Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): can assortative shoaling drive population divergence?

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Abstract

Prior studies have shown that perch (*Perca fluviatilis* L.) of Lake Constance belong to two genetically different but sympatric populations, and that local aggregations of juveniles and adults contain closely related kin. In this study we analysed the genetic structure of pelagic perch larvae to investigate, if kin structured shoals already exist during early ontogenetic development or might be the result of homing to natal sites. Analysis of the gene frequencies at five microsatellite loci revealed that 3 out of 5 pelagic aggregations of larvae showed significant accumulation of kin. To investigate possible mechanisms of shoal formation, we tested if perch use olfactory cues to recognize their kin. Choice tests in a fluviarium showed preference for odours of unfamiliar kin versus unfamiliar non-kin. Additionally, we showed that perch could differentiate between the odours of the two sympatric populations and significantly preferred unfamiliar and unrelated conspecifics of their own over the foreign population. Our results present a behavioural mechanism that could lead to the observed formation of kin structured shoals in perch. We further discuss if the ability to discriminate between the own and the foreign population could result in assortative mating within populations and thus form the basis of "socially mediated speciation" in perch.

Key words: kin recognition, population recognition, kin structure, microsatellites, relatedness

Introduction

The formation of fish shoals is a very common phenomenon and can be found throughout numerous freshwater and marine species. These shoals are often strongly assorted by species, by body size and/or colour (reviewed in Krause et al. 2000 a,b). Additionally a preference to shoal with familiar conspecifics has been shown for a variety of freshwater fish like guppies, *Poecilia reticulata*, (Griffiths and Magurran 1999), bluegill sunfish, *Lepomis macrochirus,* (Dugatkin and Wilson 1992) threespine sticklebacks, *Gasterosteus aculeatus,* (Van Havre and Fitzgerald 1988; Barber and Ruxton 2000) and brown trout, *Salmo trutta,* (Höjesjö et al. 1998). But only very little is known about the relationship of shoaling and the genetic structure of natural fish populations. Shoaling with kin can be advantageous since closely related animals are more likely to show co-operative behaviour during risky situations such as predator inspection (Milinski 1987) because helping a relative can increase the indirect fitness of an individual (Hamilton 1964). Several authors studied relatedness between shoal members using allozymes (Ferguson and Noakes 1981; Avise and Shapiro 1986; Dowling and Moore 1986; Naish et al. 1993; Hauser et al. 1998; Peuhkuri and Seppae 1998) or microsatellite markers (Pouyaud et al. 1999). The results have been very ambiguous, no indication for kin structured shoals has been found for *Anthias squamipinnis* (Avise and Shapiro 1986), common shiner, *Notropis cournutus,* (Dowling and Moore 1986), European minnow, *Phoxinus phoxinus,* (Naish et al. 1993), Tanganyikan sardine *Limnonthrissa miodon,* (Hauser et al. 1998) and threespine stickleback (Peuhkuri and Seppae 1998), while Ferguson and Noakes (1981) indicated that kin structure in the common shiner might exist. Only Pouyaud et al. (1999) found significant association of kin in shoals of the mouthbrooding tilapia, *Sarotherodon melanotheron*.

A preference to shoal with kin would imply some kind of kin recognition mechanism that allows for the differentiation of related conspecifics from non kin. There are two main types

of kin recognition, direct familiarity and indirect familiarity (phenotype matching). In the case of direct familiarity, individuals become familiar with members of the same litter or clutch during early ontogeny and prefer these individuals over unfamiliar ones (Olsén 1992). In the case of phenotype matching, an individual learns about the phenotype of close relatives or itself (self-matching) during early development and later compares unfamiliar conspecifics with this learned phenotype to identify kin (Olsén 1992; Sherman 1997). Phenotype matching implies a correlation of the phenotypic traits used for kin recognition and the genotype, because only heritable phenotypic traits can be true indicators for relatedness (Tang-Martinez 2001).

Kin recognition has been documented for several salmonid species like Arctic charr, *Salvelinus alpinus,* (Olsén 1989), Atlantic salmon, S*almo salar,* rainbow trout, *Oncorhynchus mykiss,* (Brown and Brown 1992), rainbowfish, *Melanotaenia eachamensis,* (Arnold 2000) and zebrafish, *Danio rerio,* (Mann et al. 2003). Contradictory results were obtained for coho salmon, *Oncorhynchus kisutch*, (Quinn and Busack 1985) but see (Quinn and Hara 1986), for sticklebacks (Van Havre and Fitzgerald 1988; Frommen and Bakker 2004) but see (Steck et al. 1999) and for the Trinidadian guppy, *Poecilia reticualta*, (Warburton and Lees 1996) but see (Griffiths and Magurran 1999).

However, shoaling with relatives can also be disadvantageous through the risk of inbreeding if related individuals mate. Inbreeding can increases the genetic homozygosity and thus the possible expression of recessive deleterious mutations in offspring (Charlesworth and Charlesworth 1987). Mechanisms to recognize kin can, therefore, be advantageous in two ways, an individual may profit from the benefits of co-operation with relatives and it can minimise inbreeding depression by avoiding to mate with siblings. Griffiths and Armstrong (2001) have shown however that for territorial animals kin associations can also be disadvantageous.

On the other hand, outcrossing of individuals from different populations can be disadvantageous as well due to a break-up of co adapted gene complexes or favourable epistatic relationships that have developed as an adaptation to different habitat parameters (Mayr 1963; Shields 1982). Thus with ongoing population divergence, selection should favour mechanisms that lead to a shift from outbreeding enhancement to outbreeding depression (Lynch 1991). For organisms belonging to sympatric populations, the recognition of population-specific cues provides an excellent mechanism to recognize appropriate mating partners of their own population. Species living in highly structured, dark or turbid habitats might evolve the ability to use non-visual features like e.g. acoustic or olfactory (chemical) cues to discriminate between populations. Closely related species of Hawaiian crickets find their mating partners based on calling song differences, suggesting that acoustic features could have been the first cues to change during the speciation process (reviewed in Shaw and Herlihy 2000). For the songbird greenish warbler (*Phylloscopus trochiloides*), Irwin et al. (2001) showed that sexual selection for increased complexity of song is driving population divergence. Unlike kin recognition, olfactory based population recognition in fish has, to our knowledge, only been studied in salmonids (for a review see Olsén 1992). Evidence for population recognition based on chemical cues was provided for Atlantic salmon (Stabell 1982; 1987), Arctic charr (Olsén 1986) and coho salmon (Courtenay et al. 1997). Recently, Ward et al. (2004) have shown that chemical cues play a major role in the recognition of familiar conspecifics but can be influenced by different habitat and diet experiences.

This study focuses on Eurasian perch (*Perca fluviatilis* L.). During their ontogeny, perch of Lake Constance show a typical habitat shift. Soon after hatching, larvae move to the pelagic zone to feed on zooplankton and return about one month later to the littoral (Wang and Eckmann 1994). As juveniles and adults perch remain in the littoral during summer or in close proximity to the littoral when they move to deeper waters during winter (Wang and

105 Appenzeller 1998). For perch of Lake Constance, pairwise calculations of relatedness
106 provided evidence that aggregations of juveniles and adults, caught in the littoral during
107 summer, were genetically structured, containing closely related kin within age groups
108 (Gerlach et al. 2001). This observed kin structure could be the result of homing of pelagic
109 larvae to natal sites (Aalto and Newsome 1989), or it could be based on kin preference.
110 Calculating the corrected genetic index G_{ST} of between-population divergence for perch of
111 Lake Constance, Gerlach et al.(2001) found a subdivision into two populations with restricted
112 gene flow ($G_{ST} = 0.07$). One population was found to inhabit the eastern the other the western
113 part of the lake while no obvious geographical barrier separated the two.
114 Thus, the aim of our study was twofold. Firstly, we investigated, whether kin aggregations
115 like those observed in juveniles and adults, are already present in free ranging shoals of larval
116 perch. Odour choice tests in the laboratory where conducted to test, whether the kin structured
117 perch aggregations might be based on olfactory kin recognition and kin preference. Secondly,
118 we tested, whether perch of the two sympatric populations also use olfactory recognition to
119 distinguish between conspecifics from the same versus the foreign population.
120

121 **Methods**

122 ***Microsatellite genotyping***

123 Perch larvae were sampled on one occasion from the surface in the pelagic zone of Lower
124 Lake Constance with a horizontally trawled plankton net (opening: 0.25 m^2). We used a
125 "multi-net", consisting of five conical nets of the same size that could be opened and closed
126 individually one after the other. Thus during the sampling haul of 722m, each net covered a
127 sampling transect of approx.140m length.
128 Total genomic DNA from 20 larvae per sample (19 larvae in sample 5) was extracted
129 according to standard salt extraction procedures (Sambrook et al. 1989). Larvae were

genotyped using five dinucleotide microsatellite loci comprising three isolated from walleye, *Stizostedion vitreum,* (loci SVI 6, 17, 18; GenBank Accession no.: G36962; -63; -64, Borer et al. 1999) and two from yellow perch, *Perca flavescens,* (loci PF 1,5; GenBank Accession no.: AF211826; -30, Leclerc et al. 2000). A summary of the loci characteristics is presented in Table 1. The PCR products were run individually on Spreadex gels (Spreadex ^{TM} gels, Elchrom scientific AG, Switzerland: EL 400 for SVI 6,17 and PF 1,5; EL 600 for SVI 18) using the SEA 2000 ^{TM} advanced submerged gel electrophoresis apparatus (Elchrom scientific AG, Switzerland). Gels were run at 120 V, 990 mA. Running time depended on allele sizes. Alleles were visualized by dyeing with sybr gold. The allelic size of the PCR products was determined by comparison with a standard M3 marker ladder (Elchrom scientific AG, Switzerland).

Observed and expected values for heterozygosity were determined using the GENETIX404 computer package (Belkhir et al. 1997). For calculations of genetic relatedness, the pairwise identity index I_{xy} (Mathieu et al. 1990; Castric et al. 2002) was estimated using the IDENTIX computer package (Belkhir et al. 2002), which can detect relatedness in populations using multilocus genotypic data (for details see Belkhir et al. 2002). To detect relatedness within distinct groups (aggregations of perch) belonging to the same population the statistics had to be extended (Belkhir personal communication). To test if larvae within one sample were genetically more related than expected under random distribution, the mean identity index of all perch pairs within one sample was compared with the null distribution of no relatedness. As null distribution, we calculated the distribution of identity indices of randomly generated sub-samples of the same sample size (20 individuals). Sub-samples were generated by random permutation of genotypes in 1000 randomised sub-samples using all five larvae samples as genotype pool. The statistic procedure of testing for significance was then the same as described in Castric et al. (2002).

155

156 ***Kin- and population recognition***

157 During spawning time, perch were captured with gill nets. To obtain full sibs, the egg-strand
158 of a ripe female was cut into pieces, which were then individually fertilised with the sperm of
159 the same male. For maternal half sibs the egg-strand of one female was cut into pieces, which
160 were then fertilised each by a different male. For paternal half sibs, one male was used to
161 fertilise pieces of egg-strands of different females. All pieces of egg-strands were transported
162 to the laboratory and placed separately in 9 L aquaria (constant supply of tap water, 0.1 L
163 min^{-1}, temperature 15°C, 14 h illumination). Thus, each aquarium contained a group of full-
164 sibs. After hatching, temperature was raised to 20 °C during the first week. Perch larvae were
165 fed with copepod nauplii and rotifers for the first 4 days, and afterwards with live artemia
166 nauplii and daphnids. Juveniles were fed with frozen chironomids. Preference tests were
167 carried out with fish between 3 and 6 months of age (6-9 cm total length, 2-6g wet mass).
168 The odour choice tests were carried out in a two choice fluviarium (Höglund 1961; Steck et
169 al. 1999; Atema et al. 2002, Fig. 1). The flume was divided into three compartments, the inlet
170 compartment, the test area and the outlet compartment. The inlet compartment was separated
171 in two equal halves by a PVC wall (100 cm) presenting two bodies of moving water separated
172 by a sharp boundary in the test area. Evenness of water flow on both sides of the test
173 compartment was visualised using fluorescent dye. The test area (33 cm x 25 cm) was
174 separated by screens from the other compartments. A slow but permanent flow-through of tap
175 water (4 L min^{-1}, velocity 0.6 cm s^{-1} , temperature 20 °C) ensured that no chemical
176 information about perch was initially present in the flume or was accumulating in the system
177 during the trials. Stimulus water was taken from the holding tanks of the respective stimulus
178 group and was added (7.6 ml min^{-1}) to either side of the inlet compartment using a peristaltic
179 pump. The test area was visually isolated by black cloth from all sides. The compartment was

illuminated by two halogen bulbs (same light cycle as in the holding tank). All observations were video taped for later analysis.

Since single test fish had shown erratic behaviour and no food intake during preliminary trials, we used a small group of four full sibs out of the same holding tank for the choice experiments in the flume. Acclimation time was between one and eight days, during which fish were fed daily. We defined acclimation to be completed when the fish started feeding directly after food supply. Four different trials were run, with consecutive trials separated by at least 30 min recovery time, when no odour stimulus was supplied. The following trials (1-4) were run with the order of trials set at random; (1) unfamiliar full-sibs vs. unfamiliar non-kin, n = 14; (2) unfamiliar maternal half-sibs vs. unfamiliar non-kin, n = 10; (3) unfamiliar paternal half-sibs vs. unfamiliar non-kin n = 9; (4) unfamiliar and unrelated perch from the own versus unfamiliar perch from the foreign population, n = 13. To account for potential side bias of the fish, the odour stimuli were alternated during trials between the two sides of the flume and were presented two times for three minutes on each side. The number of fish on either side of the test area (respective to the two odour stimuli) was counted every five seconds during a three minute odour supply. After the last trial all four test fish were removed from the flume and excluded from further experiments.

For each trial, we calculated the average number of test fish present on that side of the flume, where a particular stimulus was provided. One-way ANOVA (Jmp vers. 4.0) was used to test for differences between the different kin recognition trials (full-sibs, maternal or paternal half-sibs versus non-kin). Since there were no significant differences, the data was pooled for further analysis. A two tailed paired t-test, (Jmp vers. 4.0) was used to compare the average number of fish on each of the two stimulus sides (kin versus non-kin or own versus foreign population).

205 **Results**

206 ***Microsatellite genotyping***

207 We caught 404 perch larvae (mean 80 larvae, per sample) within the pelagic zone of Lower
208 Lake Constance. The degree of polymorphism at five microsatellite loci varied between 5 and
209 12 alleles per locus. Observed levels of heterozygosity were moderate and ranged from 0.404
210 to 0.687 (Table 1).

211 The I_{xy} values of randomly generated pairs (sub samples of 20 individuals) are shown as null
212 distribution in Fig 2, together with the mean I_{xy}'s for each larvae sample. The mean pairwise
213 identity index in 3 of the 5 net samples departed significantly from the null expectation of no
214 relatedness (Fig. 2, two tailed test, sample 1: p=0.01; sample 2: p=0.20; sample 3: p=0.35;
215 sample 4: p=0.01; sample 5: p=0.04).

216

217 ***Kin- and population recognition***

218 Perch showed a clear reaction to odour supply of stimulus water from conspecifics.
219 Approximately one to two minutes after the beginning of odour supply (odour plume needs
220 one min. to reach the downstream end of the test area) fish became active, swimming
221 upstream and pressing their snouts against the screen. This so called "screen-swimming" was
222 also observed in kin discrimination tests with cichlids and in population recognition tests with
223 salmonids (Barnett 1986; Courtenay et al. 1997), and was used as a measure for stimulus
224 preference.

225 Some groups of fish, although swimming actively, never changed sides during a trial and
226 where, therefore, not able to choose between odour stimuli. Additionally, some fish were
227 completely inactive during a trial (fish were resting on the bottom) and thus were not able to
228 choose between different stimuli either. To eliminate such time intervals we calculated an
229 "activity index" for each of the three minutes of a trial. We divided the observed number of

changes between the two sides of the test area during one minute of odour supply by the maximal number of side changes that are possible (four fish x 12 five sec intervals = 48 side changes possible). Minutes with activity indices lower then 0.1 (i.e. less than 10% of the maximal number of side changes were observed) were eliminated. This procedure resulted in unequal sample sizes for different stimulus trials.

Given that no significant difference in preference could be seen between the three different kin recognition tests (ANOVA, among all categories of different sibships $F_{2,30} = 2.3$, $p = 0.139$), all kin data were pooled. Perch showed a significant preference for their siblings versus non-kin (two tailed paired *t*- test, $t = 3.00$, $n = 33$, $p = 0.005$; Fig. 3A).

Given the choice between fish of the same versus the foreign population, perch significantly preferred conspecifics of the own population (two tailed paired *t*- test, $t = 2.36$, $n = 13$, $p = 0.04$, Fig. 3 B).

Discussion

Microsatellite genotyping

Our genetic data showed that larval perch stayed together in kin groups. Two out of five larvae samples that were collected in close proximity shared more and one sample shared less alleles than randomly expected, both indicating relatedness. This seems to be puzzling but the level of relatedness in a family group highly depends upon the specific genotype of the parents. If the shoal consists of only one family, the number of shared alleles between individuals would be higher than under random distribution of individuals. However, if the shoal consists of members of several families that are less related than any families taken randomly from the population, relatedness could be significantly lower than under random distribution. (Bernatchez and Castric personnel communication).

Our sampling method did not allow the identification of distinct larval shoals. Thus a sampling of two or more families within one trawl could well have occurred. Given the conservative statistical analysis (Castric et al. 2002) we assume that in reality the degree of relatedness in aggregations of perch larvae might be even higher. These kin groups may persist for years because a high degree of relatedness was found in groups of juvenile and adult perch (Gerlach et al. 2001). Therefore, we conclude that related perch stay together throughout their early development and later in life. Thus homing of perch to their natal sites, although it could occur, is not the exclusive cause for kin structure in age groups.

Kin- and population recognition

We could show that a fish species, which has been found in kin aggregations in the field (Gerlach et al. 2001 and this study), recognises and prefers chemical stimuli of siblings in laboratory experiments. Until recently the rather few studies of this type have shown contradictory results. Brown & Brown (1992; 1993; 1996) have shown in numerous laboratory experiments that salmonids including Atlantic salmon can discriminate kin and show kin-biased social behaviour by gaining benefits in higher growth rates and lesser aggressive interactions with siblings as territory neighbours. However, a field study of Atlantic salmon larvae and fry in their natural habitat showed that although numerous fish were closely related as shown by microsatellite analysis, relatives did not preferentially occupy neighbouring territories (Fontaine and Dodson 1999). Griffiths and Armstrong (2001) showed that heterogeneous advantage could outweigh the benefits of kin biased behaviour for Atlantic salmon in his natural habitat.

Studies on threespine sticklebacks show even more conflicting results. Whereas Van Havre and Fitzgerald (1988) could show stickleback fry to preferentially shoal with kin when exposed to visual and chemical stimuli in the laboratory, Steck et al. (1999) found no

preference for siblings in juvenile sticklebacks using only chemical stimuli in a flume. However, a recent analysis with adult sticklebacks revealed preference for familiar kin (Frommen and Bakker 2004). Genetic data on allozyme polymorphism in 24 free ranging stickleback shoals did not show any kin structure within fish shoals (Peuhkuri and Seppae 1998).

Our genetic analysis on the relatedness of free ranging perch larvae clearly shows that even during the mobile pelagic phase kin structure of shoals is maintained. Aggregations of newly hatched perch could be transported passively in the water column. In this case, related fish would stay together independent of any preference for distinct conspecifics. However, with ongoing ontogenetic development during the pelagic phase, perch grow to active swimmers and aggregate in free-ranging shoals (personal observations). So far, the kin structure in perch shoals could still be the result of a preference to shoal with familiar fish. However, our olfactory test shows that kin recognition is not based on familiarity, since even unfamiliar sibs were preferred over unfamiliar unrelated fish.

Helfman (1984) investigated shoaling behaviour in adult yellow perch (12-20 cm TL) of Cazenovia Lake, Madison County, New York and detected low school fidelity in this species. He argues that under high predator pressure shoal fidelity may be part of the anti-predator function of schooling and could result in strong association of individual fish. Under week predator pressure, however, other functions of schooling e.g. increased foraging efficiency could lead to decreased importance of shoaling with familiar or related individuals and thus would lead to week associations between individual fish. Larval perch in contrast to adult yellow perch in Cazenovia Lake are under much higher predation pressure. Under these conditions high shoal fidelity in Lake Constance perch and even association with familiar or related individuals could have evolved as anti-predator function based on mutualistic or even altruistic acts directed towards kin.

Olfactory recognition and preference in perch is based on phenotype matching learned from related individuals (Brown et al. 1993; Tang-Martinez 2001). Variability of major histocompatibility complex (MHC) genes might be involved in these recognition processes (Bernatchez and Landry 2003). Female sticklebacks differentiated between males according to sequence differences of their MHC alleles (Reusch et al. 2001), juvenile Arctic charr chose the water scented by fish with their own MHC type (Olsén et al. 1998; 2002).

In this study we could show for two sympatric perch populations that juveniles of one population could recognise the own versus the foreign population by waterborne chemical cues. Similar to some terrestrial species that use acoustic features as non-visual cues to discriminate between populations (Irwin et al. 2001; Shaw and Herlihy 2000), chemical cues are also an appropriate means for population discrimination especially for aquatic species .

Most experiments on population recognition in fish have been done with salmonids. Migrating salmonid species learn about the odour of their natal rivers as juveniles and use it for orientation when they return as adults to spawn in freshwater rivers (reviewed by Hasler and Scholz 1983). However it has also been hypothesized that salmonids are guided to their natal rivers by population specific odours of juveniles. Preference tests in the laboratory showed that salmonids can recognise population specific cues and mostly prefer the odour of the same over a foreign population (reviewed in Olsén 1992). We could show that juvenile perch prefer odours of the own versus a foreign population that lives in sympatry within the same lake. The ability of juvenile perch to discriminate between the own and the foreign population could be based on olfactory imprinting on population specific cues that could be used later in life for mate choice decisions. This has also been suggested for salmon fry (Courtenay et al. 1997). Further experiments are necessary to test whether perch mate assortatively within populations and prefer mating partners of the own over the foreign population, as our genetic data indicate.

330 We have shown that perch use olfactory cues in another important context i.e. for kin
331 discrimination. We hypothesize that kin recognition is the basal mechanism from which
332 olfactory based population recognition was derived. It is conceivable that the specific allelic
333 composition of MHC genes, which are known to influence the individual body odour
334 (Apanius et al. 1997), are involved in both cases. In this scenario, individuals would recognize
335 family-specific MHC alleles, as has been shown for house mice (for a review see Penn and
336 Potts 1999) and also for Arctic charr (Olsén et al. 1998; 2002), as well as population-specific
337 MHC alleles. Thus during mate choice, olfactory discrimination of adult perch could lead to
338 assortative mating within populations and avoid inbreeding at the same time. This would be a
339 perfect mechanism to reinforce reproductive isolation between the two sympatric populations.
340 By combining behavioural and genetic data, we present a possible mechanism for the
341 observed maintenance of the two genetically different populations of perch in Lake
342 Constance. We believe that ours is the first empirical study suggesting that social preferences
343 might drive population divergence.
344

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507 **Figure legends**

508

509 Figure1. Experimental choice fluviarium for odour preference tests with perch

510

511 Figure 2. Frequency distribution of coefficients of relatedness (pairwise identity index
512 calculated by the IDENTIX computer package (Belkhir et al. 2002). The distribution was
513 calculated (1000 randomizations) based on the allele frequencies of larvae caught in the
514 pelagic zone of Lake Constance assuming random association of monolocus genotypes.
515 Arrows indicate observed values of relatedness in five samples (s1-s5) of perch larvae (for
516 details see text).

517

518 Figure 3. Outcome of odour choice tests in a fluviarium. Groups of four test fish (full sibs of
519 the same holding tank) were given the choice between different odour stimuli. As measure for
520 stimulus preference, the mean number of fish (error bars: SE) on the side were the distinct
521 stimulus was added is presented a: Black bar= kin odour, light bar= non-kin odour (two tailed
522 paired *t*- test, $t = 3.00$, $n = 33$, $p = 0.005$). b: Black bar= odour of same population, light bar=
523 odour of the foreign population (paired *t*- test, $t = 2.36$, $n = 13$, $p = 0.04$)

524

525

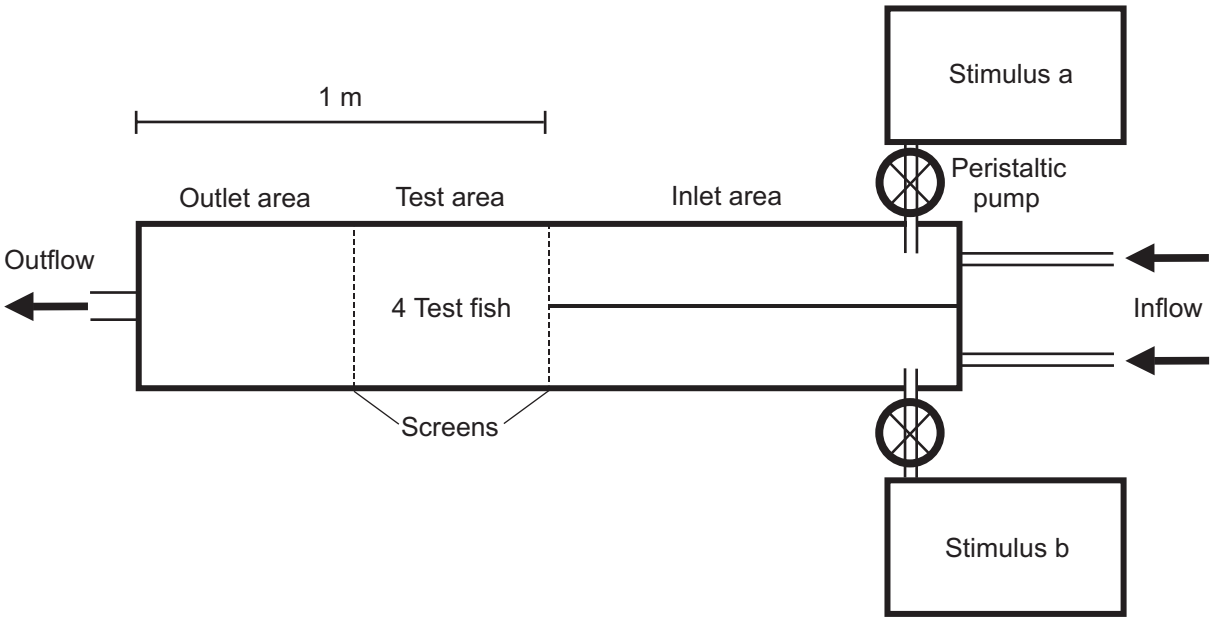
525

526 Table 1. Microsatellite characteristics. Perch larvae (n = 99) examined with five
527 microsatellites were caught in the pelagic zone of Lower Lake Constance (western
528 population). Subscripts at the locus names show the repeat motives. H_{obs} =observed
529 heterozygosity, H_{exp} = unbiased expected heterozygosity (Nei 1987).

Locus	No. of alleles	Size range (bp)	H_{obs}	H_{exp}	Primer sequences
SVI 18 $_{(AC)}$	6	160-174	0.616	0.546	GATCTGTAAACTCCAGCGTG CTTAAGCTGCTCAGCATCCAGG
SVI 17 $_{(AC)}$	6	110-142	0.475	0.550	GCGCACTCTCGCATAGGCCCTG CGTTAAAGTCCTTGGAAACC
SVI 6 $_{(AC)}$	5	106-120	0.667	0.616	CATATTATGTAGAGTGCAGACCC TGAGCTTCACCTCATATTCC
PF 1 $_{(GA)}$	12	112-140	0.687	0.737	AAGCAGCCTGATTATATATC CAGACAATTAAACATGCAAC
PF 5 $_{(GT)}$	6	134-152	0.404	0.471	TGAGAGCCCATGAATTAC GCAAACACAGCCAATTTAG

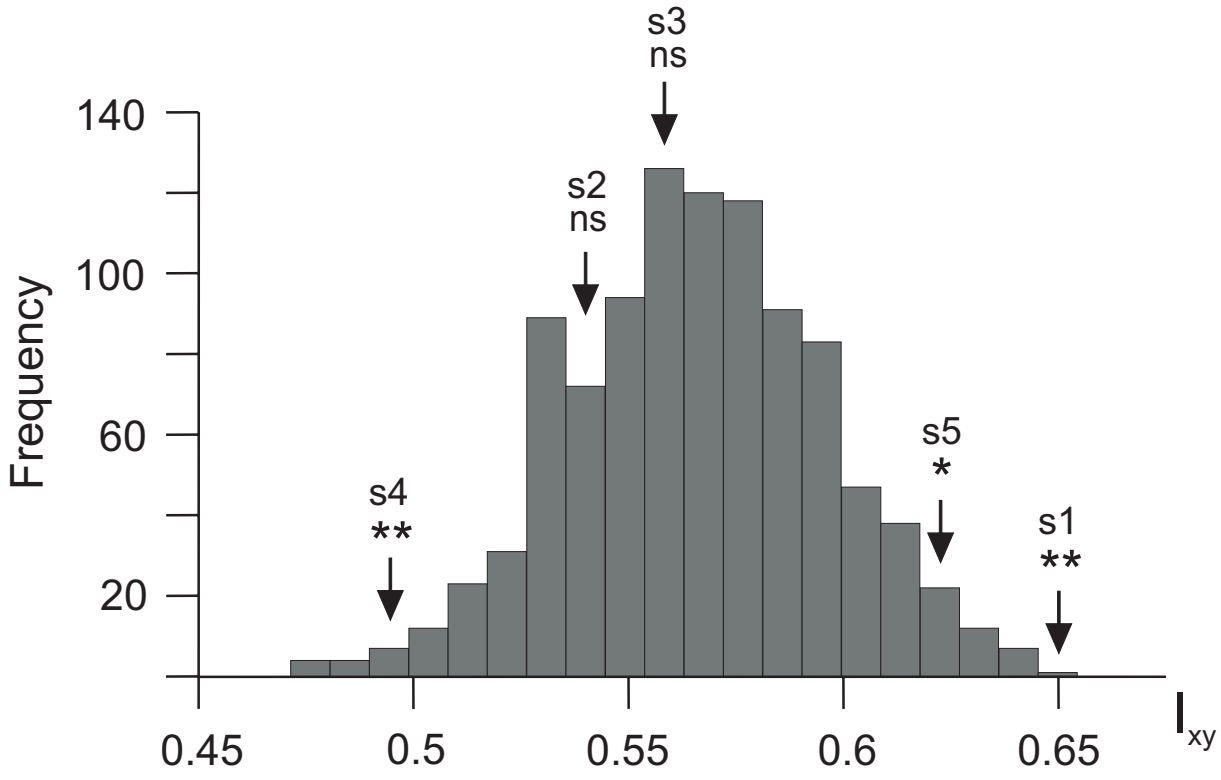
530

531



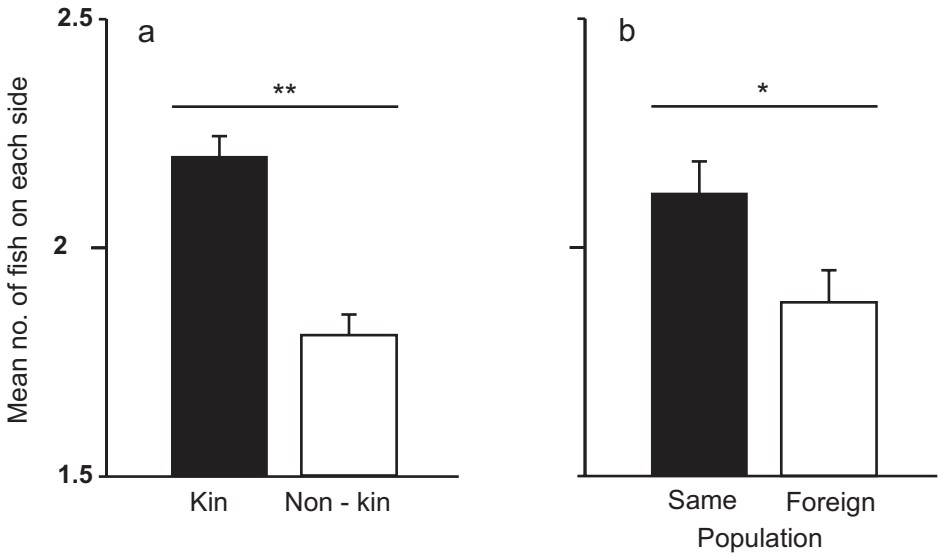
531 Figure 1 Behrmann-Godel et al.

532



532 Figure 2 Behrmann-Godel et al.

533



533 Figure 3 Behrmann-Godel et al.