

1 **First field evidence for alloparental egg care in cooperatively**
2 **breeding fish**

3

4 Dario Josi¹, Michael Taborsky¹, Joachim G. Frommen¹

5

6

7 ¹Department of Behavioural Ecology

8 Institute of Ecology and Evolution

9 University of Bern, Hinterkappelen, Switzerland

10

11

12

13 Corresponding author: Dario Josi (dario.josi@iee.unibe.ch)

14 **Abstract**

15 Helping behaviour in cooperative breeders has been intensively studied in many animal taxa, including
16 arthropods, birds and mammals. In these highly social systems, helpers typically engage in brood care
17 and the protection of dependent young. Such helping systems also exist in cooperatively breeding
18 cichlid species of Lake Tanganyika. However, breeding in these species happens in clefts, narrow holes
19 or shelters underneath stones. Therefore, direct brood care by breeders and helpers has thus far only
20 been observed under artificial laboratory conditions. Under natural conditions, brood care behaviour
21 has been estimated indirectly by determining the time spent in the breeding chamber, or by the
22 number of visits to the breeding chamber. The reliability of such substitutes needs to be validated, for
23 instance, by demonstrating alloparental egg-care of helpers through direct observations in nature.
24 Here, we describe direct egg care by a male helper of the cooperatively breeding cichlid
25 *Neolamprologus savoryi* in the field. The helper inspected and cleaned the eggs and defended them
26 against predators. By reconstructing the genetic relatedness using microsatellite markers, we show
27 that the helper was the son of the breeding male, but unrelated to the breeding female. The genetic
28 mother of the helper was defending a different territory next to the one where the helper showed
29 alloparental egg care. This indicates that the helper had dispersed inside the male territory to assist
30 another female to care for his half-siblings. These results demonstrate alloparental egg care without
31 reproductive share in a fish species under natural conditions, underlining that helping behaviour in
32 cooperatively breeding fish has a strong non-self-serving component.

33 **Introduction**

34 Cooperative breeding, where a dominant breeding pair is assisted by subordinate individuals to rear
35 their offspring, represents one of the most complex forms of sociality (Skutch, 1935; Taborsky, 1987;
36 Solomon & French, 1997; Field & Leadbeater, 2016). It evolved in a range of animal species, including
37 arthropods, mammals, birds and fishes (reviewed in: Koenig & Dickinson, 2016; Rubenstein & Abbot,
38 2017). Helping duties in cooperative breeders are highly variable between species, including vigilance
39 behaviour and food provisioning in birds and mammals (Clutton-Brock, 2016) and egg cleaning and
40 fanning, shelter digging, and antipredator defence in fishes (Taborsky, 1994, 2016). Some of these
41 behaviours, like food provisioning and care of foreign eggs or young, can be called altruistic, as they
42 involve immediate fitness costs to the alloparent without immediate fitness benefits (as defined by
43 Taborsky et al., 2016). Other behaviours, such as antipredator defence and territory maintenance (e.g.
44 shelter digging) might additionally have an immediately self-serving component, especially when they
45 are also shown in the absence of dependent young (Brouwer et al., 2005). To understand the evolution
46 of cooperative breeding systems it is important to clarify whether other individuals than the breeders
47 engage in non-immediately-self-serving helping behaviours, which are expected to increase the
48 survival of dependent young and the fitness of breeders. Care for eggs or young can be observed rather
49 easily under natural conditions in birds and mammals. It is, however, difficult to show direct brood
50 care in nature in cooperatively breeding fishes, because these species typically excavate breeding
51 shelters underneath rocks or breed in narrow clefts or holes, where direct brood care by breeders and
52 helpers cannot be observed. Therefore, researchers often use proxies of presumed brood care, like the
53 time spent in the breeding chamber (cf. Balshine et al., 2001; Tanaka et al., 2018b) or changes in
54 behaviour depending on the presence of juveniles (Brouwer et al., 2005; Bruintjes et al., 2013). Some
55 cooperatively breeding fishes are known for having only few juveniles, which is probably due either to
56 small clutch sizes (e.g. Tanaka et al., 2018a), or to high mortality of eggs and juveniles. The latter may
57 be somewhat compensated by parental and alloparental care, for example by removing fungi or
58 bacteria, or by protection from predators (Knouft et al., 2003; Brouwer et al., 2005). If egg care is

59 provided by helpers, breeders might further benefit from gaining time and energy to invest in other
60 activities. Nevertheless, individuals engaging in egg care accept energetic costs (Taborsky & Grantner,
61 1998). To the best of our knowledge, removing fungi, bacteria or debris from the eggs have not been
62 shown to provide nutritional benefits in any fish species. Such benefits would accrue when eggs were
63 cannibalised (e.g. Gomagano & Kohda, 2008; Mehlis et al., 2009). This behaviour is punished, however,
64 in cooperatively breeding fishes (Taborsky, 1985; Zöttl et al., 2013).

65 Until today, helpers engaging in direct egg care have been observed only in the *Neolamprologus*
66 *pulcher/brichardi* species complex (Duftner et al., 2007) under laboratory settings (Taborsky, 1984,
67 1985; von Siemens, 1990; Zöttl et al., 2013). Evidence for such behaviour from the field is hitherto
68 missing for any cooperatively breeding fish species. Here we provide the first evidence of alloparental
69 egg care of a helper in the cooperatively breeding cichlid *Neolamprologus savoryi* (Heg et al., 2005;
70 Garvy et al., 2015) in nature. We furthermore describe the spawning behaviour of this species and
71 apply genetic methods to elucidate the relatedness between different territory members and the
72 brood caring helper.

73 **Methods**

74 *Study species*

75 *Neolamprologus savoryi* is a cooperatively breeding cichlid fish endemic to Lake Tanganyika, East Africa
76 (Heg et al., 2005). Breeding groups are composed of a dominant male and one to several breeding
77 females (Heg et al., 2005; Garvy et al., 2015). Females defend distinct sub-territories, in which they
78 tolerate subordinate individuals of varying age, size and sex. Breeding groups cluster into colonies, and
79 each group defends the territory against conspecific and heterospecific intruders and neighbours (Heg
80 et al., 2008). Subordinates help in territory maintenance and defence (Heg et al., 2005). Furthermore,
81 they have been assumed to help in guarding and cleaning the eggs.

82 *Study site and observation period*

83 Data were collected on 24 September 2016 at Kasakalawe point at the southern tip of Lake Tanganyika,
84 Zambia. The study site was a sandy area at a depth of 10.2 m. Small groups of rocks of sizes between

85 10 and 40 cm in diameter served as shelter for the fishes. We established a 10 x 10 m grid subdivided
86 into 1 m² covering the whole focal colony. This grid allowed us to draw a detailed map of the habitat
87 inside the colony. The territory borders of the focal groups were determined by 20 min observations a
88 few days prior to the occurrence of the spawning and egg laying and plotted on the map. Based on
89 these territory borders and behavioural observations we marked all potential male and female
90 territories with numbered stones. Our focal group of *N. savoryi* was part of a colony containing 22
91 dominant males, each defending a territory containing 0 - 5 females (median = 3) and tolerating
92 between 0 and 3 large subordinate males (N = 13) in their territory (median = 0). The breeding females'
93 groups (N = 59) contained 0 to 3 helpers larger than 1.5 cm standard length (median = 1).

94 *Observations and data acquisition*

95 While conducting an experiment in the colony (Josi et al., in prep.), we haphazardly witnessed intense
96 courtship behaviour in one of our focal territories. Spawning took place in this territory at an easily
97 observable position, allowing us to record courtship, spawning and egg care. In total, we recorded 30
98 min and 16 sec of spawning behaviour. Recordings of egg care started directly after the spawning and
99 lasted for approximately two hours. Within this timeframe, we produced 3 video recordings (1: 13 min
100 13 sec; 2: 22 min 29 sec; 3: 35 min 30 sec). Video material was afterwards processed with Adobe
101 premiere pro CC and analysed for behavioural frequencies of the breeder male and female, and the
102 helper.

103 Subsequently we caught all fish of the focal male's territory (i.e., one male, 4 females, 1 helper; see
104 Fig. 1). Standard length (SL) was measured from the tip of the mouth to the posterior end of the
105 vertebral column with an accuracy of ± 1 mm using a 1mm measuring board. Further, the sex was
106 confirmed by external examination of the genital papillae. Finally, we removed a small piece of tissue
107 from the fin for genetic analyses. Afterwards, all individuals were released back to their shelter. They
108 recovered within a few minutes.

109 *Genetic relatedness analysis*

110 To scrutinize the genetic relatedness of the group members, total DNA was extracted from the ethanol
111 preserved fin-clip samples using a magnetic separation protocol (MagneSil™ Paramagnetic Particles,
112 Promega; Kotrschal et al., 2012). Fourteen polymorphic microsatellite loci were used to determine
113 relatedness (loci UNH154, UNH106 (Lee & Kocher, 1996); NP007, NP773, ULI2 (Schliewen et al., 2001);
114 Pzeb3, Pzeb4 (Van Oppen et al., 1997); TmoM11, TmoM13, TmoM25, TmoM27 (Zardoya et al., 1996);
115 UME003 (Parker & Kornfield, 1996); UNH1009 (Carleton et al., 2002), and Ppun21 (Taylor et al., 2002).
116 Some of these sequences were already optimized for the closely related species *N. pulcher*. DNA was
117 amplified using the QIAGEN® Multiplex PCR Kit (Qiagen), allowing co-amplification of several locus-
118 specific, fluorescently labelled primer pairs in one single PCR reaction. We used two different primer
119 sets containing seven primer pairs each to amplify the 14 microsatellite markers. PCR reactions were
120 attained in a 10 µl volume containing 1 µl of the genomic DNA, 5 µl 2x QIAGEN Multiplex PCR Master
121 Mix, 3 µl H₂O and 1 µl of 10 x primer mix consisting of fluorescently labelled forward and non-labelled
122 reverse primer pairs with end concentrations of 0.4 to 0.6 µM each, according to the intensity of the
123 respective amplification products. The fluorescent dyes were: 6-FAM (blue), HEX (green), Yakima
124 Yellow (green), ATTO550 (yellow), ATTO565 (red) (Microsynth), VIC (green) and PET (red) (Thermo
125 Fisher). Amplification was performed in a GeneAmp® 9700 PCR System (Applied Biosystems) using the
126 following cycling parameters: 15 min at 95°C, 35 cycles at 95°C for 30 s, 57°C for 3 min and 72°C for 60
127 s followed by a final elongation step of 72°C for 15 min. Fluorescent PCR fragments were visualized by
128 capillary electrophoresis on an ABI3100® Genetic Analyser (Applied Biosystems). GeneScan 500 LIZ
129 (Thermo Fisher) was used as an internal size standard and the fragments were analysed using the
130 GeneMarker® Analysis software version 2.4.0 (SoftGenetics). We reconstructed relatedness within the
131 focal group using the Simpson-assisted descending ratio algorithm in KINGROUP v2.1 (Konovalov,
132 2006), compared against the null hypothesis of no relatedness.

133 **Results**

134 *Group structure*

135 The breeding male (M1) of the focal group measured 60 mm SL. His territory contained 4 females
136 defending sub-territories (F1: 44 mm; F2: 45 mm; F3: 46 mm; F4: 48 mm; all measures in SL; for home
137 ranges see Fig. 1). Female F4 had a single male helper (H4; 27 mm SL) in her territory. The relatedness
138 analysis revealed that the breeding male was the genetic father of helper H4, while female F2 was its
139 genetic mother ($p < 0.01$, type II error = 0 %). Furthermore, female F3 was either the daughter or sister
140 of the breeding male, while the other females were unrelated to him ($p < 0.01$, type II error = 0 %).

141 *Spawning behaviour*

142 While female F4 showed spawning behaviour with the territory owner, she also showed 32 times
143 pseudo-spawning (behaviourally identical to spawning but without eggs being laid) with a
144 neighbouring male (M2 (61 mm SL); see video supplement material 1). Thus, she switched several
145 times between the pseudo-spawning site and the egg deposition site (see Fig. 1 and video supplement
146 material 1). During pseudo-spawning, female F4 received aggression from the breeding male M1 as
147 well as from female F2 (see video supplement material 1). The male M2 never showed any aggression
148 towards female F4, but observed or inspected her rather closely during pseudo-spawning. Based on
149 the typical male posture and behaviour during the release of sperm, we counted that male M2 released
150 9 times sperm during pseudo-spawning, while the female did not lay any eggs. At the egg deposition
151 site, she laid eggs that were fertilized directly afterwards by the dominant breeding male M1. During
152 spawning, no other individual beside the breeding male M1 and female F4 approached the egg
153 deposition site. In total, six eggs were deposited which does not seem to be an exceptional clutch size
154 for *N. savoryi*, as during a second observation in another territory a clutch of 10 eggs was recorded (DJ,
155 personal observation). After the spawning, M1 shortly inspected the eggs (0.8 seconds) while M2 never
156 inspected them. However, the breeding female (F4) and her helper (H4) inspected, cleaned, and
157 defended the eggs (see Fig. 2; video supplement material 2). During the 71 min of recordings after
158 spawning had ended, the female showed egg cleaning behaviour six times, defended the spawning site
159 against conspecific and heterospecific intruders 20 times, and inspected the eggs for a total period of
160 106 seconds. In the same time period, the helper cleaned the eggs 28 times, defended once against a

161 heterospecific egg predator (*Telmatochromis vittatus*), and inspected the eggs for a total period of 339
162 seconds. Most defence behaviour was shown by the breeding female against the facultative egg
163 predator *Telmatochromis vittatus* (twice during the spawning and 7 times afterwards), the piscivorous
164 eel *Mastacembelus moorii* (8 times during spawning and once after the spawning), and against
165 conspecifics (5 times during spawning and 12 times afterwards; see Fig. 2 and video recordings in
166 supplement material 1, 2). The breeding male M1 defended the eggs only against conspecific intruders
167 after the spawning (6 times in total), but did not engage in cleaning the eggs.

168 **Discussion**

169 To fully comprehend the occurrence of altruistic behaviour in cooperative breeders it is important to
170 show alloparental care under natural conditions. Here we provide results from the first field
171 observations of egg care behaviour by a helper in a cooperatively breeding fish. The caring helper was
172 the genetic son of the breeding male, whereas it was unrelated to the female laying the eggs. The
173 genetic mother of the helper defended the neighbouring sub-territory (F2) of the egg-laying female
174 (F4; see Fig. 1). This indicates that helpers are tolerated not only in their mothers' territory, but also in
175 other female subgroups of the breeding male. Helpers might hence be recruited from neighbouring
176 subgroups, depending on the need for help. The helper carefully inspected and cleaned the eggs and
177 showed vigilance behaviour close by. This is in accordance with the helping behaviour of *N. pulcher*
178 described from the laboratory (Taborsky, 1984, 1985; von Siemens, 1990; Zöttl et al., 2013). The
179 helper's effort cannot be explained by a share in reproduction, as it was too small to be sexually mature
180 (D. Heg, personal communication) and as it was not close to the egg laying site while spawning took
181 place. Hence, the helper might have gained indirect fitness benefits by caring for his half-siblings
182 (Bruitjes & Taborsky, 2011), and delayed direct benefits through group augmentation (Kokko et al.,
183 2001) by increased egg survival, and/or by being allowed to stay in the female's territory, where it
184 enjoys protection from predation ("pay-to-stay" Taborsky, 1985; Bergmüller & Taborsky, 2005; Zöttl et
185 al., 2013; Fischer et al., 2014). Compared to the breeding female, the helper cleaned the eggs 4.6 times
186 more often and spent 3.2 times more time with inspecting the eggs, whereas the female spent 7 times

187 more effort in defence against egg predators. These results indicate that breeding females and helpers
188 may specialize in different duties during egg care, suggesting division of labour as demonstrated in the
189 cooperatively breeding congener *N. pulcher* (Bruitjes & Taborsky, 2011).

190 The clutch had disappeared by the next morning, probably because the egg deposition site was quite
191 exposed to predators. Especially during the night, eggs may be vulnerable to predation by nocturnal
192 predators. Indeed, already during daytime the eel *Mastacembelus moorii* and the facultative egg
193 predator *Telmatochromis vittatus* tried repeatedly to approach the egg deposition site, but they were
194 chased away by the breeding female (see supplement material 1, 2). After the eggs disappeared, the
195 helper was no longer observed at the egg deposition site, indicating that he had no other interests in
196 this particular part of the female's territory.

197 The spawning was frequently interrupted by pseudo-spawning events. Such pseudo-spawning
198 behaviour has been shown in other cooperatively breeding cichlids as well (Taborsky, 1985). While the
199 function of this behaviour is not fully understood (Kohda, 1995; Heg et al., 2008), it has been
200 interpreted as evidence of mate choice (Egger et al., 2008). Alternatively, it might serve to coordinate
201 the behaviour of the spawning partners. Our observation might indicate that pseudo-spawning of the
202 female can also serve to reduce reproductive conflict through paternity insurance between breeding
203 males and the female. The female showed pseudo-spawning behaviour with the neighbouring male at
204 a different location than the egg deposition site. Additionally, the neighbouring male released sperm
205 at the pseudo-spawning site and afterwards never visited or inspected the egg deposition site.
206 However, whether such behaviour leads to a reduction of disturbances during the actual spawning
207 needs to be experimentally tested in future studies.

208 In summary, we observed for the first time direct alloparental egg care behaviour in a cooperatively
209 breeding fish in the field. These observations may enhance our appreciation of the evolutionary
210 mechanisms underlying cooperative breeding in fishes and in general.

211 **Author contributions**

212 DJ, MT and JGF conceived the study; MT and JGF organized funding; DJ conducted fieldwork, prepared
213 the video material and conducted the genetic analyses; DJ wrote the first draft of the manuscript,
214 which was edited by MT and JGF; all authors approved the final version of the manuscript

215 **Conflict of interest**

216 The authors declare no conflict of interest.

217 **Acknowledgements**

218 We would like to thank Danielle Bonfils for her help with laboratory work and Hirokazu Tanaka for his
219 help in setting up the field site. We are grateful to the Department of Fisheries, Ministry of Agriculture
220 and Livestock of Zambia, for the logistical help, especially to Taylor Banda and Lawrence Makasa for
221 their continuing support of our work. We thank Celestine and Augustin Mwewa and their team for
222 hosting us at the Tanganyika Science Lodge. Finally, we thank Redouan Bshary, Franziska C. Schädelin
223 and an anonymous reviewer for helpful comments on an earlier version of the manuscript. The work
224 was financially supported by grants of the Swiss National Science Foundation (grants 31003A_156152
225 to MT, and 31003A_144191 to JGF).

226 **Ethical Note**

227 Data collection caused minimal disturbance to the animals and followed the regulations of the Zambian
228 Prevention of Cruelty to Animals act.

229 **References**

- 230 Balshine, S., Leach, B., Francis, N., Hannah, R., Taborsky, M., & Werner, N. (2001). Correlates of group
231 size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and*
232 *Sociobiology*, *50*, 134–140. doi:org/10.1007/s002650100343
- 233 Bergmüller, R., & Taborsky, M. (2005). Experimental manipulation of helping in a cooperative
234 breeder: helpers ‘pay to stay’ by pre-emptive appeasement. *Animal Behaviour*, *69*, 19–28.
235 doi:10.1016/j.anbehav.2004.05.009
- 236 Brouwer, L., Heg, D., & Taborsky, M. (2005). Experimental evidence for helper effects in a
237 cooperatively breeding cichlid. *Behavioral Ecology*, *16*, 667–673. doi:10.1093/beheco/ari042
- 238 Bruintjes, R., & Taborsky, M. (2011). Size-dependent task specialization in a cooperative cichlid in
239 response to experimental variation of demand. *Animal Behaviour*, *81*, 387–394.
240 doi:10.1016/j.anbehav.2010.10.004
- 241 Bruintjes, R., Heg-Bachar, Z., & Heg, D. (2013). Subordinate removal affects parental investment, but
242 not offspring survival in a cooperative cichlid. *Functional Ecology*, *27*, 730–738.
243 doi:10.1111/1365-2435.12088
- 244 Carleton, K. L., Streelman, J. T., Lee, B. Y., Garnhart, N., Kidd, M., & Kocher, T. D. (2002). Rapid
245 isolation of CA microsatellites from the tilapia genome. *Animal Genetics*, *33*, 140–144.
246 doi:10.1046/j.1365-2052.2002.00817.x
- 247 Clutton-Brock, T. (2016). *Mammal societies*. Chichester: Wiley Blackwell.
- 248 Duftner, N., Sefc, K. M., Koblmüller, S., Salzburger, W., Taborsky, M., & Sturmbauer, C. (2007).
249 Parallel evolution of facial stripe patterns in the *Neolamprologus brichardi/pulcher* species
250 complex endemic to Lake Tanganyika. *Molecular Phylogenetics and Evolution*, *45*, 706–715.
251 doi:10.1016/j.ympev.2007.08.001
- 252 Egger, B., Obermüller, B., Eigner, E., Sturmbauer, C., & Sefc, K. M. (2008). Assortative mating
253 preferences between colour morphs of the endemic Lake Tanganyika cichlid genus *Tropheus*.
254 *Hydrobiologia*, *615*, 37–48. doi:10.1007/978-1-4020-9582-5_3
- 255 Field, J., & Leadbeater, E. (2016). Cooperation between non-relatives in a primitively eusocial paper
256 wasp, *Polistes dominula*. *Philosophical Transactions of the Royal Society B*, *371*, 20150093.
257 doi:10.1098/rstb.2015.0093
- 258 Fischer, S., Zöttl, M., Groenewoud, F., & Taborsky, B. (2014). Group-size-dependent punishment of
259 idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal*
260 *Society B*, *281*, 20140184. doi:10.1098/rspb.2014.0184
- 261 Garvy, K. A., Hellmann, J. K., Ligocki, I. Y., Reddon, A. R., Marsh-Rollo, S. E., Hamilton, I. M., ...
262 O’Connor, C. M. (2015). Sex and social status affect territorial defence in a cooperatively
263 breeding cichlid fish, *Neolamprologus savoryi*. *Hydrobiologia*, *748*, 75–85. doi:10.1007/s10750-
264 014-1899-0
- 265 Gomagano, D., & Kohda, M. (2008). Partial filial cannibalism enhances initial body condition and size
266 in paternal care fish with strong male—male competition. *Annales Zoologici Fennici*, *45*, 55–65.

267 doi:10.5735/086.045.0105

268 Heg, D., Bachar, Z., & Taborsky, M. (2005). Cooperative breeding and group structure in the Lake
 269 Tanganyika cichlid *Neolamprologus savoryi*. *Ethology*, *111*, 1017–1043. doi:10.1111/j.1439-
 270 0310.2005.01135.x

271 Heg, D., Heg-Bachar, Z., Brouwer, L., & Taborsky, M. (2008). Experimentally induced helper dispersal
 272 in colonially breeding cooperative cichlids. *Environmental Biology of Fishes*, *83*, 191–206.
 273 doi:10.1007/s10641-007-9317-3

274 Heg, D., Jutzeler, E., Bonfils, D., & Mitchell, J. S. (2008). Group composition affects male reproductive
 275 partitioning in a cooperatively breeding cichlid. *Molecular Ecology*, *17*, 4359–4370.
 276 doi:10.1111/j.1365-294X.2008.03920.x

277 Knouft, J. H., Page, L. M., & Plewa, M. J. (2003). Antimicrobial egg cleaning by the fringed darter
 278 (Perciformes: Percidae: *Etheostoma crossopterygion*): Implications of a novel component of
 279 parental care in fishes. *Proceedings of the Royal Society B*, *270*, 2405–2411.
 280 doi:10.1098/rspb.2003.2501

281 Koenig, W. D., & Dickinson, J. L. (2016). *Cooperative breeding in vertebrates*. Cambridge: Cambridge
 282 University Press.

283 Kohda, M. (1995). Territoriality of male cichlid fishes in Lake Tanganyika. *Ecology of Freshwater Fish*,
 284 *4*, 180–184. doi:10.1111/j.1600-0633.1995.tb00031.x

285 Kokko, H., Johnstone, R. A., & Clutton-Brock, T. H. (2001). The evolution of cooperative breeding
 286 through group augmentation. *Proceedings of the Royal Society B*, *268*, 187–196.
 287 doi:10.1098/rspb.2000.1349

288 Konovalov, D. A. (2006). Accuracy of four heuristics for the full sibship reconstruction problem in the
 289 presence of genotype errors. *Proceedings of the 4th Asia-Pacific Bioinformatics Conference*, 7–
 290 16. doi:10.1142/9781860947292_0004

291 Kotrschal, A., Heckel, G., Bonfils, D., & Taborsky, B. (2012). Life-stage specific environments in a
 292 cichlid fish: Implications for inducible maternal effects. *Evolutionary Ecology*, *26*, 123–137.
 293 doi:10.1007/s10682-011-9495-5

294 Lee, W. J., & Kocher, T. D. (1996). Microsatellite DNA markers for genetic mapping in *Oreochromis*
 295 *niloticus*. *Journal of Fish Biology*, *49*, 169–171. doi:10.1111/j.1095-8649.1996.tb00014.x

296 Mehlis, M., Bakker, T. C. M., & Frommen, J. G. (2009). Nutritional benefits of filial cannibalism in
 297 three-spined sticklebacks (*Gasterosteus aculeatus*). *Naturwissenschaften*, *96*, 399–403.
 298 doi:10.1007/s00114-008-0485-6

299 Parker, A., & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake
 300 Malawi. *Environmental Biology of Fishes*, *47*, 345–352. doi:10.1007/BF00005049

301 Rubenstein, D. R., & Abbot, P. (2017). *Comparative social evolution*. Cambridge: Cambridge
 302 University Press.

303 Schliewen, U., Rassmann, K., Markmann, M., Markert, J., Kocher, T., & Tautz, D. (2001). Genetic and
 304 ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in

- 305 Lake Ejagham, Cameroon. *Molecular Ecology*, 10, 1471–1488. doi:10.1046/j.1365-
306 294X.2001.01276.x
- 307 Skutch, A. F. (1935). Helpers at the nest. *The Auk*, 52, 257–273. doi:10.2307/4077738
- 308 Solomon, N. G., & French, J. A. (1997). *Cooperative breeding in mammals*. Cambridge: Cambridge
309 University Press.
- 310 Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and
311 benefits. *Animal Behaviour*, 32, 1236–1252. doi:10.1016/S0003-3472(84)80241-9
- 312 Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with broodcare helpers: An experimental
313 analysis. *Behaviour*, 95, 45–75. doi:10.1163/156853985X00046
- 314 Taborsky, M. (1987). Cooperative behaviour in fish: Coalitions, kin groups and reciprocity. In Y. Ito, J.
315 L. Brown, & J. Kikkawa (Eds.), *Animal Societies: Theories and Facts* (pp. 229–237). Tokyo: Japan
316 Scientific Societies Press.
- 317 Taborsky, M. (1994). Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish
318 reproduction. *Advances in the Study of Behavior*, 23, 1–100. doi:10.1016/S0065-3454(08)60351-
319 4
- 320 Taborsky, M. (2016). Cichlid fishes: a model for the integrative study of social behavior. In W. D.
321 Koenig & J. L. Dickinson (Eds.), *Cooperative breeding* (pp. 272–293). Cambridge: Cambridge
322 University Press.
- 323 Taborsky, M., & Grantner, A. (1998). Behavioural time–energy budgets of cooperatively breeding
324 *Neolamprologus pulcher* (Pisces: Cichlidae). *Animal Behaviour*, 56, 1375–1382.
325 doi:10.1006/anbe.1998.0918
- 326 Taborsky, M., Frommen, J. G., & Riehl, C. (2016). Correlated pay-offs are key to cooperation.
327 *Philosophical Transactions of the Royal Society B*, 371, 20150084. doi:10.1098/rstb.2015.0084
- 328 Tanaka, H., Kohda, M., & Frommen, J. G. (2018a). Helpers increase the reproductive success of
329 breeders in the cooperatively breeding cichlid *Neolamprologus obscurus*. *Behavioral Ecology*
330 *and Sociobiology*, 72, 152. doi:10.1007/s00265-018-2566-7
- 331 Tanaka, H., Frommen, J. G., Engqvist, L., & Kohda, M. (2018b). Task-dependent workload adjustment
332 of female breeders in a cooperatively breeding fish. *Behavioral Ecology*, 29, 221–229.
333 doi:10.1093/beheco/arx149
- 334 Taylor, M. I., Meardon, F., Turner, G., Seehausen, O., Mrosso, H. D. J., & Rico, C. (2002).
335 Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid
336 fish: a *Pundamilia pundamilia* x *Pundamilia nyererei* hybrid. *Molecular Ecology Notes*, 2, 443–
337 445. doi:10.1046/j.1471-8286.2002.00272.x
- 338 Van Oppen, M. H., Rico, C., Deutsch, J. C., Turner, G. F., & Hewitt, G. M. (1997). Isolation and
339 characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular*
340 *Ecology*, 6, 387–388. doi:10.1046/j.1365-294X.1997.00188.x
- 341 von Siemens, M. (1990). Broodcare or egg cannibalism by parents and helpers in *Neolamprologus*
342 *brichardi* (Poll 1986) (Pisces: Cichlidae): a study on behavioural mechanisms. *Ethology*, 84, 60–

343 80. doi:10.1111/j.1439-0310.1990.tb00785.x

344 Zardoya, R., Vollmer, D. M., Craddock, C., Streebman, J. T., Karl, S., & Meyer, A. (1996). Evolutionary
345 conservation of microsatellite flanking regions and their use in resolving the phylogeny of
346 cichlid fishes (Pisces: Perciformes). *Proceedings of the Royal Society B*, 263, 1589–1598.
347 doi:10.1098/rspb.1996.0233

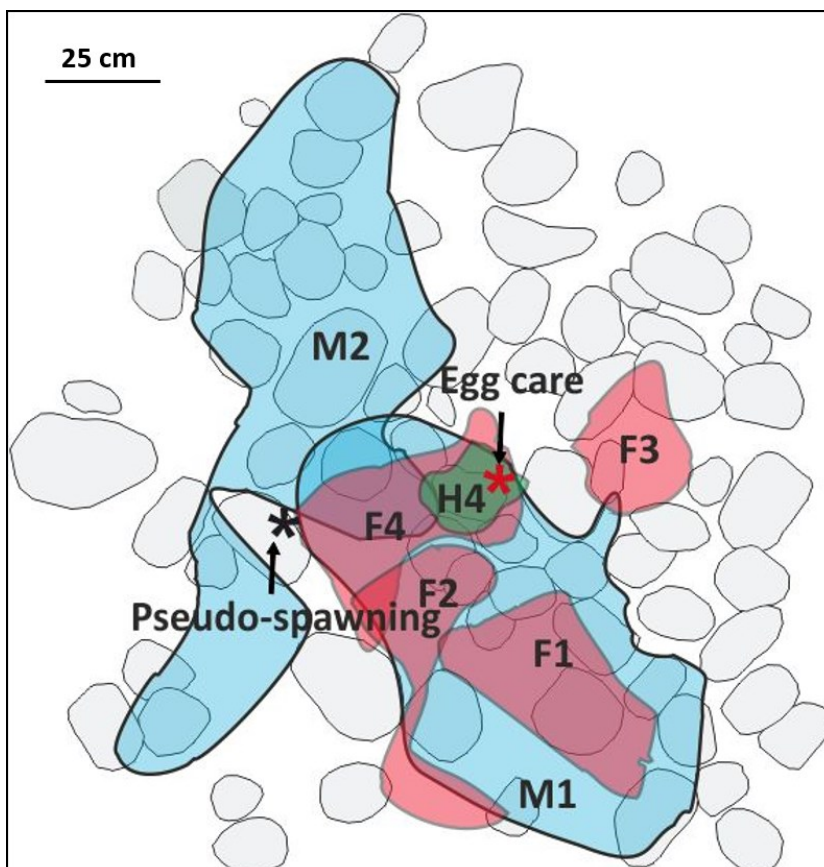
348 Zöttl, M., Heg, D., Chervet, N., & Taborsky, M. (2013). Kinship reduces alloparental care in
349 cooperative cichlids where helpers pay-to-stay. *Nature Communications*, 4, 1341.
350 doi:10.1038/ncomms2344

351

352 **Figure 1**

353 Home ranges of the fish observed in this study. Shown are territories of two neighbouring males (M1,
354 M2). M1 guarded four breeding females (F1 - F4) in his territory, and M2 monopolized three breeding
355 females (not indicated in the map). Female F4 had 1 male helper. The location of the egg deposition
356 site (red star) and the pseudo-spawning site (black star) are indicated. Grey structures indicate
357 individual rocks.

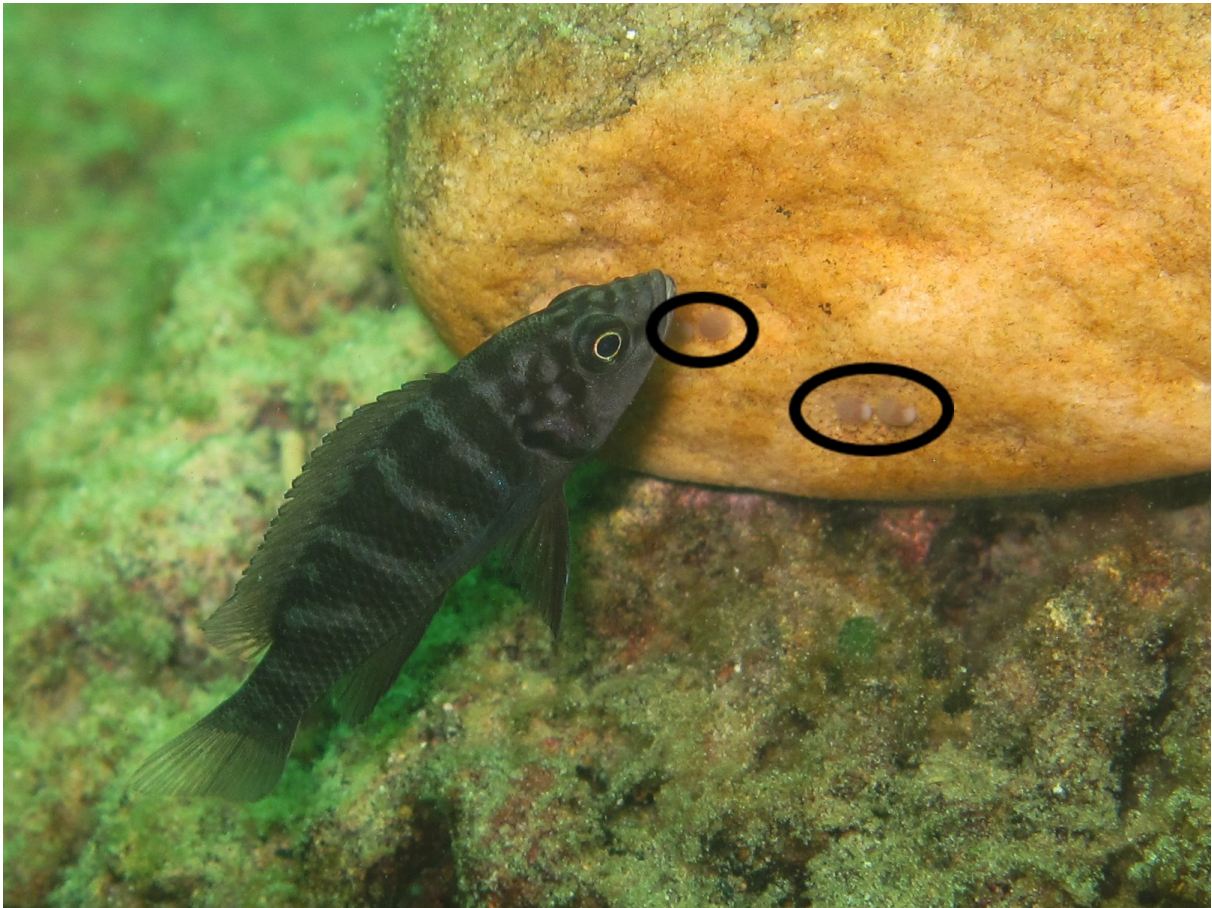
358



359

360 **Figure 2**

361 Four out of six eggs (two eggs per black circle) laid by the breeding female and inspected by the helper
362 (H4; 27 mm). The egg deposition site was on one of the stones used for marking the different
363 territories.



364