Sex biases in kin shoaling and dispersal in a cichlid fish

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Abstract

Animal dispersal is associated with diverse costs and benefits that vary among individuals based on phenotype and ecological conditions. For example, females may disperse when males benefit more from defending territories in familiar environments.

Similarly, size differences in dispersal propensity may occur when dispersal costs are size-dependent. When individuals do disperse, they may adopt behavioral strategies that minimize dispersal costs. Dispersing fish, for example, may travel within shoals to reduce predation risks. Further, kin shoaling may augment inclusive fitness by reducing predation of relatives. However, studies are lacking on the role of kin shoaling in dispersal. We explored how sex and size influence dispersal and kin shoaling in the cichlid *Neolamprologus caudopunctatus*. We microsatellite genotyped over 900 individuals from two populations separated by a potential dispersal barrier and documented patterns of population structure, migration and within-shoal relatedness. Genetic differentiation across the barrier was greater for smaller than larger fish, suggesting larger fish had dispersed longer distances. Females exhibited weaker genetic differentiation and 11 times higher migration rates than males, indicating longer-distance female-biased dispersal. Small females frequently shoaled with siblings, possibly offsetting dispersal costs associated with higher predation risks. In contrast, small males appeared to avoid kin shoaling, possibly to avoid local resource competition. In summary, long-distance dispersal in *N. caudopunctatus* appears to be female-biased and kin-based shoaling by small females may represent a behavioral adaptation that reduces dispersal costs. Our study appears to be the first to provide evidence that sex differences in dispersal influence sex differences in kin shoaling.

Introduction

Dispersal by individuals from their natal grounds to their breeding areas is a common feature of animal life (Greenwood 1980) and is associated with significant benefits and costs to dispersers (Handley and Perrin 2007; Bonte et al. 2012). By dispersing, animals may avoid the negative effects of inbreeding (e.g. Nagy et al. 2013; Banks and Lindenmayer 2014) and increase access to resources such as territories or mates (Bowler and Benton 2005). In addition, they may increase inclusive fitness, for example, by relaxing local resource competition among relatives (reviewed in Handley and Perrin 2007). However, diverse costs of dispersal also exist, including increased risks, and loss of time and energy (Bonte et al. 2012). For example, since dispersers often travel solitarily across unfamiliar and unsuitable habitat, a major cost is an increased risk of predation (e.g. Hiddink et al. 2002; Yoder et al. 2004).

The decision to disperse may accordingly depend on a tradeoff between these costs and benefits, which in turn is predicted to vary with the ecological conditions and individual phenotypes (Bowler and Benton 2005). For example, size-differences in dispersal strategies may arise when only larger individual have sufficient energy reserves to be able to move large distances or be competitive in new environments (e.g. Gundersen et al. 2002). Similarly, local mate- and resource competition may lead to sex-differences in dispersal strategies. In birds, for example, females typically disperse, as males gain more from being able to defend a familiar territory in their natal habitat (Greenwood 1980). In contrast, male-biased dispersal occurs more often in polygynous mammals as a result of females preferring immigrant males to avoid inbreeding (Handley

69   and Perrin 2007). Sex-biases in dispersal strategies in turn have important consequences

70   for the genetic structure of populations (e.g. Sheridan et al. 2010; Bisol et al. 2012).

71   Unlike the well-studied birds and mammals, the causes and consequences of

72   dispersal strategies in fish are less well-understood and often less clear-cut. For example,

73   in the Atlantic salmon (*Salmo salar*), temporal and spatial variation in sex ratios appear

74   to favor dispersal of different sexes (Consuegra and García de Leániz 2007).

75   Additionally, the biology of fish differs markedly from that of birds and mammals, which

76   may further affect dispersal strategies. For example, body size may affect the swimming

77   performance of fish during dispersal (e.g. Stobutzki and Bellwood 1997; Fisher et al.

78   2000) and their competitive ability in new environments (Rowland 1989; Koops and

79   Grant 1993). In addition, larger fish may suffer less predation from gape-limited fish

80   predators (Scharf et al. 2000). Therefore, as fish are known to continually grow

81   throughout their lives (Helfman et al. 1997), dispersal may be delayed until a threshold

82   body size is reached.

83   When individuals do disperse, they are expected to adopt behavioral adaptations

84   that minimize dispersal costs. For example, kin-based grouping is common in fish (e.g.

85   Arnold 2000; Frommen and Bakker 2004; Gerlach and Lysiak 2006) and may be

86   beneficial during dispersal by reducing stress associated with interacting with unfamiliar

87   conspecifics (e.g. Gerlach et al. 2007) or augmenting inclusive fitness via a reduction in

88   predation of relatives (Hatchwell 2010). When only one sex disperses, sex differences in

89   grouping behavior may be expected, with kin-based grouping favored in the dispersing

90   sex. For species that are non-cooperative or do not live in family groups, these kin-based

91   groups would be expected to dissolve prior to breeding to reduce kin conflict for

92   resources (Hatchwell 2010). Studies are lacking however, on the relationship between

93   dispersal and kin-grouping.

94   We characterized patterns of dispersal and kin-shoaling in *Neolamprologus*

95   *caudopunctatus*, a predominately colonial cichlid from Lake Tanganyika. Dispersal

96   strategies within the genus *Neolamprologus* appear to be highly variable, with some

97   species displaying male-biased dispersal (e.g. N. pulcher, Stiver et al. 2004), while in

98   other species females disperse (*N. multifasciatus,* Schradin and Lamprecht 2000). *N.*

99   *caudopunctatus* is a socially monogamous and sexually monomorphic substrate breeder

100   (Schaedelin et al. 2013). Individuals which are not currently breeding form large shoals

101   that are often located above breeding colonies. In contrast, breeding pairs occupy the

102   substrate, search for a nesting site together and construct nesting cavities by excavating

103   sand under stones (Ochi and Yanagisawa 1999). Nesting cavities and free swimming fry

104   are defended by both parents over a period of several weeks until fry are independent

105   (Ochi and Yanagisawa 1999). Despite both sexes being involved in nest site selection and

106   defense, males are larger than females (Schaedelin et al, unpublished data), suggesting

107   that they are more effective in territorial defense, as occurs in other fish (Rowland 1989;

108   Koops and Grant 1993). In support of this, males display a higher attack rate towards

109   territorial intruders than do females (Ochi and Yanagisawa 1999).

110   We genetically sampled over 900 breeders and shoaling fish in two distinct

111   populations. These populations are separated by a potential dispersal barrier consisting of

112   extended areas of sandy substrate where rocks are scarce, a known dispersal barrier for

113   this species (Koblmueller et al. 2006). We first quantified genetic population structure

114   within and between populations to detect evidence of sex-biased dispersal. The higher

aggressiveness of males in this species suggests that they may benefit more from

defending a territory in a familiar environment, thus favoring female dispersal

(Greenwood 1980). Our second aim was to test whether kin-based shoaling occurred in

this species as a possible strategy to reduce the costs of long-distance dispersal. Finally,

we determined whether any tendency to shoal with kin was size- or sex-dependent. As

smaller fish presumably have reduced swimming performance (Fisher et al. 2000), are

less competitive (Rowland 1989; Koops and Grant 1993; Aubin-Horth et al. 2007) and

are younger (Helfman et al. 1997), we predicted that genetic signatures of dispersal are

more likely to be detected amongst larger individuals.

Materials and methods

Field work

The study was carried out in October and November 2008 in Kasakalawe Point (S 08°

46' 46.6" E 31° 04' 44.4") on the southern shore of Lake Tanganyika, southwest of

Mpulungu, Zambia (hereafter referred to as the 'mainland site'). Clusters of *N.*

caudopunctatus defending potential nesting cavities were identified along the shore at

depths of 5 to 12m. We identified and numbered 165 breeding cavities defended by pairs

in an area of approximately 220 x 250 meters by SCUBA diving. To map the whole

population underwater (including the location of breeders and shoal fish, see below), we

constructed a coordinate system out of sisal rope and measured nest and shoal distances

to this coordinate plane using measuring tape. In addition to the main study site, we also

sampled individuals inhabiting the waters around Mbita Island (S 08° 45' 20.2" E 31° 05'

27.0"), approximately 3 km from the main site (hereafter referred to as the 'island site').

138   We collected samples along 150 meters of the shore at a depth of 1.5 to 3m. We did not

139   map the population at the island site. However, sampling at this site allowed us to explore

140   sex-biased dispersal over a relatively larger spatial scale. Both study sites comprise

141   typical *N. caudopunctatus* habitat, with a sandy substrate interspersed with many rocks

142   (Konings 1998).

143   During the study period we detected no breeding activity, although many pairs at

144   the mainland site actively defended a breeding cavity. We refer to these paired, territorial

145   individuals as breeders, whereas individuals in shoals are referred to as shoal fish.

146   Breeders regularly join shoals that are located close to their breeding cavities for short

147   periods of time, possibly for feeding (Schaedelin, personal observation). We genetically

148   sampled breeders by capturing pairs defending a breeding cavity with monofilament nets

149   and measured their standard length. No anaesthesia was used. We then clipped

150   approximately 7.5mm of the dorsal fin *in situ*. Fin clips were stored in individual water-

151   filled tubes during the diving session and transferred to Eppendorf tubes with absolute

152   ethanol once back on land. We assured that we did not resample individuals by checking

153   for evidence of our fin-clipping. Individuals were sexed by ventral inspection of their

154   genital papillae.

155   Shoal fish were captured and sampled in a similar fashion. We defined a shoal as

156   an aggregation of fish where fish were separated by a maximum of three body lengths

157   from each other (e.g. Pitcher and Parrish 1993). We estimated the approximate size of

158   each shoal at the time of sampling. The proportion of fish captured per shoal varied

159   widely depending on the size of the shoal. For small shoals (e.g. less than 20 individuals)

160   we captured the majority of the fish, while for large shoals (e.g. greater than 100

161   individuals) we typically captured less than fifty percent of the fish. As we found no pairs

162   defending a breeding cavity at the island site, we only captured shoal fish from this

163   population. However, the lack of breeders captured at the island site is unlikely to bias

164   our analyses on size-dependent dispersal in this species as we still captured many fish of

165   potential breeding size at this site (see Results). In total, we sampled 117 breeders and

166   580 shoal fish originating from 35 shoals along the mainland site (mean number of fish

167   sampled from each shoal = 16.6 ± 12.5 fish; range = 1 − 63) and 224 shoal fish from 9

168   shoals from the island (mean number of fish sampled from each shoal = 24.9 ± 9.3 fish;

169   range = 6 − 33).

170

171   *Genetic analyses*

172   DNA extraction of tissue samples was conducted using a QIAGEN DNeasy Blood and

173   Tissue Kit. All adults were genotyped at 11 microsatellite loci previously developed for

174   other species including NP773 and UNH002 (Schliewen et al. 2001), Pzeb3 (van Oppen

175   et al. 1997), TmoM5 and TmoM13 (Zardoya et al. 1996), UME003 (Parker and Kornfield

176   1996), UNH106, UNH130 and UNH154 (Lee and Kocher 1996), and UNH908 and

177   UNH1009 (Carleton et al. 2002). PCR and fragment analyses were conducted as

178   described in Schaedelin et al. (2013). Descriptive statistics were calculated for all 11

179   microsatellite loci (Online Resource 1), including the number of alleles (N_A), observed

180   heterozygosity (H_O), allelic diversity (H_S) and the inbreeding coefficient (F_{IS}) using

181   FSTAT 2.9.3 (Goudet 1995). We tested for Hardy-Weinberg equilibrium of all loci in

182   Cervus 3.0 (Kalinowski et al. 2007).

183   A principal aim of our study was to document differences in genetic structure

184   based on cichlid body size, which can provide information on dispersal strategies. Due to

185   difficulties in conducting many of the statistical analyses described below using size as a

186   continuous variable, we assigned individuals to size categories. We used the median

187   standard length of each sex as a threshold for each size category to ensure similar sample

188   sizes between size categories. We therefore classified females as small when their

189   standard length was less than 4.0cm, while small males were those shorter than 4.4cm.

190   These values were slightly larger than the minimum observed size of breeders at the

191   mainland population (males = 3.8cm, females = 3.7cm). Fish above these thresholds were

192   therefore likely able to defend territories and breed.

193

194   Population structure

195   We quantified the sex- and size-specific genetic structure of the study populations using

196   several complementary approaches (i.e. analyses of molecular variance, Bayesian cluster

197   analyses, population tree reconstruction, coalescence modeling and the characterization

198   of shoal-sharing by siblings). Using multiple analytical methods allowed us to

199   comprehensively and robustly track sex- and size-differences in genetic structure at the

200   levels of the shoal and population. Mantel tests were carried out to test for isolation by

201   distance within the mainland population (for which we had geographic distance data),

202   using GENETIX (Belkhir et al. 2004), by correlating a measure of genetic distance,

203    $\dfrac{F_{ST}}{1 - F_{ST}}$ (Rousset 1997), with geographic distance between individual shoals of fish.

204   We conducted analyses of molecular variance (AMOVA) in ARLEQUIN 3.5.1.2

205   (Excoffier and Lischer 2010) to determine whether sex and size differences in dispersal

206   affect the partitioning of genetic variation. In total, four AMOVAs were conducted. We

207   divided our total sample into sex and size specific data sets (small males, large males,

208   small females, large females) and defined the two study sites as groups and shoals within

209   the study sites as populations. For twelve shoals, we only had genetic samples for six or

210   fewer individuals. In contrast, the remainder of shoals (n = 32) contained a minimum of

211   eleven individuals. We therefore reduced sample size biases by excluding the twelve

212   small shoals from our analyses.

213   Although an AMOVA can detect population genetic differentiation, it provides no

214   information on migration rates, which are expected to be higher for the dispersing sex.

215   Therefore, we analyzed male and female data sets using an isolation with migration

216   model (IM: Hey and Nielsen 2007), which assumes a split in a parent population at some

217   time in the past, but with continued gene flow (migration) between the two resulting

218   daughter populations. Since the model is based on the coalescent, bi-directional migration

219   rates and effective population sizes can be simulated simultaneously. Thus, we used the

220   software IMa (Hey and Nielsen 2007) to test for differences in migration rates between

221   the male and female populations. IMa uses a MCMC Bayesian approach to simulate the

222   posterior distributions of migration rates between island and mainland populations (m_1

223   and m_2) and effective population sizes of the parental (θ_A) and daughter populations (θ_1

224   and θ_2, respectively). The population migration rate for each sex (i.e. the effective rate,

225   per generation, at which genes come into a population) can then be calculated as

226   migration rate$_1 = \dfrac{\theta_1 m_1}{2}$. A series of preliminary runs were first executed to determine the

227   prior parameter distributions to be used in subsequent runs. We then conducted three

228   independent runs for each sex, using 20 Markov chains with geometric heating for five

229   million generations after an initial burn-in period of one million generations. All runs

230   converged and estimates of the model parameters were the same for each run. Due to the

231   computationally demanding nature of these analyses, we did not conduct similar analyses

232   separating sexes into size classes.

233   We used STRUCTURE 2.3.3 (Pritchard et al. 2000) to estimate the number of

234   genetic clusters, K, in our data set. As we were interested in sex differences, we analyzed

235   each sex separately. Each individual was assigned to a cluster with probability, q. Five

236   independent analyses were performed for $1 \leq K \leq 10$ using a Markov chain of 600,000

237   iterations, discarding the first 100,000 as burn-in. To avoid potential biases, we assumed

238   the admixture model without including prior knowledge of the origin of samples.

239   Correlated allele frequencies were also assumed. The most likely number of clusters in

240   the data set was estimated using two methods. We first calculated ΔK following Evanno

241   et al. (2005), which estimates the most likely number of clusters based on the rate of

242   change of log-likelihood probabilities for each K. As this method cannot detect the best K

243   when K=1, we also estimated the number of clusters following Pritchard et al. (2000),

244   which uses Bayes' rule for the log-likelihood probabilities.

245   Although STRUCTURE can infer the number of genetic clusters within a dataset,

246   it cannot provide information on the relationships among populations. We therefore

247   created population neighbor joining phylogenetic trees using the $\dfrac{F_{ST}}{1 - F_{ST}}$ distance

248   calculated in ARLEQUIN to ascertain whether shoals were grouped by geographic

249   origin. The matrix was then imported into PHYLIP (Felsenstein 2005) to create

250   population phylogenies using the neighbor-joining method from Saitou and Nei (1987).

251

252   Genetic similarity

253   We calculated genetic similarity between all shoal fish and breeders within each

254   population, following Mathieu et al. (1990). Here, similarity was estimated as the

255   probability that a given pair will produce homozygous offspring (Phm). For each locus

256   (l), Phm is equal to:

257      $Phm_{xy}(l) = \dfrac{(s_{ac} + s_{ad} + s_{bc} + s_{bd})}{4}$, where s_{ij} equals 1 if alleles i and j are the same, and 0

258   otherwise. Across all loci, a weighted average was used: $Phm_{xy} = \dfrac{\sum_{l} \frac{1}{p_l} Phm_{xy}(l)}{\sum_{l} \frac{1}{p_l}}$, where

259   p_l is the probability of an individual being homozygous by chance at locus l. Therefore,

260   Phm_{xy} will be closer to 1 for more genetically similar individuals, who are thus more

261   likely to produce homozygous offspring. Belkhir et al. (2002) showed that this index of

262   genetic similarity is a superior alternative index when the number of loci used is

263   relatively low (i.e. compared to genetic similarity estimates calculated following Queller

264   and Goodnight 1989 and; Lynch and Ritland 1999)

265   We tested whether size and sex differences affected mean population-wide

266   relatedness by conducting analyses separately for shoal fish and breeders. Following

267   Taylor et al. (2003) we first created separate relatedness matrices for each sex. To avoid

268   pseudoreplication we averaged the relatedness of each individual with all other

269   individuals of the same sex. We then performed a two-sample permutation test using

270   10,000 randomizations using RUNDOM (Jadwiszczack 2002) on these averaged data to

271   test whether the difference in male and female relatedness differed significantly from

272   differences obtained by randomly assigning individuals to each sex.

273   We were also interested in whether sex and size differences affected the

274   relatedness of individuals within versus among shoals (i.e. whether fish join shoals of

275   related individuals). We therefore separated the shoals into the two size classes (i.e. small

276   and large fish) to explore whether small fish were more related within a shoal than larger

277   fish. We only conducted these additional analyses for the mainland population owing to

278   small sample sizes for the island shoals once the data were separated into size and sex

279   classes. We compared the mean relatedness of all fish within a shoal with relatedness

280   among individuals from different shoals using PERM (Duchesne et al. 2006). PERM uses

281   matrices of a pairwise relatedness statistic ("Sxy" – which corresponds to Phm in this

282   study) and calculates the sum of all Sxy values (i.e. Phm sums) within each group (shoals

283   in our study). These values are then compared with a distribution of Sxy sums generated

284   from randomly assigning individuals to shoals. One thousand randomizations were used.

285   Although the above analyses provide information on the average relatedness of

286   fish within shoals, they provide no information on the actual number of siblings that

287   shoal together. We therefore identified probable siblings based on Phm values to explore

288   what proportion of shoals consisted of siblings. To identify the minimum Phm of siblings

289   (and the maximum Phm of non-siblings) we first generated 500 offspring from matings

290   between 50 randomly selected male and female territorial pairs using HybridLab (Nielsen

291   et al. 2006). This generated a relatedness matrix of known siblings and non-siblings and

292   their corresponding Phm values. Based on these data (Online Resource 2) we

293   conservatively assigned two individuals as siblings when their Phm was greater or equal

294   to 0.57 and they were within the same size class. This value corresponds to the first

295   quartile for all siblings (i.e., 75% of all siblings have Phm values above 0.57) and is

296 greater than the maximum Phm value generated for non-siblings (maximum Phm for non-

297 siblings = 0.55). We then calculated sex and size differences in 1) the proportion of

298 individuals within a shoal that are siblings (pooling all shoals of a given sex and size

299 class and using a chi-square to test for group differences), 2) the proportion of siblings

300 that were captured within the same shoal as opposed to being captured in different shoals

301 and 3) the mean geographic distance between all sibling pairs (calculated based on the

302 position of each shoal on our coordinate system at the time of capture).

303   All non-genetic statistical analyses were conducted using SPSS 17.0 (SPSS,

304 Chicago, Illinois, USA). Non-parametric tests were used when the assumption of data

305 homoscedasticity was not met. All data are presented as mean ± SD.

306

307 **Results**

308 Shoal characteristics

309 Shoal size was highly variable, ranging from five individuals to over 200 individuals. At

310 the mainland population, where both breeder and shoal fish were sampled, breeders were

311 larger than shoal fish (mean standard length: breeders = 4.6 ± 0.4cm, shoal fish = 4.2 ±

312 0.6 cm; Mann-Whitney U = 15,349, n = 623, P <0.001). The percentage of fish within

313 shoals classified as "large" was highly variable, ranging from 0-100% (mean = 57±36%)

314 at the mainland population and 53-100% (mean = 88±17%) at the island population.

315 Among shoals, mean male and female size of each shoal was positively correlated (r =

316 0.851, n = 22, $P < 0.001$). Finally, high variation in sex ratios between shoals was

317 observed, ranging from 20-100% females per shoal (mean = 55±18%, n = 23) at the

318 mainland population and 21-67% at the island population (mean = 52±15%, n = 8).

319

Sex-size differences in overall population structure

We found no evidence of isolation by distance within the mainland population for either

males (mantel test: $Z = 70.43$, $P = 0.336$) or females (mantel test: $Z = 453.37$, $P = 0.590$).

Similarly, we found no evidence of isolation by distance when separating individuals by

sex and size (mantel test: small males - $Z = 53.61$, $P = 0.852$; large males - $Z = 151.44$, P

$= 0.188$; small females - $Z = 152.49$, $P = 0.282$; large females - $Z = 73.64$, $P = 0.608$).

Finally, we found no difference in the average relatedness between individuals within

sexes for either shoal fish or breeders, even when separate analyses were conducted for

small and large fish (Table 1).

AMOVA revealed that in both sexes the majority of genetic variation

(approximately 98%) occurred among individuals within shoals (Table 2). However,

genetic variance among shoals within study sites was over twice as high for small

females (0.83%) than for small males (0.36%: chi-squared test - $\chi^2 = 18.56$, d.f. = 1, $P <$

0.001), but similar for large females and males (0.63% and 0.55%, respectively: chi-

squared test - $\chi^2 = 0.54$, d.f. = 1, $P = 0.461$; Table 2). Furthermore, the only among-shoal

variance value (i.e. F_{SC}) that was greater than zero was for small females. Less than 2.5%

of variation was explained by differences between the mainland and island populations,

although the male populations were slightly more differentiated than the female

populations. For both sexes, genetic differentiation between the two study sites was

higher for small than large individuals (i.e. based on F_{ST} values in Table 2). An analysis

of migration rates using a coalescent IM model, revealed that migration from the island to

the shore was four times higher for females than for males and 11 times higher for

342 females than for males migrating from the shore to the island (based on $\frac{\theta_i m_i}{2}$ values in

343 Table 3).

344   Bayesian population assignment using STRUCTURE supported the one-cluster

345 (K = 1) hypothesis as most likely for females, whereas K = 2 returned the highest log-

346 likelihood for males (Fig. 1, Online Resource 3). Among males, the two clusters

347 approximately defined the mainland and island populations (mean probability of

348 assignment q(cluster 1/mainland) = 0.219 ± 0.244, q(cluster 2/island) = 0.852 ± 0.201;

349 $F_{1,387}$ = 654.36, $P < 0.001$). Forty seven percent (183/389) of the males had a q value of

350 less than 0.90, and 3.9% (8/206) of individuals with $q>0.90$ could not be assigned to the

351 population from which they were sampled. To further investigate population genetic

352 structuring of the shoals, we created sex-specific neighbor-joining phylogenies based on

353 our microsatellite data (Fig. 2). The trees supported the findings above, showing a clear

354 division between the mainland and island populations for males, but not for females.

355

356 Sex- and size-dependent relatedness among and within shoals

357 We found sex and size differences in relatedness within shoals. Within-shoal relatedness

358 was significantly higher than between-shoal relatedness only for small females (Table 4).

359 In contrast, neither large females, nor large or small males were more closely related to

360 fish of their size and sex class within their shoal than that expected by random shoaling.

361   In further support of kin-shoaling in small females, we found that small females

362 were more likely than individuals of other classes to be captured together with at least

363 one sibling (proportion of sibling pairs captured within same shoal: small females – 18/58

364 (31.0%), large females – 3/40 (7.5%), small males – 7/72 (9.7%), large males – 5/37

365 (13.5%); logistic regression – sex – Wald = 8.56, d.f. = 1, P = 0.003, size – Wald = 0.36,

366 d.f. = 1, P = 0.551, sex*size – Wald = 5.24, d.f. = 1, P = 0.022). Furthermore, small

367 females shared shoals with more siblings than did fish in the other classes (proportion of

368 siblings within shoals: small females – 16% (24/150), small males – 7% (11/165), large

369 females – 2% (3/146), large males - 4% (7/160); chi-squared test – χ^2 = 25.00, d.f. = 3, P

370 < 0.001). Short distance dispersal also involved sex- and size-specific differences (mean

371 sibling-sibling distance: small females – 72.8 ± 62.9m, large females – 90.7 ± 62.9m,

372 small males – 110.5 ± 71.5m, large males – 74.2 ± 71.0m; ANOVA: sex – $F_{1,203}$ = 1.11,

373 P = 0.293, size – $F_{1,203}$ = 0.84, P = 0.362, sex*size – $F_{1,203}$ = 7.28, P = 0.008). Small male

374 siblings were geographically more dispersed among shoals than small females ($F_{1,128}$ =

375 9.93, P = 0.002) and large males ($F_{1,107}$ = 6.32, P = 0.013). Adult male siblings were not

376 more dispersed than adult female siblings ($F_{1,75}$ = 0.99, P = 0.323).

377

378 **Discussion**

379 We have undertaken a genetic study of over 900 *Neolamprologus caudopunctatus* across

380 a localized scale of up to 3 km in Lake Tanganyika to determine factors affecting

381 population structure. Our results highlight several sex- and size-specific characteristics

382 that provide a detailed overview of the dispersal strategies adopted by this species.

383 Fundamentally, we found that females dispersed longer distances than males. There was

384 strong agreement among the several complementary analyses that we implemented to

385 detect this sex difference. Weaker genetic differentiation among females between the two

386 study sites (Table 2, Figure 1, 2), coupled with 11 times higher female migration rates

387 between the two populations (Table 3), support the conclusion that longer-distance

dispersal is female-biased. Interpopulational genetic differentiation was greater for small than large females, suggesting that larger females had dispersed longer distances than smaller females. However, the small amount of genetic variation that could be explained by population differences suggests that significant gene flow still occurs between the two populations despite the presence of the potential dispersal barrier. Although the barrier presumably consisted predominately of a sandy substrate with a scarcity of rocks, the possibility remains that suitable habitat exists within this area that acts as a stepping stone for fish dispersing between the sites. At the more localized scale of the mainland population, small female siblings tended to shoal together (Table 4). This pattern occurred despite females not being more related to each other at the population level than were males (Table 1). Longer-distance dispersal was less frequent among males, as shown by the pronounced genetic differentiation across our study area, and by lower male migration rates. Yet, at shorter distances, small male siblings were sampled in more geographically separated shoals than other size and sex classes, implying a greater propensity for kin avoidance among small males. The contrast in dispersal strategies of males and females, despite the existence of shoals of mixed sex and size, implies that shoaling behavior in *N. caudopunctatus* may be characterized by constant fission and fusion.

Size and sex differences in dispersal

Our data suggest that it is more beneficial for females to disperse than to remain on their natal grounds. This may, in part, be related to the potential advantage of males remaining in a familiar area to facilitate territory acquisition and defense, as also occurs in birds

411 (Greenwood 1980). By dispersing, females may also reduce the probability of breeding

412 with related individuals and have access to new territories (Greenwood 1980; Handley

413 and Perrin 2007). However, dispersal between the mainland and island site is also

414 presumably costly. *N. caudopunctatus* are exposed to many fish predators, including

415 species that prey on fry (e.g. *Telmatochromis vittatus* and *N. tetracanthus*; Ochi and

416 Yanagisawa 1999) and adults (e.g. *Lepidiolamprologus elongatus*; Schaedelin, personal

417 observation). These costs may be augmented by the scarcity of rocks along the dispersal

418 barrier between the two sites, making it difficult for individuals to seek shelter from

419 predators. Dispersing individuals are therefore expected to adopt strategies that minimize

420 these costs. For example, as our data suggest, individuals may benefit from dispersing

421 over long distances only when they have reached a relatively larger size, to minimise

422 predation from gape-limited fish predators and to benefit from improved swimming

423 performance (Fisher et al. 2000; Scharf et al. 2000).

424   In addition, kin-based shoaling may provide females with indirect benefits

425 associated with decreased predation of relatives during dispersal. Recent research has

426 revealed that shoaling is often kin-based (reviewed in Hatchwell 2010) , the advantages

427 of which are diverse (Ward and Hart 2003). Shoaling with kin may reduce stress, thus

428 facilitating more rapid growth (Gerlach et al. 2007). Further, Piyapong et al. (2011) found

429 that kin-based shoals of juvenile Trinidadian guppies (*Poecilia reticulate*) were more

430 likely to form in habitats with high predation pressure. This suggests that individuals may

431 gain indirect benefits by decreasing the predation of close relatives. Several studies have

432 also detected sex differences in kin-shoaling (e.g. Griffiths and Magurran 1998; Arnold

433 2000; Ruhl and McRobert 2005; Gerlach and Lysiak 2006). In the Trinidadian guppy, for

example, only females preferred to shoal with familiar kin (Griffiths and Magurran 1998), which may reflect a trade-off between the advantages of shoaling with familiar individuals and moving among shoals in search of mates. However, there has been a lack of previous studies to suggest that sex and size differences in kin shoaling may arise from differences in dispersal strategies.

In contrast to females, dispersal of male *N. caudopunctatus* appears to be more localised. This reduced dispersal propensity may result in higher competition between sibling males for territories or mates (e.g. Frommen et al. 2007). Selection may therefore favour stronger kin avoidance for males at this scale (West et al. 2002; Handley and Perrin 2007). Although large male siblings were not more dispersed than large female siblings, we found that small male siblings were more dispersed than the three other size and sex classes, suggesting that they actively avoid shoaling with kin. Given the longer-distance dispersal of large individuals, the greater localised dispersal of small males is curious and difficult to explain with our current data, thus warranting further research. However the results imply that the benefits of male kin avoidance may be size-dependant. For example, if body size is correlated with age in this species (e.g. Helfman et al. 1997) and young males search for mates in shoals, kin avoidance by small males may reduce local mate competition among siblings. In addition, males may display lower fidelity to individual shoals to permit mate searching in multiple shoals (e.g. Griffiths and Magurran 1998).

Our study highlights the complexity of dispersal strategies (short distance male-biased dispersal, but longer-distance female dispersal) and stresses the importance of exploring dispersal at multiple spatial scales. To our knowledge our study system is the

first to demonstrate sex-biased dispersal and kin-based shoaling in tandem. Our findings

may therefore provide new insights into the benefits of kin-grouping in animals.

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Table 1 Mean relatedness of individuals within age and sex categories. P values refer to

the statistical significance of the difference between two classes in within-class Phm.

Class	Class 1	Class 2	Class 1 Phm ± SD	Class 1 Phm ± SD	P
Shoal	Females	Males	0.266±0.03 (264)	0.266±0.04 (231)	0.930
Shoal	Small females	Small males	0.269±0.03 (128)	0.270±0.03 (126)	0.702
Shoal	Large females	Large males	0.267±0.03 (136)	0.267±0.04 (105)	0.894
Breeder	Females	Males	0.265±0.03 (64)	0.267±0.04 (60)	0.709

623 **Table 2** AMOVA table for *N. caudopunctatus*, separated by sex and size, outlining variation among shoals and sites.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices		P value
			Small males				
Among sites	1	15.873	0.08573	2.40	F_{ST}	0.02755	<0.001
Among shoals within sites	22	80.446	0.01272	0.36	F_{SC}	0.00365	0.522
Within shoals	322	1119.190	3.47575	97.25	F_{CT}	0.02399	<0.001
Total	345	1215.509	3.57420				
			Large males				
Among sites	1	9.782	0.04769	1.38	F_{ST}	0.01926	0.003
Among shoals within sites	13	48.237	0.01897	0.55	F_{SC}	0.00556	0.242
Within shoals	243	824.876	3.39455	98.07	F_{CT}	0.01378	0.003
Total	257	882.895	3.46122				
			Small females				
Among sites	1	13.466	0.07292	2.02	F_{ST}	0.02847	<0.001
Among shoals within sites	17	69.112	0.02982	0.83	F_{SC}	0.00843	0.01
Within shoals	347	1216.545	3.50589	97.15	F_{CT}	0.02021	<0.001
Total	365	1299.123	3.60863				
			Large females				
Among sites	1	8.837	0.04190	1.18	F_{ST}	0.01817	0.004
Among shoals within sites	17	64.273	0.02240	0.63	F_{SC}	0.00641	0.111
Within shoals	247	858.188	3.47444	98.18	F_{CT}	0.01184	<0.001
Total	265	931.297	3.53875				

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Table 3 Posterior parameter estimates for male and female cichlids after simulation using coalescent IM modelling. Refer to Methods

for explanation of model parameters. The population migration rate for each sex and population was calculated following $\frac{\theta_1 m_1}{2}$.

	Posterior model parameters					Demographic conversion	
	θ_1	θ_2	θ_A	m_1	m_2	$\frac{\theta_1 m_1}{2}$	$\frac{\theta_2 m_2}{2}$
Males	0.089±0.041	0.259±0.089	0.988±0.011	100.97±42.27	23.48±20.31	4.49	3.04
Females	0.240±0.046	0.594±0.134	0.990±0.010	147.70±31.46	112.55±30.16	17.72	33.43

627 **Table 4** Difference between observed intrashoal Phm sum and that expected due to

628 random mixing of individuals. Data are presented as mean ± SD. When Phm sums are

629 larger than expected, individuals within shoals are more related than expected by chance.

Class	Observed intrashoal Phm sum	Expected intrashoal Phm sum	N	P
Small females	180.1	172.6±2.7	11	0.001
Large females	114.4	113.6±2.2	12	0.322
Small males	116.0	117.0±2.2	17	0.625
Large males	104.6	107.6±2.4	16	0.859

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Figure legends

Fig. 1 Posterior population assignment probabilities among a) female and b) male *N. caudopunctatus*. Cluster analyses were conducted in STRUCTURE (K=1 for females, K=2 for males). Each vertical line represents one individual and the proportion of white to grey reflects the probability of belonging to each of the two clusters.

Fig. 2 Neighbour-joining trees for a) female and b) male *N. caudopunctatus* sampled in the two populations. Data are based on 11 microsatellite loci. Only shoals for which we genotyped a minimum of eight individuals of one sex were included in the analysis. Each circle represents a single shoal. Empty circles represent shoals captured at the island site and filled circles represent shoals from the mainland site.
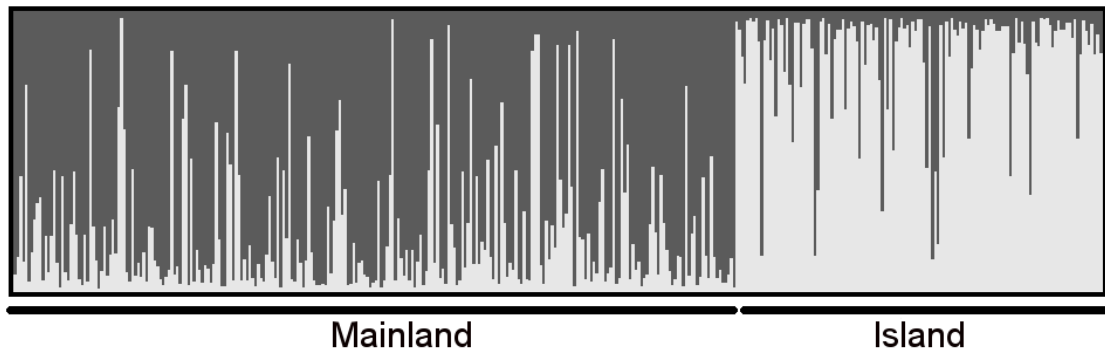
(a)

Mainland Island

(b)
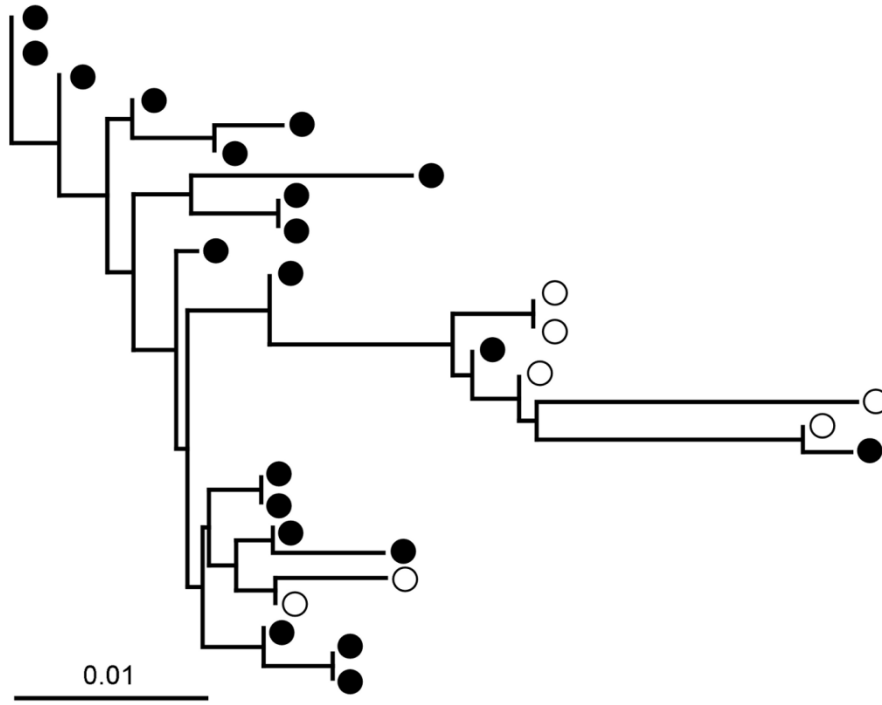
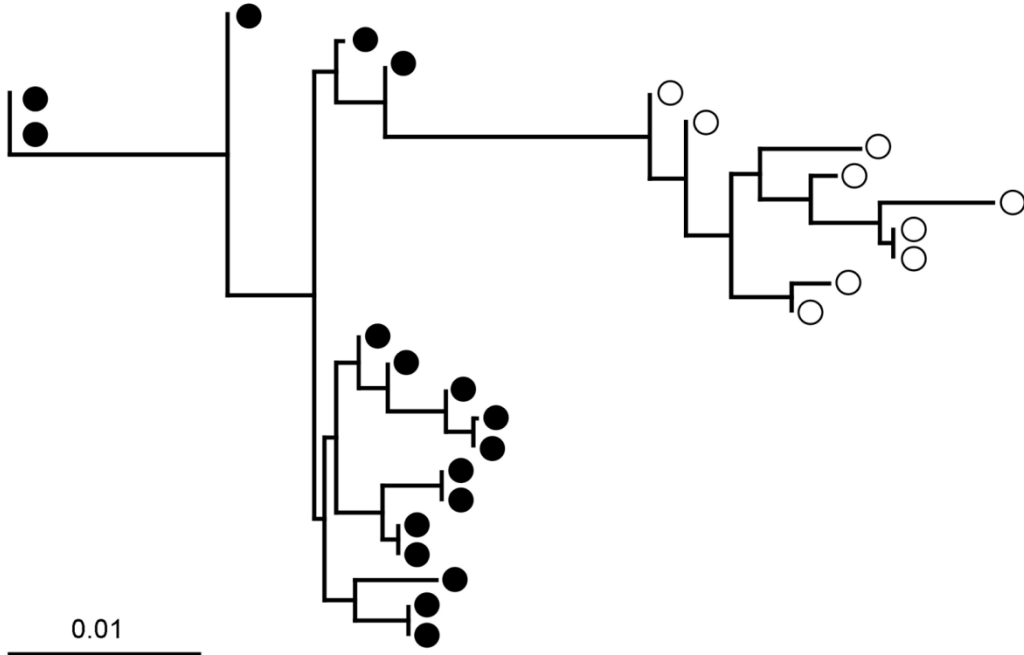Mainland Island

660

661 **Fig. 1**

(a)

0.01

(b)

0.01

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663   **Fig. 2**