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Research article

Anthropogenic activities along the Lake Nyasa catchments alter the habitat and genetic diversity of a Lake Salmon, *Opsaridium microlepis*

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ABSTRACT

The Lake Salmon, *Opsaridium microlepis* is an economically important fish along the Lake Nyasa. However, the species is under threat of extinction due to anthropogenic activities such as agriculture, mining, urbanization, and deforestation. Consequently, the fish species is currently regarded as a threatened species, and the International Union for Conservation of Nature (IUCN) has red-listed the species due to an apparent decline in abundance. The current study assesses the potential impact of human activities on the genetic diversity of *O. microlepis* using partial mitochondrial cytochrome oxidase subunit I (COI) sequences and microsatellite loci. The results indicate that genetic diversity is lower in the areas affected by human activities compared to relatively pristine areas. The results from this study may suggest that human activities taking place in the catchments are likely to contribute to the alteration of the genetic diversity of *O. microlepis* species. Thus, immediate measure is required to control anthropogenic activities in the areas to protect the species and other aquatic organisms from possible threats of extinction.

1. Introduction

Lake Nyasa, the third biggest lake in Africa, has more fish species than any other lake in the world [1] and is a major source of protein for local consumption. Ruhuhu River is the major river that discharges into Lake Nyasa and contributes about 20 % of the annual inflow of the Lake. Historically, the Lake Nyasa has supported the local communities along their shores line through fishery resources. However, the catchments of Lake Nyasa have been subjected to high pressure originating from severe environmental degradation since the 1980s [2].

Changes in land use caused by population growth in areas surrounding the lake may result in a considerable increase of nutrients entering the lake. Most of the people along Lake Nyasa rely on subsistence agriculture for food. Consequently, the increased human population has resulted in the expansion of subsistence agriculture to marginal, including wetlands and steep hill slopes that can lead to accelerated soil erosion and increase nutrients. The increased nutrients can also originate from the burning of biomass due to hunting and preparation of fields for cultivation [3]. Similarly, increasing soil exposure to wind erosion resulted from land deforestation for agriculture and overgrazing increases nutrient inputs. The effect of accelerated soil erosion can lead to increased water

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turbidity, which is reported to cause biodiversity loss among aquatic species [4].

The *Opsaridium microlepis* is one of the river-spawning cyprinids in the Lake Nyasa catchment [5,6]. Other species include *O. microcephalum* and *O. tweddleorum* [5]. *Opsaridium microlepis* and *O. microcephalum* are endemic to the Lake Nyasa catchment. However, *O. tweddleorum* is not endemic to the Lake Nyasa catchment because it is also found in tributaries of the Zambezi outside the Lake Nyasa catchment [6]. The genus *Opsaridium* is not a monophyletic group, but comprises members of the genus *Raiamas* from the broader Congo catchment, encompassing Lake Rukwa and Lake Tanganyika. The *O. microlepis* has been reported to be a sister taxon of *O. loveridgii* from the Rufiji-Ruaha basin, *O. microcephalum*, a sister taxon of *O. peringueyi* found in the eastward flowing rivers of southern Africa south of the Zambezi, and *O. tweddleorum*, a sister taxon of *O. leleupi* from the Congo [5].

The economically valuable Lake Salomon fish *O. microlepis* spawns in the river, and the fry stays near the river mouth for an extended period after reaching the Lake until moving offshore in their second year at a length of about 150–200 mm [6,7]. The spawning runs of *O. microlepis* occur during and after rainfall, with a peak in breeding activity around May–June. They spawn over a gravel substratum, often in very shallow water, during the night and early morning [7]. The population of *O. microlepis* has been dramatically declining and the *International Union for Conservation of Nature* (*IUCN*) has red-listed it as a vulnerable species [8]. The species' abundance is predicted to decrease by 30 % in ten years due to increased overfishing, continuous habitat degradation and habitat loss brought on by siltation from soil erosion, water abstraction, and farming activities [8]. Thus, the investigation of the impact of the human activities on *O. microlepis* is highly required. The assessment of genetic variation in fish species is significant because it allows researchers to understand better their ability to adapt to changing environments, which is critical for their survival [9]. Genetics has an indirect role in analysing population dynamics or ecological processes, and it can directly influence the outcome of restoration processes [10].

Estimates of genetic diversity are useful in anticipating decreases in fish species due to fishing pressures because population declines may decrease genetic diversity, which is crucial for adaptive capacity to current and future environmental changes [11]. Determining the effective population size (Ne) is an important parameter in conserving genetic diversity [12]. The potential impacts of illegal farming on the nucleotide diversity, haplotype diversity and heterozygosities of *O. microlepis* along the Lake Nyasa are reported in the current study. Additionally, the impact of farming activities on the effective population size of *O. microlepis* in the area was also investigated.

2. Material and method

2.1. Sampling

Sampling was conducted along the shores of Lake Nyasa between February and November 2020. Samples of *Opsaridium microlepis* and sediments were collected at six (6) sites (Fig. 1) which included 1. Kafyofyo, 2. Buloma, 3. Ruhuhu 4. Katumba, 5. Lituhi, and 6. Mbambabay. Three sites were affected by human activities through farming activities (Fig. 2a and b) and the other three sites were relatively pristine, with no sign of farming activities. Twenty samples of *O. microlepis* were collected from each site for molecular analysis. Fish samples were obtained at sites 1–3 during the dry season and at sites 4–6 during the wet season. This is because *O.*

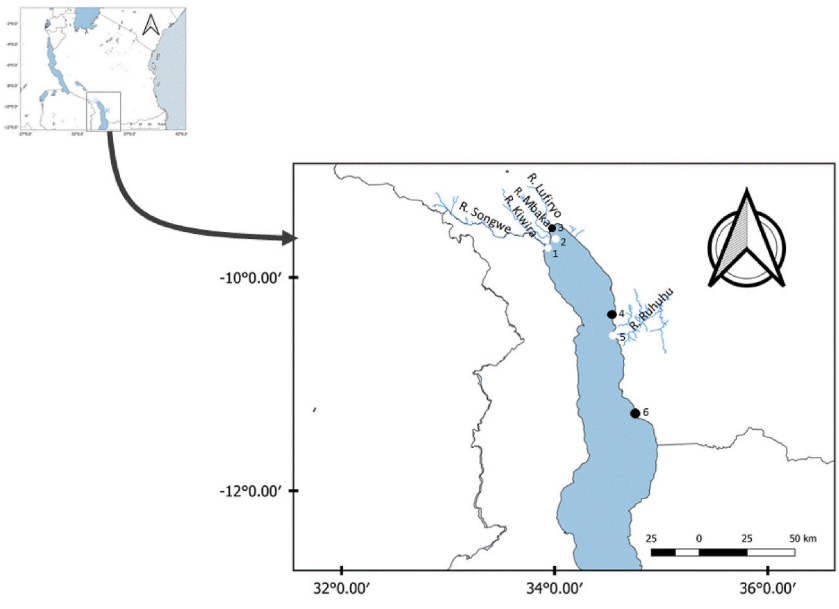

Fig. 1. Sampling sites of the *Opsaridium microlepis* along the Lake Nyasa catchments-Tanzania: black circles represent sites affected by farming activities, while the white circles represent relatively pristine sites.

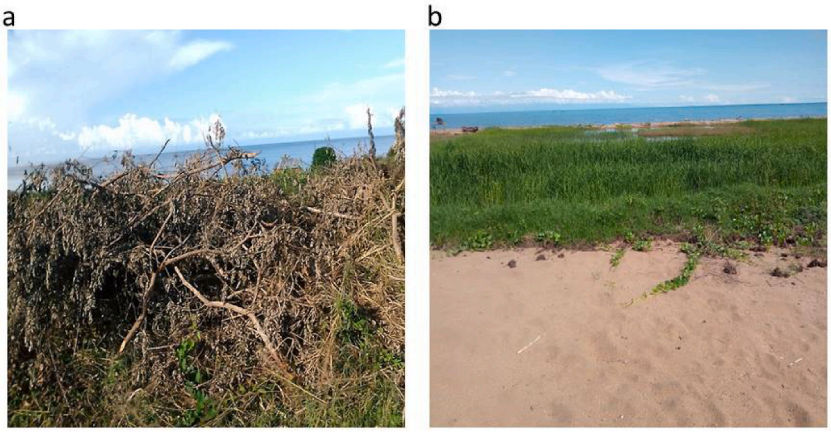

Fig. 2. a. trees cut down to prepare the land for farming and b. area planted rice along the Lake Nyasa catchments-Tanzania.

microlepis species usually is available in the northern part of the Lake (Mbeya region) during the dry season and in the southern part (Ruvuma region) during the wet season. The *O. microlepis* samples were collected in the Lake near the inflowing rivers and water distributaries. Sediment samples and data for water parameters were collected during the wet season. The northern part of the Lake is influenced by the inflows of the rivers Songwe, Kiwira, Mbaka, and Lufiryo. The River Ruhuhu and other small water distributaries influence the southern part of Lake Nyasa in Tanzania (Fig. 1). Samples of sediments were collected in triplicates and preserved in a cooler box at 4 °C for transportation to the laboratory for analysis.

2.2. Nutrient analysis

Nutrient characteristics and nutrient load estimates from the catchment areas to Lake Nyasa were conducted through an extensive field survey and an analysis of water and sediment physical-chemical characteristics. The water parameters such as pH, Electrical conductivity (EC), and Total Dissolved Salts (TDS) were measured on-site using a portable pH and conductivity meter. The total phosphorus (TP), nitrogen (TN), and organic carbon (TOC) were determined as described in the subsequent sections.

2.3. Total phosphorus (TP)

The sediment sample (2 g) was weighed and dissolved in 60 mL of a mixture of HCl and HNO_3 in a conical flask with the help of a mechanical shaker for about 7 h. The resultant mixture was filtered through a filter paper Whatman 41. The filtrate (15 mL) was then mixed with 3 mL of ammonium molybdate, and 2 mL of hydrazine sulphate and kept in a water bath for 30 min. A blue color developed by forming the posphomolydbenum complex was measured using a spectrophotometer at 830 nm [13].

2.4. Total nitrogen (TN)

The total nitrogen was determined using a modified Kjeldahl method (ISO 1995b) for extraction as previously described by others [14]. The sediment sample (0.5 g) was boiled at an extremely high temperature to produce the ammonium sulphate solution. The extracted aqueous solution was measured for TN using a calorimetric measurement at 660 n.

2.5. Total organic carbon (TOC)

The total organic carbon in the sediments was determined using the previously reported Walkley-Black procedures [15]. Potassium dichromate ($K_2Cr_2O_2$) and concentrated H_2SO_4 were added to a sediment sample (0.5 g) and the resulting mixture was gently boiled at 150 °C to oxidize organic matter. The excess $Cr_2O_7^{2}$ in the solution was then titrated against a standard ferrous ammonium sulphate solution.

2.6. DNA extraction

Tissue samples from the right fins of *Opsaridium microlepis* were collected and immediately preserved in 99.9 % ethanol. About 30 mg of tissue was taken from the parts of the fins collected and DNA was extracted following the protocol of Quick-DNA™ Miniprep Plus Kit (ZYMO Research). The extracted DNA was visualized in 2 % TBE agarose gels.

2.7. Mitochondrial DNA (mtDNA) analysis

Using the forward primer FishF1: 5′-TCAACCAACCACAAAGACAT TGGCAC-3′ and the reverse primer FishR2: 5′-ACTT CAGGGT-GACCGAAGAATCAGAA-3′ [16], a segment of the COI gene containing 629 base pairs was amplified in a T100™ Thermal cycler device (Bio-Lab Inc, GA, USA). The polymerase chain reactions (PCR) were carried out in a total volume of 35 μL, which contained 2 μL of the DNA template, 1x Multiplex PCR Master Mix, 0.4 mg of BSA, 0.3 μM of each primer, and 11.7 μL of water (RNAse free). The temperature profile consisted of an initial temperature of 94 °C for 5 min, 35 cycles of 94 °C for 40 s, 54 °C for 45 s, 72 °C for 1 min, and a final extension of 72 °C for 15 min. Using the FishF1 and FishR2 primers, the PCR products were Sanger sequenced at the Macrogen Europe Laboratory using an automated sequencer (AB 3730XL; Applied Biosystems, Foster City, USA). The sequences with bad quality were removed from the dataset. The remaining sequences were first edited by trimming the ends with MEGA 11 software, and the validation of the species identity was carried out with the help of the online program BLAST [17]. After being edited and the unreadable portions removed, the sequence's final length was 629 bp. The accession numbers for the sequences in GenBank are OQ318303-OQ318401. The sequences were converted into amino acids using MEGA 11 software [18]. This step was carried out to check for internal stop codons, which can be an indication of sequencing errors or false genes. The multiple alignments of the sequences were conducted by using CLUSTAL W [19] as implemented in the software MEGA 11. The sequences were collapsed into haplotypes by using the online FaBox 1.41. A minimum spanning haplotype network relationship of the partial mitochondrial cytochrome oxidase subunit I (COI) sequences from the *Opsaridium microlepis* was constructed by using the software Pop Art v.1.7 [20]. The software Arlequin v. 3.5.2.2 was used to calculate genetic diversity, historical demography, and neutrality parameters [21]. The within and among population differentiation was investigated by analysis of molecular variance (AMOVA) using the same software. Hierarchical AMOVA was used to determine whether catchment degradation resulted in limited or no gene flow among the subpopulations. The amplification success of sequence sample was evaluated on a 1 % agarose gel.

2.8. Microsatellites genotyping

PCR reactions were conducted in a multiplex of nine previously published microsatellite loci of the same fish species [22]. The forward primer was labelled with a fluorescent dye (Table 1). A total volume of 12 μl, containing 1 μM of Multiplex PCR 1x Master Mix, 0.4 μM of the primer mix, and 2.4 μl of DNA extracts were used to amplify the microsatellite loci. The PCR conditions were; annealing temperature 58 °C for 90 s, initial denaturation 95 °C for 5 min, denaturation 94 °C for 1 min for 35 cycles, 72 °C for 60 s, and final extension 15 min at 72 °C. Amplification success was checked on 1 % agarose gel. One sample from Kafyofyo and one from Mbambay were not successfully amplified and were thus excluded from the final analysis. The PCR products were diluted 80 times and analysed on an ABI 3730 DNA Analyser (Applied Biosystems) with a 50 cm capillary to measure the fragment size of the different microsatellite loci.

The alleles were scored manually with GeneMarker (2.4.0; SoftGenetics, State College, PA, USA). The software MICRO-CHECKER v. 2.23 [23] was used to test for the presence of null alleles, large allele dropout, or scoring errors. The software GenAlEx v. 6.5 [24] was used to estimate heterozygosities, inbreeding coefficient and test for each locus's departure from Hardy Weinberg equilibrium (HWE). The heterozygosity excess was tested for all populations using the BOTTLENECK 1.2.02 program [25] to examine possible genetic bottleneck signatures. A two-phase mutation model (TPM) was assumed, with 95 % single-step mutations, 5 % multiple-step mutations, and a variance of 12 among multiple steps. The Wilcoxon's test was used to establish the significance of heterozygosity excess across all loci. The program MIGRATE v. 3.11.6 [26] was used to determine the effect of degradation of catchment areas on the population size of fish along Lake Nyasa. After initial testing, estimates of mutation-scaled effective population size Θ ($\Theta = 4Ne\mu$) and

Table 1
Characteristics of primers used for microsatellites analysis in *Opsaridium microlepis* from Tanzania, Lake Nyasa.

Locus	Primer sequence 5' - 3′	Repeat motif	Fluorescent dye	Size range (bp)	Size range (bp) in this study
Ops3	F: ATGTTGCATGAAGCACCTG R: GGTTCGAAACGATATGAGGGTC	(AAC)11	6-FAM	396–430	393–420
Ops4	F: TCAGCAATGCATCACCCTG R: GGTTTGGACAACAACCATGC	(ATT)12	CYANINE3	273–303	247–303
Ops5	F: AATGTGTGCAGCGCTCAAG R: CAAGTTAAGGCTGCTCTGGG	(GAT)12	ROX	413–445	483–513
Ops7	F: TCATTTCTCTTGGGATTGTTTGG R: AGACACTGCTGAAGGACCG	(ATT)11	CYANINE3	385–400	333–351
Ops9	F: AGCATAGTGGACAGCTCAGT R: AGGGATCAAGAGTGTGCCTT	(ATT)13	6-FAM	270–287	273–285
Ops11	F: GGGCTGAATGACGCTCTTAA R:AGCAGCTCGACCAAACTAGT	(ATC)21	HEX	249–285	249–276
Ops16	F: GAAATGGAGTGTCATTTAGCATTAC R: CCGCTCCAATCTGGATTCAAC	(AGAT)11	HEX	313–373	321–363
Ops19	F: CATTCACACCGTTCCTCTGC R: TGTGCCCTGATAAAAGCTGC	(ATCT)11	ROX	241–277	249–279
Ops22	F: TCCGGGCCACTGAAATAGAC R:AGGAATAACACAAATTATGGGACAG	(GATT)8	HEX	384–410	382–412

Source [22].

mutation-scaled migration rates M (M = m/μ) were calculated using a Bayesian search strategy and a Brownian motion microsatellite model. The values of the parameters Θ and M were generated by F_{ST} calculation as starting parameter values with constant mutation rates for all loci. Ne represents the effective population size and μ the mutation rate per generation per locus [27]. The Metropolis sampling algorithm was used to sample from the prior distributions and generate posterior distributions. Exponential prior distribution was applied to estimate Θ (range = 0–50) and *M* (range = 0–400).A lengthy chain was set up to visit 2,500,000 parameter values with a burn-in of 100,000 for each locus and record 50,000 genealogies with a sampling increment of 50. A static heating scheme was employed, with 4 chains and temperatures set at 1, 1.5, 3 and 106.

2.9. Data analysis for environmental variables and comparisons between the habitat types

Collected data from the sediments were initially examined for normality and variance homogeneity. The data were then analysed for variations between samples. Shapiro-Wilk test was used to check for normality. Levene's test was performed to determine whether the variances were homogeneous. Both tests were carried out using R software (version 3.1.2). To increase variance homogeneity, the TN, TP, and TOC data were processed using the log (-x) function. The Wilcoxon signed-rank test was applied to data that did not meet the requirements for the parametric statistics test and the student t-test was applied to data that met the condition of the parametric statistics test as implemented in R (version 3.1.2). Wilcoxon signed rank was used to compare differences in TN, TP, and TOC between samples taken from degraded and relatively pristine catchments. A multivariate analysis using principal component analysis (PCA) was carried out through PRIMER 6 v. 2 software [28] to assess relationships between environmental variables (TN, TP, and TOC) and the habitat categories where samples were gathered. The environmental variables were normalized before the PCA. The student t-test was used to compare differences in nucleotide and haplotype diversity, observed and unbiased heterozygosity, and mutational-effective population size between degraded and relatively pristine samples. Before conducting t-tests, all dependent variables were subjected to Shapiro-Wilk, Levene's, and Fisher's F-tests to determine their normality and homogeneity of variance, respectively.

3. Results

3.1. Water and sediment assessments

Water quality parameters indicated that Mbambay (MB) and Lituhi (LH) had higher pH, Electrical Conductivity (E.C), and Total Dissolved Salts (TDS) compared to Kafyofyo (KF), Buloma (BL), Katumba (KT) and Ruhuhu (RH) (Table 2).

The results of the sediment assessment indicated that the degraded sites contained higher amounts of phosphate compared to relatively pristine sites (Fig. 3). Similarly, the degraded sites, Katumba, Lituhi, and Mbambabay, have higher total nitrogen compared to relatively pristine sites. The results indicate the potential discharge of sewage and manure into the degraded sites.

The Principal Component Analysis (PCA) also indicates that the sediments from degraded catchments are positively associated with concentrations of total phosphorus (TP), total nitrogen (TN), and negatively associated with total organic carbon (TOC), and the opposite is observed for sediments from relatively pristine catchments. The proportion of variance that explained by the first two principal components (PCA) was approximately 90 %. The variables with the highest loadings in PC1 accounted for about 60.9 % of the total variance with eigenvalues of 1.83 and PC2 accounted for about 28 % of the total variance with eigenvalues of 0.841 (Fig. 4).

3.2. Haplotypes and nucleotide diversities

The range of the mean haplotype diversity was 0.50–0.78, and the range of the nucleotide diversity was 0.08–0.19 % (Fig. 5a and b). The haplotypes and nucleotide diversities were consistently lower in degraded catchments compared to relatively pristine catchments. The haplotype number was 9, with fewer singleton haplotypes in degraded catchments compared to relatively pristine catchments (Table 3). The rare haplotypes in the minimum spanning network have only one mutational step from the central haplotype, forming a star-like structure (Fig. 6). The number of haplotypes at different sites ranged from two to six, with degraded sites having lower haplotypes compared to pristine sites. The student t-test revealed significant differences in nucleotide diversity (P = 0.01) and haplotype diversity (P = 0.02) between degraded and pristine sites.

Table 2
pH, Electronic conductivity (E.C), and Total Dissolved Solutes (TDS) along the Lake Nyasa catchments.

Sampling site	Code	pH	E.C (μS/cm)	TDS (ppm)
Kafyofo	KF	8.04 ± 0.01	82 ± 1.53	46 ± 2.52
Buloma	BL	8.06 ± 0.07	86 ± 2.52	54 ± 3.51
Ruhuhu	RH	7.75 ± 0.08	80 ± 1.53	40 ± 1.00
Katumba	KT	8.06 ± 0.04	84 ± 3.00	45 ± 1.53
Lituhi	LT	8.15 ± 0.11	144 ± 1.53	122 ± 2.00
Mbambabay	MB	8.80 ± 0.06	250 ± 4.51	261 ± 0.58

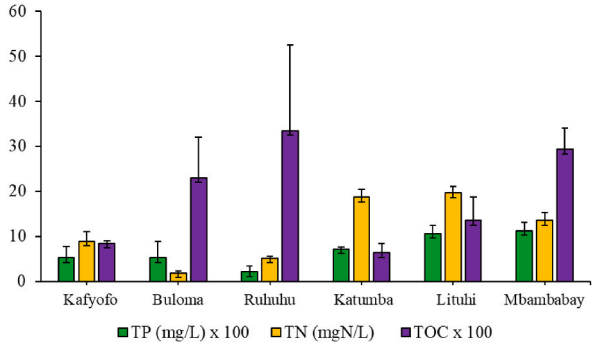

Fig. 3. Total phosphorus (TP), total nitrogen (TN), and total organic carbon (TOC) parameters along the Lake Nyasa catchments. Kafyo, Buloma and Ruhuhu represent relatively pristine sites and Katumba, Lituhi and Mbambabay represent degraded sites.

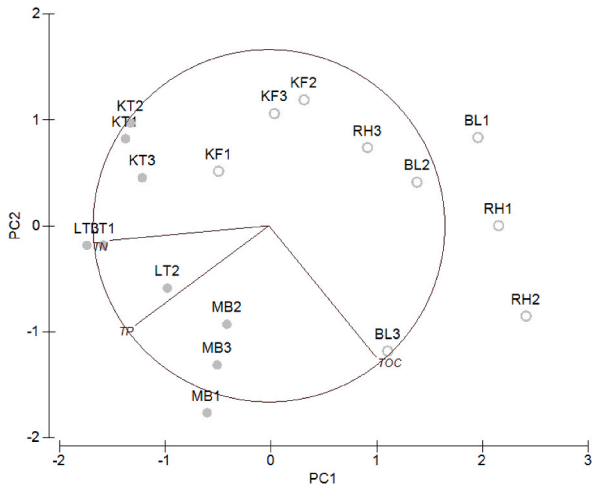

Fig. 4. Principal component analysis (PCA) showing the relationship between Total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) from sediment samples collected in the degraded and relatively pristine catchment sites along the Lake Nyasa. Samples collected from degraded catchments are represented by filled grey circles and samples collected from relatively pristine catchments are represented by open circles (The letters with numeric values represent the codes for the sites where samples were collected. The abbreviations for the codes are provided in Table 1. Each vector represents the loadings of variables (TN, TP, and TOC) in each principal component.

3.3. Heterozygosity diversities

This study also revealed that allele sizes varied from 247 to 513bp, with the highest being identified in Ops5c loci and the lowest in Ops4^{c} loci (Table 1). The mean observed heterozygosity for all populations was 0.76 ± 0.05, while the unbiased expected heterozygosity was 0.65 ± 0.04. The within-population inbreeding coefficients ranged from −0.81 to 0.57, but none was statistically significant. The overall number of alleles per locus varied from 2 to 13, with OPS11 having the highest and OPS7 having the lowest. For all groups, the locus OPS11 deviated from HWE. Other scoring errors, including stuttering and large allele dropout among the loci, were not observed. Compared to relatively pristine catchments, the amount of observed and expected heterozygosity in degraded catchments was consistently low. The highest observed heterozygosity was found in the relatively pristine sites, whereas the lowest was found in the degraded sites. Expected heterozygosity was highest in the relatively pristine Kafyofyo catchment and lowest in the degraded Mbababay catchment (Table 4).

The test performed in the software BOTTLENECK resulted in significant heterozygosity excess for Lituhi 0.68 ($p < 0.05$) and Katumba 0.67 ($p < 0.05$). The other populations exhibited no significant heterozygosity excess. The populations from degraded sites showed consistently smaller mutational-scaled effective population sizes compared to populations from relatively pristine sites. The mean mutation–scaled effective population size for samples collected in degraded sites ranged from 0.726 to 1.160 and the overall mean was 0.949. The mean mutation–scaled effective population size for samples collected in relatively pristine sites ranged from 1.633 to 3.357 with an overall mean of 3.499. The 97.5 % confidence interval mutation-scaled effective population size for samples obtained in degraded sites ranged from 0.933 to 2.400, with an overall value of 1.611. However, the 97.5 % confidence interval mutation-scaled effective population size for samples obtained in relatively pristine sites ranged from 2.800 to 3.033, with an overall 2.644.

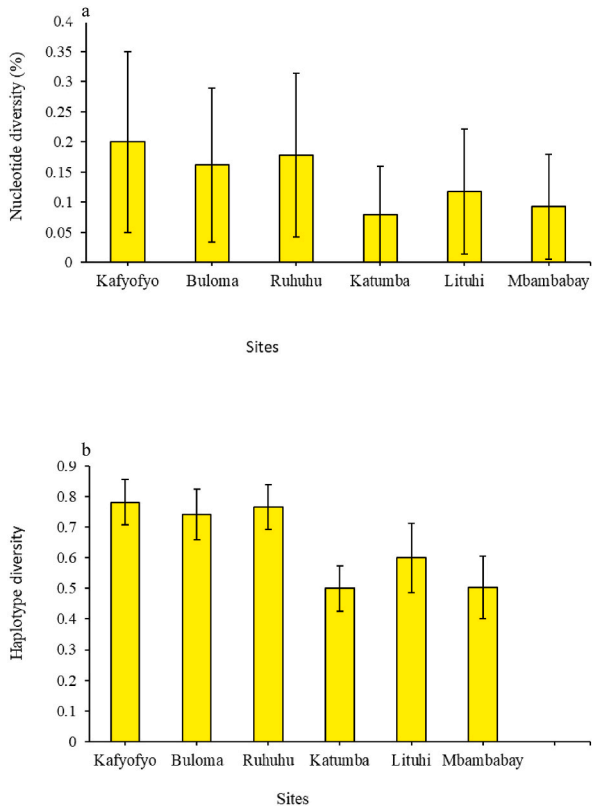

Fig. 5. Genetic diversity of *Opsaridium microlepis* from Lake Nyasa catchments in Tanzania. a: Nucleotide diversity and b: haplotype diversity. Kafyofyo, Buloma and Ruhuhu represent relatively pristine sites and Katumba, Lituhi, and Mbambabay represent degraded sites.

Table 3
The haplotype distribution of *Opsaridium microlepis's* cytochrome oxidase subunit I (COI) at Lake Nyasa in Tanzania; N is the number of samples, and Nh is the total number of haplotypes at each site.

		Haplotype									
Codes	N	1	2	3	4	5	6	7	8	9	Nh
KF	15	4	6	2	1	2					5
BL	16	5	1	1		7	1	1			6
RH	19	5	2			8	1		1	2	6
KT	16	6	10								2
LT	15	9	1	1		4					4
MB	18	5	12				1				3
Total	118	34	32	4	1	22	3	1	1	2	26

3.4. Genetic population structure and demographic history

The analysis of molecular variances (AMOVA) of the COI sequences revealed population differentiation (Overall $\Phi_{st} = 0.09$, $P < 0.001$). The AMOVA of the microsatellites also showed population differentiation ($F_{st} = 0.018$, $P < 0.001$). The hierarchical (AMOVA) analysis did not show any genetic differentiation between samples from relatively pristine and those from degraded sites for COI sequences ($\Phi_{ct} = 0.11$, $P = 0.2$) and for microsatellites ($F_{ct} = 0.02$, $P = 0.11$). After performing sequential Bonferroni correction, the majority of pairwise Φ_{st} and F_{st}-values including samples from degraded sites and relatively pristine were significantly different (Table 5).

Tajima's D values were negative for samples from Buloma, Lituhi, and Ruhuhu, but positive without being significant for samples from other sites. Only significant Fu's Fs test values were found for samples from Buloma; all other samples, except those from Katumba show negative Fu's Fs test results. The Rogers test and mismatch distribution analysis validated the theory of sudden population expansion (Table 6).

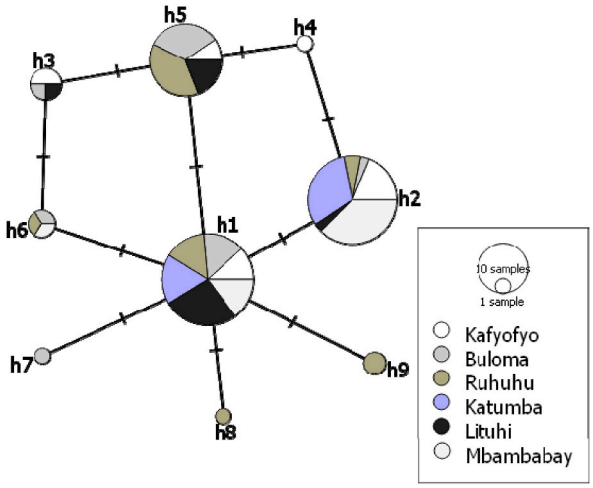

Fig. 6. A minimum spanning haplotype network of the partial mitochondrial cytochrome oxidase subunit I (COI) sequences from the *Opsaridium microlepis* in Tanzania, Lake Nyasa.

Table 4
Genetic diversity in *Opsaridium microlepis* from Tanzania, Lake Nyasa. N: number of samples; Na: number of different alleles; Ae: mean effective number of alleles; I: Shannon's information index; Ho: observed heterozygosity; uHe: unbiased expected heterozygosity; F_{is}: inbreeding coefficient.

Code	N	Na	Ae	I	Ho	uHe	F_{is}
KF	19	6.89	4.05	1.49	0.79	0.71	−0.14
BL	20	5.11	3.31	1.27	0.82	0.66	−0.27
RH	20	5.78	3.30	1.32	0.82	0.67	−0.27
KT	20	5.67	3.17	1.21	0.71	0.61	−0.17
LT	20	5.00	2.95	1.19	0.72	0.62	−0.17
MB	19	4.44	3.03	1.11	0.71	0.60	−0.22

Table 5
Pairwise Φ_{st}-values for cytochrome oxidase subunit I (COI) sequence data (above diagonal) and F_{st}-values for microsatellites (below diagonal) in the *Opsaridium microlepis* from Tanzania, Lake Nyasa. Highlighted numbers with * indicate significance value.

CODE	KF	BL	RH	KT	LT	MB
KF	-	0.097	0.070	0.102	0.089	0.104
BL	0.010	-	-0.030	0.415*	-0.026	0.424*
RH	0.026*	0.018	-	0.031*	0.013	0.348
KT	-0.006	0.009	0.331*	-	0.386*	-0.053
LT	0.035*	0.047*	-0.037	0.022*	-	0.397*
MB	0.004	0.022*	0.014	0.001	0.006	-

4. Discussion

4.1. Sediments and water quality

The average electrical conductivity and total dissolved salts in degraded sites were 159 ± 72.67 μS/cm and 142 ± 94.53 ppm, respectively. The average electrical conductivity and total dissolved salts for the relative pristine were 82 ± 3.19 μS/cm and 47 ± 6.63 ppm, respectively. These results indicate that the degraded sites have elevated electrical conductivity and total dissolved salts

Table 6
Demographic and neutrality parameters based on cytochrome oxidase I (COI) sequences from the *Opsaridium microlepis* from Tanzania, Lake Nyasa. SSD: sum of squared deviations, HRI: Harpending's raggedness index, D: Tajima's D, and Fs: Fu's Fs. * indicate significance value.

Codes	SSD	HRI	D	Fs
KF	0.006	0.100	1.078	−0.950
BL	0.022	0.160	−0.490	−2.625*
RH	0.017	0.161	−0.670	−2.000
KT	0.022	0.250	1.309	1.247
LT	0.013	0.135	−0.580	−0.986
MB	0.008	0.131	0.001	−0.005

compared to pristine sites. The elevated electrical conductivity and total dissolved salts recorded in degraded sites, particularly at Lituhi and Mbambabay, both located in the Songea region-Mbinga district, may be indicating higher inputs of nutrients from anthropogenic activities such as agriculture and mineral mining taking place in the area. At Ruhuhu, the lowest readings for pH (7.75 $\pm$ 0.08), electrical conductivity (80 $\pm$ 1.53), and total dissolved salts (40 $\pm$ 1.00) were obtained. The Katumba site had the lowest total dissolved salts and electrical conductivity compared to other degraded sites. The diluting effect of Mbaka River and Ruhuhu River water may explain for the low total dissolved salts and electrical conductivity seen at the Katumba and Ruhuhu sites, respectively. Comparing river water to lake water, it has been reported that river water has lower water quality attributes values [29].

Total phosphorus and nitrate concentrations in this study may be evidence that human activities near Lake Nyasa have led to higher nutrient inputs in the degraded areas. Total nitrogen and phosphorus concentrations were higher in degraded catchments than in relatively pristine catchments. The presence of agricultural activities along Lake Nyasa is suggested to be the reason for the elevated concentration of total nitrate and phosphorus in the degraded catchments. However, it was discovered that the TOC was lower in degraded catchments compared to relatively pristine catchments. This can be a possible outcome of long-term deforestation. Thus, the decreased percentage of TOC in the degraded catchments may be an indication that there is less leaf litter. Previous studies indicated that agriculture and mineral mining were likely to contribute to increased nutrient concentrations in catchments and cause fish biodiversity loss [30,31].

The overall nucleotide and haplotype diversity recorded in this study is higher than values previously reported for *Opsaridium microlepis* and *O. tweddleorum* but lower than that of *O. microcephalum* in the same Lake in Malawi. In this study, more haplotypes were recorded than those reported for the same species in Lake in Malawi [6]. The genetic diversity and number of haplotypes between the present study and the one carried out in Malawi may differ due to the habitat characteristics and/or number of samples collected from the Lake (The Tanzania side). Only a small number of samples from the Tanzanian shore of the Lake were collected for the study in Malawi, and the site's degree of pristine or degraded status was not a factor in the research. The current study's findings show that the relative pristine sites have consistently higher haplotype and nucleotide diversity than the degraded sites.

Genetic diversity helps organisms adapt to environmental changes and improves fitness, which are crucial for population resilience [32]. The study on an endangered Australian freshwater fish revealed low genetic diversity caused by habitat fragmentation [33]. *Opsaridium microlepis* requires well-oxygenated flowing water and silt-free gravels that mix with eggs during the spawning act. The species has shifted toward a smaller size at maturity and a higher fecundity rate, indicating that the species is adapting to survive in an unstable environment with density-independent mortalities [34]. The adults are only known from the main lake body [6], mainly where the rivers enter Lake Nyasa. However, it has been suggested that habitat loss can reduce genetic variation and change the distribution within and among populations of highly migratory fish such as salmon [35]. Farming activities in the area have fragmented and degraded some of the catchments along Lake Nyasa. Farming activities may affect *O. microlepis* habitats, which might hinder gene exchange with other subpopulations from the relatively pristine sites. Lower genetic diversity has been linked to stress during recruiting and unfavorable environmental factors [36]. These factors may result in poor dispersal potential and low connectivity due to reduced potential for recolonization and gene interchanges between populations [37].

The results indicate that all populations deviated from HWE for locus OPS11, which could be explained by the influence of null alleles. This study found consistently lower observed and expected heterozygosity in degraded sites compared to relative pristine sites. Our results suggest that the observed and expected heterozygosity has decreased in Lake Nyasa due to catchment degradation. The study conducted in a marine environment found a negative relationship between heavy metal concentrations in shrimp tissues and genetic diversity [38]. This means that anthropogenic activities such as illicit farming might harm the genetic diversity of diverse species. The significant heterozygosity excess values found in the Lituhi and Katumba subpopulations may signal a population bottleneck of *O. microlepis* in the area.

In contrast to samples from relatively pristine sites, the mean mutational-scaled effective population size for this study is consistently smaller for samples from degraded sites. This may indicate that local *O. microlepis* populations have declined due to human activity. Overfishing and other human actions that degrade habitats can alter the population size of both freshwater and marine animals, disrupting genetic diversity and population structure [39–41].

Therefore, the low genetic diversity discovered in this study for samples from catchments degraded by human activities may have been brought on by the decline in effective population size brought on by catchment degradation through human activities such as tree clearing and logging, which promotes nutrient loading and promotes fish mortalities. Habitat loss and fragmentation reduce genetic diversity in small, isolated populations due to genetic drift, which lowers adaptive potential and fitness and increases inbreeding [33]. Access to suitable habitats is crucial for settlement and recruitment, whereas habitat degradation and fragmentation can increase

subpopulation isolation and contribute to population decline [37,42]. The population from the studied degraded catchments needs proper conservation plans due to an increased risk of extinction. The threat of extinction exists for populations living under extreme environmental conditions and with decreased genetic diversity and effective population sizes [43].

The analysis of molecular variances (AMOVA) in this study revealed more significant genetic differentiation among populations compared to values found for *O. microcephalum*, but lower compared to those of *O. tweddleorum*, both endemic to Lake Nyasa [6]. However, the study in Malawi along Lake Nyasa did not find population differentiation for the same species [6]. We combined samples from pristine and degraded sites in this study, whereas samples from Malawi did not consider the characteristics of the sampling sites (relative pristine and degraded sites). These factors may account for the differences in the population differentiation observed between these studies.

Tajima's D values are non-significant in all populations studied, with populations from Buloma, Lituhi, and Ruhuhu having negative values. Only one population has significant negative Fu's Fs-values. Fu's Fs-values are said to be more powerful than Tajima's D. The population from Buloma has a negative significant Fu's Fs-value which may indicate the presence of evolutionary processes, such as a recent increase in population size following the bottleneck event, that cause deviations from the genetic marker's neutrality. Overall, Tajima's D and Fu's Fs test results in all populations were not statistically significant and were consistent with a population in drift-mutation equilibrium [44]. The non-significant sum of square deviations (SSD) may indicate that the data do not depart from what would be predicted based on the expansion model [45]. The non-significant raggedness index obtained in this study may indicate that the data has a relatively good fit to a model of population expansion [46,47]. However, we found a star-like structure in the haplotype network, which may indicate recent population growth after a decline or a bottleneck.

5. Conclusion

The degraded sites showed higher water electrical conductivity and amounts of phosphorus and nitrogen in sediments compared to pristine sites. Consequently, *O. microlepis* from the relatively pristine sites consistently exhibited higher haplotype and nucleotide diversity than those from the degraded sites. The findings in this study revealed that farming activities conducted in the catchments are likely to cause notable impacts on diversity and mutational effective population size in *O. microlepis*. Fish species with low genetic diversity and small effective populations caused by unsuitable and fragmented habitats are unlikely to disperse into new areas and adapt locally [48]. The proper management option for catchments along the Lake Nyasa is important to halt illicit human activities in the areas affected and plant trees where clearcutting has occurred. Otherwise, if habitat degradation and fragmentation persist, many species may be driven to extinction locally. However, additional research involving samples from spawning sites with a larger sample size and number of loci is required to confirm the patterns of genetic diversity and effective population sizes found in this study.

CRediT authorship contribution statement

Alex Nehemia: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alinanuswe J. Mwakalesi:** Writing – review & editing, Methodology, Investigation, Formal analysis.

Data availability statement

DNA sequences data have been deposited at National Center for Biotechnology Information (NCBI) Nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with accession numbers OQ318303-OQ318401.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Alex Nehemia reports financial support was provided by Rufford Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] O.L.F. Weyl, A.J. Ribbink, D. Tweddlel, Lake Malawi: fishes, fisheries, biodiversity, health and habitat, Aquat. Ecosys. Health Manag. 13 (3) (2010) 241–254, https://doi.org/10.1080/14634988.2010.504695.
[2] S.J. Nindi, Changing livelihoods and the environment along Lake Nyasa, Tanzania, African Study Monogr. Suppl 36 (2007) 71–93.
[3] D. Chungu, P. Ng'andwe, H. Mubanga, F. Chileshe, Fire alters the availability of soil nutrients and accelerates growth of Eucalyptus grandis in Zambia, J. For. Res. 31 (5) (2020) 1637–1645, https://doi.org/10.1007/s11676-019-00977-y.
[4] J. Gichuki, A. Getabu, C. Ezekiel, F. Wanda, O. Mkumbo, M. Njiru, An assessment of the ecosystem health of Lake victoria (east Africa): current status, trends and impacts to fishery, Tanzania J. For. Nat. Conserv. 80 (1) (2010) 36–44.
[5] H. Sungani, B.P. Ngatunga, S. Koblmüller, T. Mäkinen, P.H. Skelton, M.J. Genner, Multiple colonizations of the Lake Malawi catchment by the genus *opsaridium* (teleostei: cyprinidae), Mol. Phylogenet. Evol. 107 (2017) 256–265, https://doi.org/10.1016/j.ympev.2016.09.027.
[6] H. Sungani, B.P. Ngatunga, M.J. Genner, Migratory behaviour shapes spatial genetic structure of cyprinid fishes within the Lake Malawi catchment, Freshw. Biol. 61 (2016) 1062–1074, https://doi.org/10.1111/fwb.12767.
[7] D. Tweddle, Breeding behaviour of the mpasa, opsaridium microlepis (Gunther) (pisces:Cyprinidae), in lake Malawi, J. Limnol. Soc. South. Afr. 9 (1) (1983) 23–28, https://doi.org/10.1080/03779688.1983.9639407.

[8] D. Tweddle, Opsaridium microlepis, Lake Salmon, in: IUCN Red List Threat. Species 2018, vol. 8235, 2018.
[9] S. Mahboob, K.A. Al-ghanim, F. Al-misned, H.F.A. Al-balawi, A. Ashraf, N.M.A. Al-mulhim, Saudi Journal of Biological Sciences Genetic diversity in tilapia populations in a freshwater reservoir assayed by randomly amplified polymorphic DNA markers, Saudi J. Biol. Sci. 26 (2) (2019) 363–367, https://doi.org/10.1016/j.sjbs.2018.11.015.
[10] J.L. Mijangos, C. Pacioni, P.B.S. Spencer, M.D. Craig, Contribution of genetics to ecological restoration, Mol. Ecol. 24 (1) (2015) 22–37, https://doi.org/10.1111/mec.12995.
[11] N. Petit-Marty, et al., Declining population sizes and loss of genetic diversity in commercial fishes: a simple method for a first diagnostic, Front. Mar. Sci. 9 (May) (2022) 1–11, https://doi.org/10.3389/fmars.2022.872537.
[12] N. Nikolic, A.J. Butler, L.-J. Bagliniere, R. Laughton, G.A.I. McMyn, C. Chevalet, An examination of genetic diversity and effective population size in Atlantic salmon populations, Genet. Res. Cambridge (2009) (2010), https://doi.org/10.1017/S0016672309990346.
[13] S. Pradhan, M.R. Pokhrel, Spectrophotometric determination of phosphate in sugarcane juice , fertilizer , detergent and water, Sci. World 11 (11) (2013) 58–62.
[14] P. Sáez-Plaza, M.J. Navas, S. Wybraniec, T. Michałowski, A.G. Asuero, An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control, Crit. Rev. Anal. Chem. 43 (4) (2013) 224–272, https://doi.org/10.1080/10408347.2012.751787.
[15] T. Bhattacharyya, et al., Walkley-black recovery factor to reassess soil organic matter: indo-gangetic plains and black soil region of India case studies, Commun. Soil Sci. Plant Anal. 46 (20) (2015) 2628–2648, https://doi.org/10.1080/00103624.2015.1089265.
[16] R.D. Ward, T.S. Zemlak, B.H. Innes, P.R. Last, P.D.N. Hebert, DNA barcoding Australia's fish species, Philos. Trans. R. Soc. B Biol. Sci. 360 (1462) (2005) 1847–1857, https://doi.org/10.1098/rstb.2005.1716.
[17] D. Wheeler, M. Bhagwat, Comparative genomics volumes 1 and 2, BLAST QuickStart Example-Driven Web-Based BLAST Tutorial 395 (2007) 149–176.
[18] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (7) (2021) 3022–3027, https://doi.org/10.1093/molbev/msab120.
[19] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (12) (2013) 2725–2729, https://doi.org/10.1093/molbev/mst197.
[20] J.W. Leigh, D. Bryant, Popart : full-feature software for haplotype network construction, Methods Ecol. andEvolution 6 (2015) 1110–1116, https://doi.org/10.1111/2041-210X.12410.
[21] L. Excoffier, H.E.L. Lischer, Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows, Mol. Ecol. Resour 10 (3) (2010) 564–567, https://doi.org/10.1111/j.1755-0998.2010.02847.x.
[22] B. Goossens, Microsatellite records for volume 7, issue 4, Conserv. Genet. Resour. 7 (4) (2015) 917–944, https://doi.org/10.1007/s12686-015-0493-8.
[23] C. Van Oosterhout, W.F. Hutchinson, D.P.M. Wills, P. Shipley, MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data, Mol. Ecol. Notes 4 (3) (2004) 535–538, https://doi.org/10.1111/j.1471-8286.2004.00684.x.
[24] R. Peakall, P.E. Smouse, GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update, Bioinformatics 28 (19) (2012) 2537–2539, https://doi.org/10.1093/bioinformatics/bts460.
[25] S. Piry, G. Luikart, J.M. Cornuet, BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data, J. Hered. 90 (4) (1999) 502–503, https://doi.org/10.1093/jhered/90.4.502.
[26] P. Beerli, J. Felsenstein, Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach, Proc. Natl. Acad. Sci. U.S.A. 98 (8) (2001) 4563–4568, https://doi.org/10.1073/pnas.081068098.
[27] M. Bradic, P. Beerli, F.J. García-de León, S. Esquivel-Bobadilla, R.L. Borowsky, Gene flow and population structure in the Mexican blind cavefish complex (Astyanax mexicanus), BMC Evol. Biol. 12 (9) (2012) 1–16, https://doi.org/10.1186/1471-2148-12-9.
[28] K.R. Clarke, R.M. Warwick, "Change in marine communities: an approach to statistical analysis and interpretation, in: Primer-E, Plymouth," *Plymouth, United Kingsom*, second ed., PRIMER-E, 2001, p. 172 [Online]. Available: http://owasptop10.googlecode.com/files/OWASPTop10-2013.pd.
[29] F.M.M. Chale, Preliminary studies on the ecology of mbasa (opsaridium microlepis (gunther) in Lake Nyasa around the Ruhuhu River, J. Ecol. Nat. Environ. 3 (2011) 58–62.
[30] D.M. Njagi, et al., A century of human-induced environmental changes and the combined roles of nutrients and land use in Lake Victoria catchment on eutrophication, Sci. Total Environ. 835 (April) (2022) 155425, https://doi.org/10.1016/j.scitotenv.2022.155425.
[31] J.J. Leppänen, J. Weckström, A. Korhola, Multiple mining impacts induce widespread changes in ecosystem dynamics in a boreal lake, Sci. Rep. 7 (1) (2017) 1–11, https://doi.org/10.1038/s41598-017-11421-8.
[32] L.K. Faulks, D.M. Gilligan, L.B. Beheregaray, The role of anthropogenic vs. natural in-stream structures in determining connectivity and genetic diversity in an endangered freshwater fish, Macquarie perch (Macquaria australasica), Evol. Appl. 4 (4) (2011) 589–601, https://doi.org/10.1111/j.1752-4571.2011.00183.x.
[33] A. Pavlova, et al., Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: a call for assisted gene flow, Evol. Appl. 10 (6) (2017) 531–550, https://doi.org/10.1111/eva.12484.
[34] K. Kaunda, M. Limuwa, Wake-up call'''-Mpasa, Opsaridium microlepis released in the 2006 IUCN Red list of Threatened species: a synopsis review of studies and proposal for conservation of the threatened Species, in: 3rd Bunda College Dissemination Conference Proceedings, 2006, p. 24.
[35] M.M. McClure, et al., Evolutionary consequences of habitat loss for Pacific anadromous salmonids, Evol. Appl. 1 (2008) 300–318, https://doi.org/10.1111/j.1752-4571.2008.00030.x.
[36] J. Pini, S. Planes, E. Rochel, D. Lecchini, C. Fauvelot, Genetic diversity loss associated to high mortality and environmental stress during the recruitment stage of a coral reef fish, Coral Reefs 30 (2011) 399–404, https://doi.org/10.1007/s00338-011-0718-6.
[37] A. Garcia-Cisneros, C. Palacín, Y. Ben Khadra, R. Pérez-Portela, Low genetic diversity and recent demographic expansion in the red starfish Echinaster sepositus (Retzius 1816), Sci. Rep. 6 (August) (2016) 33269, https://doi.org/10.1038/srep33269.
[38] C. Rumisha, M. Elskens, M. Leermakers, M. Kochzius, Trace metal pollution and its influence on the community structure of soft bottom molluscs in intertidal areas of the Dar es Salaam coast, Tanzania, Mar. Pollut. Bull. 64 (3) (2012) 521–531, https://doi.org/10.1016/j.marpolbul.2011.12.025.
[39] S.M. Baillie, A.M. Muir, K. Scribner, P. Bentzen, C.C. Krueger, Loss of genetic diversity and reduction of genetic distance among lake trout Salvelinus namaycush ecomorphs , Lake Superior 1959 to 2013, J. Great Lake. Res. 42 (2) (2016) 204–216, https://doi.org/10.1016/j.jglr.2016.02.001.
[40] A. Nehemia, M. Kochzius, Reduced genetic diversity and alteration of gene flow in a fiddler crab due to mangrove degradation, PLoS One 12 (8) (2017) 1–20.
[41] A. Nehemia, F. Huyghe, M. Kochzius, Genetic erosion in the snail Littoraria subvittata (Reid, 1986) due to mangrove deforestation, J. Molluscan Stud. 83 (1) (2017), https://doi.org/10.1093/mollus/eyw040.
[42] J.D. DiBattista, Patterns of genetic variation in anthropogenically impacted populations, Conserv. Genet. 9 (1) (2008) 141–156, https://doi.org/10.1007/s10592-007-9317-z.
[43] J.A. Markert, et al., Population genetic diversity and fitness in multiple environments, BMC Evol. Biol. 10 (205) (2010) 1–13 [Online]. Available: http://www.biomedcentral.com/1471-2148/10/205.
[44] J. Joshi, R.K. Salar, P. Banerjee, S. Upasna, R.K. Tantia, M. S Vijh, Genetic variation and phylogenetic relationships of Indian buffaloes of Uttar Pradesh, AJAS (Asian-Australas. J. Anim. Sci.) 26 (2013) 1229–1236, https://doi.org/10.5713/ajas.2012.12669.
[45] A.R. Rogers, Genetic evidence for a pleistocene population explosion, Evolution 49 (4) (1995) 608–615.
[46] C.H. Harpending, Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution, Hum. Biol. 66 (4) (1994) 591–600.
[47] A.R. Rogers, H. Harpending, Population growth makes waves in the distribution of pairwise genetic differences, Mol. Biol. Evol. 9 (3) (1992) 552–569, https://doi.org/10.1534/genetics.103.024182.
[48] J.A. Huey, S.R. Balcombe, K.M. Real, D. Sternberg, J.M. Hughes, Genetic structure and effective population size of the most northern population of the Australian River Blackfish, Gadopsis marmoratus (Richardson 1848): implications for long-term population viability, Freshw. Sci. 36 (1) (2017) 113–123, https://doi.org/10.1086/690557.