STUDIES ON FIN-FISH ASSEMBLAGE AND DIVERSITY ASSESSMENT OF UPPER BONNY RIVER, USING MORPHOLOGY AND MOLECULAR METHODS OF IDENTIFICATION

CHIAHA, Emiliana Ijeoma, ANUKWU, John Uchenna, EZEOMEKE, Somadina Immaculata and NWAMBA, Helen Ogochukwu

Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.

Corresponding Author: Chiaha, E. I. Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria. **Email:** emilianachiaha@gmail.com **Phone:** +234 706 747 4994

Received *January 15, 2024;* ***Revised*** *July 14, 2024;* ***Accepted*** *July 15, 2024*

ABSTRACT

This research focuses on the critical issue of biodiversity loss, primarily caused by the misidentification of economically important fish species. Fish samples were obtained at three stations twice in a month from November 2022 to September 2023 with the aid of local fishers using gill nets and cast nets for ecological studies of important fish species of Bonny River, Okrika LGA, Rivers State, Nigeria. The sampling results showed a total composition of 23,102 individuals belonging to six (6) orders, twelve (12) families, fourteen (14) genera and nineteen (19) species which were identified using a combination of DNA barcoding using mitochondrion cytochrome oxidase subunit I (COI) gene marker and morphological method. The tissue samples for 8 species (Pseudotolithus senegallus, Pseudotolithus typhus, Pseudotolithus elongatus, Pangasius polyuranodon, Chrysichthys nigrodigitatus, Neochelon falcipinnus, Coptodon guineensis and Lutjanus aratus) were excised, DNA isolated, amplified and sequenced. Cichlidae showed the highest diversity of 31.41% with three (3) species, Sciaenidae had a diversity value of 24.42% with three species and the remaining 10 families below 10%. Fish diversity was observed to be higher in January 2023 with 19 fish species and lowest in September 2023 with 9 fish species. The Berger-Parker's dominance (d) ranged from 0.01 to 0.31 depicting the dominance of a few species. Simpson's diversity index ranged from 0.90 to 0.99. The study highlights that the high fish diversity in specific species and families within the Bonny River may be attributed to inadequate managerial practices in Bonny fisheries.

Keywords: Abundance, Composition, eDNA, Ichthyofauna, Species identification, Genetic analysis

INTRODUCTION

Nigeria is home to many natural resources, both on land and in its many water bodies. Nigeria has the highest fish diversity in all of Western Africa, with over 270 different fish species inhabiting its freshwater bodies. This abundance of aquatic life creates opportunities for Nigeria to prosper economically through fishing and other related industries. Additionally, this wealth of biodiversity contributes to the overall health and well-being of Nigerians (Adewumi *et al*., 2015; Bolarinwa *et al*., 2016; Anwadike, 2020). Fish are the most numerous vertebrate groups on the planet, accounting for half of all vertebrate species. Fish consumption is frequently a staple of the human diet due to its high digestibility and flavour. Fisheries are also important sources of income for many communities (Charlton *et al*., 2016). So

far, 33,000 fish species have been identified throughout the world (Oosting *et al*., 2019).

Approximately 268 different fish species can be found in Nigeria's freshwater areas. These fish live in over 34 well-known freshwater bodies of water, such as rivers, lakes and reservoirs, which make up around 12% of Nigeria's total surface area. This surface area is around 94, 185,000 hectares (Odo *et al*., 2009).

The inland fisheries in tropical Africa face threats both by stress from climate change and by overexploitation. Species are becoming extinct and populations are declining at an alarming but poorly understood rate (Muringai *et al*., 2022). Many species may face extinction before they can be identified or described. This presents a problem for conservation planning and prioritisation because those species that have not been identified obviously cannot be protected effectively (Costello *et al*., 2013; Sáez-Gómez and Prenda, 2022). Caddy and Garibaldi (2000) reported that only 65.09% of worldwide fishery captures reported to the FAO for the year 1996 were identified at the species level, ranging from about 90% in temperate areas to less than 40% in tropical regions (Nwakanma *et al.,* 2015). Surveys into the accuracies of species identifications have not been reported, but a significant percentage of identifications may still be erroneous (Conn *et al*., 2013; Austen *et al*., 2016).

The abundance of fish species provides a useful indication of environmental and pollution stress (Zeppilli *et al*., 2015; Zaghloul *et al*., 2020). The technology of fish exploitation in Nigeria's inland fisheries is mainly characterized by the use of simple fishing gear and techniques (Egesi, 2016). The continuing depletion of the world's marine fisheries is a key indicator of a critical decline in ocean health and a global issue of increasing concern (Manickavasagam *et al*., 2019; Zaghloul *et al*, 2020). In all these water bodies in Nigeria, the methods employed for catching fishes are broadly divided into two; the modern and crude or traditional (Olaniyan, 2015). Hence, the study was aimed at identifying the fish species abundance and causes of endangered fish species for a sustainable aquatic environment and decision-making (Quevedo *et al*., 2023).

Studies conducted by Armani *et al.* (2015) and Pollack *et al.* (2018) identified multifarious challenges in the fish market with issues of mislabeling, fraud and substitutions that prevent the expansion of the market. Some mislabeling issues are a result of the close resemblance between different fish in terms of appearance, topology, texture, taste and other morphometric characteristics (Ghouri *et al*., 2020).

DNA barcoding coupled with classical morphological description has become a promising approach for species-level identification. A wide variety of protein-based and DNA-based methods have been evaluated for the molecular identification of fish species in Africa (Iyiola *et al*., 2018). Hebert *et al.* (2003) proposed a single gene sequence to discriminate the vast majority of animal species, using a 650-bp fragment of the 5′ ends of the mitochondrial cytochrome c oxidase subunit I (COI) gene as a global bio identification sequence for animals (Nwani *et al*., 2011; Mohammed *et al*., 2021). This technology (DNA barcoding) relies on the observation that the 'barcode' sequence divergence within species is typically much lower than the divergence exhibited between species making it an effective marker for species identification and discovery (Nwani *et al*., 2011; Sonet *et al*., 2022).

The use of molecular and morphological approaches for identifying fish species has been suggested to mitigate the limitations of the lack of local fish identification expertise (Zhang and Hanner, 2011; Di Pinto *et al*., 2015). The overall aim of this research project was to determine the feasibility of using COI mitochondrial gene and morphological method for the Identification of aquatic species in Bonny River, thus promoting biodiversity and awareness. To achieve this overall aim, several study objectives were to: (i) determine the diversity of fish species in Bonny River, (ii) identify selected fish species in Bonny River using morphometric studies, (iii) determine the variations in physico-chemical parameters of Bonny River, (iv) use DNA barcoding to complement morphologically identified fish species in Bonny River and (v) establish a barcoding reference database/record of fish species in the Bonny River.

MATERIALS AND METHODS

Description of Study Area: The study area was Bonny River located at Okrika Community in Okrika Local Government Area of Rivers State, Niger Delta, Nigeria (Figure 1).

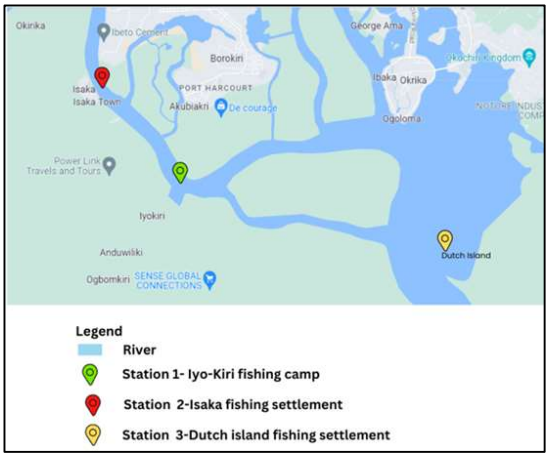

Figure 1: Study area showing the Bonny River and the sampled fishing stations (Google Map, 2024)

Bonny River lies approximately at latitude 4°27'N and longitude 7°10'E (Onuoha, 2008). The length of the river is about 150 km, the Bonny River flows through Bonny Island which is adjacent to the Okrika Local Government Area. Okrika community lies on the north bank of the Bonny River and Okrika Island, 56 km upstream from the Bight of Benin. The Bonny River's mouth opens into the Bight of Bonny in the Gulf of Guinea (Ede, 2015).

Study Design and Sampling Stations Location: The study was designed to have three sampling stations in the Bonny River. The three sample stations were established (S1 – Iyo-Kiri fishing camp Okrika, S2 – Isaka fishing settlement and S3 – Dutch island fishing settlement) along the main course of the river. The experiment lasted from November 2022 to September 2023 (10 months).

Fish Sample Collection, Treatment and Preservation: The sample stations were sampled twice every month using multiple fishing gear such as gill nets, hook and line, traps and nets of different mesh sizes. The fish samples were collected from local fishers as they landed their catch. Fish samples were preserved in ice at a controlled temperature of 0 – 4 °C, after which they were transported to the experimental station. A total of 19 fish species (Table 1) were collected from estuarine water sources (Bonny River) in Rivers State, Nigeria.

Transportation of Fish to Laboratory: The fishes (frozen) were placed in different Thermolineo Insulated Coolers and were transported to the experiment site at Heldins Fishery Unit, Old Airport Road, Thinkers Corner, Emene, Enugu for analyses.

Identification of Fish Samples: After sample collection, the fishes were morphologically identified *in situ* by visual inspection and taxonomically classified using standard guides (Paugy *et al.*, 2003; Stiassny *et al.,* 2007). This was further supported using the NCIB database that provides detailed information on various fish species worldwide, including tropical finfish. It offers descriptions, identification keys and distribution information.

Voucher specimen of fish species - *Pseudotolithus senegallus* (ESUT/ABBL/FISH0030), *Pseudotolithus typhus* (ESUT/ABBL/FISH0031), *Pseudotolithus elongatus* (ESUT/ABBL/FISH0032), *Pangasius polyuranodon* (ESUT/ABBL/FISH0033), *Chrysichthys nigrodigitatus* (ESUT/ABBL/FISH0034), *Neochelon falcipinnus* (ESUT/ABBL/FISH0035), *Coptodon guineensis* (ESUT/ABBL/FISH0036) and *Lutjanus aratus* (ESUT/ABBL/FISH0037) were kept in Applied Biology and Biotechnology Museum of Natural History, Enugu State University of Science and Technology for referral purposes.

Determination of Fish Composition: The dichotomous identification method of fish species determination (Paugy, 2010) was used to identify the fish. The meristic features of the various fishes which include dorsal, anal, caudal, pectoral, ventral fin rays and spines were considered to achieve dichotomous identification.

Table 1: Fish families in Bonny River, Port Harcourt, Niger Delta Nigeria

Species	Genus	Family	Order
Pseudotolithus elongatus	*Pseudotolithus*	Sciaenidae	Perciformes
Pseudotolithus senegallus	*Pseudotolithus*	Sciaenidae	Perciformes
Pseudotolithus typhus	*Pseudotolithus*	Sciaenidae	Perciformes
Lutjanus Aratus	*Lutjanus*	Lutjanidae	Perciformes
Lutjanus goreensis	*Lutjanus*	Lutjanidae	Perciformes
Lutjanus rivulatus	*Lutjanus*	Lutjanidae	Perciformes
Lutjanus bohar	*Lutjanus*	Lutjanidae	Perciformes
Hemichromis bimaculatus	*Hemichromis*	Cichlidae	Perciformes
Coptodon guineensis	*Coptodon*	Cichlidae	Perciformes
Polydactylus polyuranodon	*Polydactylus*	Polynemidae	Perciformes
Oreochromis niloticus	*Oreochromis*	Polynemidae	Cichliformes
Trachinotus carolinus	*Trachinotus*	Carangidae	Cichliformes
Pomadasys perotaei	*Pomadasys*	Haemulidae	Cichliformes
Sphyraena barracuda	*Sphyraena*	Sphyraenidae	Cichliformes
Cynoglossus_senegalensis	*Cynoglossus*	Cynoglossidae	Pleuronectiformes
Parabramis pekinensis	*Parabramis*	Cyprinidae	Cypriniformes
Neochelon falcipinnis	*Neochelon*	Mugilidae	Mugiliformes
Pangasianodon hypophthalmus	*Pangasianodon*	Pangasiidae	Siluriformes
Chrysichthys Nigrodigitatus	*Chrysichthys*	Claroteidae	-

The morphometric characteristics of the fishes were also measured such as the standard length, total length, caudal peduncle length, caudal peduncle depth, dorsal fin depth, dorsal fin length, body depth, predorsal depth, head length, eye diameter and head depth.

Tissue Sample Collection and Preservation: Muscle tissue was extracted from the side of each fish using a sharp blade or scalpel blade to cut about 5 mm^3 of fish muscle as described by Dowgiallo (2005). Before DNA extraction, the muscle samples were collected and preserved in 50 ml of DNA/RNA shield and transported to Inqaba Biotech Laboratory, Ibadan for further molecular analysis.

DNA Extraction: Quick DNA Mini Prep Plus kit (Zymo Research, 2023) was used for the DNA extraction. Fish muscle samples of about 5 mm^3 for each sample were used for DNA extraction. A clean plastic pestle was employed for grinding and homogenization of the tissue for about 2 minutes. The samples were then placed in clean microcentrifuge tubes of 1.5 mL and further labelled with an identification number (1 to 32) according to the number of samples. Ninety-five (95) µL of water, solid tissue buffer and ten (10) µL of proteinase k were added to the samples in the microcentrifuge tubes. The tubes were vortexed for 10 – 15 seconds and then incubated at 55^{o}C for 1 – 3 hours until the tissue was solubilized. The tubes were centrifuged at 12,000 x g for one minute to remove the insoluble debris. The aqueous supernatants were then transferred to a new microcentrifuge tube of 1.5 mL. This was followed by the addition of 400 µL of Genomic Binding Buffer and vortexing for 10 – 15 seconds. The mixture was transferred to a Zymo-Spin IIC-XLR column in a collection tube and centrifuged at ≥ 12,000 x g for 1 minute. The collection tube was then discarded with the flow through. About 400 µL of DNA *Pre*-wash Buffer was added to the spin column in a new collection tube and centrifuged at ≥ 12,000 x g for 1 minute. The collection tube was then emptied and immediately followed by the addition of 700 µL g-DNA Wash Buffer to the spin column further centrifuged at ≥ 12,000 x g for 1 minute. The collection tube was then discarded with the flow through. The spin column was then transferred to a new clean microcentrifuge tube. Fifty (50) µL of DNA Elution Buffer was added to the matrix, incubated for 5 minutes at room temperature and then centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was stored at ≥ 20^{o}C in preparation for amplification.

Amplification of DNA by PCR: For this study, a 651-bp fragment of the standard cox1 barcode region of the fish target region was amplified using the One Taq Quick Load 2X Master Mix

(NEB, Catalogue No: M9486), nuclease-free water, template DNA with two primers for forward and reverse reactions (FISH F1-TCAACCAACCACAAAGACATTGGCAC and FISH F2-TAGACTTCTGGGTGGCCAAAGAATCA). After the thermal cycling, the amplified DNA was stored at -20°C as described by (Shokralla *et al.,* 2010).

Analyzing PCR Product by Gel Electrophoresis: The gel-casting tray was used for gel electrophoresis. One (1) g of powdered agarose gel was dissolved in 100 ml of 1X Tris Acetate EDTA (TAE). It was heated until the agarose gel was completely dissolved in the buffer and allowed to cool after which 4 µL of Safeview Classic (gel stain) was added. It was allowed to cool to about 60°C and poured into the tray with casting dams fit on both ends of the tray and combs in the correct position and allowed to set. After the gel was set, the combs and casting dams were removed, while the tray was placed in the electrophoresis tank containing the buffer (TAE). The ladder and samples were carefully loaded into the wells and tank covered with its lid and connected to the negative (–ve) and positive (+ve) electrodes and power supply. The gel was kept for approximately 30 minutes at 130v and was viewed using a UV transilluminator as described by Lucentini *et al.* (2006).

DNA Sequencing: The PCR products were further purified using the EXOSAP method. The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermFisher Scientific) for each reaction and every sample. The extracted fragments were sequenced in the forward and reverse directions as described by Sanger *et al.* (1977).

Diversity Indices: Species diversity was evaluated by using Simpson's index of diversity (Simpson, 1949; Anwana and Nwosu, 2014), expressed as D=1/C, where $C = \Sigma N_i (N_i-1) / N_t(N_t-1)$. But usually: $C-\Sigma (N_i/N_t)^2$, N_i = number of individuals of the ith species, N_t = total number of individuals in the sample and Diversity index (D) can be expressed in the form 1-C (Anwana and Nwosu, 2014).

Species Dominance: This was calculated using the Berger- Parker dominance index given by: D = Nmax/NT, where NT is the proportion of total catch due to the dominant species and Nmax is the maximum number of catches.

Data Analysis: Analysis of Variance (ANOVA) was used to test for significant differences in the physical-chemical parameters in the different stations. All analysis was carried out using Excel. A total of sequences was generated from the ABI 3500xl Genetic Analyzer (Applied Biosystems). Each sample was identified to the species level using the BLAST program on the GenBank (National Center for Biotechnology Information) (Altschul *et al.,* 1997). The sequencing results were carefully trimmed and edited using the DNA subway as described by Merchant *et al.* (2016). Pairwise alignment of sequences and translation to amino acids was done using Bioedit and MEGA 11 software (Tamura *et al.,* 2021). DNASTAR was used to analyze the ab1 files generated by the ABI 3500XL Genetic Analyzer. The species diversity was analyzed using results from Simpson's Index (D), the Reciprocal of Simpson's index (Dr) and the Shannon-Weiner index of general diversity (H').

RESULTS

Species Composition: A total of 23102 individuals were caught within the fisheries. Nineteen (19) species belonging to fourteen (14) genera, twelve (12) families and six (6) orders were recorded in Bonny River, as shown in the list of fish species in Table 2. Members of the Families Sciaenidae, Lutjanidae, Pangasiidae, Mugilidae, Cichlidae, Clariidae Polynemidae, Carangidae, Clariidae, Haemulidae, Sphyraenidae, Cyprinidae, were caught in the river (Figure 2). At the family level, Cichlidae had the highest representation with three species; *Hemichromis bimaculatus*, *C. guineensis* and *Oreochromis niloticus*. This was followed by the Sciaenidae with three species; *P. elongatus*, *P. senegallus and P. typhus*. It was followed by the Clariidae, Mugilidae, Lutjanidae, Polynemidae, Pangasiidae, Cynoglossidae, Sphyraenidae, Carangidae, Cyprinidae and Haemulidae which had only one species except for Lutjanidae which had four species.

Table 2: Relative abundance of fishes caught in Bonny River

Family Species	Total Catch	Relative Abundance (%)	Family (%)
Sciaenidae			24.42
Pseudotolithus elongatus	2401	10.39	
Pseudotolithus senegallus	1940	8.40	
Pseudotolithus typhus	1300	5.63	
Lutjanidae			7.43
Lutjanus aratus	456	1.97	
Lutjanus goreensis	307	1.33	
Lutjanus bohar	565	2.45	
Lutjanus agennes	389	1.68	
Pangasiidae			2.58
Pangasius polyuranodon	595	2.58	
Mugilidae			10.42
Neochelon falcipinnis	2407	10.42	
Cichlidae			31.41
Hemichromis bimaculatus	1476	6.39	
Coptodon guineensis	2324	10.06	
Oreochromis niloticus	3456	14.96	
Claroteidae			11.02
Chrysichthys nigrodigitatus	2546	11.02	
Polynemidae			4.27
Polydactylus quadrifilis	987	4.27	
Carangidae			1.84
Trachinotus lepturus	425	1.84	
Cynoglossidae			2.31
Cynoglossus senegalensis	534	2.31	
Haemulidae			1.12
Pomadasys perotaei	258	1.12	
Sphyraenidae			1.90
Sphyraena barracuda	439	1.90	
Cyprinidae			1.29
Parabramis pekinensis	297	1.29	
Total	23102	100	100

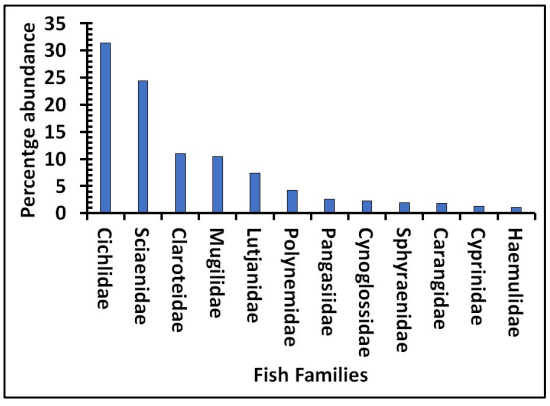

Figure 2: Percentage dominance of the different fish families in Bonny River

Species Diversity: Table 3 provide a broad overview of the ecological indices and ichthyofaunal composition of the Bonny River in Rivers State, Niger Delta, Nigeria. with further illustrations in Figure 3. Diversity indices calculated indicated a polydiverse ecosystem comprising 19 species belonging to 14 genera, 12 families and 6 orders. The different genera contained one to two species. The Berger-Parker's dominance (D) ranged from 0.01 to 0.31. Simpson's index of diversity ranged from 0.90 to 0.99 (Table 4).

Table 3: Diversity of fish species within the months in Bonny River

Months	Number of species
November	17
December	16
January	19
February	15
March	18
April	14
May	13
June	12
July	11
August	10
September	9

In terms of the orders, Perciformes comprised four families (Sciaenidae, Lutjanidae, Cichlidae and Polynemidae), followed by the Cichliformes (Carangidae, Haemulidae and Sphyraenidae) and Siluriformes (Pangasiidae, Claroteidae, Clariidae).

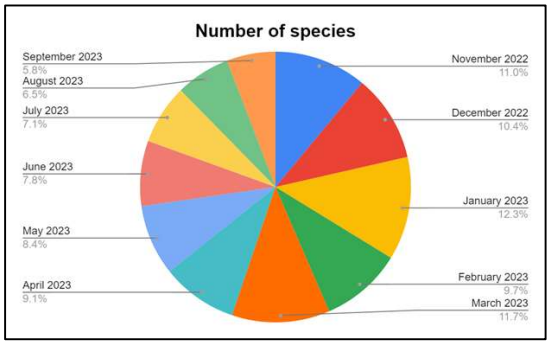

Figure 3: Diversity of fish species from November 2022 to September 2023 sampling period in Bonny River

The least occurring orders were Pleuronectiformes (Cynoglossidae), Cypriniformes (Cyprinidae) and Mugiliformes (Mugilidae) with one family each.

Table 4: The Berger-Parker dominance index, and Simpsons diversity index of some fishes from Bonny River

Fish Families	Fish Abundance	Family (%) Dominance	Berger-Parker Dominance Index	Simpsons Index
Cichlidae	7256	31.41	0.31	0.90
Sciaenidae	5641	24.42	0.24	0.94
Claroteidae	2546	11.02	0.11	0.98
Mugildae	2407	10.42	0.10	0.99
Lutjanidae	1717	7.43	0.07	0.99
Polynemidae	987	4.27	0.04	0.99
Pangasiidae	595	2.58	0.03	0.99
Cynoglossidae	534	2.31	0.02	0.99
Sphraenidae	439	1.90	0.02	0.99
Carangidae	425	1.84	0.02	0.99
Cyprinidae	297	1.29	0.01	0.99
Haemulidae	258	1.12	0.01	0.99
Total	23102	100.00		

Lutjanidae (7.43%) was the most diverse family contributing four species. The second most diverse family was the Sciaenidae (24.42%) with three species, followed by Cichlidae (31.41%) which happens to be the most abundant. Others include Pangasiidae (2.58%), Mugilidae (10.42%), Claroteidae (11.02%), Polynemidae (4.27%), Carangidae (1.84%), Cynoglossidae (2.31%), Haemulidae (1.12%), Sphyraenidae (1.90%), Cyprinidae (1.29%).

The most abundant species was the *O. niloticus* (14.96%), followed by *C. nigrodigitatus* (11.02%), *N. falcipinnus* (10.42%), *P. elongatus* (10.39%), *C. guineensis* (10.06%), *P. senegallus* (8.40%), *H. bimaculatus* (6.39%), *Polydactylus quadrifilis* (4.27%), *P. typhus* (5.63%), *P. polyuranodon* (2.58%), *Lutjanus bohar* (2.45%), *Cynoglossus senegalensis* (2.31%), *Sphyraena barracuda* (1.90%), *L. aratus* (1.97%), *Trachinotus lepturus* (1.84%), *Lutjanus rivulatus* (1.68%), *Lutjanus goreensis* (1.33%), *Parabramis pekinensis* (1.29%) and *Pomadasys perotaei* (1.12%).

Species of conservation importance in Bonny River include three polyspecific fish families, namely: Sciaenidae, Lutjanidae and Cichlidae and five monospecific fish families, namely: Pangasiidae, Mugilidae, Claroteidae Polynemidae, Carangidae, Cynoglossidae, Haemulidae, Sphyraenidae, Cyprinidae.

Species of ecological significance are *O. niloticus, C. nigrodigitatus, P. elongatus, P. senegallus, C. guineensis, P. typhus, T. lepturus, S. barracuda and Lutjanus* species (*L. bohar, L. aratus, L. rivulatus and L. goreensis*) that occurred in the river from other adjacent African rivers.

BLAST of Fish Species from NCBI Database: The BLAST results demonstrated different species of fish inherent in the samples sequenced (Table 5).

Table 5: BLAST result of sampled fish species from NCBI database

Names of Fish Sample	Percentage ID	GenBank Accession
Pseudotolithus senegallus	100	KP722769.1
Pseudotolithus typhus	90.54	NC_056258.1
Pseudotolithus elongatus	100	KY442723.1
Pangasius polyuranodon	85.78	Q151824.1
Chrysichthys nigrodigitatus	100	HG803416.1
Neochelon falcipinnus	100	HM208829.1
Coptodon guineensis	97.7	KJ938159.1
Lutjanus aratus	96.4	KJ557440.1

There are eight fish species *P. senegallus* (KP722769.1), *P. typhus* (NC_056258.1), *P. elongatus* (KY44273.1), *P. polyuranodon* (Q151824.1), *C. nigrodigitatus* (HG803416.1), *N. falcipinnus* (HM208829.1), *C. guineensis* (KJ938159.1) and *L. aratus* (KJ557440.1). A total of six families (Sciaenidae, Pangasiidae, Claroteidae, Mugilidae, Cichlidae and Lutjanidae. The highest number of families identified was Sciaenidae (number, n = 3), followed by Pangasiidae (n = 1), Claroteidae (n = 1), Mugilidae (n = 1), Cichlidae (n = 1), Lutjanidae (n = 1), while the smallest in number that had one family each were Pangasiidae, Claroteidae, Mugilidae, Cichlidae and Lutjanidae. For the total number of genera, 8 of them were found among the fish sequences.

DISCUSSION

The species composition of the river has a total of 23,102 individuals, nineteen species belonging to fourteen genera, twelve families and six orders for a ten-month sampling period indicating the presence of quite a good number of fish species in Bonny River. Ibim and Douglas (2016) recorded a total of thirty-seven species in twenty families from forty-two thousand one hundred and twenty-seven (42,127) individuals in their study on the ichthyofaunal and physico-chemical properties of upper Sombreiro River, Abua/Odual Local Government Area, Rivers State, Nigeria which is in the same ecological zone. The difference between the fish diversity in this study compared to Ibim and Douglas (2016) may be because of extended study periods and the number of researchers employed in their study. Other comparable reports include Akpan (2013) who reported a total of twenty-six species of fish belonging to fourteen families from the Uta Ewa Creek, Niger Delta Region, Nigeria.

The fish families recorded in the study were Alestidae, Citharinidae, Hepsetidae, Cichlidae, Gobidae, Claroteidae, Clariidae, Mochokidae, Schilbeidae, Malapteruridae, Palaemonidae, Physalidae, Gymnarchidae, Arapaimidae, Mormyridae, Chanidae, Cyprinidae, Mugilidae, Tetraodontidae, Ampullariidae, Clupeidae and Haemulidae. Ten families constituted over 81% of catches in number and order of descending dominance: Clupeidae, Palmonidae, Physalidae, Cichlidae, Ampullariidae, Mormyridae, Mochokidae, Schilbeidae, Claroteidae and Alestidae. Ibim and Douglas (2016) reported fish families such as Aridae, Alestidae, Alestidae, Cichlidae, Claroteidae, Eleotridae, Elopidae, Clupeidae, Sphyraenidae, Hepsetidae, Osteoglosidae, Pristigasteridae, Mugilidae, Lutjanidae, Monodactylidae, Haemulidae, Polynemidae, Sciaenidae, Clupeidae, Sphyraenidae, Paralicthyidae, Carangidae and Belonidae in the upper Sombreiro River, Abua/Odual Local Govt. Area, Rivers State, Nigeria. The most dominant fish family was the Clupeidae which is in contrast with the results of Ibim and Owhonda (2017) in which the dominant fish family in their study was the Cichlidae. Although Cichlidae is commonly found in the Niger Delta area, it was not a dominant fish family in the Sombreiro River, rather it was the Clupeidae.

Fish diversity in this study showed Cichlidae as the most diverse with four species belonging to three genera. This agreed with the diversity reported by Adaka *et al*. (2014) and Ibim and Owhonda (2017). A similar report of high Cichlid diversity was reported in the New Calabar River (Olopade and Dienye, 2018). The families recorded in this study include Cichlidae, Sciaenidae, Claroteidae, Mugildae, Lutjanidae, Polynemidae and Pangasiidae. Cynoglossidae, Sphraenidae, Carangidae, Cyprinidae and Haemulidae.

The detected total of eight species of fish (*P. senegallus, P. typhus, P. elongatus, P. polyuranodon, C. nigrodigitatus, N. falcipinnus, C. guineensis and L. aratus*) within six families (Sciaenidae, Pangasiidae, Claroteidae, Mugilidae, Cichlidae and Lutjanidae) was done using COI gene marker while other species were identified using morphological taxonomy.

Several studies have proven that DNA barcoding using the Cytochrome Oxidase Subunit 1 gene (COX 1) is effective in the identification of fish species (Shashank *et al*., 2022). This study recorded more than 95% success rate and it corresponds with other studies on DNA barcoding of freshwater fishes (Lakra *et al*., 2011; Nwani *et al*., 2011; Iyiola *et al*., 2018; Ude *et al*., 2020).

Conclusion: These studies proved that a combination of COI gene marker and morphological taxonomy is a reliable method in the identification of fish species and they aid in the biodiversity studies in Bonny River, it can therefore be concluded that Bonny River is a viable and important water body in Okrika Local Government Area of Rivers State, Niger Delta, Nigeria and can sustain a good fishery for sustainability if adequate attention is paid to it. The river contributes significantly to the commercial fishery of the community indigenes. Diversity was high in a few species/families which may be due to a lack of good managerial practices employed in the management of Bonny fisheries. Therefore, it is recommended that Bonny River should be properly managed and protected via fisheries management strategies and policies to enhance the sustainability of the

natural fishery. Also, a follow-up of continuous research surveys in Bonny River is advised as this will promote the protection and conservation of rare and endangered species found in the river to prevent such species from extinction. The following recommendations are necessary: As recommended by Edoghotu and Hart (2014), overfishing in the Bonny River should be checked regularly by controlling the use of gears. This can be done either by banning certain types of fishing methods or putting restrictions on size in the mesh-selective gears (Graham *et al*., 2007). i). The fishery community should educate fishermen on the right identification of fishes as well as their economic value to aid in the preservation of cryptic species from going extinct. ii). Mesh regulations should be encouraged to control the mesh size in mesh-selective gear where the fishery is based on a certain number of finfish species of high economic importance (Akankali and Elohor, 2023). iii). The use of illegal fishing methods such as poisons and explosives should be prohibited because it can lead to the extermination of fish communities. iv). Site control should be encouraged in the fishery of Bonny River by setting aside sufficient areas to serve as reserves that can be spawning grounds for fish. v). Season control is necessary to regulate the seasons of fishing. This will prevent fishing during spawning seasons. vi). Legislation on water quality control will help prevent pollutants in the water that may come from urbanisation and industrial development. vii). promulgation and Enforcement of Fisheries Edicts in the States. This helps in the management of fisheries resources.

ACKNOWLEDGEMENTS

The authors especially thank the management and staff of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, for their support. We thank Inqaba Biotech Laboratory for providing the necessary facilities, equipment and analytical support for this research. We also appreciate the invaluable assistance of the fishermen, Mr. Rufus Loveday, Mr. Thaddeus Everest and others, whose expertise in the field was indispensable, their help in capturing and identifying finfish specimens in their local dialect significantly contributed to the success of this study. We also thank Prof. Joseph Eyo for his guidance in the preparation of this manuscript.

REFERENCES

ADAKA, G. UDOH, J. and ONYEUKWU, D. (2014). Freshwater fish diversity of a tropical rainforest river in southeast Nigeria. *Advances in Life Science and Technology,* 23: 16 – 23.

ADEWUMI, A., IDOWU, E. and ADENIJI, A. (2015). A survey of the ichthyofauna of Ogbese River, South West, Nigeria. *International Journal of Science*, 4(7): 1 – 5.

AKANKALI, J. A. and ELOHOR, S. (2023). Fishing gears and their impacts on the environment and fisheries resources in Niger Delta Region, Nigeria. Pages 451 – 457. *In: Proceedings of the 37th Annual Conference of the Fisheries Society of Nigeria,* Held at Federal College of Education, Yola Land of Beauty 2022, 30TH October – 4TH November, 2022.

AKPAN, I. I. (2013). Species composition and abundance in Uta Ewa Creek, Niger Delta Region, Nigeria. *IOSR Journal of Agriculture and Veterinary Science*, 3(3): 56 – 60.

ALTSCHUL, S. F., MADDEN, T. L., SCHÄFFER, A. A., ZHANG, J., ZHANG, Z., MILLER, W. and LIPMAN, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17): 3389 – 3402.

ANWADIKE, B. C. (2020). Biodiversity conservation in Nigeria: perception, challenges, and possible remedies. *Current Investigations in Agriculture and Current Research*, 8(10): 1109 - 1115.

ANWANA, E. D. and NWOSU, F. M. (2014). Culturally important freshwater Lakes in the central Niger Delta, Nigeria: Fish assemblage and diversity assessment. *Standard Research Journal of Agricultural Sciences,* 2(2): 016 – 023.

ARMANI, A., GUARDONE, L., CASTIGLIEGO, L., D'AMICO, P., MESSINA, A., MALANDRA, R., GIANFALDONI, D. and GUIDI, A. (2015). DNA and Mini-DNA barcoding for the identification of *Porgies* species (Family: Sparidae) of commercial interest on the international market. *Food Control*, 50: 589 – 596.

AUSTEN, G. E., BINDEMANN, M., GRIFFITHS, R. A. and ROBERTS, D. L. (2016). Species identification by experts and non-experts: comparing images from field guides. *Scientific Reports*, 6(1): 33634. https://doi.org/10.1038/srep 33634

BOLARINWA, J. B., OGUNBONA, A. A., ISHOLA, O. J. and OGUNDANA, F. O. (2016). Socioeconomic survey and cost-benefit analysis of artisanal fisheries in Egbin Waterside, Lagos Lagoon, Lagos State, Nigeria. *International Journal of Research in Agriculture and Forestry*, 3(11): 1 – 5.

CHARLTON, K. E., RUSSELL, J., GORMAN, E., HANICH, Q., DELISLE, A., CAMPBELL, B. and BELL, J. (2016). Fish, food security and health in Pacific Island countries and territories: a systematic literature review. *BMC Public Health*, 16: 285. https://doi.org/10.1186/s12889-016-2953-9

CADDY, J. F. and GARIBALDI, L. (2000). Apparent changes in the trophic composition of world marine harvests: the perspective from the FAO capture database. *Ocean and Coastal Management*, 43(8-9): 615 – 655.

CONN, P. B., MCCLINTOCK, B. T., CAMERON, M. F., JOHNSON, D. S., MORELAND, E. E. and BOVENG, P. L. (2013). Accommodating species identification errors in transect surveys. *Ecology*, 94(11): 2607 – 2618.

COSTELLO, M. J., MAY, R. M. and STORK, N. E. (2013). Can we name Earth's species before they go extinct? *Science*, 339(6118): 413 – 416.

DI PINTO, A., MARCHETTI, P., MOTTOLA, A., BOZZO, G., BONERBA, E., CECI, E., BOTTARO, M. and TANTILLO, G. (2015). Species identification in fish fillet products using DNA barcoding. *Fisheries Research*, 170: 9 – 13.

DOWGIALLO, A. (2005). Cutting force of fibrous materials. *Journal of Food Engineering*, 66(1): 57 – 61.

EDE, P. N. (2015). Morphometric features of a low-elevation urban catchment and the implications for flooding. *Global Journal of Geological Sciences*, 13: 9 – 14.

EDOGHOTU, A. J., and HART, I. A. (2014). Feeding habit of *Heterotis niloticus* of Kugbo Creek in Niger Delta, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology*, 8(10): 26 – 29.

EGESI, O. C. (2016). Artisanal fishers and the adoption of fishing technologies in Bayelsa State, Nigeria. *IIARD International Journal of Geography and Environmental Management*, 2(1): 9 – 14.

GOOGLE MAP (2024). *Bonny River, Okrika, Rivers State, Nigeria*. https://www.google.com/maps/place/Okrika+500103,+Rivers/@4.7437942,7.0788946,15z/data

GRAHAM, N., FERRO, R. S., KARP, W. A. and MACMULLEN, P. (2007). Fishing practice, gear design, and the ecosystem approach — three case studies demonstrating the effect of management strategy on gear selectivity and discards. *ICES Journal of Marine Science*, 64(4): 744 – 750.

HEBERT, P. D., CYWINSKA, A., BALL, S. L. and DEWAARD, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 270(1): 313 – 321.

IBIM, A. T. and DOUGLAS, S. (2016). Status of the fin fish fauna of the upper Sombreiro River, Abua/Odual Local Government Area, Rivers State, Nigeria. *Journal of Agriculture and Social Research (JASR)*, 16(1): 37 – 58.

IBIM, A. T. and OWHONDA, M. (2017). Composition and diversity of the fish fauna of Omuechi Stream, Aluu, Rivers State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 11(1): 34 – 39.

IYIOLA, O. A., NNEJI, L. M., MUSTAPHA, M. K., NZEH, C. G., OLADIPO, S. O., NNEJI, I.

C. and ADEOLA, A. C. (2018). DNA barcoding of economically important freshwater fish species from north-central Nigeria uncovers cryptic diversity. *Ecology and Evolution*, 8(14): 6932 – 6951.

LAKRA, W. S., VERMA, M. S., GOSWAMI, M., LAL, K. K., MOHINDRA, V., PUNIA, P., GOPALAKRISHNAN, A., SINGH, K. V., WARD, R. D. and HEBERT, P. (2011). DNA barcoding Indian marine fishes. *Molecular Ecology Resources*, 11(1): 60 – 71.

LUCENTINI, L., CAPORALI, S., PALOMBA, A., LANCIONI, H. and PANARA, F. (2006). A comparison of conservative DNA extraction methods from fins and scales of freshwater fish, a useful tool for conservation genetics. *Conservation Genet*ics, 7(6): 1009 – 1012.

MANICKAVASAGAM, S., SUDHAN, C. and AANAND, S. (2019). Bioindicators in the aquatic environment and their significance. *Journal of Aquaculture in the Tropics*, 34(1/2): 73 – 79.

MOHAMMED-GEBA, K., OBUID-ALLAH, A. H., EL-SHIMY, N. A., MAHBOB, M. A. E. M., ALI, R. S. and SAID, S. M. (2021). DNA barcoding for scorpion species from New Valley Governorate in Egypt reveals different degrees of cryptic speciation and species misnaming. *Conservation*, 1(3): 228 – 240.

MURINGAI, R. T., MAFONGOYA, P. and LOTTERING, R. T. (2022). Sub-Saharan Africa freshwater fisheries under climate change: a review of impacts, adaptation, and mitigation measures. *Fishes*, 7(3): 131. https://www.mdpi.com/24103888/7/3/131#

NWAKANMA, C., UDE, G. and CHIEZE, O. (2015). Biodiversity - 2015: Fish species identification and biodiversification in Enugu Metropolis River by DNA barcoding. *Journal of Biodiversity and Endangered Species,* 3(2): 15 – 17.

NWANI, C. D., BECKER, S., BRAID, H., UDE., OKOGWU, O. and HANNER, R. (2011). DNA barcoding discriminates freshwater fishes from southeastern Nigeria and provides river system-level phylogeographic resolution within some species. *Mitochondrial DNA*, 22(1): 43 – 51.

ODO, G. E., NWANI, C. D. and EYO, J. E. (2009). The fish fauna of Anambra River basin, Nigeria: species abundance and morphometry. *Revista de Biologia Tropical*, 57(1-2): 177 – 186.

OLANIYAN, R. F. (2015). Fishing methods and their implications for a sustainable environment. *Fisheries and Aquaculture Journal,* 6(3): 139. https://www.longdom.org/pdfdownload.php?download=open-access/fishing-methods-and-their-implications-for-a-sustainableenvironment-2150-35081000139.pdf&ai d=58621

OLOPADE, O. A. and DIENYE, H. E. (2018). Distribution and abundance of cichlids in the New Calabar River, Nigeria. *Journal of Fisheries*, 6(2): 617 – 622.

ONUOHA, G. C. (2008). Phytoplankton productivity in the Okrika Port Harcourt sector of Bonny Estuary Nigeria. *Global Journal of Agricultural Sciences*, 7(2): 207 – 220.

OOSTING, T., STAR, B., BARRETT, J. H., WELLENREUTHER, M., RITCHIE, P. A. and RAWLENCE, N. J. (2019). Unlocking the potential of ancient fish DNA in the genomic era. *Evolutionary Applications*, 12(8): 1513 – 1522.

PAUGY, D. (2010). An historical review of African freshwater ichthyology. *Freshwater Reviews*, 3(1): 1 – 32.

PAUGY, D., LÉVÊQUE, C. and TEUGELS, G. G. (2003). *The Fresh and Brackish Water Fishes of West Africa*. Volumes 1 and 2. Muséum National D'histoire Naturelle, Paris, France.

POLLACK, S. J., KAWALEK, M. D., WILLIAMS-HILL, D. M. and HELLBERG, R. S. (2018). Evaluation of DNA barcoding methodologies for the identification of fish species in cooked products. *Food Control*, 84: 297 – 304.

QUEVEDO, J. M. D., LUKMAN, K. M., ULUMUDDIN, Y. I., UCHIYAMA, Y. and KOHSAKA, R. (2023). Applying the DPSIR framework to qualitatively assess the globally important mangrove ecosystems of Indonesia: a review

towards evidence-based policymaking approaches. *Marine Policy*, 147: 105354. https://doi.org/10.1016/j.marpol.2022.105354

SÁEZ-GÓMEZ, P. and PRENDA, J. (2022). Freshwater fish biodiversity in a large Mediterranean Basin (Guadalquivir River, S Spain): patterns, threats, status and conservation. *Diversity*, 14(10): 831. https://doi.org/10.3390 /d14100831

SANGER, F., NICKLEN, S. and COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, 74(12): 5463 – 5467.

SHASHANK, P. R., NAVEENA, N. L., RAJGOPAL, N. N., ELLIOTT, T. A., SREEDEVI, K., SUNIL, S. and MESHRAM, N. M. (2022). DNA barcoding of insects from India: Current status and future perspectives. *Molecular Biology Reports*, 49(11): 10617 – 10626.

SHOKRALLA, S., SINGER, G. A. and HAJIBABAEI, M. (2010). Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. *BioTechniques*, 48(3): 305 – 306.

SIMPSON, E. H. (1949). Measurement of diversity. *Nature*, 163: 688. https://doi.org/10.1038/163688a0

SONET, G., SMITZ, N., VANGESTEL, C. and SAMYN, Y. (2022). DNA barcoding echinoderms from the east coast of South Africa. The challenge to maintain DNA data connected with taxonomy. *Plos One*, 17(10): e0270321. https://doi.org/10.1371/journal.pone.0270321

STIASSNY, M. L., TEUGELS, G. G. and HOPKINS, C. D. (2007). *Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa.* Volumes 1 and 2, IRD Editions, Muséum National D'histoire Naturelle, Paris, France.

TAMURA. K., STECHER, G. and KUMAR, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7): 3022 – 3027.

UDE, N., IGWE, O., BROWN, C., JACKSON, M., BANGURA, A., OZOKONKWO-ALOR, O. and DAS, A. (2020). DNA barcoding for identification of fish species from freshwater in Enugu and Anambra States of Nigeria. *Conservation Genetics Resources*, 12(1): 643 – 658.

ZAGHLOUL, A., SABER, M., GADOW, S. and AWAD, F. (2020). Biological indicators for pollution detection in terrestrial and aquatic ecosystems. *Bulletin of the National Research Centre*, 44: 127. https://doi.org/10.1186/s42269-020-00385-x

ZEPPILLI, D., SARRAZIN, J., LEDUC, D., ARBIZU, P. M., FONTANETO, D., FONTANIER, C., GOODAY, A. J., KRISTENSEN, R. M., IVANENKO, V. N., SØRENSEN, M. V. and VANREUSEL, A. (2015). Is the meiofauna a good indicator for climate change and anthropogenic impacts? *Marine Biodiversity*, 45: 505 – 535.

ZHANG, J. B. and HANNER, R. (2011). DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochemical Systematics and Ecology*, 39(1): 31 – 42.

ZYMO RESEARCH (2023). *Quick-DNA™ Miniprep Plus Kit: Purify High-Quality Total DNA from a Variety of Sample Types.* Instruction Manual Version 2.0.1, Zymo Research Corporation, California, USA. https://files.zymoresearch.com/protocols/ d4068 d4069 quickdna miniprep plus kit.pdf