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Life-stage specific environments in a cichlid fish: Implications for inducible maternal effects

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Online appendix: - 1 Body size measurements of three fish species (including 1 Figure)
- 2 DNA extraction and microsatellite analysis (including 1 Table)

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Abstract

Through environmentally induced maternal effects females may fine-tune their offspring's phenotype to the conditions offspring will encounter after birth. If juvenile and adult ecologies differ, the conditions that mothers experienced as juveniles may better predict their offspring's environment than the adult females' ambient conditions. Maternal effects induced by the environment experienced by females during their early ontogeny should evolve when three ecological conditions are met: (i) Adult ecology does not predict the postnatal environmental conditions of offspring; (ii) Environmental conditions for juveniles are correlated across successive generations; and (iii) Juveniles occasionally settle in conditions that differ from the juvenile habitat of their mothers. By combining size-structured population counts, ecological surveys and a genetic analysis of population structure we provide evidence that all three conditions hold for *Simochromis pleurospilus*, a cichlid fish in which mothers adjust offspring quality to their own juvenile ecology. Adults of many species cannot predict their offspring's environment from ambient cues. Hence, we predict that life-stage specific maternal effects are common in animals. Therefore, it is important to incorporate parental ontogeny in the study of parental effects when juveniles and adults inhabit different environments.

Introduction

Environmentally induced parental effects represent a form of phenotypic plasticity spanning generations (Uller 2008) and appear to occur ubiquitously across all major taxa (Lacey et al 1998; Mousseau and Fox 1998; Räsänen et al 2008). Adaptive transgenerational plasticity is expected to be favoured when environments across generations are heterogeneous in space or time (Uller 2008). In the presence of reliable environmental cues, non-genetic maternal effects allow females to fine-tune the offspring's phenotype to the expected environmental conditions, which can confer fitness advantages for both generations (Galloway and Etterson 2007).

When maternal and offspring ecologies are correlated the environment can often provide reliable cues about the postnatal conditions offspring will experience (e.g. Galloway and Etterson 2007; Räsänen and Kruuk 2007; Badyaev 2009). In many species, however, juvenile and adult ecologies differ greatly because animals undergo ontogenetic shifts in feeding niche [reptiles (Clark and Gibbons 1969; Pough 1973; Ballinger et al 1977), fish (Werner and Gilliam 1984), annelids (Davies et al 1981), insects (Johannsson 1978; Amarillo-Suarez and Fox 2006), echinoderms (Town 1981), spiders (Turner 1979)]. Juveniles may occupy a different, often much narrower niche than adults in a common habitat [e.g. crustaceans (Lind and Welsh 1994; Dionne et al 2003)] or juveniles and adults may even be separated spatially [e.g. birds (Gillanders et al 2003), fish (Mumby et al 2004)]. In these cases, precise forecasting of offspring conditions from the ambient environment is often difficult or even impossible, whereas the conditions females experienced themselves in early life might predict their offspring's future environment quite reliably (Jonsson et al 1996; Rotem et al 2003; Taborsky 2006a). This situation can give rise to 'life-stage specific maternal effects', where

80 the environmental conditions experienced by females during a certain life stage (e.g. the early
81 juvenile stage) induce a maternal effect that affects the *same* life stage in the offspring
82 generation. To date, no study has: (a) Identified which ecological cues experienced by
83 females during their own early life are more reliable than cues experienced during
84 reproduction; or (b) Tested whether these conditions hold true in the natural habitat of
85 animals known to have life-stage specific maternal effects.
86
87 After detecting that cues perceived by females early in life can induce a maternal effect on
88 offspring performance early in life, Taborsky (2006a) proposed two necessary conditions for
89 the evolution of this life-stage specific maternal effect: (1) Ecologies across *different* life
90 stages must differ such that adults cannot predict their offspring's environment during early
91 ontogeny; and (2) Conditions for *juveniles* are correlated across successive generations. Here
92 we add a third condition, necessary for *plastic* offspring adjustment via maternal effects to
93 evolve: (3) Offspring must occasionally end up in a non-matching environment, for example,
94 due to moderate rates of dispersal to divergent habitats or because of temporal fluctuations.
95 This additional condition is important, because if the correlation between the early
96 environment of mothers and that of offspring were perfect, juveniles would always grow up
97 in the same habitat type as their mothers. In this case, a fixed egg size would perform equally
98 well as an environmentally induced anticipatory maternal effect to prepare offspring for post-
99 natal conditions.
100
101 In a laboratory experiment, females of the mouthbrooding cichlid *Simochromis pleurospilus*
102 were shown to adjust egg size to the experimental environment they experienced as juveniles,
103 and not those that they experienced during egg production. Irrespective of current food
104 availability, females reared under reduced access to food produced larger offspring (Taborsky

105 2006a). Further experiments suggest that this maternal effect prepares young for harsh post-
106 natal conditions, as larger *S. pleurospilus* young grew faster than smaller conspecific
107 competitors when food was scarce, whereas larger body size did not yield benefits when food
108 was abundant (Segers and Taborsky in revision). Thus, *S. pleurospilus* exhibits a life-stage
109 specific maternal effect and represents a suitable model to test whether the three ecological
110 conditions outlined above apply to this species.
111
112 Condition 1 demands that adults are not able to predict their offspring's post-natal
113 environment. This is relevant if adult and juvenile habitats differ or are spatially segregated.
114 To assess spatial segregation and differences in habitat we combined size-structured
115 population counts and habitat surveys of five neighbouring *S. pleurospilus* populations in
116 Lake Tanganyika. Condition 2 demands that juvenile environments are correlated across
117 successive generations. The natural habitat of *S. pleurospilus* is stable over time. Thus, a
118 correlation between the juvenile habitat of females and offspring is expected if juveniles of
119 successive generations of the same population grow up in the same area. We tested for this by
120 applying a population genetics approach predicting that genetic differentiation among
121 neighbouring populations should occur, but not genetic differentiation between adult and
122 juvenile habitats of the same population. Finally, condition 3 demands that occasional
123 mismatch between the juvenile environment of a mother and her offspring occurs, for
124 example, as a result of dispersal. To test for this, we applied a combined population genetics
125 approach with a habitat quality survey in five neighbouring populations along a continuous
126 20 km stretch of Lake Tanganyika shoreline. We investigated the genetic data for signals of
127 weak gene flow between neighbouring populations, and we tested for habitat quality
128 differences between neighbouring populations.
129

Material and Methods

Study species

S. pleurospilus is a maternally mouthbrooding cichlid of the tribe *Tropheini* endemic to Lake Tanganyika, East Africa. It inhabits the rocky shoreline between 0 to 12m depth (depending on study site; pers. obs.), where it feeds exclusively on epilithic turf algae. These algae constitute the sole food source of *S. pleurospilus* and their productivity declines exponentially with depth, differing by two orders of magnitude within the first 2 meters (Taborsky 1999). The observed reduction in algal productivity with depth correlates with a declining biomass of algae-grazing cichlids (B. Taborsky, unpub. data; this study). *S. pleurospilus* reproduces year-round, with adult males defending small, adjoining territories of 2-4 m^2 which females visit to spawn. Juveniles and females are non-territorial, but inhabit large home ranges (Kotrschal and Taborsky 2010a). After spawning, females leave the male territory immediately, and care for the clutch on their own. The young are independent after four weeks (Taborsky 2006b). The environmental conditions juveniles are exposed to after independence until they reach maturity are known to strongly influence their adult physiology (Kotrschal et al in press), behaviour (Kotrschal and Taborsky 2010b) and life history (Taborsky 2006a, b; Segers and Taborsky 2011; Segers and Taborsky in revision).

Study sites

Data were collected at the southern tip of Lake Tanganyika, Zambia; we surveyed 20 km of Lake Tanganyika coastline and found populations of *S. pleurospilus* at five sites (Figure 1) further referred to as: 'Mbete' (8°48`41.74``S, 31°02`08.56``E); 'Kasakalawe Point' (8°46`48.05``S, 31°04`58.60``E); 'Simo Paradise' (west of the town Mpulungu, 8°46`46.05``S, 31°05`47.48``E); 'Mpulungu' (near Fisheries Department, Mpulungu,

8°45`55.04``S, 31°06`10.56``E); and, 'Simo Bay' (at Nkumbula Island; 8°45`16.40``S, 31°5`28.81``E).

Population size structure along depth gradient

Previously, the size structure - depth gradient correlation was known for only one of the study populations (Kasakalawe Point, KP; Taborsky 2006a). To investigate whether the correlation applies to *S. pleurospilus* populations in general, we determined the size-frequency distribution in a second population (Simo Bay, SB), which differs most strongly from KP with regard to habitat and climate. In contrast to KP, SB is largely protected from waves (see Figure 1) and strongly affected by sedimentation, and consequently has reduced algae growth.

We conducted 100-m transect counts in parallel to the shore every 0.5 depth-meter between 0.5 and 12m depth. We repeated these transect counts three times during a 10 hour period 7.00-17.00), during daylight (which was from 6.00-18.00), on three different days, adding up to a total of 720 (3 x 10 x 24) 100-m transects. During each transect count, we dived approximately 1m above the lake bottom along the transect line and estimated the size (to the nearest 0.5 cm) of each male, female and juvenile *S. pleurospilus* occurring 2m to the left and to the right of the transect line. In the lab, *S. pleurospilus* females start to reproduce at a minimal size of 5.7 cm total length (TL) (Taborsky 2006 b). Therefore, we considered individuals <5.5 cm as 'juveniles' and individuals ≥5.5 cm as 'adults'. The transects were carried out by two observers (AK and MJ). To minimize bias both observers sampled all depths. Furthermore, when the observer was included as a factor in the statistical analyses, it was non-significant ($p>0.7$).

180 *Ecological parameters*

181 To measure habitat parameters, we conducted transects in parallel to the shore every 0.5m
182 between 0.5 and 3m depth except at Mbete and Simo Paradise, where the habitat for *S.*
183 *pleurospilus* extends only down to 1.5 and 2.5m, respectively (only sandy bottom below these
184 depths). We distinguished between 'shallow' (0.5m, 1.0m and 1.5m) and 'deep' (2.0m, 2.5m
185 and 3.0m) habitats. Since the sandy bottom began at 1.5m at Mbete, we numbered the 1.5m
186 transect among the 'deep' habitats.

187

188 *Algal cover*. At our study sites four distinct categories of substrate occurred: solid rock (the
189 lakebed consists of a flat surface of solid rock); stones (large pebbles and rounded rocks);
190 sand; and plants. We placed a weighted 2-m yardstick on the lakebed and measured the
191 distance d covered by the different classes of substrate. Starting each transect at the western
192 edge of a population and proceeding eastwards, we sampled the substrate at every second
193 2m-section and repeated this 10 times for each depth, yielding 60 samples per population
194 (except Mbete, for which there are only 30 samples). Data points of equal depth were entered
195 into the statistical analysis as independent values, since the sample sizes per depth are equal,
196 which allows for data pooling without biasing the results (Leger and Didrichsons 1994). We
197 calculated an index of algal cover by assuming that rocks have a flat surface and stones are
198 spherical. Since only the top half of a stone is exposed to sunlight and can hence be colonised
199 by turf algae we multiplied the distances covered by stones by $\frac{\pi}{2}$. For each 2m section we
200 calculated an index for algal cover as: $A = \frac{d_{rock} + d_{stones} \times \frac{\pi}{2}}{200}$ with $A \leq \frac{\pi}{2}$ cm. As the turf algae
201 used as food by *S. pleurospilus* only grow on hard surfaces, plants and sand are not included
202 in the equation. Note that A represents an index of the area available for feeding only and
203 does not incorporate depth-specific algal productivity (see 'Discussion').

Food competition. After determining algal cover, we rested motionless near the 2-m yardstick placed at the lake bottom for 5 min. During this period the fish habituated to the yardstick and to our presence and resumed feeding. We noted species, size (estimated by eye to the nearest 1.0 cm) and number of all food competitors (algae grazers) within 2m of both sides of the stick ($8m^2$). We converted length to mass using allometric relationships of all algae eating species, established during previous field studies (see Appendix 1. Finally, we converted total algae eater biomass into total metabolic rate MR_{total} (in $g \times m^{-2}$) by MR_{total} = body mass$^{0.79}$, which is the typical allometric relationship in teleost fish (Clarke and Johnston 1999). MR_{total} best reflects the amount of algae removed by food competitors.

Food availability. To obtain an estimate of food availability we built a model with log-transformed A as the dependent variable and study site and habitat (shallow or deep) as fixed factors. Because we wanted to test for potential differences in food availability, which must also take the presence of food competitors into account, we controlled for the effect of MR_{total} on A by including it as a covariate. We first tested for significant interactions between MR_{total} and the fixed factors in our model. All interaction terms with MR_{total} were non-significant ($p>0.3$) and were therefore excluded from our final model.

We log-transformed the data or used non-parametric statistics whenever the distributions violated the assumptions of parametric testing. All analyses were conducted in SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

DNA sampling and microsatellite analysis

228 In total, we collected 550 tissue samples from shallow (<1m) and deep (>1.5m depth) water
229 habitats. Sample sizes per population ranged between 85 and 146 individuals (Mbete: shallow
230 (s) 47, deep (d) 45; Kasakalawe Point: s 44, d 41; Simo Paradise: s 55, d 40, Mpulungu: s 83,
231 d 49, Simo Bay: s 54, d 92). Using hand nets each fish was driven slowly towards a fence net,
232 where the fishes fins were caught and it could be removed quickly and without damage.
233 Supported by local professional fish catchers, we managed to sample most individuals of a
234 population during a 2h session. However, in the case of very large populations, we obtained
235 only a representative subsample. Fish caught in shallow habitats were found to be mostly
236 juveniles, whereas those caught in deep habitats were exclusively adults. Fish of a specific
237 population were kept in two tanks of 200 litres separated according to habitat type (shallow
238 or deep) for up to 3 hours with frequent water exchanges. All fish survived and were released
239 after sampling. Fin clips were taken from the tip of the anal fin, and stored in 98% ethanol for
240 later processing (see Appendix 2 for details on sample collection and processing).
241
242 We used fourteen polymorphic microsatellite loci with protocols specifically adapted for *S.*
243 *pleurospilus* (loci NP007 (=UME002), NP773 (=US-758/773), NP781 (=US-781/784):
244 (Schliewen et al 2009); Pzeb2, Pzeb3, Pzeb4: (Van Oppen et al 1997); TmoM5, TmoM13,
245 TmoM25: (Zardoya et al 1996); UME003: (Parker and Kornfield 1996); UNH106, UNH130,
246 UNH154: (Lee and Kocher 1995) and UNH1009 (Carlton et al 2002); see Appendix 2 for
247 details on DNA extraction and microsatellite analysis).
248
249 *Statistical analyses of microsatellite data*
250 We computed estimates of genetic diversity including allelic richness and heterozygosity, as
251 well as tests for departure from Hardy-Weinberg proportions and linkage equilibrium for
252 each sampling locality and depth separately in Arlequin 3.1 (Excoffier and Laval 2005).

253 Overall and sample-specific inbreeding coefficients (F_{IS}) were used to assess *S. pleurospilus*
254 samples for internal kin structure or evidence of inbreeding (Schweizer et al 2007). The
255 nominal significance level of 0.05 was corrected with the sequential Bonferroni procedure
256 whenever applicable (Holm 1979).
257
258 The level of genetic differentiation among *S. pleurospilus* from different sampling depths and
259 localities was quantified separately by pairwise and overall F_{ST}-values (Weir and Cockerham
260 1984), and statistically tested with 10,000 permutations using Arlequin 3.1. Analyses of
261 molecular variance (AMOVA) (Excoffier et al 1992) were performed to assess the amount of
262 genetic variation explained by differences between the two sampling depths relative to
263 differences between sampling localities. Furthermore, we used Mantel tests (Smouse et al
264 1986) implemented in Arlequin 3.1 to test the relationship between spatial and genetic
265 distances (F_{ST}) among sites as expected under isolation by distance. We tested both the
266 logarithm of Euclidean distances and the logarithm of distances along the coastline against
267 F_{ST}. A linear relationship between F_{ST} and the logarithm of distance is expected under short-
268 distance dispersal among neighbouring populations (Rousset 1997).
269
270 *Ethical note*
271 Animal care procedures during genetic sampling are in accordance with the 'Memorandum of
272 Understanding' between the Fisheries Department of the Ministry of Agriculture and
273 Cooperatives, Mpulungu, Zambia, and the Universities of Lusaka (Zambia), Bern and Basel
274 (Switzerland) and Graz (Austria). We adhered to the "Guidelines for the treatment of animals
275 in behavioural research and teaching" published in 'Animal Behaviour' 2006, 71, 245-253.
276
277 Results

278

279 *(i) Differences between juvenile and adult habitats*

280 *Spatial segregation.* At Simo Bay the depth distributions of juveniles and adults differed
281 significantly (Chi-square test, $\chi^2_{24} = 66.66$, $p<0.001$). Juveniles occurred exclusively at
282 depths ≤ 1.5m, whereas adults were found regularly at all depths between 1.0 and 10.0m
283 (Figure 2). While this pattern resembles the size-structure at 'Kasakalawe Point' (Taborsky
284 2006a), adults inhabit a much broader depth range at Simo Bay.

285

286 *Habitat differences.* Algal cover (controlled for the effect of food competition) was higher in
287 shallow than in deep habitats (Table 1; Figure 3). Of note, the variances of algal cover and
288 food competition were greater in deep habitats (Levene's test for equality of variances: algal
289 cover, F=51.9; food competitor metabolic requirement F=42.3; both N=130 and $p<0.001$).

290

291 *(ii) Genetic differentiation within and among populations*

292 Genetic data from 550 individuals revealed very high levels of genetic diversity at the 14
293 microsatellite loci analyzed. The number of alleles per locus ranged from four for locus
294 Pzeb3 to 43 for locus TmoM5 with a mean value of 25.2. The observed heterozygosity per
295 locus and sampling locality ranged from 0.4 to 1 with a mean value of 0.84 over all sampling
296 localities. After sequential Bonferroni correction, significant deviations from Hardy-
297 Weinberg equilibrium were detected for 6/140 tests per locus and sampling locality. Locus
298 UNH130 was affected three times, locus UNH1009 twice and locus NP-007 once, but this
299 low number of significant tests suggests no overall departure from Hardy-Weinberg
300 equilibrium. Tests for linkage disequilibrium provided no evidence for physical linkage
301 among loci in the data set.

302

303 Estimates of the inbreeding coefficient F_{IS} per *S. pleurospilus* sample from different sampling
304 depths and localities provided no evidence for extensive kin substructure or inbreeding.
305 Neither the overall F_{IS}-value of 0.004 ($p > 0.2$) nor sample-specific F_{IS}-values (range: -0.016
306 to 0.029; $p > 0.05$) were significantly different from zero.
307
308 Genetic differentiation between *S. pleurospilus* sampling localities and sampling depths was
309 relatively low at this very fine geographical scale with an overall F_{ST}-value of 0.0158 ($p <$
310 0.0001). There was no evidence for genetic structure between sampling depths within
311 sampling localities. An AMOVA with each sampling locality defined as its own group
312 consisting of deep and shallow samples revealed significant variation between sampling
313 locality ($F_{CT} = 0.0149$; $p < 0.0001$) but not between deep and shallow samples ($F_{SC} = 0.0008$;
314 $p = 0.112$). Pairwise F_{ST}-values between deep and shallow samples from a locality ranged
315 between 0.0006 and 0.0019 and were all not significantly different from zero (all $p \geq 0.11$).
316
317 *S. pleurospilus* from the five sampling locations were significantly genetically structured with
318 an overall F_{ST}-value of 0.0154 ($p < 0.0001$). Pairwise genetic distances between sampling
319 locations ranged from 0.0011 for Kasakalawe Point - Simo Paradise (the only non-significant
320 comparison; $p = 0.081$) to 0.030 (Simo Bay - Mbete; $p < 0.0001$; Table 2).
321
322 Mantel tests provided no evidence for a dependence of genetic structure among *S.*
323 *pleurospilus* populations with either Euclidean distances between sampling localities ($r =$
324 0.13, $p \geq 0.2$) or distances measured along the shoreline ($r = 0.45$, $p \geq 0.2$).
325
326 *(iii) Heterogeneity of juvenile habitats*

Food availability (algae cover controlled for competitor metabolic requirement) differed greatly across the shallow habitats (ANCOVA, $F = 4.222$, $df = 4$, $N = 70$, $p = 0.006$, Figure 3) and pairwise comparisons between study sites (based on estimated marginal means) revealed significant differences between most sites (Table 3). Moreover, study sites differed distinctly in several other features including shore orientation and wave impact, sedimentation, plant coverage and turbidity (A. Kotrschal, pers. obs.).

Discussion

In *Simochromis pleurospilus* egg size is influenced by cues mothers are exposed to during their juvenile period (Taborsky 2006a). We propose three general conditions that should favour the evolution of such a life-stage specific maternal effect: (1) Juveniles and adults inhabit different ecological niches that do not allow females to predict their offspring's juvenile environment; (2) Juveniles usually grow up under similar conditions as their parents, allowing females to obtain cues that predict the juvenile conditions of their future offspring; (3) Occasionally juveniles mature in different habitats to their mothers', and hence a flexible strategy to determine egg size should be favoured. As many species occupy different ecological niches during different ontogenetic stages, often living even in spatial separation, life-stage specific maternal adjustment of offspring phenotype is likely to be common among animals. In the following sections we discuss our results in light of the three proposed conditions.

(i) Differences between juvenile and adult habitats. We found that juvenile and adult *S. pleurospilus* inhabit different water depths with only a limited overlap. As observed also in other algae grazing species (Power 1984) the shallow areas were predominantly used by

352 smaller, juvenile fish, whereas larger fish inhabited deeper areas. As this pattern is similar at
353 Kasakalawe Point (Taborsky 2006a) and at Simo Bay (this study), two qualitatively and
354 quantitatively distinct habitats, we conclude that this pattern is typical for *S. pleurospilus*. In
355 shallow depths algal cover controlled food competitor metabolic requirements were higher in
356 all but one population. Shallow areas provide better feeding grounds than deep areas because
357 a larger surface area of rocks and stone overgrown by algae is present, relative to the
358 metabolic requirements of algae eaters. In addition, deeper habitats provide worse grazing
359 grounds due to the exponential decline of sunlight with increasing depth, which corresponds
360 to an exponential decline in algae productivity with depth (Taborsky 1999). Hence shallow
361 and deep habitats usually differ with respect to the quality of feeding opportunities (cf. Figure
362 3). Most likely the disparate distribution of juvenile and adult *S. pleurospilus* across the depth
363 gradient is not solely explained by differences in feeding opportunities, but also by size-
364 dependent predation risk. Wading and diving birds, which do not prey upon fish under a
365 certain size threshold, hunt more effectively in shallow water or near the surface (Whitfield
366 and Blaber 1979; Kramer et al 1983) whereas piscivorous fish that predate on juveniles of all
367 sizes increase in number and size with increasing depth (Ruiz et al 1993). Additionally, it
368 may be risky for large fish to forage too close to the waterline because of the possibility of
369 becoming beached, while small fish may use minute quantities of interstitial water to return
370 to safer depths. In support of this consideration is the observation that the shoreline of Lake
371 Tanganyika is frequently and heavily exposed to waves.
372
373 Overall, we can conclude that juvenile and adult habitats of *S. pleurospilus* differ with respect
374 to several important ecological parameters. Here we compared only depths above 1.5m with
375 depths between 2.0 and 3.0m. Adult *S. pleurospilus* use a much broader depth range than
376 sampled by us, spanning over 9 or more meters in depth. The differences between shallow

377 waters and a habitat at 10 m depth are likely to be even more pronounced. The larger variance
378 in ecological parameters in the adult compared to the juvenile habitat indicates that adults
379 occupy a much wider niche than juveniles. Females that solely rely on cues in their current
380 (adult) habitat during egg production should therefore not be able to precisely predict juvenile
381 conditions.
382
383 *(ii) Correlation between juvenile environments across generations.* Life-stage specific
384 maternal effects can occur when successive generations of juveniles usually grow up under
385 similar ecological conditions. In the case of *S. pleurospilus*, where adults and juveniles occur
386 at different depths, this can be checked by investigating genetic structure between depths of
387 the same population and between fish of neighbouring populations. Our microsatellite
388 analyses revealed that in all populations occurring along a 20-km stretch of shoreline
389 shallow-water and deeper-water fish of the same study site were not genetically
390 differentiated. In contrast, neighbouring populations were genetically differentiated as
391 indicated by F_{ST} values significantly deviating from zero, except for the two closest
392 populations (p=0.081). Kasakalawe Point and Simo Paradise are only 1100 m apart and there
393 is no obvious dispersal barrier for *S. pleurospilus* (sand, mud, or solid rock) between them,
394 making gene flow between these populations more likely.
395
396 The observed pattern of genetic differentiation between populations despite the very fine-
397 grained, local scale of this study suggests that *S. pleurospilus* is mostly philopatric and gene
398 flow rarely occurs over large distances. This observation differs clearly for the much larger
399 congener, *S. diagramma,* which readily covers larger distances (Wagner and McCune 2009).
400 Dedicated analyses including more populations covering larger geographical scales and
401 several points in time will be necessary to provide a better understanding of dispersal patterns

and frequency in *S. pleurospilus* (e.g. Hamilton et al 2005; Heckel et al 2005; Schweizer et al 2007). This will also allow further assessment of the effects of distance and habitat structure on dispersal properties more specifically, as our analyses of isolation by distance do not detect significant patterns at this spatial scale.

(iii) Occasional mismatch between maternal and offspring juvenile habitats. S. pleurospilus occurred only along certain stretches of the lake's rocky shore, in discrete populations, separated by unoccupied habitat. Although we detected significant genetic differentiation between neighbouring populations, it is remarkable that observed F_{ST} values are low. The high levels of genetic polymorphism present within populations and relatively low levels of genetic differentiation between our five study populations are compatible with relatively large local populations with occasional dispersal between them. Occasional dispersal is crucial for a flexible egg size to be advantageous over a fixed egg size, but only if dispersing fish may encounter habitats different from their natal population. In the case of our study populations the latter is likely. Juvenile habitats of neighbouring populations differed significantly in algal cover controlled for food competitor metabolic requirement and in several other qualitative features. Remarkably, there was no gradual change of ecological parameters along the shore. Most pairwise comparisons between neighbouring populations revealed significant differences in the index of food availability. Hence, whenever fish disperse they are likely to encounter variable conditions, and so the ability of females to fine-tune offspring phenotype may therefore confer an advantage.

Conclusions

Maternal effects, by which females adjust offspring phenotype to current environmental conditions, are widespread among fish (Mousseau and Fox 1998; Einum and Fleming 1999;

427 Bashey 2006). However, the critical influence of environment during one life stage on
428 shaping a maternal effect acting on the same life stage of the following generation has only
429 been shown by one experiment so far. In a laboratory study, the food available to juvenile *S.*
430 *pleurospilus* females determined the size of eggs produced later in life (Taborsky 2006a).
431 Females that mature with experimentally manipulated high food availability produced smaller
432 eggs than females growing up in low-food conditions. Here we investigated under which
433 conditions life-stage specific maternal effects induced during the juvenile period of mothers
434 may evolve.
435
436 We demostrate that for a given study site, *S. pleurospilus* adults from deeper water and
437 juveniles from shallow water belong to the same population, and that dispersal between
438 populations differing in habitat quality occurs to a limited degree. Without dispersal between
439 populations natural selection should favour genetically determined local adaptation of egg
440 size (Kinnison et al 2001). Under the reported conditions of limited dispersal, however,
441 selection should favour the evolution of flexible egg size adjustment induced by the
442 conditions that mothers encountered as juveniles (life-stage specific cues).
443
444 In our study species, adult and juvenile ecologies differ only at a fine scale, and their habitats
445 partially overlap. Life-stage specific maternal effects should be even more important when
446 juveniles and adults have entirely different ecologies like in many metamorphosing animals
447 or anadromous fishes. There is evidence that such species do indeed adjust offspring
448 phenotype to their juvenile environment (Jonsson et al 1996; Rotem et al 2003; Amarillo-
449 Suarez and Fox 2006). In salmonids, this can be beneficial as these fish occasionally end up
450 in spawning habitats that differ substantially from their natal sites, despite high spawning site
451 fidelity (reviewed in Quinn 1993). Also, in amphibians an occasional mismatch of early

452 maternal and offspring environment is possible if females fail to return exactly to their own
453 raising ponds (reviewed in Smith and Green 2005), since these can vary greatly in their
454 climatic and ecological conditions even on small spatial scales (Van Buskirk and Arioli 2005;
455 Räsänen et al 2008). Even for species with pelagic larvae previously believed to have no
456 opportunity to obtain information on their offspring's postnatal environment, evidence of a
457 certain level of philopatry is accumulating (Gerlach et al 2007). Hence, the early maternal
458 environment may well predict the conditions experienced shortly after settlement, as many
459 juveniles of reef fish grow up in nursery grounds entirely disparate from the adult habitat,
460 such as mangroves or sea grass beds (Heck et al 2003; Mumby et al 2004).
461
462 In conclusion, we outline the conditions when maternal adjustment of offspring phenotype to
463 life-stage specific environmental differences should be beneficial. We furthermore show that
464 these conditions are fulfilled in the natural environment of a species where females do adjust
465 the offspring phenotype to their own early environment. Females thus possess a mechanism
466 that allows them, via maternal effects, to match offspring phenotype to the postnatal
467 environment, even if they are unable to obtain information about the environment that future
468 offspring will experience while producing eggs. This strongly suggests that the previously
469 detected ability of *S. pleurospilus* females to adjust offspring size according to their own
470 juvenile conditions represents a beneficial maternal effect in the natural environment of this
471 species. As in several animal species, including *S. pleurospilus,* larger offspring have survival
472 advantages under adverse growth conditions, while smaller young do equally well under
473 benign conditions (Hutchings 1991; Mousseau and Fox 1998; Einum and Fleming 1999).
474 This fine-tuning of offspring is likely to be adaptive. Finally we propose that life-stage
475 specific maternal effects should be common in animals and it therefore will often be

476 inevitable to incorporate parental ontogeny when aiming to understand the evolution of
477 parental effects.
478
479
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481
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490
491
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line figure1
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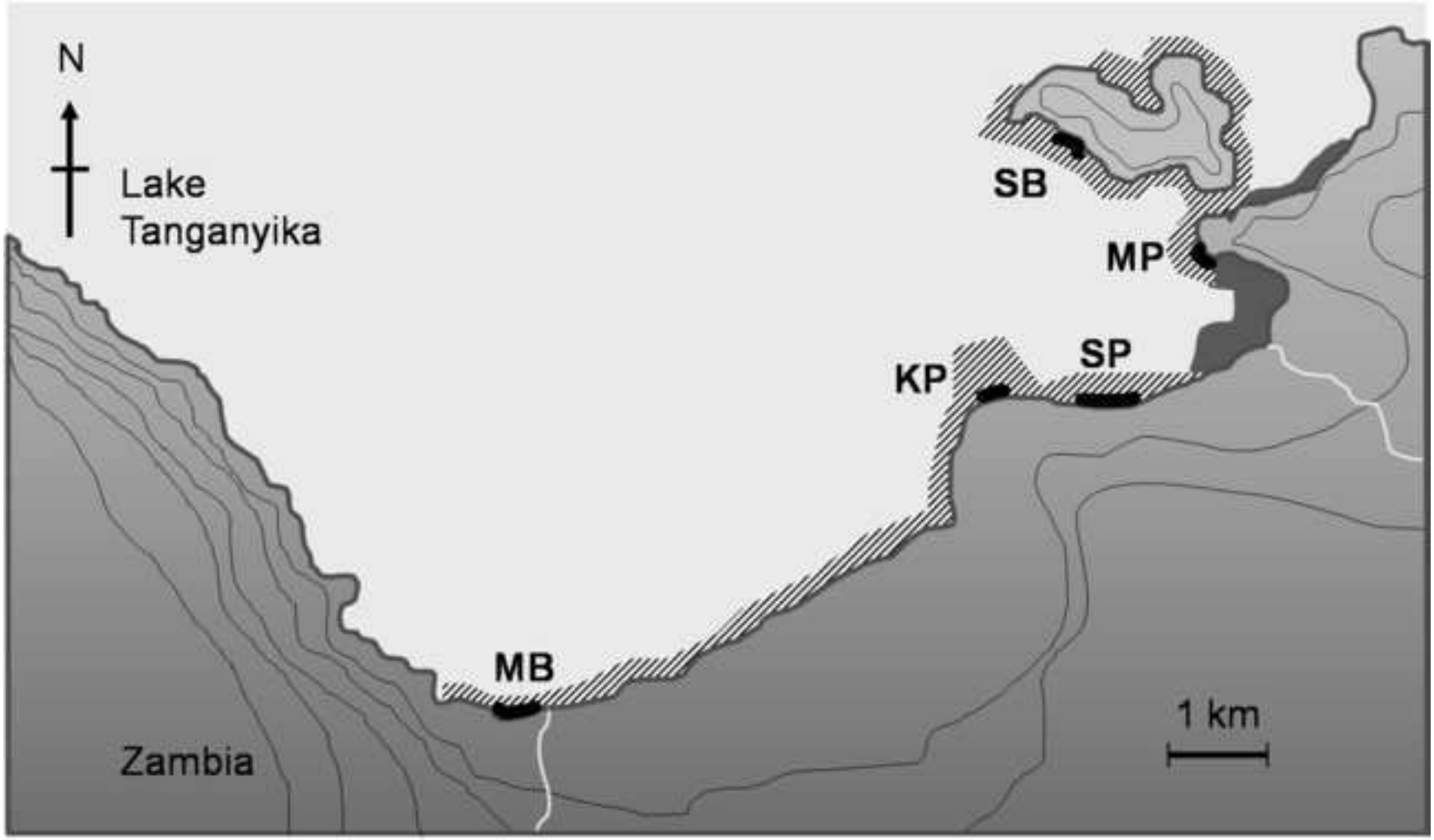

area surveyed for occurrence of *S. pleurospilus*

sampling sites = all populations of *S. pleurospilus*

unsuitably habitat for *S. pleurospilus* (mud)

colour figure2
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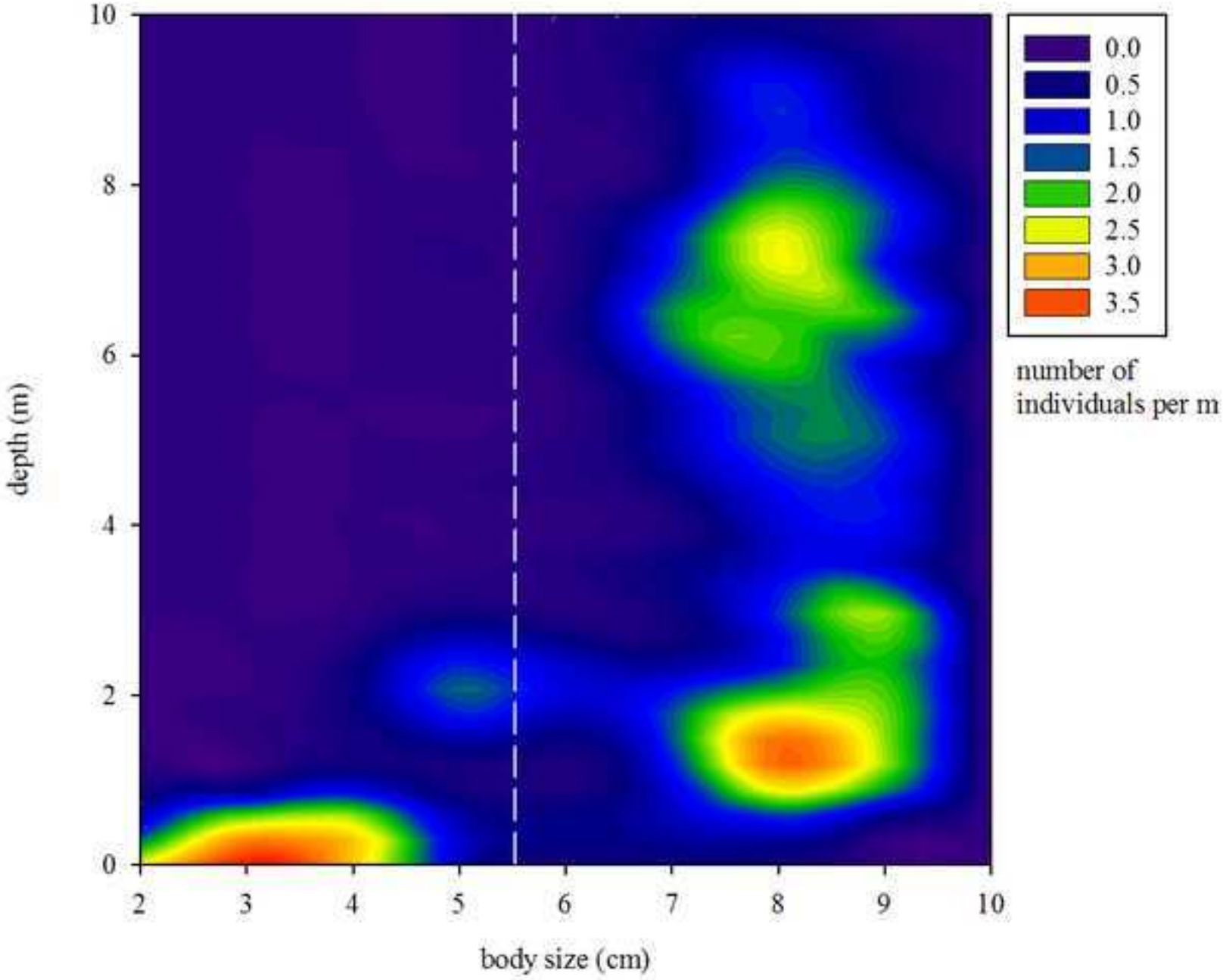

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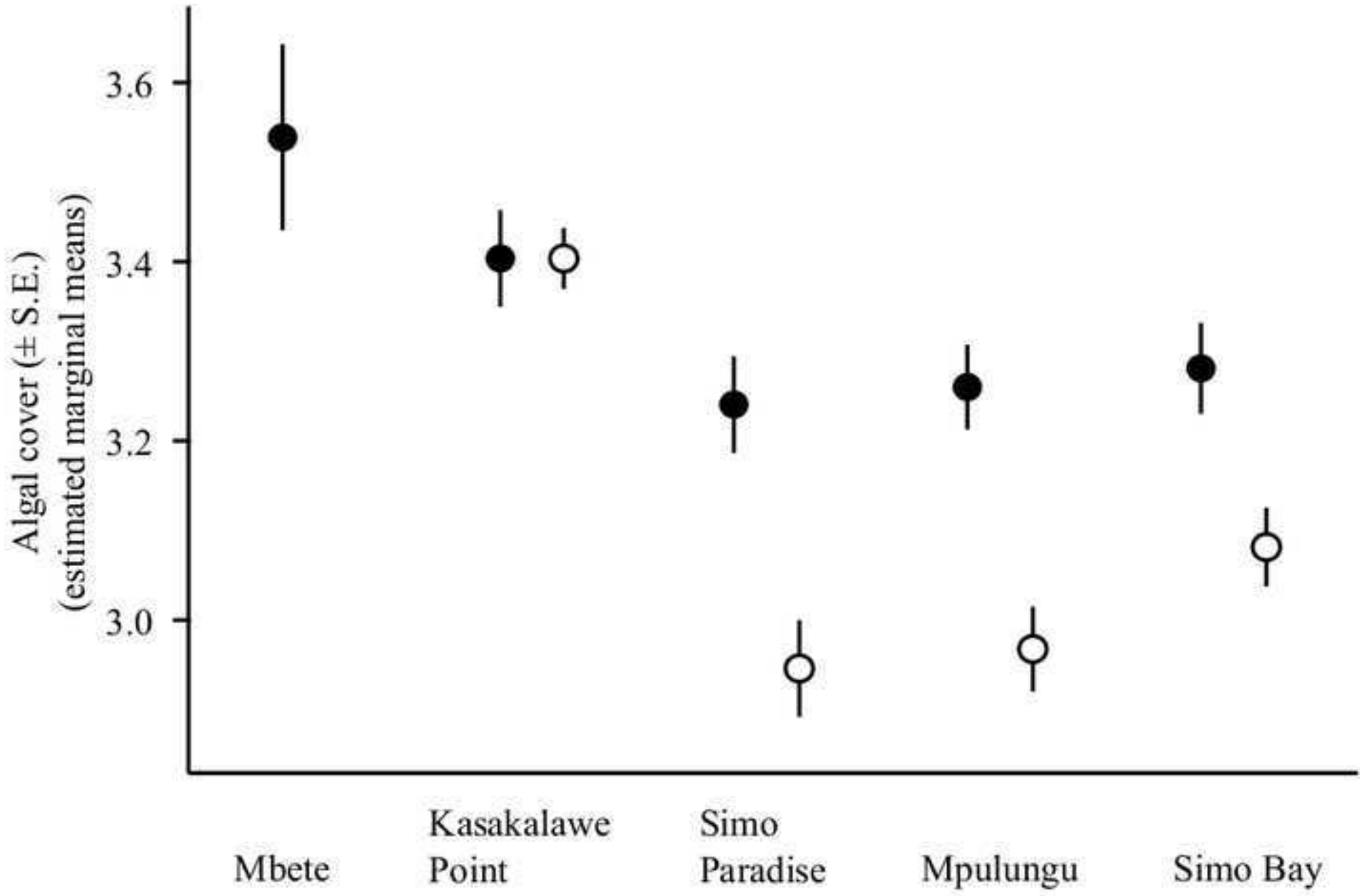


Figure 1: Study sites at the southern tip of Lake Tanganyika, Zambia. Shaded areas indicate the area surveyed for the occurrence of *Simochromis pleurospilus*, thick black lines mark the range along the shoreline inhabited by the five study populations and dark grey areas indicate habitats that are *a priori* unsuitable for *S. pleurospilus*. MB, Mbete; KP, Kasakalawe Point; SP, Simo Paradise; MU, Mpulungu; SB, Simo Bay.

Figure 2: Size-depth distribution of *Simochromis pleurospilus.* Colours indicate frequencies of fish recorded for each size class and depth (mean of 30 surveys). Dashed line indicates size at maturity.

Figure 3: Potential food availability in juvenile and adult habitats of *Simochromis pleurospilus*. Filled and empty circles represent the estimated marginal means of shallow and deep habitats respectively (± S.E.). Values are derived from a model in which we used the surface area as dependent variable, study site and habitat (shallow or deep) as fixed factors and metabolic requirement of food competitors as a covariate (see main text). No data for deep habitat in Mbete (see main text).

Table 1: Potential food availability (algae cover, controlled for the effect of food competition) compared between water depths study sites.

		Food availability	
	df	F	Sig.
Corrected Model	8	16.511	<0.001
Water depth	1	26.761	<0.001
Study site	4	10.589	<0.001
depth * site	3	4.747	0.004
Competitors MR_{total}		0.664	0.417

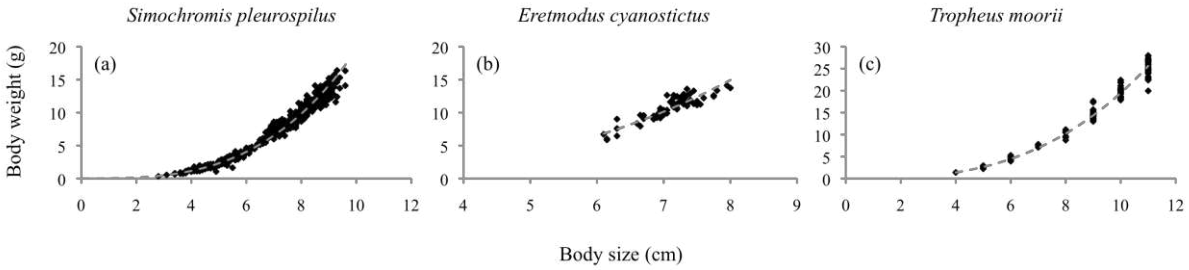
Table 2: Pairwise F_{ST}-values between different sampling localities of *Simochromis pleurospilus*. All comparisons were significantly different from zero ($p<0.002$, highlighted in bold) except for fish from the geographically closest sites Simo Paradise and Kasakalawe Point ($p=0.081$).

	Mpulungu	Simo Paradise	Simo Bay	Mbete
Simo Paradise	**0.0077**			
Simo Bay	**0.0193**	**0.0219**		
Mbete	**0.0135**	**0.0047**	**0.0301**	
Kasakalawe Point	**0.0089**	0.0011	**0.0264**	**0.0030**

Table 3: Pairwise comparison of food availability in shallow habitats of five study populations. MB, Mbete; KP, Kasakalawe Point; SP, Simo Paradise; MU, Mpulungu; SB, Simo Bay. Mean difference between study sites (mean Δ) and p-values (based on estimated marginal means) obtained from a general linear model with algae surface area as dependent variable, study site as factor and metabolic requirement of food competitors as covariate; significant p-values are highlighted in bold.

	MB		KP		SP		MU	
	mean Δ	p	mean Δ	p	mean Δ	p	mean Δ	p
KP	0.166	**0.036**						
SP	-0.341	**0.001**	-0.175	**0.003**				
MU	-0.319	**0.001**	-0.153	**0.009**	0.021	0.685		
SB	-0.307	**0.005**	-0.140	**0.030**	0.034	0.529	0.013	0.814

Appendix 1 – Body size and mass allometry of three cichlid species



Relationship of body size and mass of *Simochromis pleurospilus, Eretmodus cyanostictus and Tropheus moorii*. Data for *S. pleurospilus* were obtained when taking the fin clips during this study, and for *E. cyanostictus* and *T. moorii* during previous field projects (Taborsky 1999; Taborsky et al. 2009); body size is measured as total length (*TL*), from the tip of the snout to the end of the tail fin; body mass (*M*) as live wet weight). (a) *S. pleurospilus*: $W = 0.0198 \times TL^{2.97}$; (b) *Eretmodus cyanostictus*: $W = 0.036 \times TL^{2.89}$; (c) *Tropheus moorii*: $W = 0.035 \times TL^{2.75}$; *Petrochromis sp.*: no data available for this species; we used the equation for *S. pleurospilus*, as these species have a similar body shape.

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Appendix 2: Sample collection, DNA extraction and microsatellite analysis

Sample collection. We took fin clips from the tip of the anal fin (1-2 mm^2) with sharp dissection scissors and kept them in 98% ethanol for later processing. All instruments were daily cleaned and rinsed in 98% ethanol to avoid cross contamination between sites. Unnoticed cross contamination between samples is highly unlikely as the ratio of sample DNA to potential contamination DNA is very high. Due to the extremely variable microsatellites we should expect to find evidence of tri- or tetraploidy, if contamination had occurred. As part of our analysis protocol, we screened for these artefacts, and there was not a single one detected in our samples.

DNA extraction. Genomic DNA was extracted from ethanol-preserved fin clip samples using a manual 96 well format DNA extraction protocol on the basis of a magnetic separation technique: Tissue lysis was done in a Lysis-Buffer containing Nuclei Lysis Solution (Promega), 0.5M EDTA and Proteinase K according to the Wizard Genomic DNA Isolation Protocol (Technical Manual No. TM050, Promega). DNA was captured in solution by adding Paramagnetic Particles (MagneSil Blue, Promega) to the lysate and washed 2-3 times with 80% ethanol with the aid of a magnetic separator (MagnaBot®96 Magnetic Separation Device, Promega, Cat.# V8151) to eliminate residual contaminants. Finally, genomic DNA was eluted directly from the Paramagnetic Particles with 50-100µl of Nuclease Free Water.

Microsatellite analysis. For PCR amplification, all 14 microsatellite primer pairs (table A1) were multiplexed in one PCR reaction using the QIAGEN Multiplex PCR Kit (Qiagen). PCR reactions were carried out in 10µl volume containing 1µl of the genomic DNA, 1x QIAGEN Multiplex PCR Master Mix (consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM $MgCl_2$, dNTP mix, and HotStarTaq DNA polymerase), 0.1 µM of locus-specific 5' fluorescent labeled forward primer [fluorescent dyes: 6-FAM, HEX

(Microsynth), VIC, NED and PET (Applied Biosystems)], and non labelled reverse primer. Twelve reverse primers (NP007, NP773, UNH106, UNH130, ULI2, UME003, Pzeb2, Pzeb3, Pzeb4, TmoM5, TmoM13, TmoM25) were additionally modified by placing the nucleotide sequence GTTTCTT on the 5' end. This reverse-primer tailing results in nearly 100% adenylation of the 3' end of the forward strands, thereby facilitating accurate genotyping as a result of consistent allele calls. Amplification was achieved in a 96-well GeneAmp® PCR System 9700 (Applied Biosystems) by using the following cycling protocol: 15 min at 95°C; 35 cycles consisting of 30 sec at 94°C, 3 min at 57°C and 1 min at 72°C, followed by a final 15 min extension at 72°C. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI PRISM® 3100 Genetic Analyzer.

Genotypes were scored with the Genemapper software version 3.7 (Applied Biosystems) against an internal size standard (LIZ, Applied Biosystems). Automatic scoring was checked and revised manually to ensure consistency of genotyping (Schweizer et al. 2007).

Reference

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Table A1. Sequences of 14 primers for *S. pleurospilus* (in 5'-3' direction)

Locus	Sequences of primers
NP-007 F	TCA GAG TGC AAT GAG ACA TGA
NP-007-PT R	GTT TCT TAA TTT AGA AGC AGA AAA TTA GAC G
NP-773 F	ATC AGC ACG TCA TCT GCA TGA G
NP-773-PT R	GTT TCT TGC AAA GCA AAG CTG AGA AAC AA
NP-781 F	GAG CGA AAC CTG AAC AGA ATA C
NP-784 R	AGA GCC TGC TGG GGA CAA GAG T
Pzeb2 F	TTCGGTAGACTGATGCTTTCATA
Pzeb2-PT R	GTT TCT TAA AGC CAA AGG GTG TGA ACT GA
Pzeb3 F	GAG CCT GCA AAC CTT ACT GTA AA
Pzeb3-PT R	GTT TCT TAA GCT ACA CAA ATT CCA CTC ATA
Pzeb4 F	GCT TGT TTT GGG TTG GTT TTG T
Pzeb4-PT R	GTT TCT TAT GGA CAC GTG GAC TCA AAG AC
TmoM5 F	GCT CAA TAT TCT CAG CTG ACG CA
TmoM5-PT R	GTT TCT TAG AAC AGC GCT GGC TAT GAA AAG GT
TmoM13 F	CGC AGG GTG TTC TTC AGG TGT AT
TmoM13-PT R	GTT TCT TAA ATC ACC ATA TTC ATA TGT T
TmoM25 F	CTG CAG TGG CAC ATC AAG AAT GAG CAG CGG T
TmoM25-PT R	GTT TCT TCA AGA ACC TTT CAA GTC ATT TTG
UME003 F	GCC ACA TGT AAT CAT CTA ACT GC
UME003-PT R	GTT TCT TGA GAT TTT TTT TGG TTC CGT TG
UNH106 F	CCT TCA GCA TCC GTA TAT
UNH106-PT R	GTT TCT TGT CTC TTT CTC TCT GTC ACA AG
UNH130 F	AGG AAG AAT AGC ATG TAG CAA GTA
UNH130-PT R	GTT TCT TGT GTG ATA AAT AAA GAG GCA GAA A
UNH154 F	ACG GAA ACA GAA GTT ACT T
UNH154 R	TTC CTA CTT GTC CAC CT
UNH1009 F	CCATCTGCATGCTGTAAGACA
UNH1009-PT R	GTT TCT TTC CCA TTT GTC AGG TTC AGG