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1 **Quantifying movement of multiple threatened species to inform adaptive**
2 **management of environmental flows.**

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12 **Abstract**

13 There is a growing need for water managers to refine and optimise environmental flow strategies (e-
14 flows) to balance water requirements for humans and nature. With increasing demands for
15 freshwater and consequent declines in biodiversity, managers are faced with the problem of how to
16 adaptively manage e-flows for multiple stakeholders and species whose flow requirements may
17 overlap or vary. This study assessed the effectiveness of a regulated e-flow release strategy from a
18 dam, aimed at providing movement opportunities and facilitating reproductive processes for
19 multiple threatened species. Movements of 24 Mary River cod (*Maccullochella mariensis*), 20
20 Australian lungfish (*Neoceratodus forsteri*) and 13 Mary River turtle (*Elusor macrurus*) were
21 quantified using acoustic telemetry over a three-year period. The influence of regulated e-flow
22 releases, season, river depth, water temperature and rainfall on animal movements was assessed
23 using Generalised linear mixed models (GLMMs). Models showed that hydraulic connectivity
24 provided by both natural flows and regulated e-flow releases facilitated movement of all three
25 species between habitats, throughout the year. Mary River turtles made extensive use of regulated
26 e-flow releases when moving between habitats, whereas Mary River cod and Australian lungfish
27 required additional natural rises in river height above the regulated e-flows to trigger movements.
28 Significant movement activity was also recorded for cod and turtles during the dry season (winter
29 and spring), broadly coinciding with breeding periods for these species. The effectiveness of, and
30 potential improvements to, current e-flow strategies to sustain key life-history requirements of
31 these species is discussed. Findings suggest a revised e-flow strategy with relatively minor increases
32 in the magnitude of e-flow releases throughout winter and spring, would be effective in providing
33 movement opportunities and supporting reproductive success for all three species. This study
34 demonstrates that by quantifying movement behaviour in an e-flow context, ecological risk
35 assessment frameworks can be used to assess and provide for critical life-history requirements of
36 multiple species within the context of a highly regulated system under increasing water use
37 demands.

38 **Key words: environmental flows, adaptive management, threatened species, acoustic telemetry**

39

41 **1. Introduction**

42 Human dependence on freshwater has impacted riverine ecosystems in many ways including
43 impoundment, water extraction and flow regulation leading to the alteration, fragmentation and
44 degradation of riverine habitats (Kingsford, 2000; Tharme, 2003). Dams and associated flow regime
45 changes affect movement of aquatic species by altering key environmental factors such as
46 connectivity, hydrologic and hydraulic cues, and habitat quality and quantity (Arthington et al., 2016;
47 Crook et al., 2015). Understanding human impacts to flow regimes and habitats in relation to the
48 spatial ecology of biota allows managers to assess and prioritise water requirements and provisions
49 necessary to sustain aquatic ecosystems (Cooke et al., 2016). Furthermore, prioritising which
50 spatiotemporal movements are critical to maintain population viability and species persistence, and
51 what aspects of the flow regime are manageable considering human water security, are key to
52 developing effective strategies for conservation management.

53 Movement is essential to the long-term viability of freshwater species such as fish and turtles
54 supporting survival, dispersal, reproduction and genetic integrity over a range of spatio-temporal
55 scales (Faulks et al., 2010; Jungwirth, 1998; Micheli-Campbell et al., 2017). The triggers, purpose and
56 extent of movements are intimately tied to species life-history and physiology through the
57 environmental conditions they encounter (Young et al., 2006). These movements can be broadly
58 grouped into survival or reproductive strategies (Lucas and Baras, 2001; Northcote, 1998). Survival
59 strategies are driven by resource requirements; water, food and habitat all play a role in stimulating
60 movement for persistence, nutrition and refuge, respectively (Crook, 2004; Koehn and Nicol, 1996).
61 These movements are generally opportunistic in time and space, driven by resource limitations, and
62 support physiological condition (Koehn et al., 2009). Movements related to reproductive strategies
63 are more specific in terms of timing, duration and habitat; driven by environmental triggers that
64 initiate and facilitate the reproductive cycle (Humphries et al., 1999; Koehn and Nicol, 2016).

65 Flows of variable magnitudes, timing and duration provide differing ecosystem functions, with
66 medium to high flows eliciting dramatic ecosystem responses (Arthington and Balcombe, 2011; Junk
67 et al., 1989) including large-scale fish migrations (Crook et al., 2019; Reynolds, 1983). More recently,
68 the importance of low flows in sustaining ecosystem processes including movement has also been
69 shown (Marshall et al., 2016; Storer et al., 2021). However, not all animals move on all flows, and
70 difficulties in quantifying movements of populations has resulted in limited quantitative evidence for
71 ecologically significant movement events (Jönsson et al., 2016). This has led to a significant body of
72 literature identifying what aspects of the flow regime trigger and facilitate movement of biota, and
73 in turn, what movements are critical to sustain populations within freshwater environments subject
74 to increasing pressure from water resource development (Dudgeon et al., 2006; Tickner et al., 2020).

75 Environmental flows (e-flows) are a key strategy for conservation management of freshwater
76 ecosystems around the world. E-flows describe the quantity, timing, and quality of freshwater flows
77 and levels necessary to sustain aquatic ecosystems which, in turn, support human cultures,
78 economies, sustainable livelihoods, and well-being (Acreman et al., 2014; Arthington et al., 2018). A
79 key requirement for e-flow provision is identification and quantification of specific hydraulic habitat
80 or discharges contributing to ecological outcomes focused on water quality, aquatic refugia and
81 animal life-histories including movement. Implementation of e-flows however, can be difficult due to

82 competition for water (Poff et al., 2003), scientific uncertainty in the movement and habitat
83 requirements of species, particularly at early life stages contributing to recruitment (Gwinn et al.,
84 2016; Koehn et al., 2020); and a general lack of research, monitoring and evaluation implemented
85 within a risk-based framework (Davies et al., 2014; Mcgregor et al., 2018). Even in basins with
86 comprehensive understanding of native fish ecology, delivery of prescribed e-flows have met with
87 variable ecological responses across fish communities highlighting the need for more targeted
88 quantification of flow-ecology relationships coupled with adaptive management frameworks (Cruz et
89 al., 2020; King et al., 2010). Rare examples of targeted long-term research, monitoring and
90 management programs have successfully demonstrated e-flows as an effective tool for mitigating
91 impacts to threatened aquatic species (Koster et al., 2021, 2018, 2017, 2013).

92 The Mary River in south-east Queensland Australia supports several nationally-listed threatened
93 species including the Mary River cod (*Maccullochella mariensis*), Australian lungfish (*Neoceratodus*
94 *forsteri*) and Mary River turtle (*Elusor macrurus*) (Smith and Connell, 2018). Flow alteration and
95 habitat fragmentation from water infrastructure (dams and weirs) and extraction are major threats
96 to these species (Arthington, 2009; Clark et al., 2009; Kennard, 2003), highlighting the challenge for
97 water managers in providing water for agriculture and rapidly growing urban and rural populations,
98 whilst providing water for multiple threatened species with limited information and potentially
99 competing requirements. As part of the Queensland Government water planning process for the
100 Mary River catchment (NRM, 2003), an e-flow strategy was implemented for a dam on a major
101 tributary (Obi Obi Creek) to mitigate potential impacts to the downstream environment. Due to
102 limited ecologically relevant information, a precautionary e-flow strategy was introduced to address
103 impacts to the low flow regime including reduced magnitudes and increased dry spells.

104 Mary River cod, Australian lungfish and Mary River turtle are riverine specialists with life history and
105 habitat requirements that are critically-linked to key attributes of the flow regime (Department of
106 Natural Resources, 2018). Current knowledge indicates that reproductive cycles for all three species
107 are completed during the dry season (August – December) coinciding with low flows and rising water
108 temperatures. These species also have specific flow-related habitat and movement requirements to
109 support spawning and recruitment. Specifically, Mary River cod require connectivity for locating
110 partners (ensuring genetic dispersal) and for accessing specific nesting habitat (complex instream
111 structures) (DNRM, 2016; Simpson and Mapleston, 2002); in response to water temperatures that
112 initiate the reproductive season (Espinoza et al., 2020). For Australian lungfish, variable low flows
113 trigger spawning activity and facilitate movements to spawning habitat (dense beds of aquatic
114 plants) (Espinoza et al., 2013; Kind, 2002). Mary River turtles require connectivity for locating mates
115 (ensuring genetic dispersal) and for access to nesting habitat (sandy riverbanks) (Espinoza et al.,
116 2018).

117 Although all three species' reproductive periods coincide with the dry season, the specific timing,
118 magnitude and duration of flows that support reproduction for each species is poorly understood
119 and requires further investigation (Brooks and Kind, 2002; Micheli-Campbell et al., 2017; Simpson
120 and Mapleston, 2002). Quantification of flow-ecology relationships is essential to relate movement
121 behaviour of these species to their environments and predict responses to potential management
122 interventions. Acoustic telemetry allows researchers to passively measure and monitor animal
123 movements and behaviour in aquatic environments, whilst also overcoming some of the constraints
124 surrounding the study of threatened and cryptic species (Heupel and Webber, 2012; Hussey et al.,

125 2015). This technology involves implanting animals with acoustic transmitters that are detected by
126 hydrophones deployed across diverse habitats, over extended timeframes and has been used
127 successfully in other e-flow studies on Australian species (Grothues, 2009; Harding et al., 2017;
128 Koster et al., 2013).

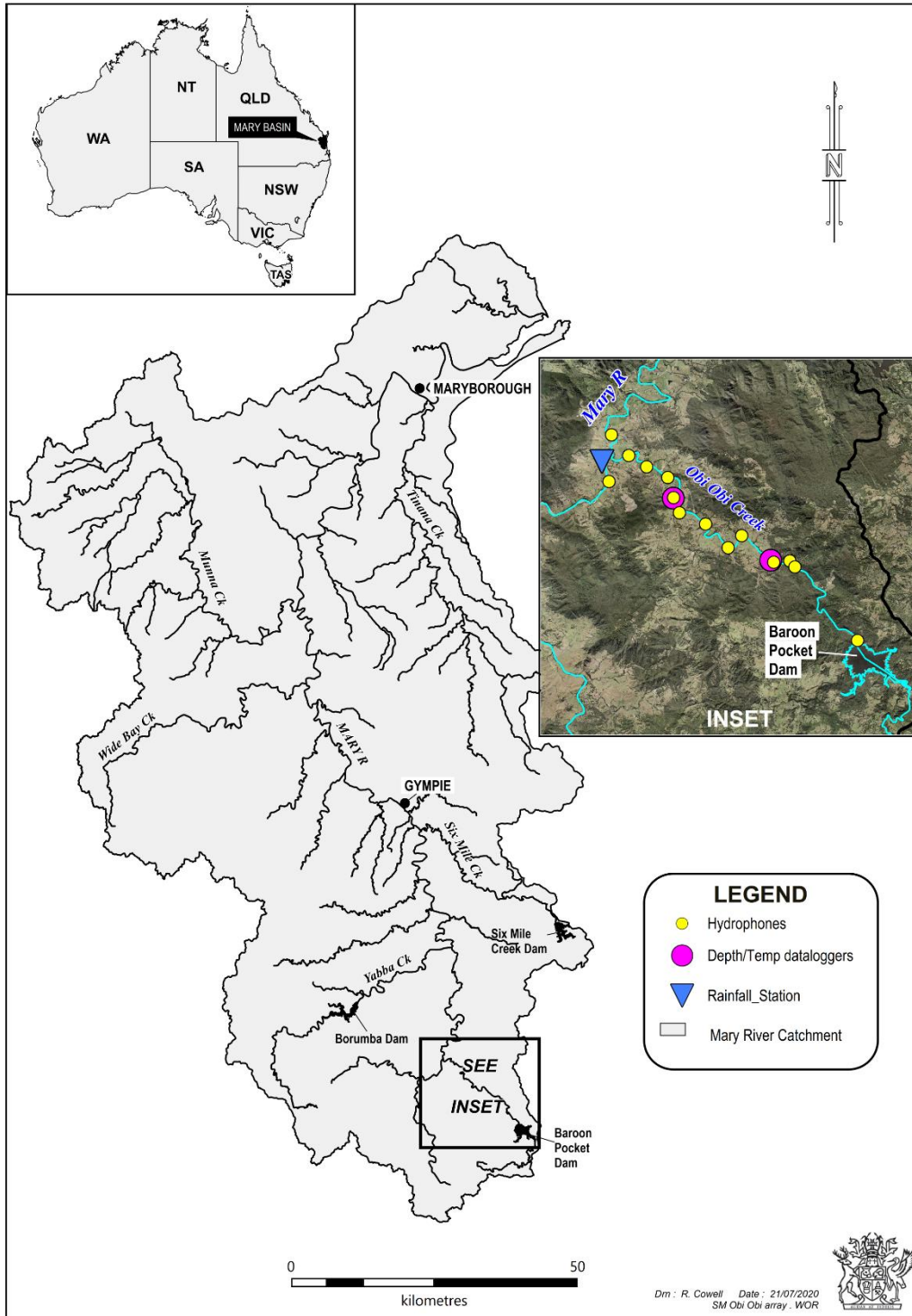
129 This three-year study quantified the movements of Mary River cod, Australian lungfish and Mary
130 River turtle, to assess the effectiveness of the current e-flow release strategy within Obi Obi Creek
131 and identify potential refinements of these strategies to better support these threatened species.
132 Movement patterns were evaluated in response to regulated e-flow releases, variations in river
133 levels and water temperatures to assess how environmental conditions influenced connectivity and
134 supported reproductive processes. We aimed to demonstrate that quantification of animal
135 movement behaviour can inform ecological risk assessments to support adaptive management of e-
136 flows. Results from this study can improve the sustainability of threatened species amidst increasing
137 human water demands and inform water managers in other regulated rivers around the world.

138

139 **2. Methods**

140 **2.1 Study area**

141 This study was undertaken between June 2013 and September 2016 within Obi Obi Creek, a
142 tributary of the Mary River in southeast Queensland, Australia (Figure 1). This watercourse is
143 approximately 57 kilometres long, drains a catchment area of approximately 202 km², and is within a
144 rapidly developing urban region with increasing water demands (Bunn et al., 2010). Baroon Pocket
145 Dam, a 61 000 ML storage intercepts flow at 26.4 km from the junction with the Mary River and
146 provides potable and agricultural water for southeast Queensland region through an interconnected
147 water grid. Below the dam wall, the creek passes through the pristine bedrock gorge and rock pools
148 (Figure 2A) before emerging through a low-lying floodplain towards the Kenilworth township
149 (Queensland Government, 2004). Catchment land use in this reach includes dairy/grazing,
150 horticulture, rural-residential, urban and forestry (Brizga et al., 2005) which has had variable impacts
151 on riparian habitat. Although some sections have very little riparian habitat (Figure 2B), other
152 previously cleared areas have established riparian canopies of native and exotic species such as
153 camphor laurel (*Cinnamomum camphora*) (Figure 2C). Obi Obi Creek has been recognised as an
154 important wetland system in Australia (DIWA) (Miller and Deacon, 2005), and supports a key
155 population of the endemic Mary River cod (Simpson and Jackson, 2000).

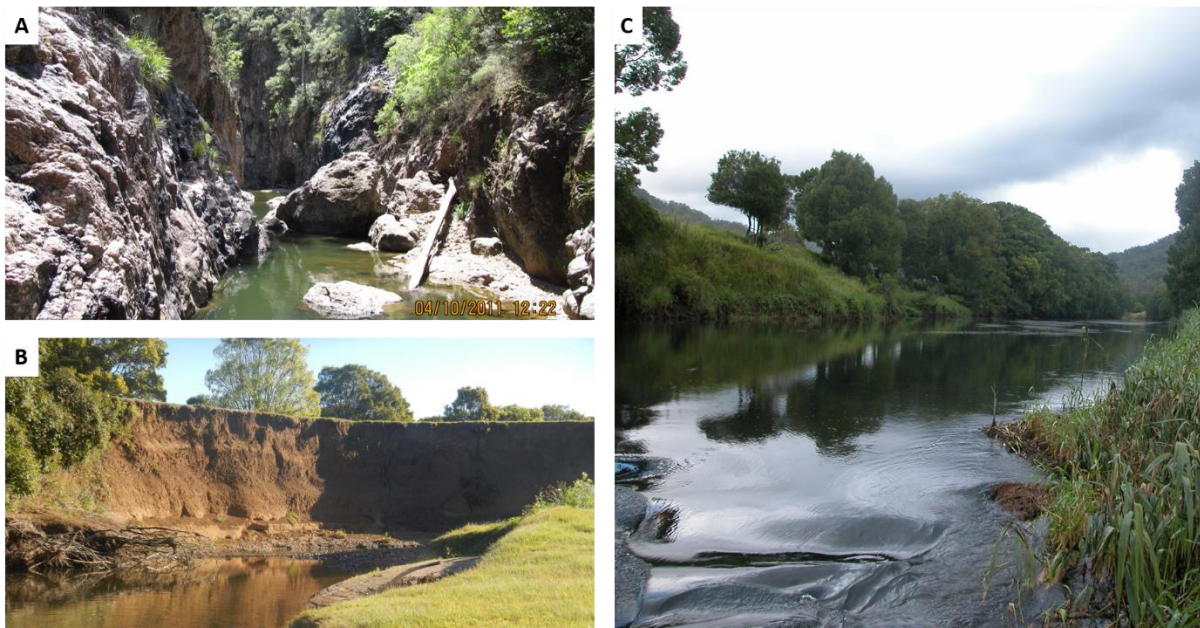


156

157 **Figure 1: Study area showing locations of hydrophones, depth/temperature dataloggers and rainfall station**

158 Obi Obi Creek has mean annual rainfall at its headwaters of ~2 000 mm providing Baroon Pocket
 159 Dam a mean annual inflow of ~70 000 ML (Queensland Government, 2004). A panel of scientific
 160 experts commissioned to advise development of the original Water Resource (Mary Basin) Plan 2006
 161 (Government, 2006) highlighted significant impacts to the natural flow regime of Obi Obi Creek
 162 downstream of Baroon Pocket Dam, particularly reductions in low flows and increased dry spells
 163 (Queensland Government, 2004). These flow alterations were determined to likely affect movement

164 and reproductive cues for key species such as Mary River cod, potentially affecting population
165 viability, therefore a mitigation strategy was recommended. Currently, an e-flow strategy consisting
166 of translucent releases (Grouns and Reinfelds, 2014) is made from Baroon Pocket Dam to Obi Obi
167 Creek for environmental purposes. This strategy aims to maintain water quality and support the
168 requirements of Mary River cod, Australian lungfish and Mary River turtle. Releases are calculated
169 based on gauged inflows to the dam with a proportion of inflows released up to a maximum of 15
170 ML.d⁻¹. Additionally, releases for downstream users and local rainfall runoff subsidise riverine flows
171 in Obi Obi Creek (Government, 2011). Rainfall was considered average to below average during the
172 study period, whilst mean temperatures were slightly above average (Figure 3) (Bureau of
173 Meteorology, Commonwealth of Australia 2020).



174
175 **Figure 2** Variable habitats of Obi Obi Creek in the (A) upper, (B) lower and (C) middle reaches.

176

177 2.2 Animal collection

178 Mary River cod and Australian lungfish were captured in June 2013 using a boat-mounted
179 electrofisher unit (2.5 GPP; Smith Root, Inc., Vancouver, WA, USA), backpack electrofishing unit (LR-
180 24 Smith-Root, Inc., Vancouver, WA, USA) or angling with lure. All fish were measured (Total Length
181 – cm) using a wetted concave fish measuring board. In total, seven pools covering 13 km of Obi Obi
182 Creek were electrofished in June 2013. Maximum depths of pools ranged from 3 - 4.5 m and were
183 considered representative of the overall watercourse.

184 Mary River turtles were captured in September 2013 from six riffle habitats on Obi Obi Creek using
185 double-winged fyke nets with 20 mm mesh, 10 m wings x 1.2 m drop, 4 round hoops with 0.9 m
186 diameter. The cylindrical section of each net was 4 m in length with two internal funnels, each
187 having a fixed opening of 0.4 m. Nets were set facing both upstream and downstream parallel to the
188 riverbank, with a maximum set time of 12 hours per net. Wings and cod-end were secured using
189 metal stakes, and polystyrene floats were inserted in the cod-end to allow air pockets for turtles and
190 other animals. Standard Carapace Length (SCL) was measured for all turtles (cm) and mature

191 specimens were sexed based on tail length with males having significantly longer and more robust
192 tails.

193 2.3 Telemetry

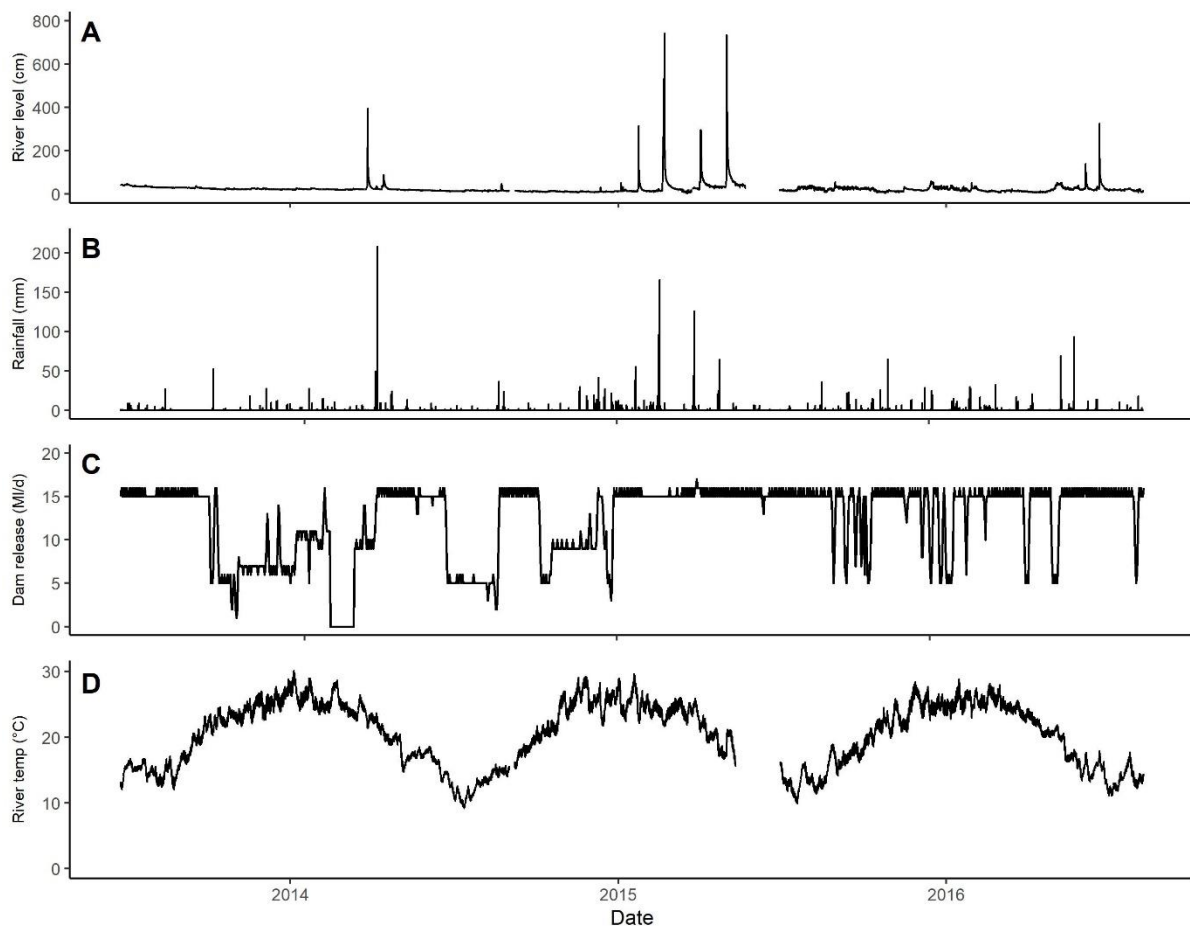
194 Acoustic telemetry was used to track animal movement and location between 2013 and 2016. In
195 total, 24 Mary River cod, 20 Australian lungfish and 13 Mary River turtles (8 males, 5 females) were
196 tagged with Vemco™ V13 acoustic transmitters. Prior to surgery, fish were held in an aerated
197 holding tank followed by an anaesthetic bath containing 50 mgL⁻¹ of AQUI-S® (AQUI-S New Zealand
198 Ltd). Once sedated, fish were placed in a V-shaped surgical cradle and measurements of total fish
199 length and body depth were recorded. Transmitters were set to a 60-180 second roving ping rate
200 and inserted into the gut cavity through a 15-20 mm incision behind the pectoral fin. Tag sizes
201 ensured the weight ratio was kept under the recommended 2.25% of total fish body weight (Butler
202 et al., 2009; Wagner et al., 2011). The incision was closed with absorbable sutures and tissue
203 adhesive (Vetbond; Provet, Brisbane Qld, Australia). All fish were allowed to recover in aerated
204 freshwater holding tanks until normal swimming behaviour was observed before release back into
205 the creek at point of capture.

206 The procedure for transmitter attachment to turtles followed Micheli-Campbell et al. (2017). Two
207 holes were drilled vertically through the posterior marginal scutes of the carapace and the
208 transmitter was secured in place using a purpose-built cap, plastic saddle, and PVC nuts and bolts
209 (1.5 mm). The nuts were secured with Loctite 243 (Henkel, Victoria, Australia), and the ends of the
210 bolts were covered in a two-part epoxy putty (KneadIt, Selleys, Padstow, Australia). Vemco™ V13
211 transmitters (36 mm L x 13 mm D) were set to a 60-180 s roving ping rate.

212 A longitudinal array of 12 hydrophones (Vemco VR2W-69 kHz) was deployed at regular intervals
213 throughout Obi Obi Creek from the Mary River junction to Baroon Pocket Dam tailwater (Figure 1).
214 Hydrophones were installed at ~2 km intervals except for a 6 km gorge section with limited access.
215 One additional hydrophone was deployed in the main Mary River channel approximately 2 km
216 downstream of the Obi Obi Creek junction to detect movement direction out of Obi Obi Creek.
217 Hydrophones were secured to a concrete anchor and tethered to stable overhanging riparian
218 vegetation using 5 mm stainless steel cable. No overlapping detection ranges occurred between
219 adjacent hydrophones. A Vemco™ VR100 portable hydrophone was also used to manually locate and
220 confirm animal positions at regular intervals (approx. 6 months) during the study period.

221 2.4 Environmental data

222 Regulated release data for Baroon Pocket Dam (e-flow) was obtained from Resource Operation
223 Licence reporting data supplied quarterly to the Department of Regional Development,
224 Manufacturing and Water (Figure 3). Data loggers (Diver, Schlumberger Ltd) recording water depth
225 and temperature were also installed in Obi Obi Creek at two locations in Obi Obi Creek (Figure 1) for
226 the entire study. Locations and heights of dataloggers were surveyed to cross-reference water levels
227 to pool cease-to-flow points and the 90th percentile river level was also calculated from level data to
228 separate baseflows provided by e-flows from natural flow events within the creek. Rainfall data was
229 obtained from Climate Data Online (Bureau of Meteorology, Commonwealth of Australia 2020) for
230 the Kenilworth Township rain gauge (site 040106, Figure 1).



231

232 **Figure 3 Summary of river level (A), rainfall (B), regulated e-flow release (C) and river temperature (D) for the study**
 233 **period.**

234 **2.5 Data analysis**

235 Movement events were initially summarised and assessed against various environmental and
 236 biological variables of interest including reported breeding periods (Table 1), changes in river levels,
 237 rainfall, movement direction, hydrograph limb, dam releases and diel periods. Here, ‘movement
 238 events’ were defined as animal movements detected across multiple hydrophones within one week,
 239 i.e. if detection between two hydrophones took longer than one week, this was considered a
 240 separate ‘movement event’. Results are presented as the percentage of movement events for each
 241 species in response to environmental variables.

242 **Table 1 Documented key periods for each species in the study. Note, breeding involves mating, nesting and paternal**
 243 **care whereas spawning is oviposition only.**

Species	Key period	Purpose	Reference
Mary River cod	Aug - Dec	Breeding	(Simpson and Jackson, 2000)
Australian lungfish	Aug - Dec	Spawning	(Brooks and Kind, 2002)
Mary River turtle	Oct - Dec	Nesting	(Micheli-Campbell et al., 2013)

244

245 The time lag of flow pulses between the upstream and downstream sections of the study reach was
 246 determined by assessing corresponding peaks in the hydrograph generated by spatially discrete river
 247 level loggers. This enabled projection of hydrographic conditions for each animal movement from

248 each respective location, at the start of a movement event; and was summarised as a cumulative
249 proportion plot of movement events versus river rise above baseflow, for all three species.
250 Cumulative distance moved and total linear range were calculated for all individuals, where total
251 linear range was the river distance between the most upstream and downstream detections for each
252 fish for the total study period.

253 A spatial object was created by digitising the study reach from satellite imagery in ArcGIS 10.4 (ESRI,
254 Redlands, CA, USA). From this, the distance between each receiver station and the most
255 downstream station was calculated and the spatial object was converted into a distance matrix in
256 the *V-Track* package (Campbell et al., 2012) in R (R Development Core Team; www.r-project.org).
257 Individual fish detections were then matched with the distance matrix and the distance between
258 detections was calculated. Likelihood of movement was quantified as a distinct function of
259 environmental and biological variables using a generalised linear mixed model (GLMM) in the *lme4*
260 package in R (Bates et al., 2015), in which movement was treated as a binary variable (presence or
261 absence of movement). We specified a logistic regression model for the binary response, with
262 predictor variables considered including: total daily dam release, average daily river level, average
263 daily river temperature, time of year (season), fish total length or turtle carapace length and sex
264 (MRT only). Continuous independent variables were centred and scaled to ensure model
265 convergence. Fish identity (ID) was included as a random intercept in the models. All possible
266 models and interactions were examined, and the best model was selected using the Akaike
267 information criterion (AIC). Following the protocol of Zuur et al. (2010), the data was checked for
268 statistical outliers and collinearity among predictor variables was assessed using variance inflation
269 factors (VIF). Model fit was assessed by comparing model binned residuals and fitted values.

270

271 **3. Results**

272 Of the 24 Mary River cod, 20 Australian lungfish and 13 Mary River turtles tagged, greater than 70%
273 of individuals across all species were recorded within the study area for at least 100 days. Only four
274 cod and one lungfish were not detected on any receiver throughout the study. One Mary River cod
275 tag was discovered (by manual tracking) in a shallow open section of a pool and had been expelled
276 by the fish soon after tagging. Manual tracking revealed tagged turtles and fish residing permanently
277 in habitats between hydrophones, and amongst complex habitats (undercut banks, hollow logs and
278 log jams) that prevented reliable detection of those individuals by the hydrophone array. Although
279 some tags ceased to be recorded without being detected at terminal hydrophones of the array
280 (which would signal movement out of the creek), no specific evidence for predation effects or
281 human interference was found for any tagged animal.

282 Mary River turtle were found to move greater cumulative distances compared to the two fish
283 species, although Australian lungfish undertook the largest linear movements (Table 2). Mary River
284 cod were the most sedentary species demonstrated through highest rates of detection, and smallest
285 linear and cumulative distance moved within the hydrophone array.

286 [Table 2 Summary statistics for Mary River cod, Australian Lungfish and Mary River turtle for the study period including](#)
287 [mean \(\$\pm\$ SE\) and range of values. Number of tags missing out of array or lost from animal from start of study in](#)
288 [parentheses.](#)

Cod (n = 24)[5]

Lungfish (n = 20)[1]

Turtles (n = 13)[0]

Metric	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Total Length (mm)	558 ± 34	(335 - 850)	955 ± 21	(780 - 1100)	350* ± 1	(260 - 410)
No. of detections	102321 ± 19375	(0 - 321691)	60592 ± 17490	(0 - 279773)	41773 ± 17678	(0 - 230158)
Days detected in array	421 ± 69	(0 - 1116)	194 ± 41	(0 - 542)	222 ± 50	(0 - 680)
No. of movements	1 ± 0	(0 - 8)	3 ± 1	(0 - 8)	14 ± 3	(0 - 36)
Cumulative dist. moved (km)	1 ± 1	(0 - 17)	6 ± 1	(0 - 23)	21 ± 5	(0 - 53)
Linear range (km)	1 ± 0	(0 - 6)	5 ± 1	(0 - 19)	5 ± 1	(0 - 10)

289 * carapace length

290 3.1 General movement patterns

291 Male Mary River turtles moved frequently between pools with a preference for nocturnal
 292 movements during river rises, particularly regulated e-flow releases, followed by return movements
 293 back to home pools (Table 3). Female Mary River turtles also made extensive use of regulated e-flow
 294 releases during nocturnal periods, with a greater proportion of movements during the reported
 295 breeding season. Although Mary River cod generally resided within a single 'home' pool, movements
 296 generally coincided with all flow-related environmental variables (i.e. river rise, regulated e-flow
 297 release and rainfall), with a high relative proportion of movements during the reported breeding
 298 season (August to December). Mary River cod also preferred nocturnal movements between pools
 299 and tended to utilise hydraulic conditions provided by the rising limb of the hydrograph. Australian
 300 lungfish also generally resided within home pools throughout the study period although movements
 301 coincided with all flow-related variables, particularly natural flow events with a rising and falling
 302 limbs (i.e. not regulated e-flow releases). Movements during the reported breeding season were not
 303 observed for Australian lungfish.

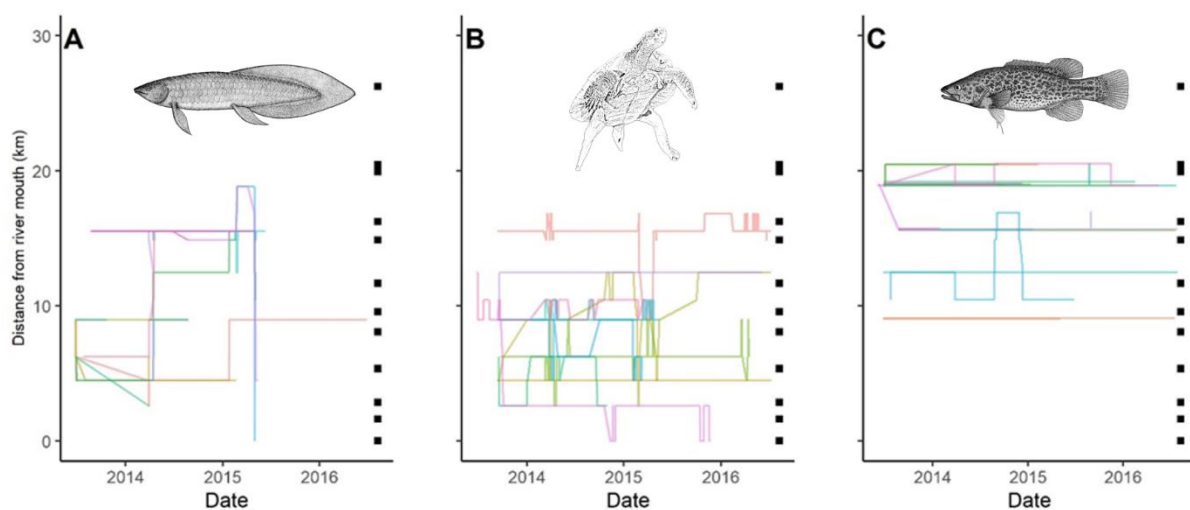
304 **Table 3 Summary of animal movements over the study period. Results presented as percentage of animal movements in**
 305 **response to environmental variables.**

Species	Sex	Period	River rise	Rain	Direction	Return	Hydrograph	E-flow release	Nocturnal
Mary River turtle	Male (n=8)	Overall	45% yes	60% yes	53% downstream	45% yes	13% rising 25% falling 62% steady	100% yes	70% yes
		Breeding season (3%)	100% yes	100% yes	100% upstream	0% yes	100% rising 0% falling 0% steady	100% yes	0% yes
	Female (n=5)	Overall	32% yes	77% yes	55% downstream	9% yes	14% rising 14% falling 72% steady	100% yes	82% yes
		Breeding season (60%)	15% yes	69% yes	54% upstream	8% yes	0% rising 8% falling 92% steady	100% yes	85% yes
Mary River cod		Overall	100% yes	100% yes	62% upstream	25% yes	81% rising or peak 19% falling 0% steady	100% yes	88% yes

	Breeding season (50%)	100% yes	100% yes	50% upstream	25% yes	87% rising or peak 13% falling 0% steady	100% yes	88% yes
Australian lungfish	Overall	96% yes	92% yes	56% downstream	20% yes	56% rising or peak 40% falling 4% steady	100% yes	52% yes
	Breeding season (0%)	0% yes	0% yes	n/a	n/a	n/a	0% yes	0% yes

306

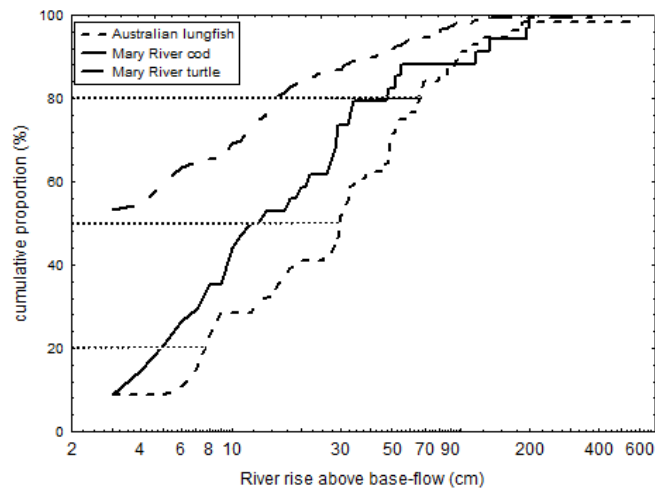
307 Movement plots for all three species were plotted as distance from river mouth to provide a
 308 comparable reference point for all species and identified distinct patterns across the study period
 309 (Figure 4). Male Mary River turtles frequently undertook short distance movements within defined
 310 home ranges throughout Obi Obi Creek, although longer distance movements were recorded on
 311 large natural flow events (Figure 3). A significant overlap in habitat use was observed within a 5 km
 312 reach of Obi Obi Creek between AMTD 5 km and 10 km for both males and females (Figure 4).
 313 Female Mary River turtles generally remained within home pools except during the reported nesting
 314 season, where repeated movements during spring to habitats in the vicinity of the junction with the
 315 Mary River, followed by return movements to home ranges, were recorded. In addition, one female
 316 Mary River turtle moved a cumulative distance of over 52 km during the study period. A majority of
 317 Mary River cod tagged in this study stayed within resident pools for the entire study period however,
 318 repeated return movements to and from home pools were observed during the late winter - early
 319 spring period for some individuals. Although largely sedentary, Australian lungfish movements were
 320 characterised by long distance movements during high flow events, often without return to previous
 321 home ranges. Synchronised movements in both upstream and downstream directions were
 322 detected, including three Australian lungfish with significant downstream movements on the same
 323 flood of which two lungfish exited Obi Obi Creek without return (Figure 4).



324

325 **Figure 4** Distance from the river mouth (km) of individual Australian lungfish (A), Mary River
 326 turtle (B) and Mary River cod (C) throughout the study array, presented as unique colours. Blocks on the right denote the hydrophone positions
 327 along Obi Obi Creek. Cod have been jittered 0.02 to reduce visual overlap. Fish drawings by Pusey et al. (2004), turtle
 328 photograph by John Cann and converted to drawing (all images used with permission).

329 Assessment of cumulative movements for all three species in relation to river rises above regulated
 330 e-flow releases also revealed important relationships (Figure 5). More than 50% of Mary River turtle
 331 movements occurred solely on regulated e-flow releases from Baroon Pocket Dam, with 80% of
 332 movements accounted for by a further 15 cm river rise above regulated releases. In contrast, for
 333 Mary River cod and Australian lungfish, a low proportion (< 15%) of movements coincided with
 334 regulated e-flow releases alone. Small river rises of less than 30 cm during rainfall-driven flow events
 335 however, facilitated a significant proportion of movements for Mary River cod and Australian
 336 lungfish.



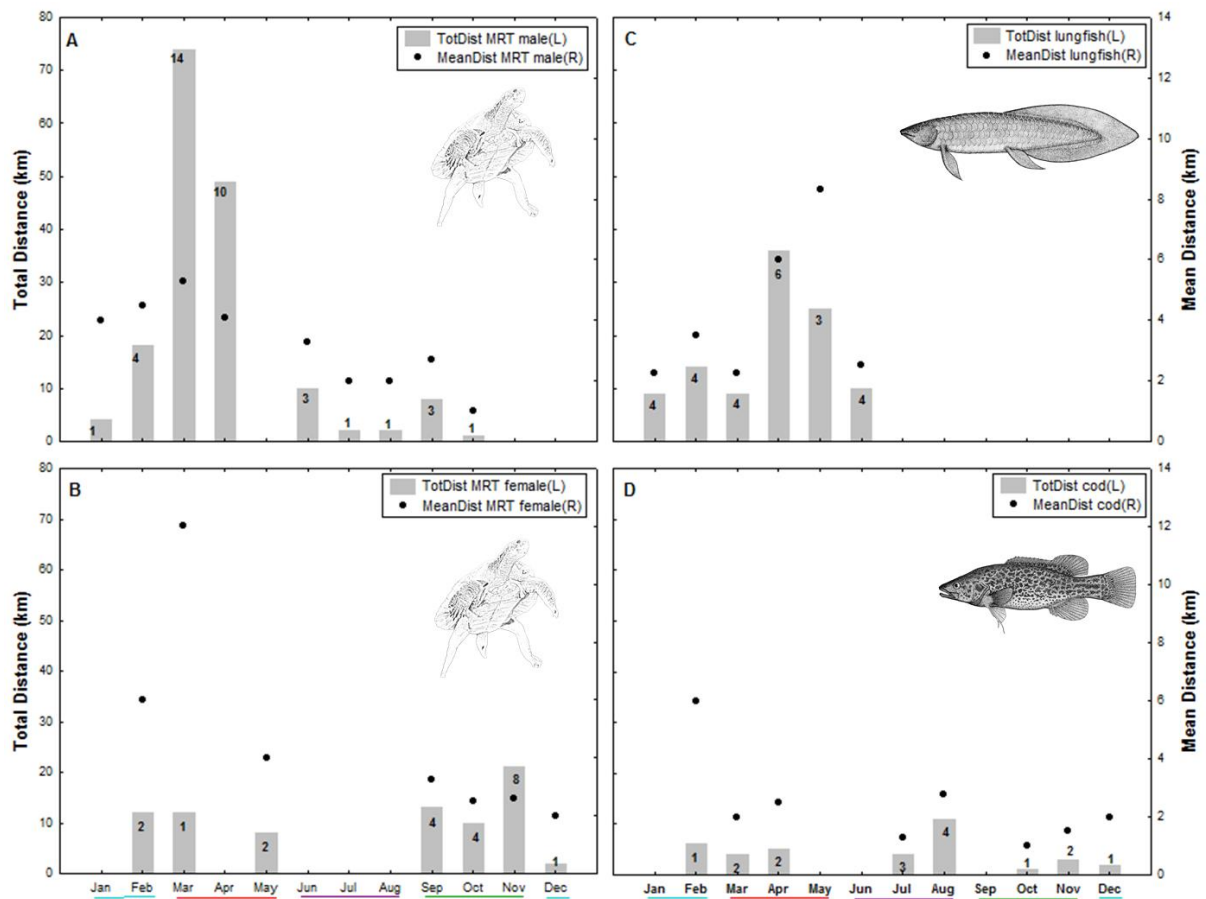
337

338 **Figure 5 Cumulative proportion of movements for river rises above base-flow (regulated e-flow releases). Horizontal**
 339 **dashed lines indicate 20%, 50% and 80% of cumulative movements.**

340

341 3.2 Seasonal movement patterns

342 Monthly summary plots of movements across the study period identified important seasonal
 343 patterns for each species in relation to documented reproductive periods (Figure 6 and Table 1). All
 344 three species moved frequently during large natural flow events in late summer and early autumn
 345 (February – May; Figure 3). Male Mary River turtles and Australian lungfish were particularly active
 346 during these periods, although one female Mary River turtle and one Mary River cod also made
 347 relatively long-distance movements during this time. Mary River turtles were active during the
 348 winter – spring period with males predominantly moving in winter, females predominantly in spring,
 349 and movements for both males and females overlapping in September. Mary River cod were
 350 relatively active during the late winter and spring period with July and August noted for movements
 351 across multiple individuals. Australian lungfish movements coincided with all wet season months
 352 covering summer and early autumn for multiple individuals.



353

354 **Figure 6** Monthly movement summaries for total distance (grey bars), mean distance (black dots) and number of
 355 individuals contributing to monthly movements. Mary River turtle (male – A, female – B), Australian lungfish (C) and
 356 Mary River cod (D). Summer (light blue), Autumn (orange), winter (purple) and spring (green) also shown on x axis.

357

358 3.3 Effects of environmental variations on probability of movement

359 Generalised Linear Mixed Modelling (GLMM) of animal movements in relation to environmental
 360 variability further highlighted contrasting relationships across all three species (Table 4; Figure 7).
 361 The probability of Mary River turtle movement increased significantly with increases in dam release
 362 magnitudes and river level. Winter, summer and spring were also associated with increased
 363 probabilities of movement; however, increases in regulated e-flow releases during spring did not
 364 increase the probability of movement for turtles. In addition, although higher river temperatures
 365 were also associated with increased probabilities of movement, the cooler periods in spring and
 366 summer were preferred. Finally, male Mary River turtle were significantly less likely to move in
 367 spring compared to females.

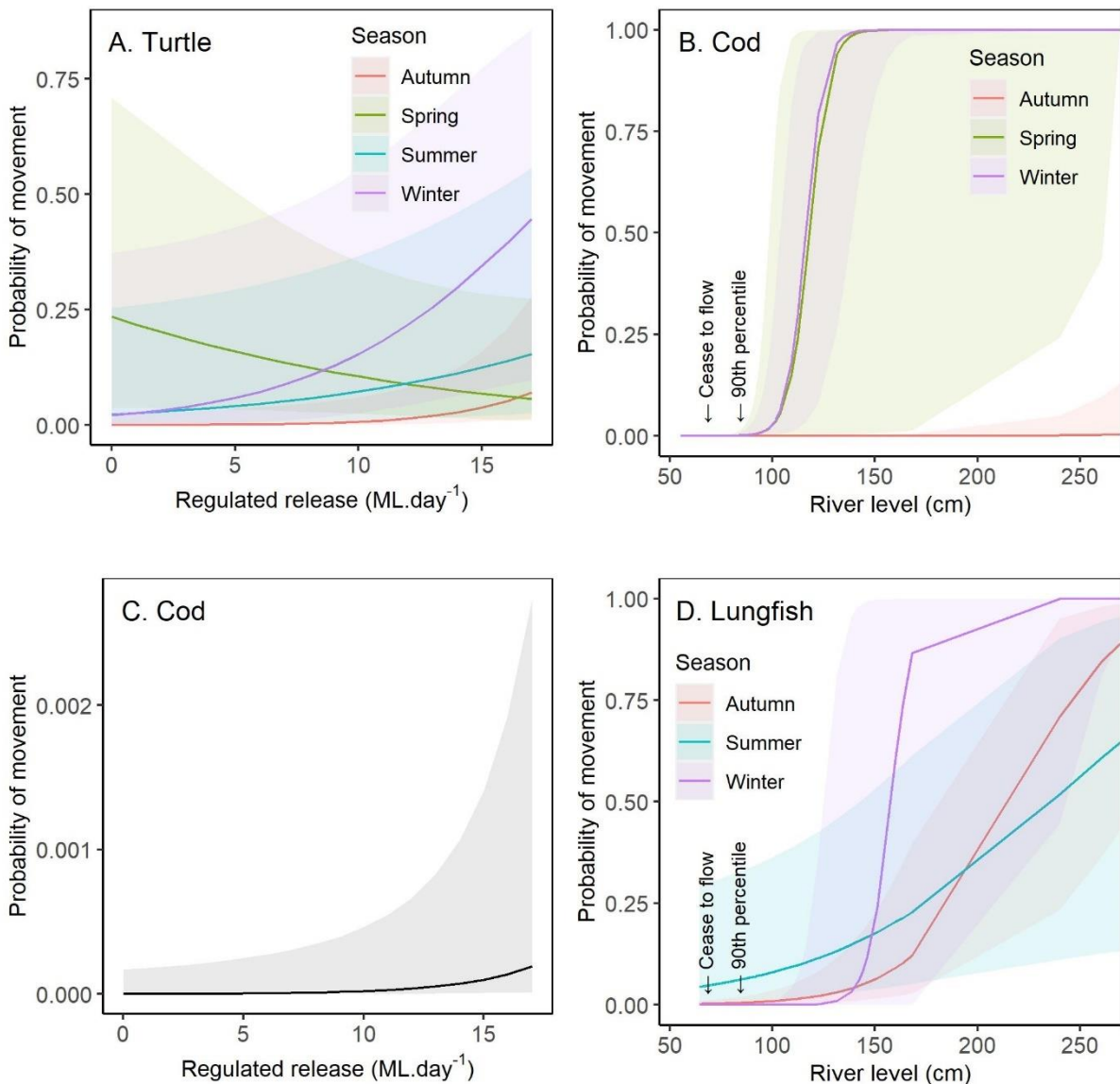
368 **Table 4** Parameter estimates (\pm SE) and significance levels from binomial GLMM relating to environmental variables and
 369 morphology, and animal movements for three species. Continuous predictor variables are scaled. Also shown are
 370 attributes of the random effects from each model, including number of IDs and years of release, among-ID and release
 371 year standard deviation and number of observations.

Fixed effect	Mary River turtle		Mary River cod		Australian lungfish	
	Est. \pm SE	P	Est. \pm SE	P	Est. \pm SE	P
River level (cm)	0.2 \pm 0.07	<0.001	0.35 \pm 0.12	<0.001	0.62 \pm 0.09	<0.001

Regulated release (ML day ⁻¹)	1.43 ± 0.6	0.02	1.51 ± 0.71	0.03	1.29 ± 0.52	0.01
River temperature (°C)	1.29 ± 0.25	<0.001			1.27 ± 0.49	0.01
Spring	1.81 ± 0.68	0.01	1.94 ± 1.1	0.08	-17.63 ± 6388	0.99
Summer	1.88 ± 0.85	0.03	-16.53 ± 191.21	0.93	2.8 ± 0.93	<0.001
Winter	2.92 ± 0.87	<0.001	1.62 ± 0.91	0.08	-8.89 ± 2.85	<0.001
River level : Spring			2.45 ± 1.14	0.03	-0.73 ± 15150	0.99
River level : Summer			-0.65 ± 389.2	0.99	-0.34 ± 0.16	0.03
River level : Winter			2.68 ± 0.6	<0.001	2.17 ± 0.8	0.01
Reg release : Spring	-1.84 ± 0.68	0.01				
Reg release : Summer	-0.91 ± 0.65	0.16				
Reg release : Winter	-0.51 ± 0.77	0.51				
River temperature : Spring	-1.83 ± 0.53	<0.001			-1.14 ± 8150	0.99
River temperature : Summer	-2.86 ± 0.71	<0.001			-6.16 ± 1.87	<0.001
River temperature : Winter	0.49 ± 0.61	0.43			-6.1 ± 1.41	<0.001
Autumn : Male	1.13 ± 0.96	0.24				
Spring : Male	-4.52 ± 1.38	<0.001				
Summer : Male	0.86 ± 0.98	0.38				
Winter : Male	-1.26 ± 1.03	0.22				
Random effect	ID	Year	ID		ID	
N	11	4	17		19	
Std. Dev.	1.3797	0.1539	2.172		1.624	
Observations	2708	2708	9362		3791	

372

373 Probability of movement of Mary River cod increased in response to increases in river level,
374 particularly during winter and spring (Figure 7B) however, regulated e-flow releases alone did not
375 increase the probability of movement for this species (Table 4; and Figure 7C). Probability of
376 movement of Australian lungfish increased significantly with increases in river level, river
377 temperature and regulated e-flow releases (Table 4). Australian lungfish were also most likely to
378 move in summer (Figure 7D) at cooler river temperatures; and much less likely to move in spring or
379 winter. Increased river levels in winter however, increased probability of movement.



380

381 **Figure 7** Predicted probability of movement for (A) Mary River turtle, (B, C) Mary River cod and (D) Australian lungfish as
 382 a function of dam release and river level (+/- 95% CI) across season. Probabilities were generated from the binomial
 383 model and all other covariates held at their mean values.

384

385 Discussion

386 This study quantified movements of multiple individuals of three threatened species in relation to
 387 environmental conditions, and an existing e-flow strategy, demonstrating that targeted monitoring
 388 of e-flows provides an improved understanding of flow-ecology relationships in regulated rivers
 389 (Davies et al. 2014; King et al. 2010; Scheele et al. 2018). We found increased probability of
 390 movements for three threatened species in response to both natural flows and regulated e-flow
 391 releases, coupled with seasonal changes in environmental conditions. By understanding the specific
 392 timing, minimum discharges and environmental conditions correlated to movements of all three
 393 species; risks from changes in the flow regime can be better quantified through ecological risk
 394 assessments that consider not only the frequency and timing of these flows for each species, but

395 also the corresponding consequences for the life-history for each species (Mcgregor et al. 2018).
396 Assessment of e-flows within an adaptive management framework not only helps identify key
397 aspects of the flow regime required to support the requirements of target species (Crook et al.,
398 2015; Robinson et al., 2018), but also provides an opportunity to refine and improve the current e-
399 flow strategy within the socio-economic constraints of regulated river systems (Webb et al., 2017,
400 2010).

401 For Mary River cod and Australian lungfish, correlations between movement and discharge have
402 been recorded in response to natural flow events in unregulated watercourses (Brooks and Kind,
403 2002; Simpson and Mapleston, 2002); whereas although Mary River turtles have shown a high
404 propensity to move (Micheli-Campbell et al., 2017), this is the first record of movements in response
405 to flow. Extensive Mary River turtle movements in response to regulated e-flow releases, and
406 preference of Mary River cod and Australian lungfish for river rises above these discharges,
407 demonstrate the importance of specific hydraulic cues and habitat for these species. Mary River
408 turtles do not traverse dry habitats between waterholes (M. Connell pers. comm.) and although no-
409 flow periods were rare during this study, our results support the requirement for hydraulic
410 connectivity to facilitate Mary River turtle movements. In contrast, the lack of Mary River cod and
411 Australian lungfish movement observed on regulated e-flow releases alone, even though suitable
412 water depths were recorded (i.e. greater than maximum depth of fish), suggests other
413 environmental variables or hydraulic cues associated with natural flow events trigger these species
414 to move. Changes in water level (flow variability) and water quality (water temperature and water
415 chemistry) have been previously shown to elicit movement responses for many Australian fish
416 species (Amtstaetter et al., 2021; Harding et al., 2017; Thiem et al., 2018).

417 Movements related to reproduction have been previously observed in spring (September-
418 November) for all three species in this study (Kind, 2002; Micheli-Campbell et al., 2017; Simpson and
419 Mapleston, 2002). Flows that facilitate reproductive processes of aquatic species are often targeted
420 as measurable indicators for long-term viability through links to animal condition, spawning,
421 recruitment and movement (King et al., 2009; Koehn et al., 2014; Mcgregor et al., 2018). In this
422 study, female Mary River turtle and Mary River cod were more likely to move when connectivity was
423 provided during spring, with repeated and return movements recorded across individuals and
424 breeding seasons. Both of these species are also known to seek suitable nesting habitat during this
425 period (Micheli-Campbell et al., 2013; Simpson and Jackson, 2000) with flows facilitating this
426 impetus. No movements were recorded for Australian lungfish during spring, in contrast to previous
427 studies which recorded targeted movements during this period, particularly upstream movements
428 from impounded to riverine habitats (Kind, 2002). This is an unexpected result for Australian lungfish
429 and we speculate this may be due to flood-related loss of food resources (scouring of macrophytes
430 and associated benthic fauna) in Obi Obi Creek prior to our study period which potentially affected
431 lungfish condition, breeding potential and availability of preferred spawning habitat.

432 Outside of the spring period, increased movement was recorded for Australian lungfish and male
433 Mary River turtle during high flow events which is common for many Australian freshwater species
434 (Ocock et al., 2018; Pusey et al., 2004; Roe and Georges, 2008). Movement during winter for all
435 three species, however, is a novel finding of this study. Probability of movement for Mary River
436 turtle increased significantly during winter, and monthly movement summaries highlighted this to be
437 driven primarily by males. Mary River cod and Australian lungfish were also more likely to move

438 during winter on natural flow events. Mary River cod have also recently been shown to increase
439 movement activity prior to breeding (Espinoza et al., 2020). Whether these movements are related
440 to pre-conditioning in anticipation of breeding (Arthington et al., 2014; Rolls et al., 2013), or
441 representative of actual reproductive activity (Mary River cod – nest location, Australian lungfish –
442 spawning, and Mary River turtle - mating) is unknown, however, the provision of low flows in winter
443 is a key finding in the development of effective e-flow strategies for all three species.

444 Outside of movements during seasonal flow events, we found all three species to demonstrate an
445 affinity for home pools, rather than a propensity to move. Quantitative evidence for ecologically
446 significant movement events across populations is not common (Jønsson et al., 2016), and although
447 advances in tagging technologies have addressed issues of low sample sizes and cost-effective
448 monitoring of more individuals over larger spatial scales; this does not necessarily apply to
449 endangered species due to restricted distributions and increased ethical limitations for research
450 (Cooke et al., 2012). Regardless, quantitative movement data from even small proportions of
451 populations has important implications for population viability through dispersal, range shifts,
452 climate change adaptability and genetic mixing (Cooke et al., 2016; Faulks et al., 2010; Reside et al.,
453 2017). Two factors that may have affected movement activity during our study include the relatively
454 optimal instream and riparian habitat that occurs within Obi Obi Creek compared to the rest of the
455 catchment (T. Espinoza pers. observations); and record floods prior to our study period (early 2011
456 and 2013) which caused extensive geomorphic changes and removal of aquatic plants within the
457 watercourse. Increased habitat availability and environmental predictability learned by animals over
458 various spatial scales has been shown to limit movement across species and habitats (Riotte-
459 Lambert and Matthiopoulos, 2020).

460 ***Management Implications***

461 Key lessons for water management from this study include: (1) hydraulic connectivity provided by
462 either natural flows or regulated releases facilitate movement of Mary River cod, Australian lungfish
463 and Mary River turtle throughout the year; (2) current regulated e-flow releases from Baroon Pocket
464 Dam provide baseflows that are particularly important for Mary River turtle; (3) small river rises
465 above these regulated baseflows significantly increase the probability of movement of Mary River
466 cod and Australian lungfish; and, (4) e-flow strategies that include flow provision during winter (in
467 addition to spring) months will be most effective for all three species in this study.

468 Winter and spring (May – December), however, represent the Queensland dry season (Klingaman,
469 2012), which coincides with increased water demands and increased potential conflict between
470 environmental and human water use. For example, although flows found to facilitate movement
471 have the potential to be delivered from existing infrastructure due to small magnitudes, the current
472 maximum e-flow release is restricted by the need to maintain reliability of water for human use.
473 With better understanding of the hydrologic requirements of Mary River turtle, Mary River cod and
474 Australian lungfish provided by this study, ecological risk assessments can be used to ensure the
475 long-term viability of these species through improved opportunities for movement within
476 reproductive periods throughout the Mary water plan area (Mcgregor et al., 2018).

477 ***Conclusions***

478 The current e-flow strategy within Obi Obi Creek is providing opportunities for movement for key
479 threatened species including Mary River cod, Australian lungfish and Mary River turtle. E-flow

480 releases alone are used extensively by Mary River turtles, and also by Mary River cod and Australian
481 lungfish when small additional river rises are provided by natural flow events. Refinement of the
482 current e-flow strategy to target winter and spring with increased flow variability and small increases
483 in discharges, will improve its effectiveness. This study will inform future ecological risk assessments
484 to review the efficacy of e-flow releases within Obi Obi Creek and develop suitable e-flow strategies
485 for these threatened species in the broader Mary River catchment.

486

487

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496

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