between locations with distinct water ⁸⁷Sr/⁸⁶Sr, or to seasonal variation in water ⁸⁷Sr/⁸⁶Sr at

the same location (or perhaps some combination of both). Recent analyses in the Daly River 338 demonstrated strong seasonal variation in freshwater ⁸⁷Sr/⁸⁶Sr due to changes in the relative 339 contribution of groundwater versus surface runoff, with low ⁸⁷Sr/⁸⁶Sr in the dry season due to 340 groundwater input from aquifers that interact with geological formations of marine origin, 341 and higher ⁸⁷Sr/⁸⁶Sr in the wet season due to surface runoff from catchments with more 342 radiogenic geology (Crook et al. in press). Given the potential for strong seasonal variation in 343 water ⁸⁷Sr/⁸⁶Sr in the study rivers, we were not able to determine whether annual oscillations 344 in the otolith ⁸⁷Sr/⁸⁶Sr profiles of barramundi represent regular movements within freshwater 345 346 or temporal water chemistry variation. Fortunately, the telemetry data fills this knowledge gap, at least for the South Alligator River, by demonstrating high rates of movement within 347 freshwater during the wet season (including use of inundated floodplain habitat) followed by 348 homing to previous residence locations prior to the dry season. 349

Oscillations in some of the otolith ⁸⁷Sr/⁸⁶Sr profiles also indicated annual alternations in 350 ambient salinity of fish residing in the estuary (Fig. 4 a, b, g, h, i, r). Again, this pattern might 351 either be attributed to return movements among regions of different salinity or to seasonal 352 variation in ambient water ⁸⁷Sr/⁸⁶Sr; in this case due to the influx of fresh water into the 353 estuary during the wet season. The estuarine zones of Northern Territory rivers often become 354 entirely fresh during high flow periods in the wet season (D. Williams, Australian Institute of 355 Marine Science, unpubl. data), so regular oscillations between ⁸⁷Sr/⁸⁶Sr values associated 356 with saline/brackish water (~ 0.709) and freshwater (≥ 0.713) would be expected for fish that 357 358 remain in the estuary throughout the year.

The telemetry data provided little evidence of regular movements between fresh water and saline estuary habitats by barramundi in the South Alligator River. Although many of the tagged fish moved between the billabongs and upstream reaches of the main channel during the wet season, salinity measurements taken 85 km from estuary mouth (0.19 on 12

December 2013; 0.36 on 28 April 2014) suggest that the upper main channel was fresh during 363 the period when these movements were undertaken. Only two tagged fish undertook return 364 movements between the billabongs and potentially saline/brackish reaches of the estuary 365 (Fig. 6 c, m). One of these fish (Fig. 6 m, 550 TL) was detected 36 km upstream of the 366 estuary mouth on 18 December 2013. Salinity measurements of 20.4 on 10 December 2013 367 and 5.1 on 10 January 2014 taken 50 km upstream of the estuary mouth confirm that this fish 368 369 had entered brackish water during this time. Salinity data were not available for the 2014/15 wet season; however, given the lower rainfall in 2014/15, it appears likely that the second 370 371 fish (Fig. 6 c, 720 mm TL) had also entered brackish water when it was detected 55 km from the estuary mouth on 19 January 2015, before moving back upstream to the freshwater 372 billabongs. Consistent with these data, several barramundi (Fig. 4 b, n, S1) had distinct 373 troughs in their otolith ⁸⁷Sr/⁸⁶Sr profiles that may reflect short-term excursions into brackish 374 water. 375

376 In combination, these results suggest that return movements between fresh and saline/brackish water occur occasionally in the study rivers, but are relatively uncommon and 377 appear unlikely to represent key aspects of the reproductive behaviour of barramundi. This is 378 consistent with conclusions from previous otolith chemistry studies of barramundi 379 populations in Papua New Guinea and Queensland (Milton and Chenery 2005; Milton et al. 380 2008), but differs from the interpretation of Halliday et al. (2012) who attributed oscillations 381 in otolith Sr/Ca and Ba/Ca to annual migrations across the salinity gradient. Given that we 382 383 only tagged fish from main channel billabong with acoustic transmitters, there remains a need for further detailed information on the movements of barramundi collected from estuarine 384 habitats to develop our understanding of the level of connectivity between estuarine and 385 386 freshwater populations.

387 Life history implications

The current study has significant implications for our understanding of barramundi life 388 history. Firstly, our results show that only small minority of fish exhibited movements 389 consistent with the generalised life history model for the species (Fig 1). Of the 48 fish >5 390 years of age analysed for otolith chemistry, only 9 had otolith ⁸⁷Sr/⁸⁶Sr profiles consistent 391 with this model (Fig. 4; S1). The otolith chemistry data also show that a proportion of fish did 392 not exhibit extended residence in fresh water at any time during the life history. This finding 393 394 agrees with previous conventional tagging (Moore and Reynolds 1982) and scale and otolith chemistry studies in Australia (Pender and Griffin 1996; Cappo et al. 2005; McCulloch et al. 395 396 2005; Milton et al. 2008; Halliday et al. 2012) and Papua New Guinea (Milton and Chenery 2005), which identified the existence of estuarine/marine contingents within barramundi 397 populations. 398

A particularly significant finding of the current study was the identification of a proportion of 399 400 fish that remained resident in fresh water until 6–11 (or more) years of age. The protandrous 401 transformation from male to female in Northern Territory barramundi populations generally occurs at 840-970 mm TL (Davis 1982) at an average age of 5.4 years (range 4-8 years) 402 403 (Davis 1982; Davis and Kirkwood 1984). Bearing in mind that barramundi spawn near estuary mouths in salinities of ~28-36 (Grey 1987), our findings suggest that at least some 404 freshwater residents had developed into females without ever spawning as males. This 405 suggestion is supported by confirmation that a 6-year old fish which had lived continuously 406 in the freshwater reaches of the South Alligator River since the early life history was a mature 407 408 female. Furthermore, two 11-year old fish collected from freshwater had lived continuously in freshwater since the early life history and another two were 8 and 10 years old when they 409 migrated back to the estuary after living within fresh water since the early life history. 410

One alternative to this interpretation is that barramundi undertake extremely rapid return
migrations between freshwater and the estuarine spawning grounds that are not reflected in

the otolith chemistry data. Delays of 10-40 days for otolith Sr to reach equilibrium with 413 ambient water Sr have been reported (e.g., Macdonald and Crook 2010), so it is possible that 414 very rapid return migrations would not be detected in our analyses. However, this explanation 415 seems unlikely for several reasons. Firstly, barramundi in northern Australia exhibit a 416 prolonged breeding season that extends from September to March (Davis 1987; Garrett 417 1987). Observations of the spawning behaviour of barramundi suggest they spend a 418 419 significant amount of time on the spawning grounds during this time, with larger female fish spawning multiple times over a season and males fertilising the eggs of multiple females 420 421 (Moore 1982; Garrett 1987). Consistent with these observations, the three tagged fish that moved to the lower saline reaches of the South Alligator estuary during the spawning season 422 continued to reside in that area for the remainder of the study without returning to freshwater. 423 424 Whilst two tagged fish undertook rapid downstream return movements into brackish water 425 during the spawning season, these are unlikely to have been spawning migrations because the two fish spent only a few days in brackish water and neither moved into the putative 426 spawning grounds of the lower estuary. Based on this evidence, we suggest the most 427 parsimonious explanation for our results is that a proportion of barramundi transform into 428 females whilst resident in freshwater, without returning to the estuary to spawn as males. 429 Whether such fish mature initially as males but then fail to spawn, or develop directly into 430 females, requires further investigation. 431

Our observations of extended freshwater residency in some barramundi are comparable to the
findings of Milton and Chenery (2005), who examined otolith ⁸⁷Sr/⁸⁶Sr and Sr/Ca in
barramundi in southern Papua New Guinea. Milton and Chenery (2005) sampled fish from
the fresh water reaches of several rivers, as well as the coastal marine spawning grounds.
Similar to the current study, they found that a proportion of mature female fish collected from
fresh water were >8 years of age and had not migrated back to the spawning grounds since

the early life history. Based on this observation, Milton and Chenery (2005) suggested that 438 such fish may not contribute to spawning during their lives and that "freshwater fisheries for 439 barramundi may have less effect on the spawning population than those in coastal regions and 440 thus be able to sustainably remove a greater proportion of the population." In contrast, our 441 results show that fish that had been resident in freshwater for up to 10 years eventually 442 returned to the estuary where they presumably joined the spawning population. These 443 444 delayed migrants are likely to be large, highly fecund females, suggesting that freshwaterresident fish may in fact be important contributors to spawning and would, therefore, warrant 445 446 the same level of protection as estuarine fish. Further analysis is required to determine the extent to which the different interpretations between studies represent differences in 447 sampling/methodology versus variation in the life history of Papua New Guinea and northern 448 Australian barramundi populations. 449

450 Based on the current study's findings and information contained in previous studies of barramundi movement and life history (Moore and Reynolds 1982; Moore 1982; Davis and 451 Kirkwood 1984; Davis 1986; Grey 1987; Russell and Garrett 1988; Pender and Griffin 1996, 452 McCulloch et al. 2005; Cappo et al. 2005; Milton and Chenery 2005; Halliday et al. 2012), a 453 revised conceptual model of barramundi life history that defines distinct migratory 454 contingents is presented (Fig. 7). Although previous studies have recognised the existence of 455 migratory contingents in barramundi populations, they have concentrated on broad 456 457 differences in movement and residence patterns among individuals, rather than explicitly 458 linking migration strategies to life history stage. For example, Pender and Griffin (1996) used cluster analysis to classify individual barramundi in the Mary River, Northern Territory, into 459 "marine", "billabong" and "mixed" groups based on chemical analysis of whole scales. 460 McCulloch et al. (2005) classified barramundi in Queensland as "freshwater migratory", 461 "landlocked", "estuarine" and "marine" based on otolith chemistry analysis, whilst Cappo et 462

al. (2005) used a statistical classification technique to group barramundi from northern
Queensland into "freshwater" and "marine" groups based on chemical analysis of whole
scales.

The life history model presented here differs from these previous classifications by focussing 466 on migration and residence as a function of the stage of sexual development. Three 467 contingents are recognised in the model. The "estuarine" contingent consists of non-468 diadromous individuals that undertake their life cycles (including sequential sex change) 469 primarily within coastal or estuarine habitats. It is possible that some fish in this contingent 470 undertake occasional or annual upstream forays into freshwater habitats, but freshwater 471 residence in such individuals is short-lived in comparison to the other two contingents. The 472 "catadromy, sequential hermaphrodism" contingent consists of individuals that migrate into 473 freshwater as juveniles and then move at the onset of male maturation (3-5 years of age) back 474 475 to the estuary, where they spawn initially as males and then later as females (i.e., the "classic" life history strategy; Fig. 1). The "catadromy, delayed female spawning" contingent consists 476 of individuals that migrate into freshwater as juveniles and remain within freshwater without 477 returning to the estuary to spawn as males. Sexual inversion from male to female (or possibly 478 direct female development) in these fish occurs within freshwater and an eventual 479 downstream migration to the estuary is undertaken at a large body size. 480

Although the proposed life history model identifies three discrete contingents, we recognise the strong individual variability in movement behaviour of barramundi and the fact that our classification may not adequately describe the movements of all individuals. The proposed life history model is an attempt to synthesise the main patterns of barramundi movement and life history into a coherent framework with utility for management and future research.

486 *Conclusions*

Our findings suggest that barramundi populations in northern Australia are comprised of 487 migratory contingents and that these may represent different tactics within a conditional 488 strategy (see Gross 1996). If this is the case, the decisions individuals make regarding 489 migratory tactics would be conditional on factors such as the individual's status (e.g., body 490 condition, growth rate), interactions with other organisms (e.g., conspecifics, predators) and 491 ambient environmental conditions (e.g., habitat availability, river flow). Because the choice 492 493 of migratory tactics is, at least in part, dependent on environmental conditions, anthropogenic disturbance to aquatic ecosystems has the potential to influence the frequencies of migratory 494 495 contingents within barramundi populations, and thus affect a range of demographic processes. 496

Previous studies suggest that barramundi populations are especially vulnerable to hydrologic 497 disturbance, with recruitment and growth strongly and positively correlated with river 498 499 discharge (Staunton-Smith et al. 2004; Robins et al. 2005, 2006; Halliday et al. 2010; 2012). 500 It has also been shown that barramundi tend to grow faster when living in freshwater than when residing in saline estuarine habitats (Anas 2008; Milton et al. 2008) and that a higher 501 proportion of juvenile fish enter fresh water during high flow years than low flow years 502 (Halliday et al. 2012). Decisions by individual barramundi about whether or not to migrate 503 into freshwater during the juvenile phase, or to migrate to the estuary to spawn as males, may 504 therefore represent trade-offs between the reproductive benefits of growing faster versus the 505 energetic costs and mortality risk associated with migration (Milton 2009). The availability of 506 507 high-quality freshwater habitat, such as undisturbed floodplains that become inundated during the wet season, is likely to be a key factor affecting these decisions. If more fish adopt 508 slower-growing, estuarine life histories in systems with heavily disturbed hydrology and 509 510 freshwater habitat, the productivity of barramundi fisheries and their ability to sustain fishing pressure may be adversely impacted. 511

512 Understanding the drivers of life history variation and the potential outcomes of 513 anthropogenic disturbance in a sequentially hermaphroditic species such as barramundi is 514 particularly complex because reproductive tactics that are beneficial for male reproduction in 515 early adulthood may not necessarily maximise reproductive success following sex inversion 516 into the female form later in life. On the other hand, this complexity makes barramundi a 517 fascinating model species for future research on the evolution of intra-specific life history 518 variation within populations.

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| River | Site | Date | EC (ms/cm) | Salinity | Sr (mg/L) | ⁸⁷ Sr/ ⁸⁶ Sr |
|---------------|-----------------|------------|------------|----------|-----------|------------------------------------|
| Daly | Galloping Jacks | 12/03/2014 | 0.082 | 0.05 | 0.009 | 0.74390 |
| Mary | Arnhem Hwy | 2/11/2014 | 0.059 | 0.04 | 0.017 | 0.75850 |
| Sth Alligator | Yellow Water | 19/11/2013 | 0.047 | 0.03 | 0.009 | 0.74501 |
| Roper | Elsey Nat. Park | 11/11/2014 | 1.370 | 0.90 | 0.384 | 0.71748 |

Table 1: Freshwater samples used to generate water ⁸⁷Sr/⁸⁶Sr mixing models.

683

684 **Figure legends**

Figure 1: Conceptual models of barramundi life history. (a) generalised barramundi life
history model from Grey (1987), (b) current Northern Territory life history model (source:
NT Fisheries).

Figure 2: Map of the study area. (a) Locations of the four rivers from which barramundi

otoliths and water samples were collected for chemical analysis. (b) South Alligator River

690 showing the locations where barramundi were tagged with acoustic transmitters (YW:

691 Yellow Water Billabong, HB: Home Billabong, Mardugul Billabong. Filled black circles

692 show locations of acoustic listening stations.

Figure 3: Mixing models of water ⁸⁷Sr/⁸⁶Sr across the salinity gradient in the four study

rivers (Daly = black line, Mary = grey line, South Alligator = dashed grey line, Roper =
dashed black line).

696 Figure 4: Core-to-edge transects of otolith ⁸⁷Sr/⁸⁶Sr in barramundi collected from the Daly

697 River (a-f), Mary River (g-l), South Alligator River (m-o), and Roper River (p-r). The black 698 broken line represents seawater 87 Sr/ 86 Sr and the grey broken line represents water of salinity 699 1. The black diamonds show the locations of annual increments along each transect. The total 700 length (mm) and location of collection (E = estuary and F = freshwater) for each fish is 701 shown.

Figure 5: (a) Large barramundi (1030 mm TL) collected from Yellow Water Billabong,

703 Kakadu National Park, Northern Territory, (b) photograph of the same fish with abdominal

cavity dissected to show developed female gonads. This fish was 6 years of age when

collected and had been continuously resident in fresh water since 1 year of age (see Fig. 4 m).

Figure 6: Graphs of movements of the 20 tagged barramundi for which data was collected for more than 2 months. Small black symbols represent detections and lines have been drawn between detections for clarity. The total length (mm) at the time of tagging is shown for each fish. Grey bars show total monthly rainfall recorded at Jabiru 33 km from the South Alligator

710 River (Source: Bureau of Meteorology).

711 Figure 7: Proposed "three-contingent" conceptual life history model for Australian

712 barramundi (see text for detailed explanation).