

1 **Sex biases in kin shoaling and dispersal in a cichlid fish**

2

3 Wouter F.D. van Dongen^{1*†}, Richard H. Wagner¹, Yoshan Moodley¹ and Franziska C.
4 Schaedelin¹

5

6 ¹*Konrad Lorenz Institute of Ethology, Department of Integrative Biology and Evolution,*
7 *University of Veterinary Medicine Vienna, Vienna, Austria*

8

9 *email: wouter.v.dongen@gmail.com

10

11 † Current address: *Applied Ecology Research Group and Institute for Sustainability and*
12 *Innovation, College of Engineering and Science, Victoria University, Melbourne,*
13 *Australia*

14

15

16 Author contributions: FS and RH conceived and designed the study. FS performed the
17 fieldwork and WvD performed the genetics work. WvD and YM analyzed the data. WvD
18 wrote the manuscript; all other authors provided editorial advice.

19

20 **Abstract**

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

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

43

44 **Keywords** Animal movements · Fish shoals · Grouping behavior · Kin selection ·

45 Population genetics

46

47 **Introduction**

48 Dispersal by individuals from their natal grounds to their breeding areas is a common
49 feature of animal life (Greenwood 1980) and is associated with significant benefits and
50 costs to dispersers (Handley and Perrin 2007; Bonte et al. 2012). By dispersing, animals
51 may avoid the negative effects of inbreeding (e.g. Nagy et al. 2013; Banks and
52 Lindenmayer 2014) and increase access to resources such as territories or mates (Bowler
53 and Benton 2005). In addition, they may increase inclusive fitness, for example, by
54 relaxing local resource competition among relatives (reviewed in Handley and Perrin
55 2007). However, diverse costs of dispersal also exist, including increased risks, and loss
56 of time and energy (Bonte et al. 2012). For example, since dispersers often travel
57 solitarily across unfamiliar and unsuitable habitat, a major cost is an increased risk of
58 predation (e.g. Hiddink et al. 2002; Yoder et al. 2004).

59 The decision to disperse may accordingly depend on a tradeoff between these
60 costs and benefits, which in turn is predicted to vary with the ecological conditions and
61 individual phenotypes (Bowler and Benton 2005). For example, size-differences in
62 dispersal strategies may arise when only larger individual have sufficient energy reserves
63 to be able to move large distances or be competitive in new environments (e.g.
64 Gundersen et al. 2002). Similarly, local mate- and resource competition may lead to sex-
65 differences in dispersal strategies. In birds, for example, females typically disperse, as
66 males gain more from being able to defend a familiar territory in their natal habitat
67 (Greenwood 1980). In contrast, male-biased dispersal occurs more often in polygynous
68 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 (Koblmüller et al. 2006). We first quantified genetic population structure
114 within and between populations to detect evidence of sex-biased dispersal. The higher

115 aggressiveness of males in this species suggests that they may benefit more from
116 defending a territory in a familiar environment, thus favoring female dispersal
117 (Greenwood 1980). Our second aim was to test whether kin-based shoaling occurred in
118 this species as a possible strategy to reduce the costs of long-distance dispersal. Finally,
119 we determined whether any tendency to shoal with kin was size- or sex-dependent. As
120 smaller fish presumably have reduced swimming performance (Fisher et al. 2000), are
121 less competitive (Rowland 1989; Koops and Grant 1993; Aubin-Horth et al. 2007) and
122 are younger (Helfman et al. 1997), we predicted that genetic signatures of dispersal are
123 more likely to be detected amongst larger individuals.

124

125 **Materials and methods**

126 *Field work*

127 The study was carried out in October and November 2008 in Kasakalawe Point (S 08°
128 46' 46.6" E 31° 04' 44.4") on the southern shore of Lake Tanganyika, southwest of
129 Mpulungu, Zambia (hereafter referred to as the 'mainland site'). Clusters of *N.*
130 *caudopunctatus* defending potential nesting cavities were identified along the shore at
131 depths of 5 to 12m. We identified and numbered 165 breeding cavities defended by pairs
132 in an area of approximately 220 x 250 meters by SCUBA diving. To map the whole
133 population underwater (including the location of breeders and shoal fish, see below), we
134 constructed a coordinate system out of sisal rope and measured nest and shoal distances
135 to this coordinate plane using measuring tape. In addition to the main study site, we also
136 sampled individuals inhabiting the waters around Mbita Island (S 08° 45' 20.2" E 31° 05'
137 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 $\frac{F_{ST}}{1 - F_{ST}}$ (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 rate₁ = $\frac{\theta_1 m_1}{2}$. 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 $\frac{F_{ST}}{1 - F_{ST}}$ 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 $Phm_{xy}(l) = \frac{(s_{ac} + s_{ad} + s_{bc} + s_{bd})}{4}$, 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: $Phm_{xy} = \frac{\sum_l \frac{1}{p_l} Phm_{xy}(l)}{\sum_l \frac{1}{p_l}}$, 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 RNDOM (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 \pm 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 ± 0.4 cm, shoal fish = $4.2 \pm$
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 = $57 \pm 36\%$)
314 at the mainland population and 53-100% (mean = $88 \pm 17\%$) 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 = $55 \pm 18\%$, n = 23) at the
318 mainland population and 21-67% at the island population (mean = $52 \pm 15\%$, n = 8).

319

320 Sex-size differences in overall population structure

321 We found no evidence of isolation by distance within the mainland population for either
322 males (mantel test: $Z = 70.43$, $P = 0.336$) or females (mantel test: $Z = 453.37$, $P = 0.590$).
323 Similarly, we found no evidence of isolation by distance when separating individuals by
324 sex and size (mantel test: small males - $Z = 53.61$, $P = 0.852$; large males - $Z = 151.44$, P
325 $= 0.188$; small females - $Z = 152.49$, $P = 0.282$; large females - $Z = 73.64$, $P = 0.608$).
326 Finally, we found no difference in the average relatedness between individuals within
327 sexes for either shoal fish or breeders, even when separate analyses were conducted for
328 small and large fish (Table 1).

329 AMOVA revealed that in both sexes the majority of genetic variation
330 (approximately 98%) occurred among individuals within shoals (Table 2). However,
331 genetic variance among shoals within study sites was over twice as high for small
332 females (0.83%) than for small males (0.36%: chi-squared test - $\chi^2 = 18.56$, d.f. = 1, $P <$
333 0.001), but similar for large females and males (0.63% and 0.55%, respectively: chi-
334 squared test - $\chi^2 = 0.54$, d.f. = 1, $P = 0.461$; Table 2). Furthermore, the only among-shoal
335 variance value (i.e. F_{SC}) that was greater than zero was for small females. Less than 2.5%
336 of variation was explained by differences between the mainland and island populations,
337 although the male populations were slightly more differentiated than the female
338 populations. For both sexes, genetic differentiation between the two study sites was
339 higher for small than large individuals (i.e. based on F_{ST} values in Table 2). An analysis
340 of migration rates using a coalescent IM model, revealed that migration from the island to
341 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 $q(\text{cluster 1/mainland}) = 0.219 \pm 0.244$, $q(\text{cluster 2/island}) = 0.852 \pm 0.201$;
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 – $\chi^2 = 25.00$, 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 ± 62.9 m, large females – 90.7 ± 62.9 m,
372 small males – 110.5 ± 71.5 m, large males – 74.2 ± 71.0 m; 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

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

406

407 Size and sex differences in dispersal

408 Our data suggest that it is more beneficial for females to disperse than to remain on their
409 natal grounds. This may, in part, be related to the potential advantage of males remaining
410 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. *Lepidolamprologus 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 reticulata*) 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

434 example, only females preferred to shoal with familiar kin (Griffiths and Magurran
435 1998), which may reflect a trade-off between the advantages of shoaling with familiar
436 individuals and moving among shoals in search of mates. However, there has been a lack
437 of previous studies to suggest that sex and size differences in kin shoaling may arise from
438 differences in dispersal strategies.

439 In contrast to females, dispersal of male *N. caudopunctatus* appears to be more
440 localised. This reduced dispersal propensity may result in higher competition between
441 sibling males for territories or mates (e.g. Frommen et al. 2007). Selection may therefore
442 favour stronger kin avoidance for males at this scale (West et al. 2002; Handley and
443 Perrin 2007). Although large male siblings were not more dispersed than large female
444 siblings, we found that small male siblings were more dispersed than the three other size
445 and sex classes, suggesting that they actively avoid shoaling with kin. Given the longer-
446 distance dispersal of large individuals, the greater localised dispersal of small males is
447 curious and difficult to explain with our current data, thus warranting further research.
448 However the results imply that the benefits of male kin avoidance may be size-dependant.
449 For example, if body size is correlated with age in this species (e.g. Helfman et al. 1997)
450 and young males search for mates in shoals, kin avoidance by small males may reduce
451 local mate competition among siblings. In addition, males may display lower fidelity to
452 individual shoals to permit mate searching in multiple shoals (e.g. Griffiths and Magurran
453 1998).

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

457 first to demonstrate sex-biased dispersal and kin-based shoaling in tandem. Our findings
458 may therefore provide new insights into the benefits of kin-grouping in animals.

459

460 **Acknowledgements** We thank Florian Sammer for conducting the laboratory work for
461 this study, Valeria Montana for assistance with the IM analyses and Marlene Mann for
462 helping with many aspects of the project. We are grateful to Stefan Fischer, Stefanie
463 Schwamberger, Peter Turai and Hartmut Lemmel for their great field assistance, John,
464 Enoch, and Maxwell Juma for their support in the field, and Bornfirst and family for
465 managing the Tanganyika Lodge. We thank Michael and Barbara Taborsky for the
466 provision of diving bottles and Drs Harris Phiri, Patrick Nagalda, and Justina Kasabila of
467 the Zambian Ministry of Agriculture, Food and Fisheries, as well as Rueben Shappola
468 and the Department of Fisheries, in Mpulungu for their logistical support of our research.
469 Thank you to Andrea Manica and three anonymous referees for their comments on earlier
470 versions of this manuscript. This project was funded by the Austrian Academy of
471 Sciences, the Veterinary University of Vienna and by the Austrian Science Fund (FWF;
472 projects P17468 and P20401).

473

474 **References**

475 Arnold KE (2000) Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex, sibs
476 and shoaling. Behav Ecol Sociobiol 48:385-391.

477 Aubin-Horth N, Desjardins JK, Martei YM, Balshine S, Hofmann HA (2007)
478 Masculinized dominant females in a cooperatively breeding species. Mol Ecol 16:1349-
479 1358.

480 Banks SC, Lindenmayer DB (2014) Inbreeding avoidance, patch isolation and matrix
481 permeability influence dispersal and settlement choices by male agile antechinus in a
482 fragmented landscape. *J Anim Ecol* 83:515-524.

483 Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05.
484 Université de Montpellier II

485 Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in
486 a population using permutation methods. *Mol Ecol Notes* 2:611-614.

487 Bisol GD, Capocasa M, Anagnostou P (2012) When gender matters: new insights into the
488 relationships between social systems and the genetic structure of human populations.
489 *Mol Ecol* 21:4917-4920.

490 Bonte D, Van Dyck H, Bullock JM, Coulon A, Delgado M, Gibbs M, Lehouck V,
491 Matthysen E, Mustin K, Saastamoinen M, Schtickzelle N, Stevens VM,
492 Vandewoestijne S, Baguette M, Barton K, Benton TG, Chaput-Bardy A, Clobert J,
493 Dytham C, Hovestadt T, Meier CM, Palmer SCF, Turlure C, Travis JMJ (2012) Costs
494 of dispersal. *Biol Rev* 87:290-312.

495 Bowler DE, Benton TG (2005) Causes and consequences of animal dispersal strategies:
496 relating individual behaviour to spatial dynamics. *Biol Rev Camb Phil Soc* 80:205-225.

497 Carleton KL, Streelman JT, Lee BY, Garnhart N, Kidd M, Kocher TD (2002) Rapid
498 isolation of CA microsatellites from the tilapia genome. *Anim Genet* 33:140-144.

499 Consuegra S, García de Leániz C (2007) Fluctuating sex ratios, but not sex-biased
500 dispersal, in a promiscuous fish. *Evol Ecol* 21:229-245.

501 Duchesne P, Étienne C, Bernatchez L (2006) PERM: A computer program to detect
502 structuring factors in meaningful social units. *Mol Ecol Notes* 6:965-976.

503 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals
504 using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611-2620.

505 Excoffier L, Lischer HEL (2010) Arlequin suite version 3.5: a new series of programs to
506 perform population genetics analyses under Linux and Windows. *Mol Ecol Res* 10:564-
507 567.

508 Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by
509 the author. Department of Genome Sciences, University of Washington, Seattle

510 Fisher R, Bellwood DR, Job SD (2000) Development of swimming abilities in reef fish
511 larvae. *Mar Ecol Prog Ser* 202:163-173.

512 Frommen JG, Bakker TCM (2004) Adult three-spined sticklebacks prefer to shoal with
513 familiar kin. *Behaviour* 141:1401-1409.

514 Frommen JG, Luz C, T.C.M. B (2007) Nutritional state influences shoaling preference
515 for familiars. *Zoology* 110:369-376.

516 Gerlach G, Hodgins-Davis A, MacDonald B, Hannah RC (2007) Benefits of kin
517 association: related and familiar zebra fish (*Danio rerio*) show improved growth. *Behav*
518 *Ecol Sociobiol* 61:1765-1770.

519 Gerlach G, Lysiak N (2006) Kin recognition and inbreeding avoidance in zebrafish,
520 *Danio rerio*, is based on phenotype matching. *Anim Behav* 71:1371-1377.

521 Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-Statistics. *J*
522 *Hered* 86:485-486.

523 Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals.
524 *Anim Behav* 28:1140-1162.

525 Griffiths SW, Magurran AE (1998) Sex and schooling behaviour in the Trinidadian
526 guppy. *Anim Behav* 56:689-693.

527 Gundersen G, Andreassen HP, Ims RA (2002) Individual and population level
528 determinants of immigration success on local habitat patches: an experimental
529 approach. *Ecol Lett* 5:294-301.

530 Handley LJJ, Perrin N (2007) Advances in our understanding of mammalian sex-biased
531 dispersal. *Mol Ecol* 16:1559-1578.

532 Hatchwell BJ (2010) Cryptic kin selection: kin structure in vertebrate populations and
533 opportunities for kin-directed cooperation. *Ethology* 116:203-216.

534 Helfman GB, Collette BB, Facey DE (1997) *The diversity of fishes*. Blackwell Science,
535 Malden, USA

536 Hey J, Nielsen EE (2007) Integration within the Felsenstein equation for improved
537 Markov chain Monte Carlo methods in population genetics. *Proc Nat Acad Sci*
538 104:2785-2790.

539 Hiddink JG, Kock RP, Wolff WJ (2002) Active pelagic migrations of the bivalve
540 *Macoma balthica* are dangerous. *Mar Biol* 140:1149-1156.

541 Jadwiszczak P (2002) Rndom projects: An application for randomization and bootstrap
542 testing. <http://pjadw.tripod.com/soft.htm>

543 Kalinowski S, Taper M, Marshall T (2007) Revising how the computer program
544 CERVUS accommodates genotyping error increases success in paternity assignment.
545 *Mol Ecol* 16:1099-1106.

546 KoblmueLLer S, Sefc KM, Duftner N, Warum M, Sturmbauer C (2006) Genetic
547 population structure as indirect measure of dispersal ability in a Lake Tanganyika
548 cichlid. *Genetica* 128:121-131.

549 Konings A (1998) Tanganyika cichlids in their natural habitat. Cichlid Press, El Paso,
550 Texas

551 Koops MA, Grant JWA (1993) Weight asymmetry and sequential assessment in convict
552 cichlid contests. *Can J Zool* 71:475-479.

553 Lee WJ, Kocher TD (1996) Microsatellite DNA markers for genetic mapping in
554 *Oreochromis niloticus*. *J Fish Biol* 49:169-171.

555 Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers.
556 *Genetics* 152:1753-1766.

557 Mathieu E, Autem M, Roux M, Bonhomme F (1990) Preuves de validation dans l'analyse
558 de structures génétiques multivariées: comment tester l'équilibre panmictique? *Rev*
559 *Statist Appl* 38:47-66.

560 Nagy M, Gunther L, Knornschild M, Mayer F (2013) Female-biased dispersal in a bat
561 with a female-defence mating strategy. *Mol Ecol* 22:1733-1745.

562 Nielsen EG, Bach LA, Kotlicki P (2006) Hybridlab (version 1.0): a program for
563 generating simulated hybrids from population samples. *Mol Ecol Notes* 6:971-973.

564 Ochi H, Yanagisawa Y (1999) Sand-transfer behavior outside the nest by guarding
565 parents of the Tanganyikan cichlid, *Neolamprologus caudopunctatus*. *Ichthyol Res*
566 46:419-422.

567 Parker A, Kornfield I (1996) Polygynandry in *Pseudotropheus zebra*, a cichlid fish from
568 Lake Malawi. *Environ Biol Fish* 47:345-352.

569 Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ
570 (ed) The behaviour of teleost fishes, 2nd ed. Croom Helm, London

571 Piyapong C, Butlin RK, Faria JJ, Scruton KJ, Wang J, Krause J (2011) Kin assortment in
572 juvenile shoals in wild guppy populations. *Heredity* 106:749-756.

573 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
574 multilocus genotype data. *Genetics* 155:945-959.

575 Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers.
576 *Evolution* 43:258-275.

577 Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics
578 under isolation by distance. *Genetics* 145:1219-1228.

579 Rowland WJ (1989) The effects of body size, aggression and nuptial coloration on
580 competition for territories in male threespine sticklebacks, *Gasterosteus aculeatus*.
581 *Anim Behav* 37:282-289.

582 Ruhl N, McRobert SP (2005) The effect of sex and shoal size on shoaling behaviour in
583 *Danio rerio*. *J Fish Biol* 67:1318-1326.

584 Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing
585 phylogenetic trees. *Mol Biol Evol* 4:406-425.

586 Schaedelin FC, van Dongen WFD, Wagner RH (2013) Non-random brood mixing
587 suggests adoption in a colonial cichlid. *Behav Ecol* 24:540-546.

588 Scharf FS, Juanes F, Rountree RA (2000) Predator size - prey size relationships of marine
589 fish predators: interspecific variation and effects of ontogeny and body size on trophic-
590 niche breadth. *Mar Ecol Prog Ser* 208:229-248.

591 Schliewen U, Rassmann K, Markmann M, Markert J, Kocher T, Tautz D (2001) Genetic
592 and ecological divergence of a monophyletic cichlid species pair under fully sympatric
593 conditions in Lake Ejagham, Cameroon. *Mol Ecol* 10:1471-1488.

594 Schradin C, Lamprecht J (2000) Female-biased immigration and male peace-keeping in
595 groups of the shell-dwelling cichlid fish *Neolamprologus multifasciatus*. *Behav Ecol*
596 *Sociobiol* 48:236-242.

597 Sheridan CM, Spotila JR, Bien WF, Avery HW (2010) Sex-biased dispersal and natal
598 philopatry in the diamondback terrapin, *Malaclemys terrapin*. *Mol Ecol* 19:5497-5510.

599 Stiver KA, Dierkest P, Taborsky M, Balshine S (2004) Dispersal patterns and status
600 change in a co-operatively breeding cichlid *Neolamprologus pulcher*: evidence from
601 microsatellite analyses and behavioural observations. *J Fish Biol* 65:91-105.

602 Stobutzki I, Bellwood DR (1997) Sustained swimming abilities of the late pelagic stages
603 of coral reef fishes. *Mar Ecol Prog Ser* 149:35-41.

604 Taylor MI, Morley JI, Rico C, Balshine S (2003) Evidence for genetic monogamy and
605 female-biased dispersal in the biparental mouthbrooding cichlid *Eretmodus cyanostictus*
606 from Lake Tanganyika. *Mol Ecol* 12:3173-3177.

607 van Oppen MJH, Rico C, Deutsch TC, Turner GF, Hewitt GM (1997) Isolation and
608 characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Mol*
609 *Ecol* 6:387-388.

610 Ward AJW, Hart PJB (2003) The effects of kin and familiarity on interactions between
611 fish. *Fish Fish* 4:348-358.

612 West SA, Pen I, Griffin AS (2002) Conflict and cooperation – cooperation and
613 competition between relatives. *Science* 296:72-75.

614 Yoder JM, Marschall EA, Swanson DA (2004) The cost of dispersal: predation as a
615 function of movement and site familiarity in ruffed grouse. *Behav Ecol* 15:469-476.
616 Zardoya R, Vollmer DM, Craddock C, Streelman JT, Karl S, Meyer A (1996)
617 Evolutionary conservation of microsatellite flanking regions and their use in resolving
618 the phylogeny of cichlid fishes (Pisces: Perciformes). *Proc Roy Soc Lond B* 263:1589-
619 1598.
620

621 **Table 1** Mean relatedness of individuals within age and sex categories. P values refer to
 622 the statistical significance of the difference between two classes in within-class Phm.

Class	Class 1	Class 2	Class 1 Phm ± SD	Class 2 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

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

Small males							
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	P value	
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				

625 **Table 3** Posterior parameter estimates for male and female cichlids after simulation using coalescent IM modelling. Refer to Methods
 626 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 \pm 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 \pm 2.7	11	0.001
Large females	114.4	113.6 \pm 2.2	12	0.322
Small males	116.0	117.0 \pm 2.2	17	0.625
Large males	104.6	107.6 \pm 2.4	16	0.859

630

631

632

633

634

635

636

637

638

639

640

641

642

643

644

645 **Figure legends**

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

650

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

656

657

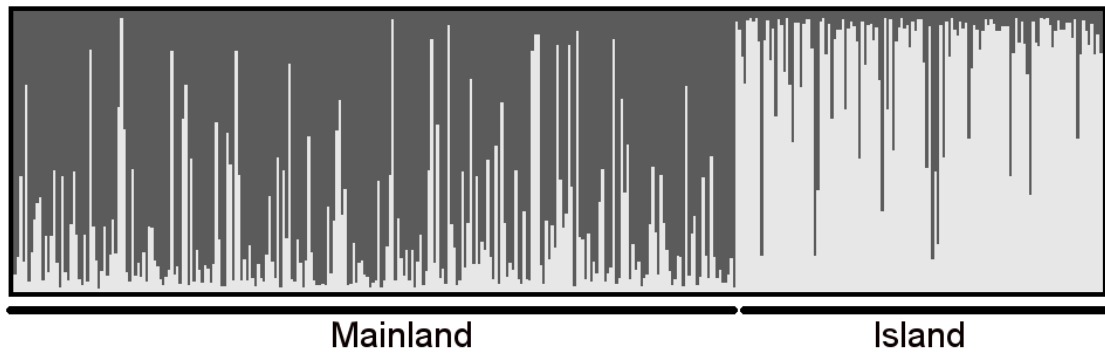
658

659

(a)



(b)

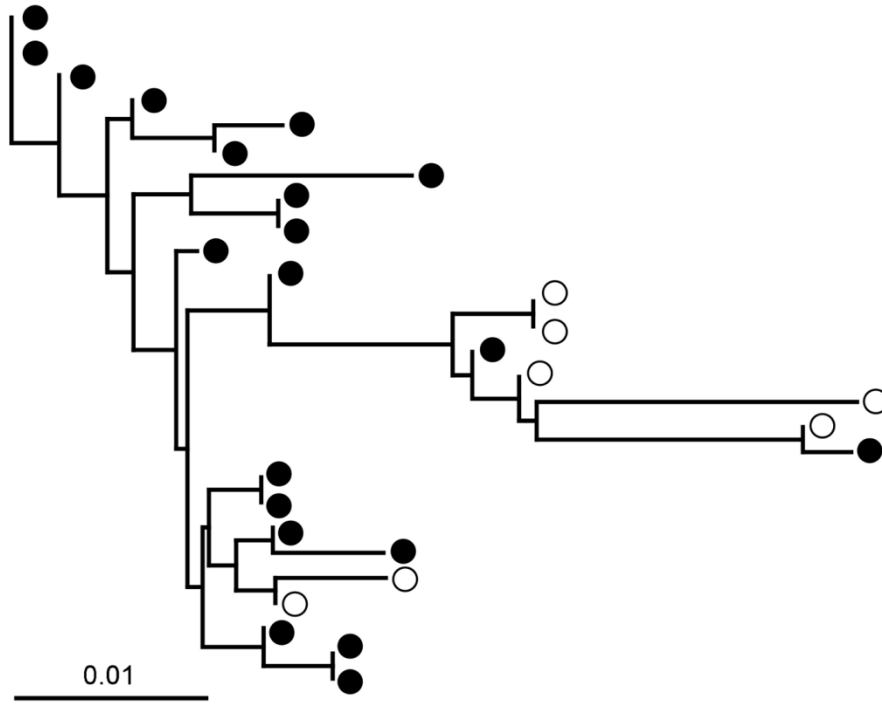


660

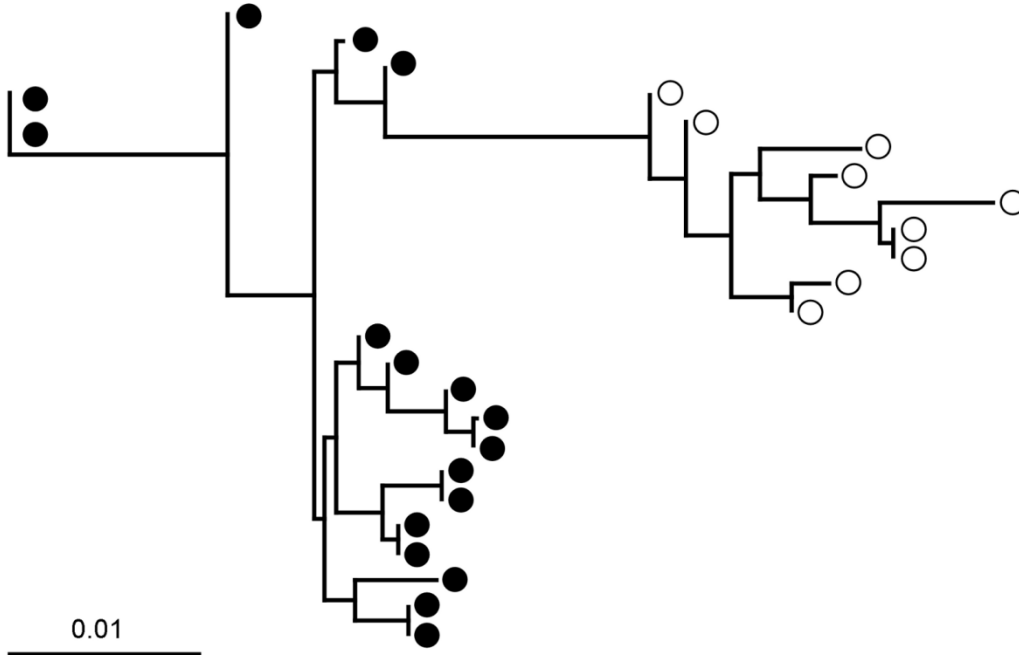
661

Fig. 1

(a)



(b)



662

663

Fig. 2