Revista Eletrônica Científica Ensino Interdisciplinar
Mossoró, v. 6, n. 19, outubro/2020 (Edição especial)

POPULATION ANALYSIS IN ATLANTIC THREAD HERRING FROM THE BRAZILIAN COAST

ANÁLISE DE POPULAÇÕES DA SARDINHA BANDEIRA DA COSTA ATLÂNTICA BRASILEIRA

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ABSTRACT
The Atlantic thread herring *Opisthonema oglinum*, also known as Bermuda herring belongs to the family Clupeidae (Clupeiformes). Because of their economic and ecological importance, this species has been the target of several scientific studies. However, this is the first study of population genetics in O. oglinum. We sampled individuals of O. oglinum from seven localities along the Brazilian coast in South Atlantic, totaling 225 DNA sequences of control region in mitochondrial DNA (mtDNA). The present results revealed no deviations from Hardy-Weinberg equilibrium as well as the presence of a single gene pool since high gene flow and non-significant population structure have been detected among populations. The D (Tajima) and Fs (Fu) neutrality tests were negative. In spite of non-significant D values, all Fs estimates were significant, indicating population expansion.
KEYWORDS: *Opisthonema oglinum*; Genetic diversity; Population expansion; Control Region; mtDNA

RESUMO
A sardinha-bandeira *Opisthonema oglinum*, também conhecida vulgarmente como, sardinha-laje, manjuba, arenque fio, pertence à família Clupeidae (Clupeiformes). Devido sua importância econômica e ecológica, a espécie vem sendo alvo de vários estudos científicos. Porém, este é o primeiro estudo genético populacional de O. oglinum. Foram investigados indivíduos de O. oglinum de sete diferentes localidades da costa atlântica brasileira, com 225 sequencias geradas a partir da região controle do DNA mitocondrial. Os resultados mostraram que a espécie encontra-se em equilíbrio genético e evidenciaram a existência de um único pool gênico, pois não observamos estruturação genética significativa, o fluxo gênico entre as sete populações analisadas é altíssimo. Os testes de neutralidade D (Tajima) e Fs (Fu) foram negativos, D não significativo e Fs significativo, indicando expansão populacional.
PALAVRAS-CHAVE: *Opisthonema oglinum*; Diversidade genética; Expansão populacional; Região controle; mtDNA

DOI: 10.21920/recei720206193140
http://dx.doi.org/10.21920/recei720206193140

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INTRODUÇÃO

Fishes first arise and diverged more than 500 million years ago, resulting in a remarkable diversity that has been useful to infer the evolutionary history and the biological classification of life (Nelson et al., 2016). Taking into account the worldwide ecological and economical relevance of sardines, several molecular studies have been carried out in this fish group in order to elucidate their genetic patterns of differentiation.

The sardines and anchovies belong to the order Clupeiformes and, based on molecular data, they are divided into five families (Lavoué et al., 2013; Queiroz et al., 2020). The family Clupeidae comprises 218 species and about 70% of them inhabit marine habitats (Nelson et al., 2016). The Atlantic thread herring Opisthonema oglinum, also known as Bermuda herring, is a representative of this family, being characterized by the thread-like shape of the last ray in dorsal fin (Whitehead, 1985).

Because of their biological importance, O. oglinum has been a target of several scientific studies over the last three decades (Finucane and Vaught, 1986; Patterson and Santos, 1992; Smith, 1994; Segura, 1995; Vega-Cendejas et al., 1997; García-Abad et al., 1998; Lino, 2003; Teixeira et al., 2014). Nonetheless, no studies of population genetics are available for this species along the Brazilian Atlantic coast so far. The genetic variation can be estimated from the control or D-loop region of mitochondrial DNA (mtDNA), as commonly reported in analyses of population genetics of fishes (Speller et al., 2013; Mamzunder and Alam, 2009; Debes et al. 2008; Kristoffersen and Magoulas 2008; Atarhouch et al., 2006; Liu et al., 2006; Avise, 2004). Therefore, this mtDNA marker has been selected to the present analyses.

Our goal was to evaluate the genetic structure and estimate the levels of intra and interpopulation variation in Atlantic thread herring. Furthermore, we aimed to verify whether the population from Brazilian coast a single and genetically homogeneous stock or not.

MATERIALS AND METHODS

The specimens of *O. oglinum* were collected along the Brazilian Atlantic coast in the states of Pará, Maranhão, Ceará, Rio Grande do Norte, Bahia, Rio de Janeiro and Santa Catarina. After fishing and identification of specimens based on Whitehead (1985), fragments of the muscle tissue were removed and stored in 70% ethanol at -20°C up to DNA extraction. It should be pointed out that according to the Brazilian legislation this is not regarded as overfished.

DNA isolation

The DNA was isolated from 50 mg of muscle tissue using the phenol: chloroform method described by Sambroock *et al.* (2001) after treatment with proteinase-K and RNAse. Afterwards, the DNA was precipitated in isoamyl alcohol and sodium chlorite. The quality of DNA samples was verified after electrophoresis in 1% agarose gel.

DNA amplification and sequencing

The control or D-loop region of mtDNA was amplified via PCR (Polymerase Chain Reaction), using the primers Dloop L1 5'-CCTAACTCCCAAAGCTAGGTATTC-3' (forward) and Perc12S2R 5'-CGGTCGGTGGCGCCAATATG-3' and H2 5'-CCGGCAGCTCTTAGCTTTAACTA-3' (reverse). Each PCR comprised Taq polymerase buffer (10x), $MgCl_2$ (25 mM), primers (200 ng/µL), template DNA (200 ng/µL), 1.25 mM of

dNTPs (dATP, dCTP, dGTP, dTTP), Taq DNA polymerase (2 U/μL) and ultrapure water to a final volume of 25 μL. The PCR parameters encompassed a first denaturation step at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, extension at 72°C for 2 minutes, plus a final extension step at 72°C for 7 minutes. The PCR products were checked after electrophoresis in 1% agarose gel.

Afterwards, 2.5 μL of the amplified DNA samples were purified using the ExoSAP-IT enzyme (Amersham Pharmacia Biotech Inc.) and sequenced in ABI 3500 automatic sequencer using the dideoxy method (Sanger *et al.*, 1977) and the ABI Prism TM Dye Terminator Cycle Sequencing Ready Reaction (Perkin Elmer) kit.

Genetic analysis

The nucleotide sequences were edited in the software BIOEDIT 7.0.9 (Hall, 1999) and aligned using Clustal W (Thompson *et al.,* 1994). The genetic diversity was estimated based on the haplotype diversity index (*h* – probability that two random haplotypes are different between individuals) and the nucleotide diversity index (π – probability that two homologous nucleotides selected randomly are different between individuals) (Nei, 1987) using the software DNASP 6.12.3 (Rozas and Librado, 2009).

Both Fu (*Fs*) (Fu, 1997) and Tajima (*D*) (Tajima, 1989) neutrality tests were performed in the software ARLEQUIN 3.5 (Excoffier and Lischer, 2010) to check putative neutrality deviation and to infer the demographic history of populations. Values of D and Fs that are significantly different from zero indicate neutrality deviation. When several recent mutations have taken place, both D and Fs values are usually negative, representing population growth. On the other hand, positive values are indicators of balancing selection, population subdivision or bottleneck effects (Tajima, 1989).

The Analysis of Molecular Variance (AMOVA) was carried out in ARLEQUIN (Excoffier and Lischer, 2010). This method is used to test the geographic structure of population into distinct clusters, thus providing insights about the presence or not of population differentiation. The AMOVA was estimated based on pairwise Fst values from each population. According to these results, we estimated the genetic diversity and structure of Atlantic thread herring.

The demographic history of populations was inferred from the mismatch distribution (differences between pairs of haplotypes) and according to the parameters of demographic expansion (t, q0 and q1) based on minimum squares as reported by Schneider and Excoffier (1999). The validation of the stepwise expansion model was tested using the parametric bootstrap estimated from the significance values in the sum of square deviations (SSD) and Raggedness (Rg) index (Harpending, 1994), also in the software ARLEQUIN (Excoffier and Lischer, 2010).

The software Network 5 (Bandelt *et al.,* 1999) was used to generate the haplotype network to visualize the distribution and the relatedness of population haplotypes.

RESULTS

A total of 313 specimens of the Atlantic thread herring was collected along the Brazilian coast, encompassing the states of Pará, Maranhão, Bahia, Ceará, Rio Grande do Norte, Rio de Janeiro and Santa Catarina. After alignment and edition, the dataset comprised 225 sequences of 649 base pairs (bp), since PCR has failed in 88 samples. The mean nucleotide composition was: 36.28% A, 30.29% T, 16.29 %G and 17.14% C.

The lowest number of polymorphic sites was found in the samples from the coast of Santa Catarina while the highest values was detected in the samples from Maranhão (Table 1). The number of transitions ranged from 54 in the specimens from Santa Catarina to 82 in the samples from Rio Grande do Norte. The number of transversions varied from 7 (Santa Catarina and Rio de Janeiro coast) to 41 (Ceará). The presence of indels ranged from 3 in the sample Santa Catarina to 10 in Ceará.

Table 1. Genetic diversity and neutrality test in the Atlantic thread herring.

						Molecular Diversity		Neutrality Test	
POPULATION	N	NS	H	Hu	S	h	π	*D* (Tajima)	*Fs* (Fu's)
Pará	25	18	17	12	71	**1.0000 +/- 0.0185**	**0.090025 +/- 0.046894**	-0.77733 (0.23130)	-6.62806 (0.00560)*
Maranhão	50	34	29	26	98	0.9982 +/- 0.0077	0.077963 +/- 0.039567	-1.30536 (0.07680)	-18.27843 (0.00000)*
Ceará	50	33	33	30	97	1.0000 +/- 0.0075	0.078780 +/- 0.040221	-0.58198 (0.03520)*	-22.30295 (0.00000)*
Rio G. do Norte	50	32	32	18	93	1.0000 +/- 0.0078	0.073265 +/- 0.037341	-1.32849 (0.07300)	-20.38203 (0.00000)*
Bahia	63	49	44	31	74	0.9966 +/- 0.0053	**0.058294 +/- 0.029779**	-1.09888 (0.12840)	-24.00000 (0.00000)*
Rio de Janeiro	50	35	32	25	62	0.9983 +/- 0.0074	0.061943 +/- 0.032103	-1.14840 (0.11310)	-24.45232 (0.00000)*
Santa Catarina	25	24	22	14	59	**0.9964 +/- 0.0133**	0.065661 +/- 0.034176	-0.80224 (0.22320)	-11.16440 (0.00080)*

N= Number of collected specimens; NS= Number of sequenced samples; H= Number of haplotypes; Hu= Number of unique haplotypes; S= Number of polymorphic sites; h= Haplotype diversity; π= Nucleotide diversity. *P<0.05.

A total of 180 haplotypes were detected, being 163 of them exclusive to each locality and 17 haplotypes shared between, at least, two populations along the Brazilian coast. The lowest and the highest percentage of unique haplotypes per population was observed in the samples from Rio Grande do Norte and Ceará, respectively. Considering the high number of unique haplotypes in the D-loop region of the mtDNA in *Opisthonema oglinum*, the haplotype network is not shown. The values of nucleotide (π) and haplotype (h) diversity were remarkably high in all samples of the Atlantic thread herring (Table 1). The highest π value was observed in the samples from the state of Pará (π= 0.090025 +/-0.046894 or 9 %), while the lowest value was found in Bahia (π= 0.058294 +/-0.029779 or 6%). As for the haplotype diversity, the highest value was observed in Pará (h= 1.0000 +/-0.0185) and the lowest value was detected in Santa Catarina (h= 0.9964 +/-0.0133).

Negative values were obtained in both Tajima D and Fu's Fs tests for all sampled localities. Nonetheless, the Tajima D value was significant only for the population from Ceará ($p = 0.03520$), while Fu's Fs values were significant for all samples ($p < 0.01$) (Table 1). The demographic history of the Atlantic thread herring was inferred from the mismatch distribution, resulting in non-significant Rg values from 0.00220 to 0.03207 (PRg > 0.05) that indicate population expansion (. The SSD values also indicated population expansion for all sampled localities (PSSD > 0.05) (Table 2).

Table 2. Parameters of mismatch distribution in the Atlantic thread herring.

POPULATION	τ	$\theta 0$	$\theta 1$	SSD (P*SSD*)	Rg (PRg)
Pará	8.00000	17.99992	6834.95675	0.01770 (0.20000)	0.03207 (0.45000)
Maranhão	11.00000	10.10381	79.29593	0.00819 (0.21000)	0.00569 (0.75000)
Ceará	10.00000	6.82909	97.03141	0.00140 (0.96000)	0.00220 (1.00000)
Rio Grande do Norte	11.01000	7.57433	91.43841	0.00212 (0.92000)	0.00278 (0.98000)
Bahia	10.80000	2.61210	61.46357	0.00153 (0.87000)	0.00323 (0.98000)
Rio de Janeiro	9.90000	1.49765	71.56156	0.00390 (0.37000)	0.00646 (0.65000)
Santa Catarina	13.40000	1.47997	55.97245	0.00291 (0.87000)	0.00727 (0.88000)
Mean	10.59684	6.87098	1041.67430	0.00539 (0.60286)	0.00724 (0.81286)

τ= parameter of expansion; $\theta 0$= parameter of mutation before population expansion; $\theta 1$= parameter of mutation after population expansion; SSD (P*SSD*) = sum of square deviation; Rg (PRg) = Raggedness index (Raggedness statistical probability).

The genetic structure inferred from AMOVA was carried out considering the presence of a single gene pool along the Brazilian coast, revealing that most of genetic variation is found within the overall set of specimens instead of among samples per locality. The variation within this large population was nearly equal to 98% when compared to 2% among the populations from the seven Brazilian states, with significant Fst values ($P < 0.05$) (Table 3). Therefore, the AMOVA revealed that the specimens of *Opisthonema oglinum* along the Brazilian coast represent a single and genetically homogeneous stock.

Table 3. AMOVA based on pairwise distance considering the presence of a single population of the Atlantic thread herring.

	Components of variation	Percentage of variation	FST index
Among local samples	0.14570 Va	2.10	0.02*
Within population	6.79440 Vb	97.90	

Values calculated at random for 1.023 permutations. *$P < 0.05$ (significant).

DISCUSSION

Our results revealed extremely high levels of genetic variation in *O. oglinum* from the Brazilian Atlantic coast. Similar results have been previously reported in other clupeids (Speller *et al.,* 2013; Mamzunder and Alam, 2009; Debes *et al*, 2008). The negative values obtained by the Tajima D and Fu's Fs tests indicated that the population of *O. oglinum* from the Brazilian coast are under genetic equilibrium. Apparently, no selective pressure is present over the haplotypes in the D-loop region of mtDNA in the Atlantic thread herring. Accordingly, high

values of haplotype and nucleotide diversity are found in large populations, particularly those widespread over large geographic distances (Gao *et al.,* 2018). As a matter of fact, increased genetic diversity has been observed in migratory and panmictic populations of fishes (Santos *et al.,* 2007).

A large amount of unique haplotypes was detected in the Atlantic thread herring suggesting past drastic fluctuation in population number as a result of bottleneck or founder effects followed by fast population growth. During this process, new alleles should have been created by mutation and determined the high frequency of unique haplotypes (Watterson, 1984; Rogers and Harpending, 1992), being compatible with the lack of dominant haplotypes (Baisvar *et al.,* 2019). Therefore, we infer that population of *O. oglinum* from the Brazilian coast have undergone recent and temporal environmental fluctuations, resulting in a single and large population of Atlantic thread herring in Western South Atlantic.

Likewise, the AMOVA indicated the presence of a single and homogeneous gene pool in *O. oglinum* widespread along the Brazilian coast since most of genetic variation was found within the entire set of specimens but not among samples per locality. The FST values revealed that gene flow among specimens of the Atlantic thread herring in the Brazilian coast are high enough to prevent subpopulation structuring. Local populations that are connected by some degree of migration and gene flow are known as metapopulations. In this sense, the spatial distribution of individuals and populations play a key role in the growth rate and the dynamics of metapopulations, particularly in aquatic organisms (Hanski and Gaggiotti, 2004).

The non-significant values obtained in Raggedness and sum of square deviation tests supported that *O. oglinum* has been facing recent population expansion after a putative bottleneck event. Therefore, the hypothesis of recent expansion in the population of the Atlantic thread herring was corroborated by our analyses. A similar scenario was reported in the clupeid *Ethmalosa fimbriata* (Durant *et al.,* 2005). On the other hand, this genetic pattern might be related to the ecological traits of *O. oglinum*, since this is an estuarine-dependent species that feeds over large geographic areas and presents an extended reproductive period (García-Abad *et al.,* 1998).

Our results revealed that *O. oglinum* from the Brazilian Western Atlantic coast in a panmictic population or a metapopulation inasmuch as a single gene pool was observed across the Brazilian coast. Nonetheless, population genetic studies based on less variable molecular markers in relation to the control region of mtDNA should be performed to validate this hypothesis.

CONCLUSION

This study represents a pioneer contribution to the knowledge of population genetics of *O. oglinum* along the Brazilian Atlantic, revealing high levels of intrapopulation genetic variation. AMOVA indicated a single gene pool in the Atlantic thread herring along their range without evidence of genetic structure. Apparently, events of population expansion and gene flow between the sampled regions have taken place, resulting in the formation of a large population tolerant to distinct climate conditions along the Brazilian coast.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Horacio Schneider (*in memoriam*) for all the knowledge and advice to his students. His loss represented a huge gap in the scientific community. We would also like to thank Dr. Diego Carvalho for his classes about scientific writing. Funding for this research was provided by CNPq (grants 306233/2009-6 to IS and 306233/2009-6 to HS) and

FAPESPA (PRONEX 2007 to HS). Cleonilde Queiroz was supported by a PhD scholarship from CAPES.

REFERENCES

ATARHOUCH, T.; RÜBER, L.; GONZALEZ, E. G.; ALBERT, E. M.; RAMI, M.; DAKKAK, A.; ZARDOYA, R. Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). **Molecular Phylogenetics and Evolution**, v. 39, p. 373–383, 2006.

AVISE, J. C. **Molecular markers, natural history, and evolution.** Sunderland, Massachusetts: Sinauer Associates, Inc. 684 p. 2nd ed. 2004.

BAISVAR, V. S.; SINGH, M.; KUMAR, R. Population structuring of *Channa striata* from Indian waters using control region of mtDNA. **Mitochondrial DNA Part A**, v. 30, n. 3, p. 414-423. 2019.

BANDELT, H. J.; FORSTER, P.; ROHL, A. A Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution**, v. 16, p. 37-48. 1999.

DEBES, P. V.; ZACHOS, F. E.; HANEL, R. Mitochondrial phylogeography of the European sprat (*Sprattus sprattus* L., Clupeidae) reveals isolated climatically vulnerable populations in the Mediterranean Sea and range expansion in the northeast Atlantic. **Molecular Ecology**, n. 17, p. 3873-3888, 2008.

DURAND, J. D.; TINE, M.; PANFILI, J.; THIAW, O. T.; LAE, R. Impact of glaciations and geographic distance on the genetic structure of a tropical estuarine fish, *Ethmalosa fimbriata* (clupeidae, S. Bowdich, 1825). **Molecular Phylogenetics and Evolution**, v. 36, n. 2, p. 277-287, 2005.

EXCOFFIER, L.; LISCHER, H. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources**, v. 10, p. 564-7, 2010.

FINUCANE, J. H.; VAUGHT, R. N. **Species profile of Atlantic thread herring, *Opisthonema oglinum* (Le Sueur 1818).** NOAA Technical Memorandum, NMFS-SEFC- 182. 1986.

FU, Y. X. **Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection.** Genetics, 147:915-925. 1997.

GAO, T. X.; YANG, T. Y.; YANAGIMOTO, T.; XIAO, Y. S. Levels and patterns of genetic variation in Japanese whiting (Sillago japonica) based on mitochondrial DNA control region. **Mitochondrial DNA Part A**, v. 30, n. 1, p. 172-183, 2018.

GARCÍA-ABAD, M. C.; YÁÑEZ-ARANCIBIA, A.; SÁNCHEZ-GIL, P.; TAPIA-GARCÍA, M. Distribución, abundancia y reproducción de *Opisthonema oglinum* (Pisces: Clupeidae) en. la plataforma continental del sur del Golfo de México. **Revista de Biologia Tropical**, v. 46, n. 2, p. 257-266, 1998.

HALL, T. A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucleic Ácids Symposium**, v. 41, p. 95-98, 1999.

HANSKI, I.; GAGGIOTTI, O. **Ecology, Genetics and Evolution of Metapopulations.** 2004. In: Metapopulation biology: past, present and future. p. 3-22. Elsevier Academic Press.

HARPENDING, H. C. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. **Human Biology**, v. 66, p. 591-600, 1994.

KRISTOFFERSEN, J. B.; MAGOULAS, U. M. A. Population structure of anchovy *Engraulis encrasicolus* L. in the Mediterranean Sea inferred from multiple methods. **Fisheries Research**, v. 19, p. 187-195, 2008.

LAVOUÉ, S.; MIYA, M.; MUSIKASINTHORN, P.; CHEN, W. J.; NISHIDA, M. Mitogenomic evidence for an Indo-West Pacific origin of the Clupeoidei (Teleostei: Clupeiformes). **Plos One**, v. 8, p. 1-15, 2013.

LIU, J. X.; GAO, T. X.; ZHUANG, Z. M.; JIN, X. S.; YOKOGA,W. A. K.; ZHANG, Y. P. Late pleistocene divergence and subsequent population expansion of two related fish, japanese anchovy (*Engraulis japonicus*) and australian anchovy (*Engraulis australis*). **Molecular Phylogenetics and Evolution**, v. 40, p. 712-723, 2006.

MAZUMDER, S. K.; ALAM, S. High levels of genetic variability and differentiation in hilsa shad, Tenualosa ilisha (Clupeidae, Clupeiformes) populations revealed by PCR-RFLP analysis of the mitochondrial DNA D-loop region. **Genetics and Molecular Biology**, v. 32, n. 1, p. 190-196, 2009.

NELSON, J. S.; GRANDE, T. C.; WILSON, M. V. H. **Fish of the world.** Fifth edition. Hoboken, New Jersey : John Wiley & Sons, 2016.

NEI, M. **Molecular Evolutionary Genetics.** University Press, New York, pp 176-207. 1987.

PATTERSON, K. R.; SANTOS, M. The thread-herrings *Opisthonema* spp. off Ecuador: review and population dynamics. **Original Research Article Fisheries Research**, v. 14, n. 4, p. 273-294, 1992.

QUEIROZ, C.; SOUZA, R.F.C.; SILVA, S. S.; DIAS, C. A. G. M.; FECURY, A. A.; OLIVEIRA, E.; CUNHA, D. B.; SCHNEIDER, H.; SAMPAIO, I. Molecular phylogeny of Clupeiformes and the placement of some Western Atlantic and Amazonian Taxa. **Biota amazonia**, v. 10, n. 2, p. 14-19, 2020.

ROGERS, A. R.; HARPENDING, H. Population growth makes waves in the distribution of pairwise genetic differences. **Molecular Biology Evolution**, v. 9, p. 552-569, 1992.

ROZAS, J.; LIBRADO, P. DnaSP v5: A software for comprehensive analysis of DNA polimorphism data. **Bioinformatics**, v. 25, p. 1451-1452. 2009.

SANGER, F.; NICHLEN, S.; COULSON, A. R. **DNA Sequencing with chain-termination inhibitors.** Proceeding of the National Academy Science of the USA 74: 5463-5468. 1977.

SAMBROOK, J.; MACCALLUM, P.; RUSSEL, D. **Molecular cloning: A laboratory manual,** 3nd ed. Cold Springs Harbour Press, NY, ISBN 0-87969-577-3, 2344 p. 2001.

SANTOS, M. C. F.; RUFFINO, M. L.; FARIAS, J. P. High levels of genetic variability and panmixia of the tambaqui *Colossoma macropomum* (Cuvier, 1816) in the main channel of the Amazon River. **Journal of Fish Biology**, v. 71, p. 33-44, 2007.

SCHNEIDER, S.; EXCOFFIER, L. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. **Genetics**, v. 152, p. 1079-1089, 1999.

SEGURA, L. S. C. Interaccion trofica entre dos especies de sardina *Opisthonema oglinum* (LeSueur, 1818) y *Harengula jaguana* Poey, 1865 (Osteichthyes: Clupeidae) que coexisten en las Costas de Celestun, Yucatan, Mexico. **Tese**. Instituto Politecnico Nacional, Centro Interdisciplinario De Ciencias Marinas. La Paz. 86 p. 1995.

SMITH, J. W. Biology and Fishery for Atlantic Thread Herring, *Opisthonema oglinum,* along the North Carolina Coast. **National Marine Fisheries Service**, v. 56, n. 4, p. 1-7, 1994.

SPELLER, C. F.; HAUSER, L.; LEPOFSKY, D.; MOORE, J.; RODRIGUES, A.T.; MOSS, M.L.; MCKECHNIE, I.; YANG, D.Y. High Potential for Using DNA from Ancient Herring Bones to Inform Modern Fisheries Management and Conservation. **PLOS ONE**, v. 8, n. 7, p. 10-1371, 2013.

TAJIMA, F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. **Genetics**, v. 123, p. 585-595, 1989.

TEIXEIRA, S. R. A.; SAMPAIO, L. A. S. F.; MARINHO, R.; A. Study of the fisheries biology of Atlantic thread-herring,*Opisthonema oglinum*, in Cascavel county, Ceará State, Brazil. **Arquivos de Ciências do Mar**, v. 47, n. 2, p. 31-38, 2014.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. CLUSTAL W: improving the sensitivity of progressive multiples sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Research**, v. 22, p. 4673–4680, 1994.

VEGA-CENDEJAS, M. E.; MEXICANO-CINTORA, G.; ARCE, A. M. Biology of the thread herring *Opisthonema oglinum* (Pisces: Clupeidae) from a beach seine fishery of the Campeche Bank, Mexico**. Fisheries Research**, v. 30, p. 117-126, 1997.

WATTERSON, G. A. Allele frequencies after a bottleneck. **Theoretical Population Biology**, v. 26, p. 387-407, 1984.

WHITEHEAD, P. J. P. **FAO species catalogue: Clupeoid fishes of the world an annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads united nations development programme food and agriculture organization of the united nations anchovies and wolf-herrings.**

FAO fisheries synopsis. Part 1 - chirocentridae, clupeidae and pristigasteridae (suborder clupeoidei). vol 7. Rome FAO, 303 pp. 1985.

Submetido em: junho de 2020
Aprovado em: outubro de 2020