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1 **Migratory behaviour shapes spatial genetic structure of cyprinid fishes within the**
2 **Lake Malawi catchment**

3
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13

14 **Key words.** Microsatellite DNA, mitochondrial DNA, river fishes, conservation genetics, stock
15 structure

16

SUMMARY

1. Genetic differences among freshwater fish populations are dependent on life history characteristics of the species, including the range of adult dispersal and the extent of homing to natal breeding grounds. However, the effects of variation in such characteristics on population genetic connectivity are rarely studied comparatively among closely related species.

2. We studied population genetic structure within three fish congeneric cyprinid species from the Lake Malawi catchment that differ substantially in life history traits and conservation status, using a combination of microsatellite and mitochondrial DNA markers. Mpasa (*Opsaridium microlepis*) is a large (70 cm total length) migratory species that spawns in rivers, but as an adult is exclusively known from the main lake body. Sanjika (*Opsaridium microcephalum*), is medium size (30 cm total length) species that exists in lake breeding, river-lake migratory and apparently landlocked populations. Dwarf sanjika (*Opsaridium tweddleorum*) is a small non-migratory species (15 cm total length) that persists in small tributaries surrounding the main lake and adjoining rivers.

3. The results revealed striking differences among the three species in spatial genetic structuring. The river-lake migratory mpasa showed only weak yet significant population genetic structure within the main Lake Malawi catchment, suggesting that there is no strong natal homing. The habitat generalist sanjika showed only weak spatial genetic differentiation at microsatellite loci within the Lake Malawi catchment, but moderate structure in mitochondrial DNA, potentially reflecting male biased dispersal. The river restricted dwarf sanjika showed strong genetic structure in both microsatellite and mitochondrial DNA, suggesting strictly limited dispersal at both adult and juvenile stages.

4. We conclude that contrasting migration life-histories have resulted in dramatically different patterns of population genetic structure among these congeneric species. The observed patterns demonstrate how divergent life history evolution may strongly influence broader patterns of population genetic connectivity in freshwater fish, with consequences for management and conservation. Specifically the results suggesting gene flow among Lake Malawi populations of mpasa, an IUCN red-listed “Endangered” species endemic to the lake catchment, implying that conservation initiatives operating at both local and catchment scales are needed to reverse local population decline.

46 **Introduction**

47

48 Conservation and management of freshwater fish species can benefit from an understanding of the
49 genetic connectivity of populations which is dependent on multiple factors, including the rates of
50 migration between populations and effective population sizes (Palumbi, 2003). Migration rates are
51 fundamentally governed by movement behaviour throughout the life history of individuals. In many
52 pelagic freshwater fish species an important dispersal phase is present during the egg and post-hatching
53 larval stages. However, in most benthic freshwater or anadromous species dispersal is primarily
54 restricted to adult phases. Thus, in freshwater riverine species that have benthic spawning habitats we
55 may expect the degree of adult movement to be closely-linked to the extent of spatial population genetic
56 structuring. However, it is also notable that in migratory species there may be cases where adults freely
57 mix on lacustrine or marine feeding grounds, but return to natal riverine breeding grounds (Hedgecock
58 *et al.*, 2007). There are potentially strong benefits to breeding in rivers, due to a fundamentally lower
59 predation risk to eggs and fry (Waldman *et al.*, 2008).

60

61 There is evidence that life history traits are closely correlated with the extent of population genetic
62 connectivity. In multi-species analyses of marine fish data collected over large spatial scales, for
63 example, the presence of a pelagic larval dispersal stage leads to lower population genetic structuring
64 (Riginos *et al.*, 2011; Selkoe *et al.*, 2014). Over narrower taxonomic and spatial scales a potentially
65 powerful method of inferring the role of life histories in determining patterns of population genetic
66 structure is to directly compare closely related species with contrasting life history traits. In fishes,
67 evolutionary switches between migratory and non-migratory behavioural patterns have occurred
68 multiple times. Such switches have been particularly common in salmonids and clupeids (e.g. alosines),
69 which can have both migratory and resident populations of the same species (Fleming 1996, Roff 1988).
70 In these species groups, it is common for the populations containing migratory individuals to exhibit
71 reduced genetic structuring relative to the exclusively non-migratory populations (e.g. Hindar *et al.*,
72 1991; Jolly *et al.*, 2011).

73

74 Lake Malawi (= Lake Nyasa) in East Africa is an ancient lake estimated to have formed following
75 rifting that initiated 8-12 million years ago (Mortimer *et al.*, 2007; Danley *et al.*, 2012). It has a
76 maximum depth of 700 m, is 580 km long and 30–80 km wide. The lake has multiple inflowing rivers
77 and a single outflow into the Zambezi drainage. A series of waterfalls along this outflow, the Shire
78 River, form a biogeographic barrier between the Lake Malawi and Zambezi fish faunas (Tweddle &
79 Willoughby 1979). We studied three species of river-spawning cyprinid from the Lake Malawi
80 catchment that differ substantially in life history traits and conservation status. Mpasa (*Opsaridium*

microlepis) is a large sized (70cm maximum total length) migratory species that spawns in rivers but as an adult is otherwise exclusively found in the main lake body (Tweddle, 1987). The species is endemic to Lake Malawi catchment and is IUCN red-listed as “Endangered” due to apparent declines in abundance linked to fishing and alteration of spawning habitat (Kazembe *et al.*, 2006a). A second species, sanjika (*Opsaridium microcephalum*), is medium sized species (30cm maximum total length) that exists in lake breeding, river-lake migratory and apparently landlocked populations (Tweddle, 1983; Tweddle & Turner 2014). The species is also endemic to Lake Malawi and has a conservation status of “Vulnerable” (Kazembe *et al.*, 2006b). A third species, *Opsaridium tweddleorum*, is a small bodied species (15 cm maximum total length) that persists only in streams and small rivers (Skelton 2001). It is not endemic to Lake Malawi, as it is also found in tributaries of the Zambezi outside the Lake Malawi catchment. It has a conservation status of “Least Concern” (Vreven, 2006). Here we used a combination of microsatellite and mitochondrial DNA markers to quantify the extent of population connectivity in these species with contrasting migration behaviour, with a view to providing data to inform management and conservation of these species and critical habitats.

METHODS

Study sites, sample collection and DNA extraction.

Sixteen sites were sampled across the Lake Malawi catchment between 4th of May 2010 and 28th of January 2014 (Figure 1). Samples were obtained by fishing using seine nets, purchasing from artisanal fishermen, or from local markets (Tables 1 & 2). DNA samples were collected as either fin clips or whole juvenile fish, and were preserved in 100% ethanol. Total genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega), following the protocol of the manufacturer.

Microsatellite DNA analyses

PCR reactions were performed in a multiplex of ten microsatellite loci (Sungani & Genner 2015; Table S1). Each PCR contained 5 µL of QIAGEN Multiplex mix, 1 µL of DNA, 0.5 µL of each primer (10 µM concentration), and 3 µL of RNase-free water. PCR conditions were as follows: 5 min at 95°C, then 35 cycles of 94°C for 1 min, 58°C for 90 s and 72°C for 1 min, followed by 72°C for 15 min. Amplification success was checked on 1% agarose gel. Amplified fragments were separated on an ABI3500 sequencer alongside a Genescan500 size standard. Alleles were identified and sizes checked using GeneMapper4.0 (Applied Biosystems Inc., Foster City, CA, USA).

Allele frequencies, observed (H_o) and expected (H_e) heterozygosity (Nei 1987) were calculated with Arlequin 3.5 (Excoffier & Lischer 2010). Deviations from Hardy-Weinberg equilibrium (HWE) were

116 examined for each population at each locus by using Exact tests with Markov chain permutations (chain
 117 length 1,000,000 with 100,000 dememorization steps) in Arlequin 3.5. Microchecker 2.2.3 (van
 118 Oosterhout *et al.*, 2004) was used to check for the presence of null alleles. Genetic differentiation
 119 between populations was estimated by calculating Wright's F_{ST} statistic (Weir & Cockerham 1984)
 120 and the significance of population genetic differences was tested using Exact with 1000 permutations
 121 in Genepop 4.2 (Rousset 2008). Tests of the power of the combined microsatellite loci for each species
 122 to detect significant spatial genetic differences were conducted using Powsim (Ryman & Palm, 2006).
 123 Specifically, we used the proportion of Fisher's tests that were significant ($P < 0.05$) following 500
 124 runs, effective population sizes of 100 or 500, and parameters that provided estimated $F_{ST} = 0.005$ and
 125 $F_{ST} = 0.010$. We simulated tests of differences between two populations, each with 22 individuals which
 126 was the mean sample size of our study, and using the total allele frequencies observed across all
 127 populations. The extent of genetic differences among populations was visualised using
 128 multidimensional scaling in the MASS package (Ripley *et al.* 2015) in R 3.2.1 (R Development Core
 129 Team 2015). Population structure was analysed in each species independently using a model-based
 130 clustering algorithm with STRUCTURE 2.3 (Pritchard *et al.*, 2000). Ten runs were applied for each K .
 131 We applied a model that allowed for admixture with correlated allele frequencies, and conducted runs
 132 both with and without the Location Prior employed. Each run had a burn-in of 100,000 iterations
 133 followed by 100,000 MCMC replicates. STRUCTURE HARVESTER (Evanno *et al.*, 2005) and
 134 CLUMPAK (Kopelman *et al.*, 2015) were used to calculate summary data from the STRUCTURE
 135 runs.

136

137 ***Mitochondrial DNA analyses***

138 The primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) were used to amplify a ~700bp fragment
 139 of the *cytochrome oxidase subunit 1 (COI)* gene. PCR was carried out in 25 µl volumes, including 12.5
 140 µl of MyTaq (Bioline), 0.5 µl of each primer, 10.5 µl of H₂O, and 1 µl of DNA. PCR conditions were
 141 as follows: 4 min at 95 °C; then 34 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30s, followed
 142 by 72 °C for 7 min. Amplification success was checked on 1% agarose gel Sequencing of the PCR
 143 products was outsourced to Macrogen (Seoul, Korea). Sequences were aligned using ClustalW in
 144 DAMBE 5.3.32 (Xia, 2013). Haplotype networks were constructed using Hapstar (Teacher & Griffiths
 145 2011), based on minimum spanning trees generated in Arlequin 3.5. Haplotype (h) and nucleotide (π)
 146 diversities were estimated per locality with DNASP 5.0 (Librado & Rozas, 2009). Genetic
 147 differentiation among pairs of populations was estimated using Φ_{ST} , and the significance of differences
 148 was tested using 1000 permutations in Arlequin 3.5, while multidimensional scaling as described above
 149 was used to visualise Φ_{ST} among populations. New sequences generated have the Genbank Accession
 150 numbers KU941588 to KU941831.

Results

Microsatellite data

Loci that were monomorphic within a species were excluded from further analyses, namely Ops20 in *O. microlepis*, and Ops 5 and Ops7 in *O. microcephalum* (Table S1). In *O. microlepis* across the 6 populations and 9 loci, we conducted 44 population-level tests of deviation from HWE (Table S1). Of these seven were significant ($P \leq 0.05$), five of which were at locus Ops16 and two were at locus Ops3. In *O. microcephalum* across 11 populations and 8 loci, we conducted 53 population-level tests of deviation from HWE (Table S1). Of these eight were significant ($P \leq 0.05$), six of which were at locus Ops16 and two at Ops3. In *O. tweddleorum* across 6 populations 10 loci, we conducted 42 population-level tests of deviation from HWE. Of these 11 were significant ($P \leq 0.05$), three of which were at locus Ops16 (Table S1). Significant deviations from HWE at Ops16 and Ops3 were in all cases associated with the potential presence of null alleles, as indicated by Microchecker (Table S2).

In *O. microlepis*, 202 tests of linkage disequilibrium between pairs of loci were performed, of which nine were significant ($P \leq 0.05$). In *O. microcephalum*, 106 tests of linkage disequilibrium between pairs of loci were performed, of which 1 was significant ($P \leq 0.05$). In *O. tweddleorum*, 116 tests of linkage disequilibrium between pairs of loci were performed, of which 4 were significant ($P \leq 0.05$). Given a lack of evidence for consistent patterns of linkage among loci across species all remaining loci were retained (Table S3). There were substantial differences among species in the power of exact tests on microsatellite data to detect significant differences among populations. At F_{ST} of 0.005 and 0.01, all species had low probability of rejecting the null hypothesis of panmixia (< 0.80). At F_{ST} of 0.05, *O. microlepis* and *O. tweddleorum* were able to reliably reject panmixia (probability > 0.99), while *O. microcephalum* performed less well (probability ~ 0.82)(Table S4).

A global test revealed an overall significant genetic difference among *O. microlepis* populations ($F_{ST} = 0.063$, Exact test, $P < 0.001$), and there were significant differences ($P \leq 0.05$) among all pairs of populations with the exception of populations from the North Rukuru and Songwe rivers, and the North Rukuru and Linthipe rivers (Table 3). The Lake Malombe population differed substantially from all main Lake Malawi populations (F_{ST} range = 0.172 to 0.217), while there was considerably less genetic structure present among the Lake Malawi populations (F_{ST} range 0.009 to 0.055; Fig 2a; Table 3). One locus (Ops11) showed strong F_{ST} among populations, while at the other eight loci F_{ST} was less than 0.01 (Figure S1). Structure resolved $K = 3$ as the most likely number of populations for analyses with

186 and without the location prior (Table S5). This analysis showed that the Lake Malombe population
187 represented a genetically different population to the five from the main Lake Malawi catchment (Fig.
188 3a), but there was no clear pattern of structuring within the Lake Malawi catchment. There was no
189 significant genetic isolation by distance ($r = 0.34$, $P = 0.25$).

190

191 A global test revealed an overall significant difference among *O. microcephalum* populations ($F_{ST} =$
192 0.012 , Exact test $P < 0.001$), and there were 17 significant differences ($P \leq 0.05$) in the 55 pairwise
193 tests (Table 4). The Kamuzu Dam and Lake Malombe populations were significantly different from
194 each other and most populations in the Lake Malawi catchment. F_{ST} values ranged between -0.0215
195 and 0.183 (Fig. 2c, Table 4). Mean F_{ST} among populations was greatest at Ops11, Ops 16 and Ops19,
196 but all loci showed mean $F_{ST} < 0.01$ (Fig. S1). Structure resolved $K = 2$ as the most likely number of
197 populations for analyses with the location prior (Table S4), but there was no evidence of two discrete
198 groups of individuals in the Structure output (Fig. 3b). Analyses without the location prior indicated K
199 $= 1$ as the most likely number of populations (Table S5). There was no evidence of significant genetic
200 isolation by distance ($r = 0.005$, $P = 0.387$).

201

202 A global test revealed an overall significant difference among *O. tweddleorum* populations ($F_{ST} =$
203 0.437 , Exact test $P < 0.001$), and there were significant differences ($P \leq 0.05$) among all pairs of
204 populations (Table 5). F_{ST} values ranged between 0.139 and 0.609 (Fig. 2e, Table 5). Mean F_{ST} was
205 high (> 0.1) at all loci, with the exceptions of Ops3 and Ops20 (Fig. S1). Structure resolved $K = 6$ as
206 the most likely number of populations for analyses with and without the location prior (Fig. 3c; Table
207 S5). Under both sets of analyses all populations were largely distinct, with the exception of the
208 neighbouring Lingadzi and Lumbadzi populations. There was no significant genetic isolation by
209 distance ($r = 0.23$, $P = 0.23$).

210

211 Mitochondrial DNA data

212 The length of the *COI* sequences screened were 541 bp for *O. microlepis*, 579 bp for *O. microcephalum*
213 and 574 bp for *O. tweddleorum*. In *O. microlepis* only two haplotypes differing by 1 bp were identified
214 from the 98 individuals sequenced (Fig. 4a). There were no overall significant genetic difference among
215 *O. microlepis* populations ($\Phi_{ST} = 0.002$, $P = 0.320$). Pairwise comparisons of populations revealed Φ_{ST}
216 values ranging between -0.081 and 0.157 , and only one significant difference was present ($P < 0.05$),
217 between the Bua and North Rukuru populations (Fig. 2b, Table 3). In *O. microcephalum* a total of 26
218 haplotypes were found among the 88 individuals sequenced, with several populations exhibiting private
219 haplotypes (Fig. 4b). Overall population genetic differentiation was highly significant in *O.*
220 *microcephalum* ($\Phi_{ST} = 0.088$, $P < 0.001$). Pairwise comparisons of populations revealed Φ_{ST} values

221 ranging between -0.021 and 0.259, and six of the ten pairwise comparisons were significant ($P < 0.05$),
222 all involving either the Linthipe or Chia rivers (Fig. 2d, Table 4). In *O. tweddleorum* five haplotypes
223 were identified among the 57 individuals sequenced (Fig. 3), with only one haplotype shared between
224 multiple populations, all of which were geographically located in the south-east of the Lake Malawi
225 catchment. In a global analysis there was a highly significant difference identified among *O.*
226 *tweddleorum* populations ($\Phi_{ST} = 0.416$, $P < 0.001$). Ten of the fifteen pairwise comparisons were
227 significant ($P < 0.05$), with Φ_{ST} values ranging between 0 and 1 (Fig 2f, Table 5). There were no
228 significant associations between genetic distance (Φ_{ST}) and geographic distance for any of the species
229 (Mantel tests: *O. microlepis*, $r = 0.645$, $P = 0.148$; *O. microcephalum* $r = 0.386$, $P = 0.253$; *O.*
230 *tweddleorum* $r = 0.238$, $P = 0.123$).

231 232 Discussion 233

234 The results revealed striking differences among the three focal species in spatial genetic structuring.
235 The large bodied river-lake migratory mpasa (*O. microlepis*) showed weak yet significant genetic
236 structuring among populations from the main Lake Malawi catchment in microsatellite DNA, but no
237 evidence of structuring in mitochondrial DNA. The biological relevance of low yet statistically
238 significant genetic structuring is an open to debate, but such patterns are consistent with partial
239 reproductive isolation (Knutsen et al. 2010). At the very least the results are suggestive of a lack of
240 strong natal homing in mpasa. Nevertheless there may be some dispersal limitation among populations,
241 perhaps related to adult mpasa remaining in the vicinity of natal sites as adults.

242
243 Notably, we did find some evidence of relatively strong spatial population genetic structure between
244 the Lake Malawi and Lake Malombe mpasa populations sampled. This is indicative of a lack of
245 dispersal through the connecting Upper Shire River (Fig. 1), however we note that our sample size from
246 the Lake Malombe population was relatively small ($n = 14$). Moreover, all individuals sampled were
247 juveniles collected from the same location on the same day, and the population contained low
248 heterozygosity relative to the Lake Malawi populations. Thus, we cannot rule out the possibility that
249 the observed structure is a result of sampling only very closely related individuals. Notably mpasa is
250 very rare in the vicinity of Lake Malombe, although juveniles have previously been observed in a
251 tributary of Shire River at Liwonde (D. Tweddle, pers. comm). It is possible that there is a breeding
252 population within Lake Malombe, but further work is required to determine if this is genetically distinct
253 from those in Lake Malawi.

254

255 The weak population genetic structure among Lake Malawi mpasa populations in our results contrasts
256 with results of Changadeya *et al.*, (2013) who also used microsatellite loci to study population genetic
257 differences among mpasa populations from the Linthipe, Bua, Dwangwa and North Rukuru systems.
258 Although qualitatively similar in demonstrating significant genetic differences among populations,
259 Changadeya *et al.*, (2013) reported consistently high F_{ST} values among all populations (mean 0.17,
260 range 0.14 to 0.19). They also reported highly significant deviations from HWE in all 20 loci they
261 studied, and in all populations. They observed excess heterozygosity relative to expected values, which
262 the authors proposed may be due to outbreeding. Our data suggest are not consistent with this
263 interpretation, and instead suggest that populations of mpasa largely conform to HWE expectations.

264
265 We found only very weak population genetic structure within sanjika (*O. microcephalum*) using
266 microsatellite makers, but some evidence of structure in mitochondrial DNA. The absence of genetic
267 structure among many populations using microsatellite markers is likely to be linked to a low power of
268 resolution (Table S4), driven by a low allelic diversity at most loci, when compared with mpasa and
269 dwarf sanjika (Table S1). However, it is also potentially linked to broad adult dispersal and an
270 opportunistic breeding strategy, as sanjika are known to spawn in both river and lacustrine
271 environments (Tweddle & Turner, 2014). In contrast to the microsatellite diversity, mitochondrial DNA
272 diversity was comparatively high in sanjika relative to other *Opsaridium* species studied. Significant
273 population structure was present, but there was no evidence of a pattern of genetic isolation by distance.
274 This result is partially compatible with male-biased dispersal in this species. Male-biased dispersal has
275 been widely reported in fish using both genetic (Prugnolle & de Meeus, 2002) and tagging methods
276 (Croft *et al.*, 2003), including migratory salmonids (Hutchings & Gerber, 2002). It is generally thought
277 that the strategy has evolved to weaken male-male competition among siblings, and reduce probability
278 of mating with closely-related females. Our data suggest there is scope to further investigate sex biases
279 in the migratory behaviour of this species using molecular genetic markers, or perhaps trace-element
280 analyses of otoliths (e.g. Walther & Thorrold, 2008).

281
282 The sampled populations of dwarf sanjika (*O. tweddleorum*) typically exhibited very strong evidence
283 of genetic differentiation, and there was a significant pattern of genetic isolation by distance in the
284 microsatellite data. These results strongly suggest that gene-flow among populations is infrequent and
285 that the main lake is only very rarely used as a conduit for dispersal. It is interesting to speculate on
286 reasons why dwarf sanjika does not possess an adult lacustrine dispersal phase equivalent to mpasa and
287 sanjika. It is possible that the small body size enables year-round exploitation of smaller riverine food
288 resources. This in turn may enable larger populations to persist in rivers than congeneric sympatric
289 species meaning the costs of inbreeding are reduced.

290

291 The apparent contrast in mtDNA and microsatellite diversity in the large bodied *Opsaridium* species is
292 intriguing. Mpasa had low mtDNA diversity, yet relatively high microsatellite diversity. Sanjika had
293 comparatively higher mtDNA diversity, yet low microsatellite diversity. Such patterns may be
294 reflective of chance events during population bottlenecks during megadroughts that have repeatedly
295 desiccated Lake Malawi over the last 1.3 million years (Lyons *et al.*, 2015). However further
296 investigations using many more markers are required to determine if the patterns are consistent across
297 the genome.

298

299 ***Connectivity and conservation***

300 Integrating information on evolutionary connectivity between populations in the design of a network
301 of designated areas can, in principle, increase the efficiency of biodiversity protection. Management
302 priorities can be based on their degree of diversity and evolutionary uniqueness with respect to other
303 populations (Woodruff, 1989; Crozier & Kusmierski, 1994). Specifically, in terms of fisheries
304 management, information on the extent of genetic connectivity can help to reveal aspects of the
305 underlying dispersal biology of the species and help to identify management units (Palsbøll *et al.*,
306 2007). Our data strongly imply that three *Opsaridium* species have different genetic structures governed
307 by their life histories and ecological preferences. Management therefore needs to recognise these issues,
308 in addition to threats from fisheries, habitat fragmentation, pollution and water abstraction (Vörösmarty
309 *et al.*, 2010).

310

311 The IUCN ‘Endangered’ mpasa and ‘Vulnerable’ sanjika have traditionally been exploited during the
312 annual spawning runs, and both species continue to be the focus of fisheries in rivers in both Malawi
313 and Tanzania. Although few quantitative data are available on catches, there is a collective opinion that
314 catches of both have declined (Kazumbe *et al.*, 2006a, 2006b). Mpasa, in particular are suggested to
315 have declined substantially in all major rivers studied between the 1940s and 1970s, based on
316 interviews with fishers (Tweddle, 1981). Specific threats include overfishing, degradation of spawning
317 habitat, weir construction and fish poisoning (Tweddle, 2001). An important challenge for the long-
318 term conservation of both species is to protect the remaining intact river habitat. Sampling for this study
319 has demonstrated that number of rivers including the Bua, Linthipe North Rukuru and Songwe still
320 have spawning runs with intact breeding habitat for adult fish of both species. Our results are suggestive
321 of partially restricted gene flow among populations in both species and imply that conservation
322 initiatives that protect spawning and juvenile habitat in individual rivers may have both local and
323 broader geographic benefits for rebuilding of the total Lake Malawi spawning stocks of these species.
324 Such habitat protection should be coupled with regular monitoring of abundance of these species within

325 rivers. Since juvenile *Opsaridium* are sensitive to changes in water quality, food resources, river flow,
326 turbidity and temperature (Tweddle, 1983), flourishing populations of juveniles could be useful
327 indicators of river ecosystem health.

328

329 Finally, our results are consistent with field observations that the dwarf sanjika is river-restricted and
330 non-migratory. It is therefore representative of many freshwater fish species of small rivers and
331 ephemeral habitats across Africa, and notably the high degree of genetic structure in *O. tweddleorum*
332 is mirrored over similar spatial scales in many other fish groups including cyprinids (*Pseudobarbus*
333 sp.; Swartz *et al.*, 2009), catfishes (*Chiloglanis* sp., Schmidt *et al.*, 2014), killifishes (*Nothobranchius*
334 *furzeri*; Bartáková *et al.*, 2013) and cichlids (*Astatotilapia calliptera*, Nichols *et al.*, 2015;
335 *Pseudocrenilabrus* sp., Katongo *et al.* 2008; Egger *et al.* 2015). Together these results demonstrate a
336 general pattern of only occasional dispersal and low genetic connectivity among many river systems in
337 Africa, suggesting that local disturbance events or habitat alteration could strongly impact on the
338 ecology of individual water bodies and lead to a loss of unique genetic diversity.

339

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346

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485 **Figure Legends.**

486

487 **Fig. 1.** Sampling locations of the populations of three species *O. microlepis*, *O. microcephalum* and *O.*
488 *tweddleorum* within the Lake Malawi catchment. Colour shading indicates the species sampled from
489 each location (Table 1).

490

491 **Fig. 2.** Multidimensional scaling plots of genetic differences between populations, based on
492 microsatellite F_{ST} and mitochondrial Φ_{ST} values, for a) *O. microlepis*, b) *O. microcephalum* and c) *O.*
493 *tweddleorum*. of *O. microcephalum* data without a location prior indicated a single panmictic
494 population ($K = 1$).

495

496 **Fig. 3.** STRUCTURE assignment of individuals to populations using microsatellite data for a) *O.*
497 *microlepis*, b) *O. microcephalum* and c) *O. tweddleorum*. Analyses

498

499 **Fig. 4.** MtDNA (*COI*) haplotype network of sampled populations for a) *O. microlepis* b) *O.*
500 *microcephalum*, and c) *O. tweddleorum*.

Table 1: Sampled locations and sample size of populations of *O. microlepis*, *O. microcephalum* and *O. tweddleorum* analysed for nuclear (microsatellite) DNA

Species	Sampling Date	Location name	Sampling method	Latitude (S)	Longitude (E)	N individuals microsatellites
<i>O. microlepis</i>	28-01-2014	Songwe*	Seine nets	09°35.24'	033°46.16'	15
	28-01-2014	North Rukuru	Market	09°55.03'	033°55.68'	48
	25-01-2014	Dwangwa*	Seine nets	12°30.81'	034°06.97'	19
	24-01-2014	Bua	Artisanal fishers	12°47.19'	034°11.76'	19
	22-01-2014	Linthipe	Artisanal fishers	13°47.33'	034°26.09'	38
	04-05-2010	Malombe*	Seine nets	14°32.58'	035°12.66'	14
<i>O. microcephalum</i>	28-01-2014	Songwe*	Seine nets	09°35.24'	033°46.16'	5
	28-01-2014	North Rukuru	Seine nets	09°55.03'	033°55.68'	34
	26-01-2014	Ngala	Market	12°21.30'	034°03.57'	8
	25-01-2014	Dwangwa*	Seine nets	12°30.81'	034°06.97'	6
	24-01-2014	Bua	Artisanal fishers	12°47.19'	034°11.76'	56
	23-01-2014	Chia	Artisanal fishers	13°07.55'	034°19.64'	18
	23-08-2013	Lumbadzi*	Seine nets	13°47.64'	033°59.30'	18
	23-08-2013	Kamuzu Dam*	Artisanal fishers	14°10.47'	033°38.57'	20
	22-01-2014	Linthipe	Artisanal fishers	13°47.33'	034°26.09'	74
	20-01-2014	Monkey Bay	Market	14°04.17'	034°55.03'	16
	04-05-2010	Malombe*	Seine nets	14°32.58'	035°12.66'	6
<i>O. tweddleorum</i>	27-01-2014	North Rumphi	Seine nets	10°41.109'	034°10.883'	16
	09-09-2012	Ruhuhu	Seine nets	10°35.650'	034°39.20'	18
	26-01-2014	Khuyu	Seine nets	12°16.735'	033°59.939'	26
	23-01-2014	Lingadzi	Seine nets	13°32.061'	034°17.064'	10
	23-08-2013	Lumbadzi	Seine nets	13°47.64'	033°59.30'	8
	12-01-2011	Mtakataka	Seine nets	14°12.855'	034°30.819'	14

*Sample of juveniles, all other individuals of *O. microlepis* and *O. microcephalum* were adult size.

Table 2: MtDNA (*COI*) variation within populations of *O. microlepis*, *O. microcephalum* and *O. tweddleorum*.

Species	Population	N individuals mtDNA	Polymorphic sites	Number haplotypes	Haplotype diversity	Nucleotide diversity
<i>O. microlepis</i>	Linthipe	20	1	2	0.337	0.00062
	Bua	16	1	2	0.125	0.00023
	North Rukuru	47	1	2	0.459	0.00085
	Dwangwa	9	1	2	0.222	0.00041
	Malombe	6	0	1	0	0
	All 5 populations	98	1	2	0.352	0.00066
<i>O. microcephalum</i>	Linthipe	22	19	13	0.905	0.00613
	Chia	17	8	3	0.581	0.00593
	Bua	20	7	4	0.284	0.00198
	Ngala	7	8	4	0.714	0.00592
	North Rukuru	22	14	8	0.732	0.00544
	All 5 populations	88	31	25	0.726	0.00681
<i>O. tweddleorum</i>	Lumbadzi	8	0	1	0	0
	North Rumphu	16	1	2	0.462	0.00080
	Khuyu	26	0	1	0	0
	Lingadzi	10	0	1	0	0
	Mtakataka	14	0	1	0	0
	Ruhuhu	18	0	1	0	0
	All 6 populations	92	5	5	0.771	0.00314

Table 3: Genetic differences among *Opsaridium microlepis*. Below the diagonal are F_{ST} and Φ_{ST} values for microsatellite and mitochondrial data, respectively. Above the diagonal P -values

Microsatellite	Bua	Dwangwa	Linthipe	North Rukuru	Songwe	Malombe
Bua	-	< 0.001	0.006	< 0.001	0.006	< 0.001
Dwangwa	0.025	-	0.002	< 0.001	0.002	< 0.001
Linthipe	0.002	0.027	-	0.038	0.004	< 0.001
North Rukuru	0.018	0.055	0.009	-	0.371	< 0.001
Songwe	0.024	0.036	0.014	0.002	-	< 0.001
Malombe	0.172	0.217	0.200	0.211	0.198	-
MtDNA	Bua	Dwangwa	Linthipe	North Rukuru	Malombe	
Bua	-	0.455	0.630	0.040	0.999	
Dwangwa	0.023	-	0.940	0.075	0.999	
Linthipe	-0.012	-0.048	-	0.110	0.651	
North Rukuru	0.140	0.105	0.040	-	0.187	
Malombe	-0.079	-0.051	-0.081	0.157	-	

Table 4: Genetic differences among *Opsaridium microcephalum*. Below the diagonal are F_{ST} and Φ_{ST} values for microsatellite and mitochondrial data, respectively. Above the diagonal P -values.

Microsatellite	Bua	Chia	Dwangwa	Kamuzu Dam	Linthipe	Lumbadzi	Monkey Bay	Ngala	North Rukuru	Songwe	Malombe
Bua	-	0.139	0.780	0.033	0.585	0.140	0.112	0.668	0.915	0.468	0.015
Chia	0.005	-	0.759	0.025	0.166	0.193	0.250	0.496	0.929	0.318	0.016
Dwangwa	-0.005	-0.017	-	0.006	0.974	0.875	0.796	0.287	0.893	0.985	0.034
Kamuzu Dam	0.049	0.039	0.094	-	0.077	< 0.001	0.139	0.004	0.038	0.039	< 0.001
Linthipe	-0.003	0.005	-0.014	0.048	-	0.032	0.238	0.265	0.490	0.752	0.004
Lumbadzi	0.006	0.015	-0.019	0.087	0.010	-	0.291	0.013	0.288	0.514	0.004
Monkey Bay	0.013	0.001	-0.001	0.017	0.009	0.019	-	0.087	0.190	0.618	0.003
Ngala	0.029	-0.011	0.049	0.035	0.029	0.067	0.026	-	0.771	0.225	0.257
North Rukuru	-0.005	-0.010	-0.007	0.046	0.002	0.013	0.009	0.001	-	0.576	0.042
Songwe	-0.014	-0.008	-0.031	0.057	-0.022	-0.009	-0.009	0.018	-0.020	-	0.428
Malombe	0.081	0.090	0.107	0.183	0.079	0.078	0.134	0.041	0.069	0.050	-

MtDNA	Bua	Chia	Linthipe	Ngala	North Rukuru
Bua	-	< 0.001	< 0.001	0.587	0.277
Chia	0.066	-	< 0.001	0.256	0.001
Linthipe	0.181	0.118	-	0.022	< 0.001
Ngala	-0.021	0.011	0.144	-	0.172
North Rukuru	0.004	0.081	0.259	0.041	-

Table 5: Genetic differences among *Opsaridium tweddleorum*. Below the diagonal are F_{ST} and Φ_{ST} values for microsatellite and mitochondrial data, respectively. Above the diagonal P -values.

Microsatellite	Khuyu	Lingadzi	Lumbadzi	North Rumphu	Mtakataka	Ruhuhu
Khuyu	-	0.005	0.033	< 0.001	< 0.001	0.085
Lingadzi	0.485	-	0.190	0.001	< 0.001	0.002
Lumbadzi	0.453	0.139	-	0.014	0.011	< 0.001
North Rumphu	0.609	0.362	0.274	-	0.007	< 0.001
Mtakataka	0.562	0.326	0.267	0.274	-	< 0.001
Ruhuhu	0.528	0.396	0.318	0.445	0.390	-

MtDNA	Khuyu	Lingadzi	Lumbadzi	North Rumphu	Mtakataka	Ruhuhu
Khuyu	-	< 0.001	0.009	< 0.001	< 0.001	< 0.001
Lingadzi	1	-	0.991	< 0.001	0.991	< 0.001
Lumbadzi	1	0	-	< 0.001	0.991	0.234
North Rumphu	1	1	1	-	< 0.001	< 0.001
Mtakataka	1	0	0	1	-	0.315
Ruhuhu	0.313	0.122	0.084	0.196	0.083	-

Fig. 1

531 Fig. 2
532

a) *O. microlepis*

b) *O. microcephalum*

c) *O. tweddleorum*

Fig. 3

a) *O. microlepis*

b) *O. microcephalum*

c) *O. tweddleorum*

537
538
539 Fig. 4