Aquaculture and Fisheries 8 (2023) 689–694

Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries

Original research article

A genetic linkage map of the threatened catfish *Lophiosilurus alexandri*: Inferences on effective population size

M. Raquel M. Coimbra [a,*], Renata da S. Farias [a], Bruno C.N.R. da Silva [a], Andres Blanco [b], Miguel Hermida [b], Armando Caballero [c], Michael Bekaert [d], Paulino Martinez [b]

[a] Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco, Rua Dom Manuel de Medeiros, S/n, Dois Irmãos, Recife, PE, 52171-900, Brazil
[b] Department of Zoology, Genetics and Physical Anthropology, ACUIGEN Group, Faculty of Veterinary, University of Santiago de Compostela, Campus of Lugo, Lugo, 27002, Spain
[c] Center of Marine Research, University of Vigo, Faculty of Biology, Vigo, 36310, Spain
[d] Institute of Aquaculture, University of Stirling, Scotland, FK9 4LA, United Kingdom

A B S T R A C T

We report the first genetic linkage map of the catfish *Lophiosilurus alexandri*, a South American threatened and promising aquaculture catfish species. Using the progeny of three full-sib families, with 141, 74, and 49 offspring, respectively, we could genotype 2351 SNP markers using the ddRAD technology, shared by the male and the female maps constructed. The averaged, female and male maps spanned a total length of 2201.3 cM, 2481.9 cM, and 1872.8 cM, respectively, and comprehended the expected 27 linkage groups according to the karyotype information of the species (2n = 54). The recombination rate was nearly twice higher in the female than in the male map. The average map was used to estimate the historical effective population size (N_e) of the species from linkage disequilibrium between pairs of SNPs using parental individuals and revealed a remarkable drop in N_e about 20–25 generations in the past. The construction of the biggest artificial reservoir in Latin America and associated nutrient retention is pointed as a possible reason for such a reduction and suggests a reevaluation of the conservation status of the species. The current map lays the groundwork for understanding the genetic basis of economically important traits in breeding programs and will be useful for the genome assembly of this important commercial species.

1. Introduction

The order Siluriformes is a group of ray-finned catfishes of relevance in the aquaculture industry. Around 40% of the catfish families are native to Central and South America (Malabarba & Malabarba, 2020); however, Asia produces over 90% of the world aquaculture of this group, followed by the African continent and North America, while South America accounts for less than 0.5% of the total production (Gisbert et al., 2022). A Brazilian native catfish candidate species for aquaculture diversification is the *Lophiosilurus alexandri* (Pseudopimelodidae), a sedentary carnivorous species endemic from the São Francisco River that can reach up to 8 kg (Sato et al., 2003). It inhabits sandy bottoms where buries itself waiting for its preys, rarely showing displacement. It shows progeny parental care, which is carried by males (Costa et al., 2015; Sato et al., 2003). Gonadal maturation takes place before two years old in males, while females mature later, but before three years old (Melillo Filho et al., 2020) and spawning occurs all year around except for the cold months (Campeche et al., 2011, p. 20). Sex determination in juveniles is not trivial and careful coeliotomy is the most efficient method for sexing (Melillo Filho *et al.*, 2016). Further, it is a threatened species classified as Vulnerable (IUCN, 2018) and subjected to restocking hatchery actions.

This species is known for successful artificial spawning and larviculture, low dissolved oxygen requirements, acceptance of formulated diets, tolerating high stocking densities, and showing high consumption acceptance (Melillo Filho *et al.*, 2014; Costa et al., 2015; Porto et al., 2021; Silva et al., 2014; Cordeiro et al., 2016; Campeche et al., 2011, p. 20). *L. alexandri* has been experimentally reared in ponds and in recirculating aquaculture system (Melillo Filho *et al.*, 2020; Mello et al., 2015), nevertheless, there are still desired traits to improve the rearing conditions of this species, such as growth. Developing genomic resources and appropriate tools is crucial to improve production and aid in the management of wild resources (Houston et al., 2020; Liu & Cordes, 2004; Yue, 2014). In this sense, genetic mapping has become an important cost-efficient tool for understanding the genetic basis of commercially valuable traits to be further applied in breeding programs (Yue, 2014). Moreover, mapping the genetic markers used for population screening has been instrumental in the identification of genes or

* Corresponding author.
E-mail address: maria.rmcoimbra2@ufrpe.br (M.R.M. Coimbra).

https://doi.org/10.1016/j.aaf.2023.02.003
Received 16 November 2022; Received in revised form 12 January 2023; Accepted 15 February 2023
Available online 21 March 2023

genomic regions related to local adaptation (Hoban et al., 2016), which is of utmost importance in genetically structured species under restocking actions, as reported in this species by Farias et al. (2020). Finally, genetic maps are also very helpful for improving the assembly of genomes at the chromosome level to provide a robust genomic tool for future investigation in aquaculture production (Yue & Wang, 2017).

Additionally, linkage maps allow the estimation of the historical effective population size (N_e) using linkage disequilibrium between pairs of SNPs across the genome (Hayes et al., 2003; Santiago et al., 2020). The effective population size is an essential parameter in population genetics and conservation, as its magnitude reflects genetic drift and inbreeding occurring in the population (Caballero, 2020). An estimate of the changes in N_e across time is important, not only to detect drops in N_e because of domestication and or past population bottlenecks, but also for making decisions in the conservation of threatened species by inferring the timeframe and thus, the possible reasons causing drastic declines in population size.

We presented here the first genetic linkage map of the moderate density of *L. alexandri* as a valuable genomic resource not only for understanding the genomic basis of economically important traits in aquaculture systems but also for conservation management purposes.

2. Material and methods

2.1. Ethical statement

All sample collection and animal handling in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the Federal Rural University of Pernambuco (Permit Number: CEUA/UFRPE 24/2017).

2.2. DNA samples

Initially, five full-sib families of *L. alexandri* were generated using five males and five females at the Aquaculture Station of Bebedouro from Brazil's Sao Francisco and Parnaiba Valleys Development Company (Codevasf) in Pernambuco (Brazil), located in the submiddle stretch of this river system, where offspring grew up for 45 days in separate gutters. DNA was extracted from breeders and offspring (eight/family) following a standard phenol/chloroform extraction method (Sambrook & Russell, 2006).

2.3. Library protocols

A first set of libraries was prepared with the 10 breeders and 8 individuals from each family to explore the success of the ddRAD protocol and the polymorphism degree in families to be further used for mapping. Then, a second set of libraries was built with the three most polymorphic families. Both libraries followed the same protocol detailed below and were sequenced at Novogene (Hong Kong) on an Illumina HiSeq platform (150 bases paired-end reads).

Library construction followed the general protocol described in Brown et al. (2016), a variant of the original ddRAD methodology (Peterson et al. 2012). Approximately 20 ng of genomic DNA of each sample was digested by two high-fidelity restriction enzymes: *Sbf*I (CCTGCA|GG recognition site) and *Nla*III (CATG| recognition site), both sourced from New England Biolabs, (NEB) UK. Digestions were incubated at 37 °C for 50 min, using 20 U of each enzyme per μgDNA in 1 × CutSmart Buffer (NEB) for a 6 μL total reaction volume. The restriction enzymes were inactivated by incubation at 65 °C for 20 min. After cooling the reactions to room temperature, 3 μL of a premade barcode/adapter mix was added to the digested DNA of each sample and incubated at room temperature for 10 min. This adapter mix comprised individual-specific barcoded combinations of P1 (*Sbf*I-compatible) and P2 (*Nla*III-compatible) adapters at 6 nM and 720 nM concentrations, respectively, in 1 × Reaction Buffer 2 (NEB). Adapters were compatible with Illumina sequencing chemistry and included an inline five- or seven-base barcode for sample identification. Ligation was performed over 3 h at 18 °C by the addition of a further 3 μL of a ligation mix comprising 4 mM rATP (Promega, UK), and 2000 cohesive-end units of T4 ligase (NEB) in 1 × CutSmart Buffer.

The ligated samples were then heat denatured at 65 °C for 20 min, cooled, and combined into a single pool. The pooled sample was column-purified (MinElute PCR Purification Kit, Qiagen, UK), and eluted in 55 μL EB buffer (Qiagen, UK). Size selection of fragments, ranging from approximately 450 bp to 650 bp, was performed by agarose gel separation (1.1% agarose; 0.5 × TAE buffer). Following gel purification (MinElute Gel Extraction Kit, Qiagen, UK), the eluted size-selected template DNA (60 μL in EB buffer) was PCR amplified in 12.5 μL reactions containing 1 μL template DNA using a high fidelity *Taq* polymerase (Q5 Hot Start High-Fidelity DNA Polymerase, NEB). The PCR reactions were combined in a single volume (350 μL total) and column-purified (MinElute PCR Purification Kit). The 55 μL eluate in EB buffer was then subjected to a further size-selection cleanup using an equal volume of AMPure magnetic beads (PerkinElmer, UK) to maximize the removal of small fragments (less than ca. 200 bp). The final libraries were eluted in EB buffer and sequencing took place at Stirling Institute of Aquaculture using an Illumina MiSeq sequencer.

In order to obtain the highest possible density of markers, a second set of libraries was built, as outlined above, with the progeny of three families showing the highest number of SNPs. They were sequenced at Novogene (Hong Kong) on an Illumina HiSeq platform (400 million read capacity, 150 base paired-end reads).

2.4. RAD alleles genotyping

The sequence data of breeders from the three families and their respective offspring were processed to discard low quality (quality scores below 25) and ambiguous reads (no recognizable barcode or restriction site). Reads were processed using Stacks v 2.3e (Catchen et al., 2013) considering paired-end reads as separate loci. These sequences were assigned to loci and genotypes using the Stacks 'denovo_map.pl' component. The key parameters employed in identifying marker loci were a maximum of three mismatches allowed in a locus within individuals and up to two mismatches per locus among individuals when building the catalog (n). Informative RAD markers were kept only when presenting at least two alleles with a MAF >0.05 among breeders and progenies and when genotyped in the three families in at least 25% of the samples and with depth coverage ≥8.

2.5. Construction of the linkage maps

Based on SNP genotyping, a linkage map was constructed with LepMap (Rastas, 2017). SNPs deviating from the expected Mendelian segregation ($P < 0.001$) in any of the three families were excluded. The number of linkage groups found was 27 (LOD = 10), which agrees with the available karyotype information of the species ($2n = 54$; Marques et al., 2008). Within each linkage group, the order of SNPs was obtained using the OrderMarkers module. The total length of the map in centimorgans (cM) was estimated using the Kosambi mapping function. Three maps were generated, a male, a female and a sex-averaged one and the map figures were constructed using MapChart (Voorrips, 2002).

2.6. Estimation of historical effective population size

Estimates of the historical effective population size (N_e) were obtained from linkage disequilibrium between pairs of SNPs using the software GONE (Santiago et al., 2020). Squared correlations between allele frequencies were obtained for all pairs of SNPs within each of the 27 linkage groups (2351 SNPs in total) using the six parental individuals. The analysis was made assuming the default parameters of the software.

3. Results

The initial single MiSeq run of the first set of samples (10 breeders and 8 progenies/family) yielded ~15.8 million paired-end reads. Following barcode demultiplexing and filtering for quality ~12.6 million reads were available for Stacks analysis. Nearly 20,000 robust RADtags were identified per individual, yielding between 650 and 884 SNPs (3.7% of the total) per individual. From the first progeny set (8 individuals/family), the majority of identified loci segregated in a Mendelian fashion, providing confidence in the ddRAD approach for generating robust SNP calls for linkage mapping in *L. alexandri*.

Surprisingly, the proportion of polymorphic RADtags observed across all parental samples was 3–6 times lower than that observed for most other fish species studied by the same methodology (Maroso et al., 2018). As the potential of the mapping panel was lower than originally anticipated, to maximize the number of mapped loci, we decided to screen progeny from three different family pedigrees in the large scale-mapping panel. The three selected families that became the reference-families were named Family 1 (141 offspring), Family 2 (74 offspring), and Family 3 (49 offspring) and were used for linkage mapping.

For the second set of libraries, high throughput sequencing (Illumina HiSeq platform) of the fish from Fam1, Fam 2, and Fam 3 produced 390,701,491 paired-end reads in total. After the removal of low-quality and incomplete reads, 59.9% of the total reads were retained (234,182,556 paired-end reads) and archived at the NCBI Sequence Read Archive (SRA) under accession number PRJNA923021. The reads were *de novo* assembled and genotypes for all samples were obtained, yielding 2,510,030 unique loci. Among them, 5271 loci were bi-allelic SNP markers shared by at least 25% of each family member and showed an allele frequency ≥0.05. All these markers were subsequently used to construct the genetic linkage maps and a total of 2351 informative SNPs were mapped (1,467, 1037 and 765, respectively, for Fam1, Fam 2, and Fam 3, respectively) to the 27 linkage groups (each comprising at least 10 SNPs). The maps spanned a total distance of 2201.3 cM, 2481.9 cM, and 1872.8 cM for the averaged, female and male maps, respectively (Fig. 1 and Supplementary material 1). The genetic distances between SNPs varied from 0 to 17 cM (sex-average map), from 0 to 64 cM (female map), and from 0 to 32 cM (male map), with an average distance of 0.91 cM in the male map, 1.35 cM in the female map, and 1.09 cM in the sex-average map (Table 1). For most of the linkage groups, the recombination rate was nearly twice higher in the female than in the male map.

Fig. 2 shows the estimated changes in effective population size (N_e) across generations back in time (the current generation is generation 0). Estimates of ancestral N_e suggested very large census sizes (tens of thousands of individuals), and a remarkable drop was estimated to occur about 20–25 generations in the past, leading to a small N_e of around one hundred individuals.

4. Discussion

Currently, a few genetic linkage maps are available for catfish species: *Clarias fuscus* (Lin et al., 2022), *Ictalurus punctatus* (Zhang et al.,

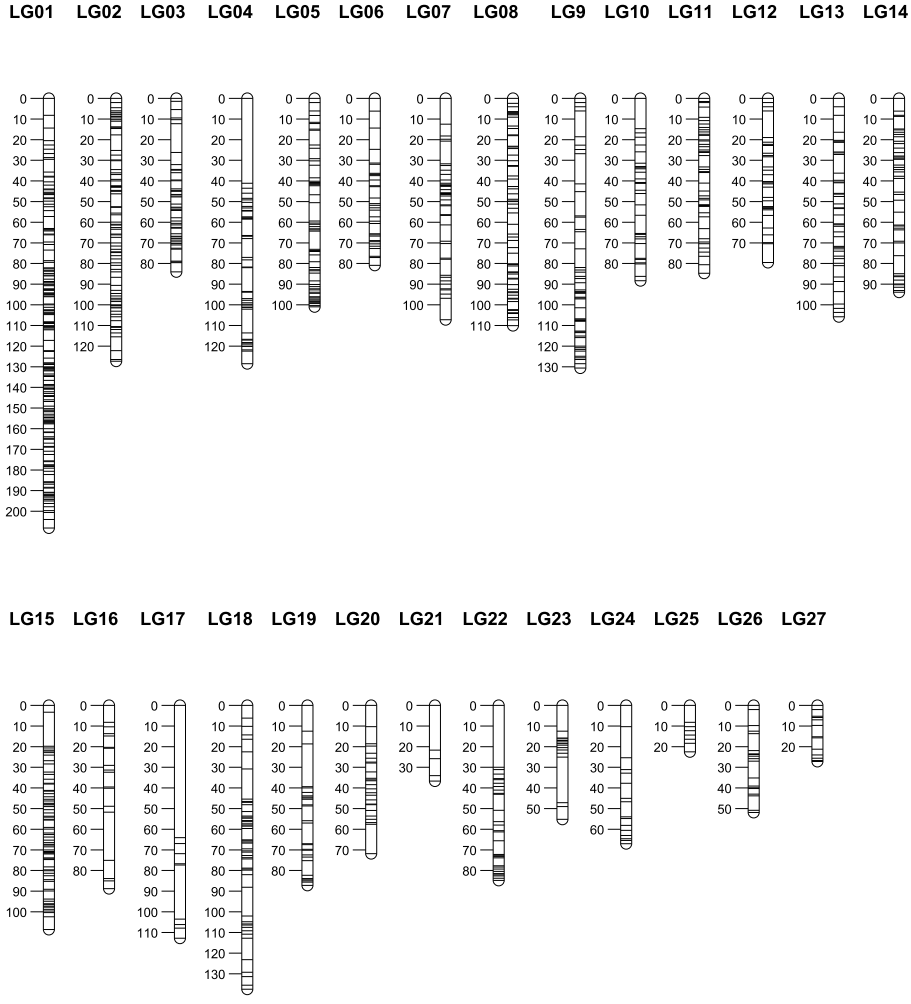

Fig. 1. Linkage groups length (in cM) and marker distribution (black horizontal bars) of the male/female average linkage map of *Lophiosilurus alexandri*.

Table 1
Information on the genetic linkage maps of *Lophiosilurus alexandri*.

LG	#SNPs	SEX AVERAGE			FEMALE			MALE		
		Size (cM)	Av. Dist. (sd)	Max. Dist	Size (cM)	Av. Dist. (sd)	Max. Dist.	Size (cM)	Av. Dist (sd)	Max. Dist
1	414	177.162	0.429 (0.726)	5.272	208.082	0.504 (1.069)	8.237	157.340	0.381 (0.969)	8.245
2	141	112.466	0.803 (1.456)	10.259	127.253	0.909 (1.333)	7.448	91.025	0.650 (1.461)	10.739
3	117	83.757	0.722 (1.505)	10.055	84.007	0.724 (1.742)	13.972	63.062	0.544 (1.563)	12.446
4	90	73.988	0.831 (1.574)	8.245	128.535	1.444 (4.796)	41.056	80.489	0.904 (2.473)	13.314
5	135	132.068	0.986 (1.735)	11.972	100.992	0.754 (1.597)	8.834	89.553	0.668 (2.256)	23.352
6	84	90.442	1.090 (1.827)	10.349	80.893	0.975 (2.009)	10.350	79.752	0.961 (2.332)	12.719
7	90	100.693	0.999 (1.699)	10.056	107.194	1.204 (2.526)	12.499	85.053	0.956 (2.260)	16.947
8	124	116.432	0.947 (1.438)	8.388	110.058	0.895 (1.352)	5.470	86.557	0.704 (1.953)	14.393
9	101	96.044	0.960 (1.413)	5.864	130.574	1.306 (2.684)	14.610	89.151	0.892 (2.125)	12.499
10	75	71.259	0.963 (1.512)	8.834	88.288	1.193 (2.463)	14.695	41.167	0.556 (1.137)	5.863
11	87	89.686	1.043 (1.384)	6.154	84.722	0.985 (1.461)	5.716	124.388	1.446 (3.919)	24.172
12	79	70.014	0.898 (2.225)	16.947	79.484	1.019 (2.138)	12.843	35.859	0.460 (1.525)	8.237
13	79	87.660	1.124 (2.118)	15.188	105.754	1.356 (2.044)	9.047	62.183	0.797 (2.130)	10.665
14	87	89.803	1.044 (1.928)	11.585	93.923	1.092 (1.882)	8.646	86.337	1.004 (2.405)	18.283
15	116	105.041	0.913 (1.184)	5.272	108.583	0.944 (1.827)	16.530	107.955	0.939 (1.895)	12.718
16	34	58.322	1.767 (2.518)	10.822	88.848	2.692 (4.777)	23.435	69.638	2.110 (5.854)	32.482
17	36	50.322	1.438 (3.561)	18.511	112.788	3.223 (11.538)	64.124	35.150	1.004 (2.192)	9.966
18	114	155.240	1.374 (2.561)	14.415	137.512	1.217 (2.572)	14.610	104.097	0.921 (2.588)	19.513
19	78	80.864	1.050 (2.374)	14.677	87.223	1.133 (3.177)	20.794	90.252	1.172 (1.999)	7.448
20	48	62.639	1.333 (1.774)	8.245	71.787	1.527 (2.804)	14.190	76.263	1.623 (2.783)	12.499
21	28	22.938	0.850 (2.133)	10.349	36.703	1.359 (4.436)	21.670	14.321	0.530 (1.344)	6.153
22	55	85.783	1.589 (1.915)	8.834	84.812	1.571 (4.290)	30.019	83.669	1.549 (2.582)	10.350
23	27	31.704	1.219 (2.303)	11.532	55.151	2.121 (4.833)	21.928	10.237	0.394 (1.299)	6.153
24	39	80.371	2.115 (3.522)	15.717	67.045	1.764 (3.352)	15.075	51.794	1.363 (3.921)	19.626
25	16	11.235	0.749 (1.124)	4.090	22.537	1.502 (2.261)	8.237	0.000	0.000 (0.000)	0.000
26	39	44.013	1.158 (1.937)	9.047	51.919	1.366 (2.429)	8.245	43.106	1.134 (1.799)	5.426
27	18	21.353	1.256 (1.272)	4.641	27.234	1.602 (1.811)	5.486	14.370	0.845 (2.188)	8.237

LG: Linkage group; Av. Dist (sd): average distance in centimorgan and standard deviation in parenthesis; Max dist: Maximum distance in centimorgan. The minimum distanced were equal 0 in all cases.

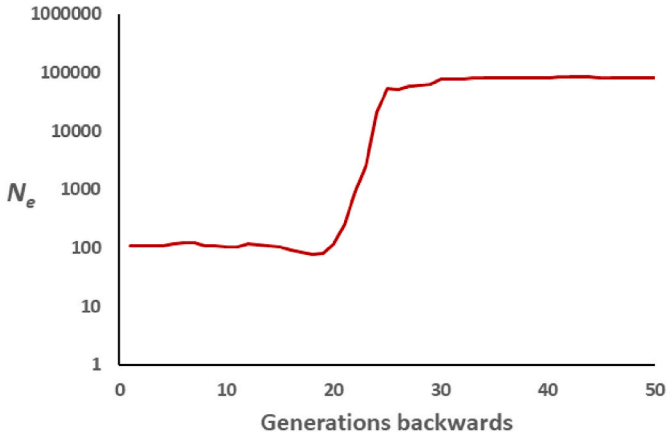

Fig. 2. Estimates of historical linkage disequilibrium N_e of *Lophiosilurus alexandri* based on six individuals and 2351 SNPs in the submiddle stretch of the São Francisco River.

2019), and the hybrid *I. furcatus* × *I. punctatus* (Liu et al., 2016) and a dozen of genome assemblies are available in the National Center of Biotechnology Information (2022, November 13th), half of them belonging to Asian aquaculture species, and the others to North American, African, and European species. This is the first study where a genetic linkage map has been constructed for a Brazilian threatened catfish species subjected to restocking actions and a potential candidate for aquaculture. Three maps were constructed, species-average, male, and female maps spanning above 1800 cM, all of them comprehending the 27 linkage groups corresponding to the haploid chromosome number of the species (Marques et al., 2008). Differences in recombination rate between the sexes or heterochiasmy are common in vertebrate species (Burt et al., 1991) and females' genetic linkage maps are often longer than males. This trend has also been recently confirmed in fish, where recombination and mapping information on 61 species was evaluated, with authors suggesting potential for adaptive processes related to sexual selection or sexual conflict (Cooney et al., 2021). The ratio found in our study of almost 2:1 is compatible with the 1.7:1 found in the channel catfish (Yun et al., 2015).

Interestingly, the proportion of RADtags observed across all parental samples was 3–6 times lower than that observed for other fish species by the same methodology, which suggests a much lower genetic diversity in *L. alexandri* (Maroso et al., 2018). Some characteristics of the species, such as sedentary habits with limited displacements and parental care could contribute to an intrinsically low level of variation. Farias et al. (2020) detected a low genetic diversity using microsatellite markers in wild individuals in *L. alexandri* (average number of alleles of 4.2) and observed a reduction of 20% in the genetic diversity in breeders from hatcheries when compared to their wild conspecifics.

Despite the low number of individuals used (only six) and the not-too-large set of SNPs available (2,351), the estimation of historical N_e strongly suggests a drastic reduction of effective population size around 20–25 generations ago. Considering an approximate generation interval of 2 years for this species (Melillo Filho *et al.*, 2020) this implies that the N_e drop would have occurred 40–50 years ago. The construction in 1979 of a 4214 km^2 surface area reservoir with a storage capacity of 34.1 km^3 could be associated with this observation. The Sobradinho reservoir retains high rates of sedimentation, nutrients, and turbidity, making significant modifications to primary productivity, with severe implications for fish communities downstream (Santos et al., 2020). The breeders used in the construction of the map were originally collected in the submiddle stretch of the São Francisco River, immediately downstream of this reservoir. Thus, it can be speculated that such a putative drastic reduction in N_e could be associated with the construction of the Sobradinho reservoir. The GONE estimation method can detect drastic changes in N_e, such as that observed in Fig. 2, although the time of the change in N_e is difficult to be established with precision.

In addition, the method can be reliably applied even with small sample sizes (see Fig. 2(J) of Santiago et al., 2020). However, it is more

accurate for the most contemporary estimates than for the oldest ones, and computer simulations show that the method tends to overestimate the ancestral estimates of N_e when the true values are large (Saura et al., 2021). This may imply that the estimates of N_e detected before 25 generations ago can be largely overestimated and the results should be taken with caution. Nevertheless, the results suggest that the N_e values were much larger in the past than at present, and an estimate of N_e as low as one hundred individuals in the recent generations is compatible with the low diversity found in relation to other species.

The map here presented paves the way to progress in aquaculture breeding programs for ascertaining the genetic basis of economically important traits in *L. alexandri.* Moreover, our results reinforce the necessity of urgent actions to protect the species and review its conservation status based on the estimated effective population size.

CRediT authorship contribution statement

M. Raquel M. Coimbra: Conceptualization, Funding acquisition, Writing – original draft, Project administration. **Renata da S. Farias:** Methodology, Investigation. **Bruno C.N.R. da Silva:** Methodology, Investigation. **Andres Blanco:** Data curation, Formal analysis. **Miguel Hermida:** Data curation, Formal analysis. **Armando Caballero:** Formal analysis, Writing – original draft. **Michael Bekaert:** Data curation. **Paulino Martinez:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by Grupo Boticário de Proteção à Natureza (1076_20162) and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (grants 407789/2016–2 and 202015/2020–3).

The sixth and eighth authors were funded by grants PID 2020-114426 GB-C21 (MCIN/AEI/10.13039/501100011033), Xunta de Galicia (ED431C 2020–05), Centro Singular de Investigación de Galicia (2019–2022) and "ERDF A way of making Europe".

The authors are grateful to Miguel Arcanjo dos Santos Neto from Paulo Afonso Hatchery of Chesf, to Mateus Felix from Itiúba Hatchery of Codevasf, and to Rozzanno Figueiredo from Bebedouro Hatchery of Codevasf for providing biological material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aaf.2023.02.003.

References

Brown, J. K., Taggart, J. B., Bekaert, M., Wehner, S., Palaiokostas, C., Setiawan, A. N., Symonds, J. E., & Penman, D. J. (2016). Mapping the sex determination locus in the hāpuku (Polyprion oxygeneios) using ddRAD sequencing. *BMC Genomics, 17*, 448. https://doi.org/10.1186/s12864-016-2773-4

Burt, A., Bell, G., & Harvey, P. H. (1991). Sex differences in recombination. *Journal of Evolutionary Biology, 4*, 259–277.

Caballero, A. (2020). *Quantitative genetics* (1st ed.). Cambridge University Press (Chapter 5)).

Campeche, D. F. B., Balzana, L., Figueiredo, R. C. R., Barbalho, M. R. S., Reis, F. J. S., & Melo, J. F. B. (2011). *Peixes nativos do rio São Francisco adaptados para cultivo*. Petrolina: EMBRAPA Semiárido. p. Available on: http://ainfo.cnptia.embrapa.br/digital/bitstream/item/54780/1/SDC244.pdf.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology, 22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Cooney, C. R., Mank, J. E., & Wright, A. E. (2021). Constraint and divergence in the evolution of male and female recombination rates in fishes. *Evolution, 75*(11), 2857–2866. https://doi.org/10.1111/evo.14357

Cordeiro, N. I. S., Costa, D. C., Silva, W. S., Takata, R., Miranda Filho, K. C., & Luz, R. K. (2016). High stocking density during larviculture and effect of size and diet on production of juvenile Lophiosilurus alexandri steindachner, 1876 (Siluriformes: Pseudopimelodidae). *Journal of Applied Ichthyology, 32*, 61–66. https://doi.org/10.1111/jai.12963

Costa, D. C., E Silva, W. S., Melillo Filho, R., Miranda Filho, K. C., Dos Santos, J. C. E., & Luz, R. K. (2015). Capture, adaptation and artificial control of reproduction of Lophiosilurus alexandri: A carnivorous freshwater species. *Animal Reproduction Science, 159*, 148–154. https://doi.org/10.1016/j.anireprosci.2015.06.009

Farias, R. S., Silva, B. C. N. R., Nascimento, W. V. G., Silva, G. C., Luz, R. K., Prosdocimi, F., & Coimbra, M. R. M. (2020). Genetic diversity and aquaculture conservation for a threatened Neotropical catfish. *Neotropical Ichthyology, 18*. https://doi.org/10.1590/1982-0224-2020-0028

Gisbert, E., Luz, R. K., Fernández, I., Pradhan, P. K., Salhi, M., Mozanzadeh, M. T., … Darias, M. J. (2022). Development, nutrition, and rearing practices of relevant catfish species (Siluriformes) at early stages. *Reviews in Aquaculture, 14*(1), 73–105. https://doi.org/10.1111/raq.12586

Hayes, B. J., Visscher, P. M., McPartlan, H. C., & Goddard, M. E. (2003). Novel multilocus measure of linkage disequilibrium to estimate past effective population size. *Genome Research, 13*, 635–643. https://doi.org/10.1101/gr.387103

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., … Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions. *The American Naturalist, 188*(4), 379–397. https://doi.org/10.1086/688018

Houston, R. D., Bean, T. P., Macqueen, D. J., Gundappa, M. K., Jin, Y. H., Jenkins, T. L., Selly, S. L., Martin, S. A. M., Stevens, J. R., Santos, E. M., Davie, A., & Robledo, D. (2020). Harnessing genomics to fast-track genetic improvement in aquaculture. *Nature Reviews Genetics, 21*(7), 389–409. https://doi.org/10.1038/s41576-020-0227-y

2018 IUCN. (2018). Lophiosilurus alexandri: Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio): The IUCN Red List of Threatened Species 2021: e. T186399A1812477. In *IUCN Red List of Threatened Species*. Retrieved from https://doi.org/10.2305/iucn.uk.2021-1.rlts.t186399a1812477.pt. (Accessed 22 February 2023).

Lin, X., Tan, J., Shen, Y., Yang, B., Zhang, Y., Liao, Y., Wang, P., Zhou, D., Li, G., & Tian, C. (2022). A high-density genetic linkage map and QTL mapping for sex in Clarias fuscus. *Aquaculture, 561*, Article 738723. https://doi.org/10.1016/j.aquaculture.2022.738723

Liu, Z. J., & Cordes, J. F. (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture, 238*(1–4), 1–37. https://doi.org/10.1016/j.aquaculture.2004.05.027

Liu, S., Li, Y., Qin, Z., Geng, X., Bao, L., Kaltenboeck, L., Kucuktas, H., Dunham, R., & Liu, Z. (2016). High-density interspecific genetic linkage mapping provides insights into genomic incompatibility between channel catfish and blue catfish. *Animal Genetics, 47*(1), 81–90. https://doi.org/10.1111/age.12372. Epub 2015 Nov 4. PMID: 26537786.

Malabarba, L. R., & Malabarba, M. C. (2020). Phylogeny and classification of Neotropical fish. In *Biology and physiology of freshwater neotropical fish* (pp. 1–19). Academic Press.

Maroso, F., Hillen, J. E. J., Pardo, B. G., Gkagkavouzis, K., Coscia, I., Hermida, M., Franch, R., Hellemans, B., et al.Ogden, R. (2018). Performance and precision of double digestion RAD (ddRAD) genotyping in large multiplexed datasets of marine fish species. *Marine Genomics, 39*, 64–72. https://doi.org/10.1016/j.margen.2018.02.002

Marques, M. B. A., et al. (2008). Cytogenetic analyses of two endemic fish species from the São Francisco River basin: Conorhynchus conirostris and Lophiosilurus alexandri (Siluriformes). *Genetics and Molecular Biology*, 215–221. https://doi.org/10.1590/S1415-47572008000200008 [online]. v. 31, n. 1 suppl [Accessed 19 May 2022] pp.

Melillo Filho, R., Bazzoli, N., e Silva, W. D. S., Costa, D. C., Boaventura, T. P., da Costa Julio, G. S., & Luz, R. K. (2020). Rearing and maturation of Lophiosilurus alexandri (Steindachner, 1876) in controlled conditions: First reports and future perspective. *Animal Reproduction Science, 214*, Article 106311. https://doi.org/10.1016/j.anireprosci.2020.106311

Melillo Filho, R., Gheller, V. A., Chaves, G. V., e Silva, W. D. S., Costa, D. C., Figueiredo, L. G., … Luz, R. K. (2016). Early sexing techniques in Lophiosilurus alexandri (Steindachner, 1876), a freshwater carnivorous catfish. *Theriogenology, 86*(6), 1523–1529. https://doi.org/10.1016/j.theriogenology.2016.05.011

Melillo Filho, R., Takata, R., Santos, A. E. H., Silva, W. S., Ikeda, A. L., Rodrigues, L. A., & Luz, R. K. (2014). Draining system and feeding rate during the initial development of Lophiosilurus alexandri (Steindachner, 1877), a carnivorous freshwater fish. *Aquaculture Research, 45*, 1913–1920. https://doi.org/10.1111/are.12139

Mello, G. C. G. D., Santos, J. E. D., Guimarães-Cruz, R. J., Godinho, A. L., & Godinho, H. P. (2015). Allometric larval growth of the bottom-dwelling catfish Lophiosilurus alexandri Steindachner, 1876 (Siluriformes: Pseudopimelodidae). *Neotropical Ichthyology, 13*, 677–684. https://doi.org/10.1590/1982-0224-20140161

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One, 7*(5), Article e37135. https://doi.org/10.1371/journal.pone.0037135

Porto, L. A., Melo, R. M. C., Beier, S. L., Luz, R. K., & Favero, G. C. (2021). Lophiosilurus alexandri, a sedentary bottom fish, adjusts its physiological parameters to survive in hypoxia condition. *Fish Physiology and Biochemistry, 47*(6), 1793–1804. https://doi.org/10.1007/s10695-021-00996-9

Rastas, P. (2017). Lep-MAP3: Robust linkage mapping even for low-coverage whole genome sequencing data. *Bioinformatics, 33*(23), 3726–3732. https://doi.org/10.1093/bioinformatics/btx494

Sambrook, J., & Russell, D. W. (2006). *Purification of nucleic acids by extraction with Phenol:Chloroform*. CSH Protocols, 2006, 4455.

Santiago, E., Novo, I., Pardiñas, A. F., Saura, M., Wang, J., & Caballero, A. (2020). Recent demographic history inferred by high-resolution analysis of linkage disequilibrium. *Molecular Biology and Evolution, 37*, 3642–3653. https://doi.org/10.1093/molbev/msaa169

Santos, N. C. L., Dias, R. M., Alves, D. C., De Melo, B. A. R., Ganassin, M. J. M., Gomes, L. C., & Agostinho, A. A. (2020). Trophic and limnological changes in highly fragmented rivers predict the decreasing abundance of detritivorous fish. *Ecological Indicators, 110*, Article 105933. https://doi.org/10.1016/j.ecolind.2019.105933

Sato, Y., Fenerich-Verani, N., & Godinho, H. P. (2003). Reprodução induzida de peixes da bacia do São Francisco. In H. P. Godinho, & A. L. Godinho (Eds.), *Águas, peixes e pescadores do São Francisco das Minas Gerais. Belo Horizonte, Editora PUC Minas* (pp. 275–289).

Saura, M., Caballero, A., Santiago, E., et al. (2021). Estimates of recent and historical effective population size in turbot, seabream, seabass and carp selective breeding programmes. *Genetics Selection Evolution, 53*, 85. https://doi.org/10.1186/s12711-021-00680-9

Silva, W. D. S., Cordeiro, N. I. S., Costa, D. C., Takata, R., & Luz, R. K. (2014). Feeding frequency and rate during feed training of pacamã juveniles. *Pesquisa Agropecuária Brasileira, 49*, 648–651. https://doi.org/10.1590/S0100-204X2014000800009

Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity, 93*(1), 77–78. https://doi.org/10.1093/jhered/93.1.77

Yue, G. H. (2014). Recent advances of genome mapping and marker-assisted selection in aquaculture. *Fish and Fisheries, 15*(3), 376–396. https://doi.org/10.1111/faf.12020

Yue, G. H., & Wang, L. (2017). Current status of genome sequencing and its applications in aquaculture. *Aquaculture, 468*, 337–347. https://doi.org/10.1016/j.aquaculture.2016.10.036

Yun, L., Shika, I. L., Zhenkui, Q., Waldbieser, G., Wan, G. R., Sun, L., Bao, L., Danzmann, R. G., Dunham, R., & Liu, Z. (2015). Construction of a high-density, high-resolution genetic map and its integration with BAC-based physical map in channel catfish. *DNA Research, 22*(1), 39–52. https://doi.org/10.1093/dnares/dsu038

Zhang, S., Zhang, X., Chen, X., Xu, T., Wang, M., Qin, Q., Zhong, L., Jiang, H., Zhu, X., Liu, H., Shao, J., Zhu, Z., Shi, Q., Bian, W., & You, X. (2019). Construction of a high-density linkage map and QTL fine mapping for growth-and sex-related traits in channel catfish (Ictalurus punctatus). *Frontiers in Genetics, 10*, 251. https://doi.org/10.3389/fgene.2019.00251