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RESEARCH ARTICLE

Historical and recent reductions in genetic variation of the *Sarotherodon galilaeus* population in the Sea of Galilee

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Abstract
The Sea of Galilee has great significance as a natural habitat and a freshwater source for Israel. Anthropogenic impacts have been placing significant pressure on the species inhabiting this lake, among which is *Sarotherodon galilaeus*, an omnivorous fish with a relatively large population and significance for commercial fishing. An alarming decline in annual catch towards 2008 suggested that this unique population might be at risk. With that in mind, we characterized the current genetic variation of this species in Israel with reference to fish from Ghana, based on D-loop and microsatellite markers. Genetic variation and differentiation were found mostly among fish from Ghanaian localities and between fish from Israel and Ghana, whereas fish from all Israeli localities had uniform and limited variation, a signature compatible with historical founder effect followed by local adaptations. Such historical processes could leave a population vulnerable as reflected in the sudden and recent population decline. Comparing genetic variation between archived 30 year-old scales and modern lake fish revealed further reduction in genetic variation coincident with the recent population decline. Thus, a recently occurring genetic bottleneck had placed this unique and isolated population at an even higher risk. We carefully discuss the events leading to the current risk status for *S. galilaeus* in Israel and highlight the need for vigilant monitoring and active management to support a more sustainable future for this and other fish communities in this important habitat.

Keywords Cichlid fish · St. Peter's fish · Lake Kinneret · Genetic bottleneck · Isolated population · Archived biological samples

Introduction

Freshwater habitats can support rich aquatic biodiversity, but this capacity is delicate and can be easily disturbed. Pressures placed on freshwater habitats deplete endemic fish communities and could drive them to the brink of extinction (Ryman 1991; Frankham et al. 2002; Allan et al. 2005; Dudgeon et al. 2006; Allendorf et al. 2008; Lévêque et al. 2008; Pinsky and Palumbi 2014). Decreasing population size of imperiled species has been clearly associated with decreased genetic variation and increased species vulnerability (Nei et al. 1975; Lande and Barrowclough 1987; Leberg 1990; Frankham 1996; Laikre 2010; Markert et al. 2010; Bijlsma and Loeschcke 2012; Allendorf et al. 2014). Additionally, once disturbed, the ecological balance in isolated habitats with unique conditions is hard to recover. Therefore, responsible ecological management of imperiled species should monitor changes in population size and genetic

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diversity while considering their potential consequences (Lande and Barrowclough 1987; Schwartz et al. 2007).

The Sea of Galilee (Lake Kinneret) is the biggest surface freshwater reservoir in the Middle East. Located in the northeastern part of Israel, this lake is an important link in the country's eastern watershed (Serruya 1978; Berman 1998; Por 2012; Berman et al. 2014). Due to low rainfall characterizing this fast-developing region, freshwater has always been a precious national resource. Despite the regulation on water level and quality (Markel et al. 2014), varying precipitation levels and changing water demands have been causing significant fluctuations of the surface level of the lake.

About 19 native and eight exotic species are documented in the Sea of Galilee (Goren and Ortal 1999; Ostrovsky et al. 2014; Gophen et al. 2015). Native species are dominated by cyprinids and cichlids that are of mainly Asian (Mesopotamian) and African origins, respectively (Goren and Ortal 1999; Werner and Mokady 2004). Among the native lake cichlids, *Sarotherodon galilaeus* (Linnaeus 1758), also known as Galilee tilapia and St. Peter's fish, is the most abundant in the lake and it holds economic and cultural importance (Ostrovsky et al. 2014; Zohary et al. 2014). Israel is at the northern edge of the species distribution range. In contrast, *S. galilaeus* is widespread in equatorial Africa, from west to east and to northern Africa along the Nile river. As such, it is native to the Volta river system in Ghana (western part of the distribution range), where it also has a commercial value (Béné 2007; Lemoalle and de Condappa 2010; Lind et al. 2012).

To enhance fishing and support the native population in the Sea of Galilee, the Fishery and Aquaculture Division of the Israeli Ministry of Agriculture and Rural Development produces *S. galilaeus* in captivity and has stocked fry into the lake on an annual basis since the 1950s. The *S. galilaeus* lake population size was reported to change in cycles of about 10 years, cycles that are not explained well by fishing efforts (Pisanty et al. 1987). Regardless of these cycles and despite this stock enhancement, a steep decline in population size was observed recently. The commercial catch of *S. galilaeus* has declined from an annual average of 300 tons during 1980–2005 to less than 10 tons in 2008—the lowest harvest ever recorded (Ostrovsky et al. 2014). The collapse of the *S. galilaeus* population was hypothetically attributed to several possible factors (Zohary et al. 2008), including reduction in appropriate spawning grounds during low water levels, overfishing, predation by birds and food scarcity (Ben-Tuvia 1960; Zohary and Ostrovsky 2011; Gophen et al. 2015). This decline raised deep concerns as to the sustainability of this population and populations of other native cichlids that even if not commercially fished, are still sharing with *S. galilaeus* similar ecological needs for food sources and spawning grounds.

In this study, the genetic variation of *S. galilaeus* in Israel was analyzed with reference to fish from the Volta River in Ghana. The level of variation and the pattern of genetic differentiation in modern and archived samples were analyzed to elucidate what processes may have led to the situation we see today and to suggest future measures to improve sustainability of fish communities in the Sea of Galilee.

Materials and methods

S. galilaeus fish sampling

Sampling in Israel was done between 2010 and 2015 with an emphasis on the Sea of Galilee (32.821120N, 35.591699E), but also from Beit She'an valley streams (32.498870N, 35.460219E) south of the lake and part of the eastern watershed of Israel, and from Ein Afek spring (32.848177N, 35.111908E), close by Acre (Akko) and part of the western watershed of Israel. Samples were taken also from the Ginosar broodstock (located next to the lake) used for the stock enhancement program. Sampling sites are mapped in Fig. 1 and listed in Table 1. Fresh fin clips were taken from all samples. Additionally, dry fin clips were obtained from fish sampled along the Volta river system in Ghana. Sampling sites in Ghana were: North—in the White Volta River at Nawuni (9.65934N, 1.05079W), and two southern locations in the Volta lake at the South—Dzemeni (6.558386N, 0.138228E) and South West—Akaten (6.51888N, 0.15291W). Finally, dry scales were obtained from *S. galilaeus* fish that lived in the Sea of Galilee between 1977 and 1989.

DNA extraction

DNA from modern samples was extracted using a modified protein salting-out method (Martínez et al. 1998). In brief, about 5 mg of dry fin sample was digested at 50 °C with 1% SDS and Proteinase K solution (20 mg/mL). To precipitate the proteins, 300 µL of 5 M NaCl were added and the sample was vortexed and centrifuged. DNA pellet was obtained by mixing with 900 µL of freezer-cold 100% isopropanol, 2 h incubation at − 20 °C and centrifugation. The pellet was washed with 700 µL of 70% ethanol, dried for 15 min and dissolved overnight in 100 µL of double-distilled water at room temp. DNA samples were diluted to a concentration of 25 ng/µL and stored at − 20 °C for further analysis.

For dried archived scales, the entire process of DNA extraction took place inside a clean hood in a clean pre-PCR area to avoid contamination by modern DNA. About 2–3 scales from a single fish were placed into 500 µL of GuSCN solution (4 M guanidinium thiocyanate, 0.1 M Tris–HCl pH 6.4, 0.02 M EDTA pH 8, 1.3% V/V Triton X-100). After adding 10 µL Proteinase K (20 mg/mL, Promega), samples

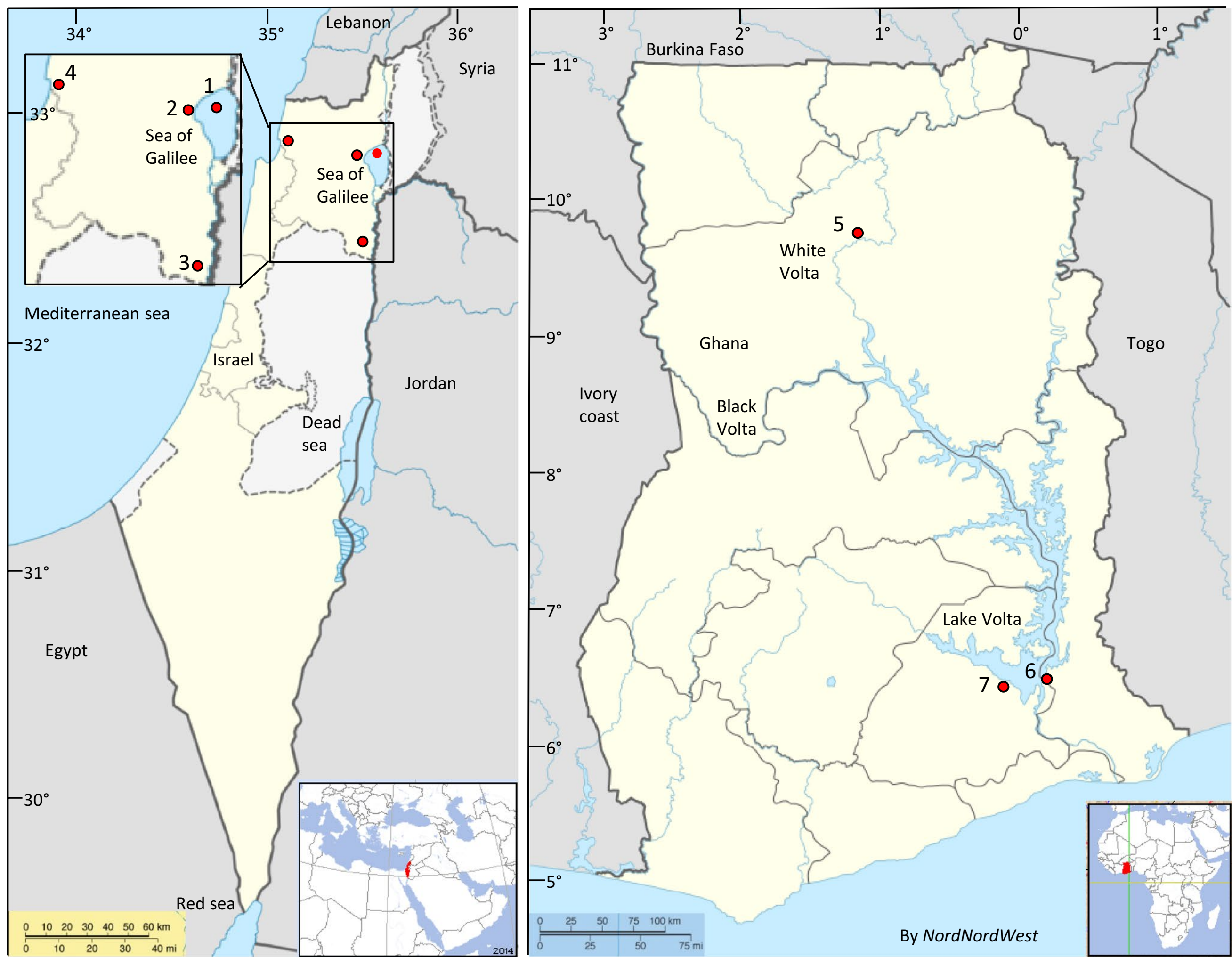

Fig. 1 Maps showing sampling locations in the regional context. Sampling sites (red dots) on the map of Israel are Sea of Galilee (1), Ginosar broodstock (2), Biet She'an valley streams (3) and Ein Afek spring (4), whereas on the map of Ghana are North (5), South (6) and Southwest (7)

Table 1 Diversity indices of populations based on D-loop and microsatellite analyses

Sampling point	D-loop		Microsatellites		
	N samples	Theta (π)	N samples	N alleles (mean/locus)	Observed heterozygosity
Sea of Galilee	120	0.69	24	2.35	0.39
Ginosar broodstock	25	0.33	20	2.59	0.42
Beit She'an streams	11	0.55	15	2.76	0.49
Ein Afek spring	19	1.34	19	2.59	0.47
Total Israel	175	0.95	78	3.94	0.42
Ghana North	20	8.17	5	NA	NA
Ghana South	27	4.94	3	NA	NA
Ghana Southwest	26	5.06	10	NA	NA
Total Ghana	73	7.91	18	5.59	0.55

NA data not presented due to small sample size

were agitated at 56 °C over two nights and then boiled at 94 °C for 10 min and centrifuged (13,000 rpm, 3 min). The supernatant was transferred and supplemented by 1 mL of 6 M NaI solution, 15 µL silica beads (Sigma) and 7 µL of linear acrylamide (Ambion). Samples were placed on ice for 60 min with occasional shaking. After a quick spin, the supernatant was carefully discarded, and 500 µL of cold washing buffer (0.01 M Tris–HCL pH 7.5, 1 mM EDTA pH 8, 0.05 M NaCl, 50% EtOH) was added, tubes were shaken and after a quick spin (5000 rpm, 30 s), the supernatant was carefully discarded. Two rounds of washing were done by adding 200 µL of ethanol, shaking, quick spinning and discarding the supernatant, after which the DNA-containing bead pellet was allowed to air-dry and dissolved in 100 µL of ultrapure ddH_2O at 56 °C for 15–30 min. If used within the following week, samples were stored at 4 °C, or at −20 °C for longer storage.

PCR procedures

Two regions of mitochondrial DNA (mtDNA) were PCR amplified; the non-coding D-loop and the coding *Cytochrome C oxidase subunit I* (COI). Henceforth, positions on the mtDNA sequence are reported relative to the reference sequence of *Oreochromis aureus* strain America (Genbank accession GU477630.1); positions 15,644–16,579 for D-loop and 5697–6176 for COI. PCR primers were adopted (Ward et al. 2005; Ivanova et al. 2007) and their sequences are listed in Online resources (Table S1). As DNA template, 50 ng of purified DNA was used in a total reaction volume of 20 µL. PCR mix contained: 1.66 µM of primers, 0.25 mM $MgCl_2$, 0.66 mM dNTP mix, 2 µL 10× PCR buffer, 0.5 µL *Taq* DNA polymerase and water to complete the volume. PCR profile included an initial step of 94 °C for 10 min; followed by a touchdown profile of 94 °C for 30 s, annealing from 60 to 53 °C for 1 min with a decrease of 0.5 °C per each of 14 cycles, and extension at 72 °C for 2 min; further 23 cycles at the lower annealing temperature and final elongation step at 72 °C for 10 min. PCR products were verified by electrophoresis using 1.5% TBE Agarose gel containing ethidium bromide.

For microsatellites, the PCR method used two site-specific primers and a third common fluorescently-labeled primer, as previously described (Rodriguez et al. 2003). The PCR profile included an initial step at 94 °C for 10 min; 35 cycles of 94 °C for 30 s, 48–56 °C (depending on the specific primer pair) for 1 min, and 72 °C for 30 s; and a final step of 72 °C for 10 min. PCR products were separated using an ABI PRISM 3730xl DNA Analyzer (The Center for Genomic Technologies, The Hebrew University of Jerusalem) and analyzed for the allele size by GeneMapper and Peak Scanner software (Applied Biosystems).

Sequencing of PCR products

PCR products were cleaned using ExoSAP-IT (USB, Cleveland, OH) and Sanger sequenced using BigDye chemistry on an ABI PRISM 3730xl DNA Analyzer. D-loop sequences of modern fish are given in Online resources (Data File S1) and were deposited in the GenBank database under accession numbers: KY940659–KY940710.

For archived scales, due to the low amount and fragmented nature of the DNA, only shorter fragments covering polymorphic regions were amplified and sequenced by specific primers (Online resources, Table S1). Fragments spanning COI gene positions 5576–5801 and 5889–6125 were sequenced from five samples (Online resources, Data file S2), and fragments spanning D-loop positions 15,737–16,050 were sequenced from 35 samples (Online resources, Data file S3). To impute haplotypes from the discontinuous sequences, gaps were filled with homologous sequence from the most common modern haplotype. The frequency of polymorphisms initially found in the archived samples was kept the same also in the imputed haplotypes (Online resources, Data file S3). In this way, the complementation by the modern sequence did not bias the variation found in archived samples on one hand and on the other, using only one common modern haplotype for complementation yielded a conservative estimate of the variation that existed in the past.

Data analysis: D-loop and COI

mtDNA sequence files were assembled, aligned to a reference sequence and polymorphisms were extracted and checked using the SeqScape software (Applied Biosystems). For modern samples, an estimated gene tree based on D-loop haplotypes was constructed utilizing the Molecular Evolutionary Genetics Analysis (MEGA7) freeware (Kumar et al. 2016), using the HKY + G + I model, number of differences distance, and the neighbor-joining tree construction method with 1000 bootstrap replicates. The tree with the strongest bootstrap support was chosen for representation. A haplotype network was generated using the parsimony method implemented in the TCS software (Clement et al. 2000). Modern D-loop haplotype frequencies by populations are given in online resources (Table S2). For both modern and archived samples, diversity indices (Theta π), F_{ST} and pairwise differences calculations, Tajima's D test for neutrality and comparisons between populations were done using Arlequin (Excoffier and Lischer 2010). Statistical analysis comparing haplotype frequencies among populations were done using JMP13 software (SAS institute, NC).

Data analysis: microsatellites

To complement the D-loop results with nuclear markers, markers from *Oreochromis niloticus* (Lee et al. 2005) were adapted to use in *S. galilaeus*, (Online resources, Table S1). A selection of 96 fish, representing the D-loop haplotypes, were genotyped, including 78 from Israel and 18 from Ghana (Table 1). Given this limited and non-random sample selection with respect to D-loop variation, restricted analyses were done with the microsatellite data. After testing for null alleles, large-allele dropout and stuttering peaks using Micro-Checker (Van Oosterhout et al. 2004), a final dataset from 17 markers was used for the analyses (Online resources, Table S3). Numbers of alleles, observed and expected frequency of heterozygotes, deviations from Hardy–Weinberg equilibrium, detection of marker linkage-disequilibrium, calculation of F_{ST} and Analysis of Molecular Variance (AMOVA) were calculated by the Arlequin program. The genetic structure was analyzed using the Structure program (Pritchard et al. 2000). For each K from 2 to 6, 100 runs were done with parameters set to: Admixture Model, 10,000 MCMC Reps for the Burn-in Period and 50,000 for the run. Output data was further analyzed by CLUMPAK (Kopelman et al. 2015) to account for possible label-switching, calculate the average solution and frequency of different solutions. The most likely number of genetic clusters (K) solution was selected based on the highest log-likelihood and based on the Evanno-method (Evanno et al. 2005) calculating Delta K values using Structure Harvester (Earl and vonHoldt 2012).

Results

Genetic variation in *S. galilaeus* populations

About 250 samples, representing four locations from Israel and three from Ghana (Table 1; Fig. 1), were analyzed for variation at the mitochondrial DNA (mtDNA) D-loop region. No differences were found among samples from different parts of the Sea of Galilee indicating a single population across the whole lake. Comparing the 1004-bp D-loop sequences across all samples identified 39 polymorphic positions, which constituted 52 haplotypes, 19 in Israeli and 33 in Ghanaian fish (Online resources, Table S2). From the Israeli haplotypes, two that differed by only one nucleotide at position 16,200, were very common and found in 82% of fish from all locations (Fig. 2 and Online resources, Table S2). The rest of the 17 Israeli haplotypes were rare, with frequencies of 4% or less, and differed from the most common haplotype by five bases at most (Online resources, Fig. S1). Genetic diversity (Theta π) was highest (1.34) and lowest (0.33) in Ein Afek spring and Ginosar broodstock fish, respectively (Table 1). The Tajima's D index (neutrality test) was not significant except for the Sea of Galilee population ($D = -1.57$, $P = 0.034$), a finding consistent with the excess of rare variants found there (Fig. 2).

In contrast, the frequency distributions of the 33 haplotypes in populations from Ghana were more even (Fig. 2 and Online resources, Table S2) and the most divergent ones differed by 19 positions (Online resources, Fig. S1). Theta π values were 8.17, 4.94 and 5.06 for Ghana North, South and Southwest, respectively, and significantly ($P < 0.05$) higher than in each of the Israeli populations. Also when locations were combined together (Table 1), the genetic diversity in Israeli fish (0.95) was about eight times, and significantly ($P < 0.05$), lower than in Ghanaian fish (7.91).

To substantiate the D-loop findings, 17 polymorphic microsatellite markers were used to genotype a subset of 96 fish with a focus on Israeli fish (Table 1 and Online resources, Table S3). The proportion of marker pairs that showed signs of linkage disequilibrium (LD) was 0.11, 0.08, 0.10 and 0.06 in Ginosar broodstock, Sea of Galilee, Ein Afek spring and Beit She'an valley streams fish, respectively. However, the LD pairs varied among populations suggesting that the lower polymorphism level explains this observation better than actual genetic linkage between loci. A total of 112 microsatellite alleles were found, yielding an average of 6.6 alleles per marker. Among the Israeli populations, fish from Beit She'an valley streams were the most polymorphic (mean number of alleles = 2.76 and observed heterozygosity = 0.49, Table 1), whereas Sea of Galilee fish were the least (mean number of alleles = 2.35 and observed heterozygosity = 0.39). Combining locations together, fish from Israel were less polymorphic than fish from Ghana, with mean number of alleles of 3.94 vs. 5.59 and observed heterozygosity of 0.42 vs. 0.55, respectively (Table 1). Analysis of Hardy–Weinberg equilibrium (HWE) in Israeli populations identified minor proportions (up to 0.27) of deviating markers, with slightly more negatively than positively deviating markers (Online resources, Table S4). Negative deviations might suggest fewer heterozygotes than expected in Israeli populations.

Taken together, for all metrics of genetic variation and consistently for D-loop and microsatellite polymorphisms, considerably less genetic variation was found in fish from Israel than in fish from Ghana.

The structure of *S. galilaeus* populations

The variation data were further analyzed to characterize genetic differences and population structure. None of the D-loop haplotypes was shared between Israeli and Ghanaian fish and the most similar ones differed from each other by seven sites (Online resources, Fig. S1). For Israeli populations, significant F_{ST} differences ($P < 0.05$), based on both

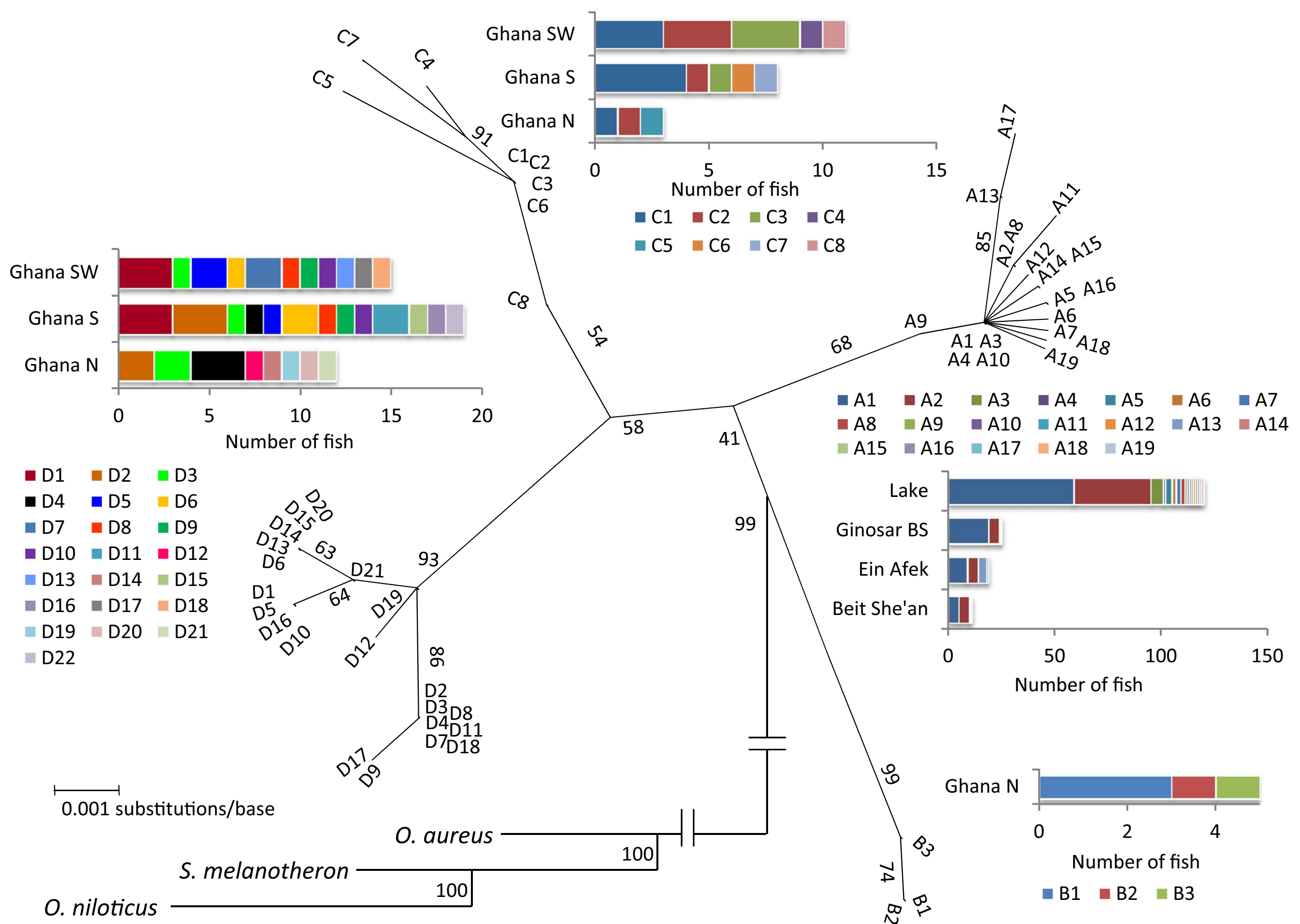

Fig. 2 D-loop gene tree of *S. galilaeus*. Constructed based on 52 *S. galilaeus* D-loop haplotypes from this study and sequences of three outgroup species: *Sarotherodon melanotheron* (GenBank: JF894132.1), *Oreochromis aureus* (GU477630.1) and *Oreochromis niloticus* (GU370126.1). *S. galilaeus* haplotypes clustered into four clades (A–D) and accordingly, haplotype designations are given at the tips of the edges. Percent bootstrap support higher than 50% (out of 1000 randomizations) is shown next to respective bifurcations. Bar graphs show the haplotype count distributions by sampling locations for each clade. Note that the outgroup species branches were artificially truncated to fit into the figure and are not to scale because their divergence from *S. galilaeus* was about four to nine times more than that between *S. galilaeus* clades

Table 2 Differentiation among populations based on D-loop haplotypes (below diagonal) and microsatellites (above diagonal)

	1	2	3	4	5	6	7
1. Sea of Galilee		0.041	0.049**	0.159**	0.310**	0.357**	0.354**
2. Ginosar broodstock	0.010		0.120**	0.179**	0.323**	0.399**	0.385**
3. Beit She'an streams	0.000	0.063		0.058*	0.264**	0.249**	0.292**
4. Ein Afek spring	0.162**	0.148*	0.102		0.321**	0.335**	0.360**
5. Ghana North	0.792**	0.635**	0.538**	0.560**		0.030	0.031
6. Ghana South	0.839**	0.732**	0.665**	0.673**	0.073*		0.063
7. Ghana Southwest	0.832**	0.722**	0.651**	0.660**	0.093*	0.000	

Significance levels (permutation test) are denoted by *($0.05 > P > 0.001$) and **$P < 0.001$. Note that differentiation from Ghanaian populations based on microsatellites should be taken cautiously since sample sizes were small

D-loop haplotypes and microsatellites markers, were found between Ein Afek spring and each of the Sea of Galilee and Ginosar broodstock fish (Table 2), whereas for Ghanaian populations, based on D-loop haplotypes, between North and each of South and Southwest fish (Table 2). Additionally, F_{ST} differences between each pair of Israeli and Ghanaian populations, based on both D-loop haplotypes and microsatellites markers, were much larger and more significant ($P<0.001$) than between populations within each country (Table 2). In the AMOVA analysis, based on microsatellites, 31.3% of the genetic variation was found between Israeli and Ghanaian fish, 5.0% among populations within each country and 65.3% within populations. This allocation of variation corresponds to a significant F_{ST} value of 0.31 between fish from the two countries.

For an overview of the genetic differentiation of *S. galilaeus*, a gene tree was constructed based on D-loop haplotypes with *Sarotherodon melanotheron, Oreochromis aureus* and *O. niloticus* as outgroup sequences (Fig. 2). Four separated clades were formed; one for all Israeli haplotypes (clade A) and three for Ghanaian haplotypes (clades B, C and D). Furthermore, distribution of haplotype frequency by localities was plotted for each clade. Similar distributions (χ^2, $P>0.05$), dominated by the two common haplotypes A1 and A2, were found for all Israeli populations. Two haplotypes, A13 and A17, which differed by one base from each other and by three and four bases from the common Israeli haplotype, respectively, were found only in Ein Afek spring, in 26% of the fish sampled there. For the Ghanaian samples, haplotypes from clades C and D were found in all three populations of the Volta River system (Southwest, South and North). The population from the distant northern sampling point had significantly different D clade haplotypes frequency distribution and only there, in 25% of the fish, B clade haplotypes were found (Fig. 2).

Additionally, the microsatellite data were analyzed for population genetic structure using the Structure assignment algorithm. The solution of three genetic clusters (K = 3) had the highest likelihood. Integrating locality information post-hoc showed that the first genetic division (K = 2) was between Israeli and Ghanaian fish (Fig. 3). Then, for K = 3, additional division in Ghanaian fish occurred in 60% of the runs. In 40% of the K = 3 runs, consistent with the F_{ST} values, further division occurred that incompletely distinguished Ein Afek spring and Beit She'an streams fish from Sea of Galilee and Ginosar broodstock fish. The solution of K = 4 essentially integrated the divisions found in the two K = 3 solutions. Finding a higher fraction of markers deviating from HWE in all Israeli fish combined compared to each population alone (Online resources, Table S4) is consistent with the slight differentiation among Israeli populations found by F_{ST} and Structure analyses.

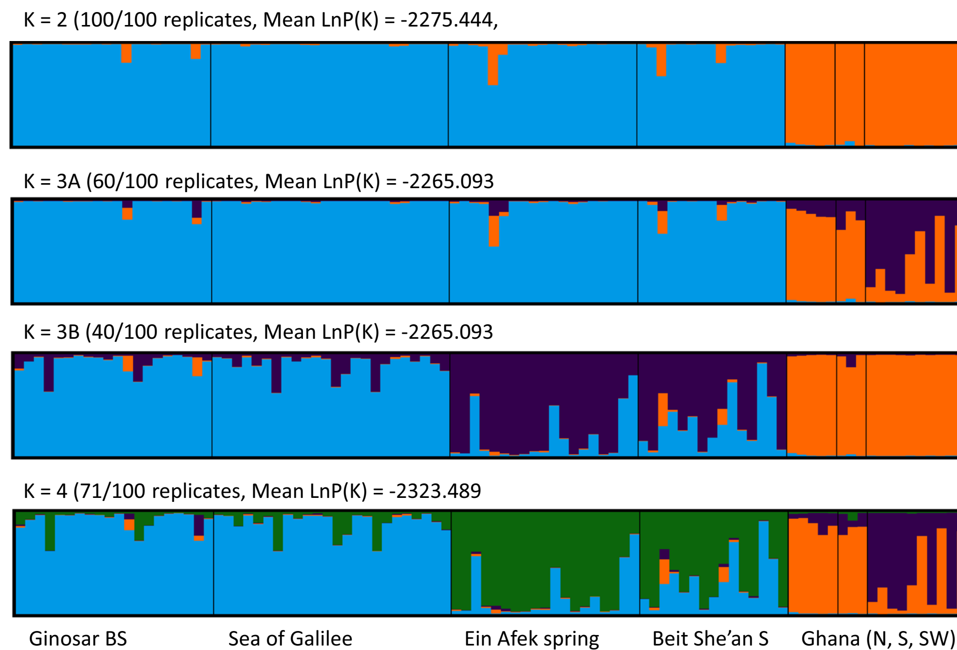

Fig. 3 Genetic structure of *S. galilaeus* gene pool based on microsatellite markers. Genetic clusters are represented by different colors. Shown from top to bottom are the mean solutions of division into K = 2, 3 and 4 clusters. Given above each plot are the number of times this solution repeated out of 100 runs and its mean Ln(likelihood). On the X-axis, each bar represents one individual and the individuals were grouped post-hoc by locations. On the Y-axis, the proportion of the individual's genome that belonged to each of the genetic clusters. Note that while the genetic makeup of some individuals belonged to a single genetic cluster (single color bars), others were admixed in varying proportions (multiple color bars)

Taken together, smaller differentiation was observed among Israeli populations compared to larger differentiation between Israeli and Ghanaian populations.

Genetic variation in old *S. galilaeus* scales

D-loop variation was studied in dry *S. galilaeus* scales archived from 1977 to 1989 and compared to variation in the modern Sea of Galilee population. Due to the type, age and condition of the samples, the extracted DNA allowed PCR amplification and sequencing of merely short fragments from 35 samples, intermittently overlapping positions 15,737–16,050 of the D-loop region (Online resources, Data File S3). Additionally, short sequences overlapping the mtDNA *Cytochrome c oxidase subunit 1* (COI) were obtained from five DNA samples. Aligning these latter sequences to publically available COI sequences of cichlids, ascertained that the archived scales indeed belonged to *S. galilaeus* (Online resources, Data File S2).

Next, the discontinuous D-loop sequence of each archived sample was complemented by a modern sequence to create a 314 bp long alignment of archived and modern haplotypes. From the 35 samples, 18 shared the A1 haplotype with modern samples. The rest 17 archived samples included five haplotypes that were not found in modern ones (Online resources, Data File S3). Genetic diversity in archived samples (Theta $\pi = 1.41$) was considerably higher than in the modern lake fish (Theta $\pi = 0.15$). The Tajima's D index (neutrality test) of archived samples was not significant (0.6, $P = 0.74$). Highly significant F_{ST} value (0.44, $P < 0.001$) and differentiation (exact test, $P < 0.001$) were found between old and modern samples. Therefore, the analyses of archived samples indicated that genetic variation in the lake population had recently reduced considerably.

Discussion

Differentiation patterns among *S. galilaeus* populations

Based on both mtDNA and nuclear variation, two groups of geographically separated Israeli populations were identified, which are slightly yet significantly differentiated. One includes lake and broodstock fish and the other Ein Afek spring and Beit She'an streams fish. This slight differentiation was also hinted at the larger deviation from HWE for all Israeli locations together compared to each location separately. No differentiation could be found between the lake population and the Ginosar broodstock fish, suggesting that either the broodstock has been founded from lake fish or the stock enhancement had made a major contribution to the lake population. Most notably, the Ein Afek spring population had D-loop haplotypes that were not found elsewhere. Ein Afek spring is the only location belonging to the western coastal streams that are physically separated from the eastern locations for an estimated time of 20,000 years (Por 2012).

Among Ghanaian fish, three separated genetic clusters (B, C and D) were found, however, the genetic differences did not overlap with geographic ones. Fish at all three sampling points were a mixture of at least two different ancestral populations represented by haplotypes from clades C and D. Additional Ghanaian ancestry found only in the northern sampling point was represented by clade B fish. Accordingly, significant differentiation was found between the northern and either of the southern locations.

Most differentiation was found between fish from Israel and Ghana at both mtDNA and nuclear polymorphisms. A few cichlid species native to Israel had probably migrated from Africa and inhabited western coastal streams first, and then further migrated to the eastern watershed. Also, it is likely that the population of origin was from the northeastern part of Africa rather than from the Volta river system (Goren and Ortal 1999; Werner and Mokady 2004; Por 2012). Nonetheless, common ancestry of Israeli and Ghanaian fish could be assumed because most of the individual D-loop variants were shared and thus, likely to be ancestral. These ancestral variants include also the variants that differentiate the Ein Afek spring population from the other eastern Israeli fish, supporting the hypothesized migration route. Given this common ancestry, the differentiation found between Israeli and Ghanaian fish had probably resulted from a combination of possible processes following separation like, genetic drift, adaptation to local conditions and emergence of new variants.

S. galilaeus populations in Israel have low genetic variation

The clearest and pivotal finding of this study is the low level of genetic variation found in *S. galilaeus* in Israel. This finding was consistent across all metrics analyzed by two independently inherited types of polymorphisms and holds true for all samples together as much as for each population alone.

Since most D-loop variants found in Israeli fish are ancestral, finding less such variants suggested that a founder effect and genetic drift might have reduced the variation of the Israeli populations. This scenario is supported also by the ancestral variants found in Ein Afek spring but not in the eastern populations. In addition, given that Israel is at the northern boundary of the species' distribution and that African cichlids are generally sensitive to colder temperatures (Trewavas 1983; Chakrabarty 2004), this and other environmental conditions might have imposed selection

on the colonizing founders, reducing the genetic variation of this population further. The Tajima's D neutrality test indicated an excess of rare variants, a situation consistent with the beginning of recovery from past genetic bottleneck or founder effect. Taken together, historical factors, including the carrying capacity limitation on population size, the likely founder effect and local adaptations have all likely contributed to the low level of genetic variation seen today in Israeli fish.

Further and recent reduction in genetic variation of the lake population

Finding only subtle genetic differences among fish from different locations in Israel suggested that their genetic makeup and low variation are mostly ancestral. However, the recent decline in the lake population size suggests that the low genetic variation observed today is a result not only of the historical processes discussed so far. Inferences on temporal changes in biological attributes of populations are mainly made based on models and simulations, but archives of old biological materials, when available, offer the advantage of empirically analyzing temporal changes (Purcell et al. 1996; Shaffer et al. 1998; Hutchinson et al. 2003; Wandeler et al. 2007). Here, the past genetic variation was empirically analyzed in archived scales of lake fish. Comparing diversity based on D-loop haplotypes revealed that there used to be significantly more genetic variation in the lake population 30–40 years ago. Therefore, there happened further, recent reduction in genetic variation of *S. galilaeus* in the Sea of Galilee. It is hard to determine whether the recent reduction in population size slightly predated the reduction in genetic variation, vice versa, or if both reductions co-occurred. Nevertheless, the recent decline is consistent with a genetic bottleneck effect (Nei et al. 1975; Bouzat 2010) occurring on the background of a population with already-limited genetic variation.

Conclusions

The small, isolated populations inhabiting the Sea of Galilee lives under unique conditions, and thus, various pressures could risk their sustainability. Following the sudden decline in commercial catch of *S. galilaeus* from the lake, studying the genetic variation of this species in Israel with reference to fish from Ghana provided the following model to explain the observations (Fig. 4). A population was founded in Israel by migrants from Africa, containing a subset of the genetic variation compared to the original

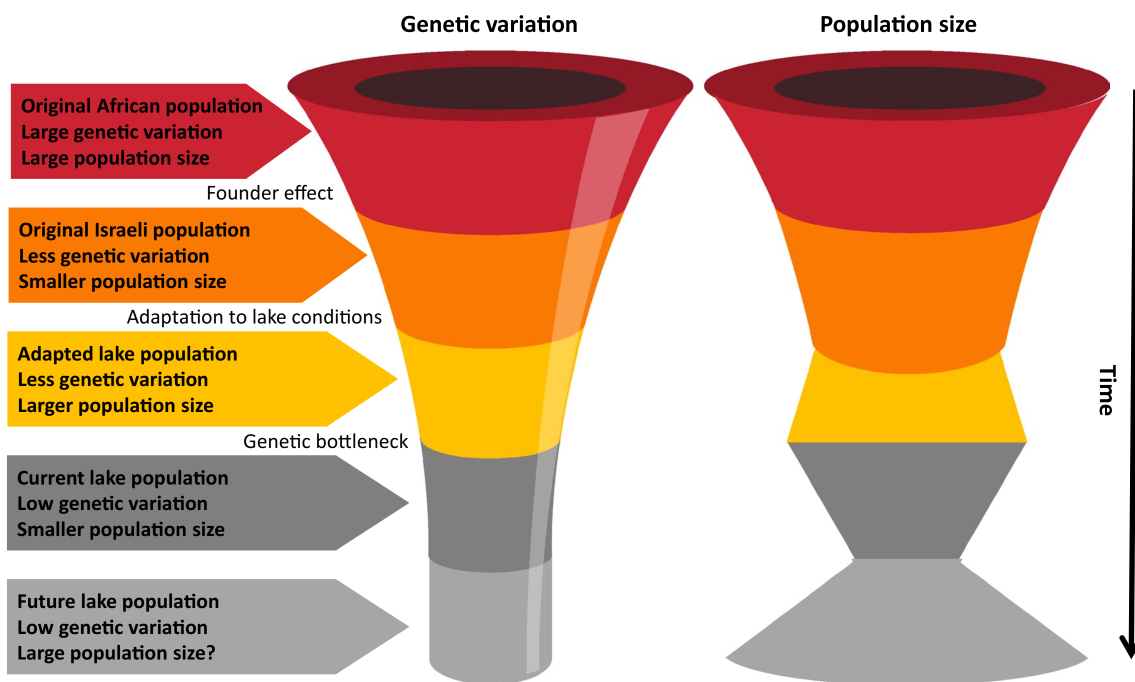

Fig. 4 A model for the timeline and processes affecting genetic variation and population size of *S. galilaeus* in the Sea of Galilee. The original lake population was founded by fish that migrated from the African population of origin (resembling the Ghanaian fish). In the process, the population experienced a founder effect and possibly also adaptation to the conditions in the Sea of Galilee. The founder effect reduced both genetic variation and population size, however, the adaptation, while further reducing genetic variation had probably allowed expansion in population size. The recent genetic bottleneck reduced further both genetic variation and population size. While population size could possibly recover in the future, genetic variation will remain restricted to what was found today for a long time

population. This founder effect has probably been followed by a stage of adaptation to the new habitat, which also reduced genetic variation. The species progressively colonized different places and habitats, expanding in numbers, but not so much in genetic variation. This historically limited variation may have rendered the lake population more vulnerable, and due to one or more changes in conditions which are still not fully understood, the population recently went through a genetic bottleneck that reduced further its genetic variation and increased further population vulnerability.

This study is stressing the importance of monitoring population size and genetic variation for the sake of fish communities sustainability (O'Brien 1994; Schwartz et al. 2007; Bouzat 2010; Frankham et al. 2014). The recent reduction in genetic variation is alarming because even if population size will revert to its former state of 30 years ago, the low genetic variation will remain much like it is today for many centuries ahead (Ryman and Laikre 1991), as will the vulnerability of the population to future changes. Greater enhancement of the lake population by fry of the available broodstock fish might increase the population size but not its genetic variation. Enhancement of genetic variation by fish from other places should be very cautiously considered due to the unique conditions and sensitive ecological balance of this habitat. Therefore, as exemplified here, the tools to manage the sustainability of isolated populations living in unique habitats are limited, stressing even more the importance of monitoring actual population size and genetic variation in order to have the information in time and place so that management actions would be taken to actively prevent the next crisis and support the recovery of this unique population.

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Data accessibility D-loop sequences by haplotype names are given in Online resources (Data File S1) and were deposited in the GenBank database under the accession numbers: KY940659-KY940710. Alignment of sequences from old scales to publically available COI sequences and to D-loop haplotypes are given in Online resources (Data File S2 and S3, respectively). Tables containing D-loop haplotype frequency and microsatellite genotypes by fish and population are given in Online resources (Tables S2 and S3, respectively).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Fish sampling was done under special permits 2012/38733 and 2014/40233 for sampling protected wildlife as reviewed and approved by the Israel Nature and National Parks Protection Authority.

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