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Fish diversity and environmental factors at the Don Hoi Lot Ramsar Site, Thailand

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Abstract. *Siriwattanarat R, Wongroj W, Ngamniyom A, Vilasri V, Chungthanawong S, Punnarak P. 2024. Fish diversity and environmental factors at the Don Hoi Lot Ramsar Site, Thailand. Biodiversitas 25: 1394-1403.* The Don Hoi Lot mudflat, recognized as a Ramsar site, is a globally acclaimed wetland located in the Khlong Khon Sub-district of Samut Songkhram Province, Thailand. This research aims to study fish diversity and environmental variables at the Don Hoi Lot Ramsar Site; fish samples were collected using cast nets (mesh size 1.5 cm and 2.5 cm) and gill nets (mesh size of ¾ inch, 1.5 inches, and 2 inches) by local fisheries from four sampling stations which represented upstream (KK1), midstream (KK2), downstream (KK3), and estuary (KK4) zones in Khlong Khon Bay during January to April 2023. A total of 1,389 fish specimens were collected, representing 36 species belonging to 26 families. Higher diversity in number of species (28-35 species), Shannon index (2.90-2.98), and species richness (10.40-13.11) were found at downstream and estuary stations, while the lowest number of fish species (three species), Shannon index (0.75), and species richness (0.68) were recorded at the upstream station. Similarly, the higher relative abundance of fish (84-173 specimens) was observed at downstream and estuary stations, and the lowest relative abundance (29-50 specimens) was also reported at the upstream station. The blackchin tilapia (*Sarotherodon melanotheron* Rüppell 1852) was the dominant species at the Don Hoi Lot Ramsar Site during the study periods, followed by the Mozambique tilapia (*Oreochromis mossambicus* Peters 1852)). Principal component analysis (PCA) further revealed that altitude, temperature, pH, salinity, transparency, ammonia, phosphate and silicate had significant relationships within fish assemblages, whereas nitrite and nitrate were less important in this study. Nonetheless, the rapid expansion of the blackchin cichlid population has had an adverse impact on native fish populations, which could pose a real threat to fish diversity and population, and other aquatic organisms.

Keywords: Don Hoi Lot Ramsar Site, environmental factors, fish diversity

INTRODUCTION

Don Hoi Lot plays an important ecological role and also has economic value, as attested by its registration in 2001 according to the Ramsar International Wetlands Treaty (ONEP 2008). The Don Hoi Lot mudflat, recognized as a Ramsar site, is a globally acclaimed wetland located in the Khlong Khon sub-district of Samut Songkhram province, Thailand. The area is located about 100 km west of Bangkok, in the upper Gulf of Thailand, encompassing an area of 24.09 square kilometers (Worrapimphong et al. 2010; OEPP 2002). This wetland receives and accumulates sediments from the Mae Klong River, resulting in its rich organic nutrient content. The exceptional character of Don Hoi Lot extends beyond the biodiversity and range of species found in its wetland habitats, as it also features a sandbar at the mouth of the Mae Klong River. This sandbar serves as a critical habitat for the locally renowned tubeworm, *Solen regularis* Dunker 1862. Furthermore, this site represents the sole habitat within Samut Songkhram Province, where substantial populations of razor clams and other threatened or near-threatened fish species can be found. During the past two decades, Don Hoi Lot has faced problems due to unsustainable development in terms of land development and infrastructure construction, e.g., the changing of mangroves in the coastal zone to aquaculture, which has resulted in the nursing ground and productivity in the estuary ecosystem decreasing, caused by the spread of blackchin cichlids in this area (Worrapimphong et al. 2010; Ordoñez et al. 2015). The blackchin cichlid (*Sarotherodon melanotheron* Rüppell 1852) is known to be invasive, i.e., it has the potential to spread widely, reproduce rapidly and persist in a new environment with a large range of impacts (Ordoñez et al. 2015; Cassemiro et al. 2017). Consequently, to better understand the importance of monitoring changes in fish communities and resource management in ensuring the conservation of the environment, more scientific studies focused on classifying fish species into categories or guilds and analyzing the fish community have emerged (Elliott et al. 2007).

There are only a few studies documenting the aquatic diversity within this region (Yoshida et al. 2013; Petsut et

al. 2017; Chamason and Phenpraphai 2020; Vilasri et al. 2023). Approximately 372 fish species from 109 families in the Gulf of Thailand were listed during 2009-2012 (Yoshida et al. 2013). Later, 16 fish species from 14 families were recorded in the mangroves and estuaries of Phetchaburi Province and Prachuap Khiri Khan Province, western Thailand, and it was found that most fish permanently existing in mangrove forests were economically important, juvenile fish, e.g., *Ellochelon vaigiensis* Quoy and Gaimard 1825, *Moolgada cunnesius* Valenciennes 1836, *Terapon jarbua* Forsskål 1775, *Lutjanus russellii* Bleeker 1849, *Gerres erythrourus* Bloch 1791, *Sillago sihama* Forsskål 1775 and *Equulites oblongus* Valenciennes 1835 (Petsut et al. 2017). On the contrary, a total of 29,287 fish individuals belonging to 33 families and 54 species were collected from coastal land used in the mangrove estuary of Ban Laem District, Phetchaburi Province, inner Gulf of Thailand, from December 2012 to October 2013 (Premcharoen and Kiat-amornwet 2016). Recently, 936 fish specimens, which were identified as belonging to 131 species from 50 families, were collected using a bottom trawling technique (otter trawling) from the upper Gulf of Thailand by the MV-SEAFDEC 2 cruise in December 2019, and two species, namely, lizardfish, *Saurida fortis* sp. nov, and lefteye flounder, *Engyprosopon keliaoense* Amaoka and Ho 2022, were first recorded in the Gulf of Thailand (Vilasri et al. 2023).

We focused on the mangrove and estuary area as the number of fish species generally increases from upstream to downstream. The lower reach of the canal is typically a vast area incorporating the seasonal floodplain and also under marine influence, and it consequently supports a complex fish composition comprising freshwater, brackish water and marine fish species (Jutagate et al. 2010, 2011). The importance of estuaries is well understood in many parts of the world as they are breeding and nursery grounds for a wide variety of fish. Although estuaries provide a rather harsh environment because of changes in salinity, many species of fish have found them to be highly advantageous areas in which they can spawn offspring and allow them to develop and grow during early life; therefore, productivity tends to be high. Estuarine environments are among the most productive ecosystems in the world, producing more organic matter annually than forests, grasslands, or agricultural land of equal size, and providing important commercial value through economic benefits for tourism and fisheries. The estuary's protected coastal waters also support critical public infrastructure, such as harbors and ports for shipping and transportation (Hossain et al. 2012). The estuaries display a high level of geomorphological and environmental complexity, resulting in significant physicochemical variability by supporting the basic requirements of the life cycle for various aquatic organisms (McLusky and Elliott 2004; Franco et al. 2008; Molina et al. 2020). The shallow turbid waters of estuaries provide a haven for the juveniles of marine fish species by protecting them from predators and providing food (Thiel et al. 2003). In order to understand estuarine water quality along with its diversity in terms of fish species, the estuarine fish taxa can be used as an indicator of the ecological health of the estuaries, informing about environmental changes and potentially aiding in managing the impacts of anthropogenic activities on these transitional systems (McLusky and Elliott 2004; Elliott et al. 2007). The impact of environmental variability on the structure of fish communities in estuaries at different scales has been extensively studied (Molina et al. 2020). Additional environmental variables have been investigated to reflect the level of anthropogenic stress in the estuarine water (Teichert et al. 2017), and this study partially addresses that. Estuaries play a significant role not only as spawning grounds influencing the distribution of fish but also due to the abundant food resources they offer. Additionally, estuaries serve as essential nursery habitats and provide shelter during various life stages, resulting in changes in species composition, density, and biomass along the estuary (Ferreira et al. 2016; Chen et al. 2023). Furthermore, resource availability and competition, as well as tolerance or preference for specific environmental conditions, also influence the utilization of estuaries by fish, resulting in spatial and temporal variations in fish communities. All these processes and patterns of estuary utilization are regulated by changes in environmental conditions, particularly hydrological patterns (Molina et al. 2020). However, due to the complexity of estuarine ecosystems, understanding how fish respond to environmental fluctuations remains an ongoing endeavor (Sheaves et al. 2016; Teichert et al. 2017)

Many studies have evaluated the importance of different environmental variables in assemblages of estuarine fishes at different spatial scales, concluding that variations in their specific environmental and morphological characteristics generate unique dynamics in the assemblages of fishes in each estuary, which must be understood within patterns at larger scales (Paumier et al. 2018; Molina et al. 2020; Romero-Berny et al. 2020; Chen et al. 2023). For instance, the unique rivers in Nepal are abundant in aquatic biodiversity, hosting about 252 fish species from different climatic zones (Adhikari 2013; Shrestha 2019). Based on this, it is necessary to study the specific ecological dynamics of each estuary at different area scales and in different seasons. In the case of tropical estuaries, the study of these variations is important for the recognition of their high species richness throughout these scales (Chase et al. 2019).

Diversity and distribution of fish communities are significantly determined by the biochemical and environmental parameters of the system. A better understanding of these factors and the response of the ecosystem towards the environmental variables have become the central area of interest for environmentalists over time (Odulate et al. 2014). Therefore, the objective of this research is to investigate the diversity and environmental factors of fish assemblages in the Don Hoi Lot estuary. By employing comprehensive sampling techniques and analyzing environmental parameters, we aim to unravel the interactions between the diversity of fish species and water quality. This study will provide valuable scientific knowledge to support conservation initiatives, aid in the sustainable management of the Don Hoi Lot estuary,

and contribute to the broader understanding of estuarine ecology worldwide.

MATERIALS AND METHODS

Ethical statement

This research project has been approved according to the Ethical Principles and Guidelines for the Use of Animals, No. IACUC 65-004/2022, of Suan Sunandha Rajabhat University, Thailand. The project leader holds a license according to the Ethical Principles and Guidelines for the Use of Animals, No. U1-10659-2565, from the Institute of Animals for Scientific Purposes Development (IAD), National Research Council of Thailand (NRCT), Thailand.

Study area

The study was conducted between January and April 2023 at the Don Hoi Lot Ramsar site, an internationally renowned wetland situated in Samut Songkhram province, Thailand. Spanning an area of 24.09 km^2 at coordinates 13°21'N latitude and 099°59'E longitude (Figure 1), this site encompasses the Don Hoi Lot mudflat. The mudflat consists of expansive intertidal mudflats and sandbars located at the mouth of the Mae Klong River, making it an exceptionally productive habitat for the razor clam. This species holds significant economic value and is unique to this particular region. The Don Hoi Lot Ramsar site exhibits dynamic coastal features within the Bight of Bangkok, which is a part of the Gulf of Thailand. These features are a result of the interaction between the river and marine sediments, extending approximately 8 kilometers from the shoreline into the sea, with a gentle slope of less than 1%.

Sampling sites

A total of four sampling sites were chosen, encompassing the upstream, midstream, and downstream areas of the canal, as well as the Khlong Khon Bay estuary (Table 1). These selections were based on the extraction of the study area, and the precise geographic coordinates of the sampling points were plotted using Google Earth (version 6.0.3). The upstream site of KK1 (13°21'10.0"N, 99°55'59.5"E) represented a small canal, the midstream site of KK2 (13°20'09.2"N, 99°57'43.5"E) represented wetlands connected to ditches, the downstream site of KK3 (13°18'44.1"N, 99°56'48.4"E) was a small brackish water canal in a mangrove forest, and the Khlong Khon Bay estuary and shoreline site of KK4 (13°18'09.6"N, 99°59'50.6"E) was in a mudflat habitat, which is frequently influenced by the accumulation of sediment and the tide found on the coast (Figure 1). Sampling sites (approximately 200 m long) were selected to include multiple representative habitats: estuary, shoreline, small canal, mangrove forest, and wetlands connected to ditches.

Table 1. Sampling sites at the Don Hoi Lot Ramsar site, Thailand

Site location	Sampling points	Latitude	Longitude
Saeng Tai Amphawa	KK1	13°21'10.0"	99°55'59.5"
Ban Khlong Khon	KK2	13°20'09.2"	99°57'43.5"
Mangrove Forest Conservation Center	KK3	13°18'44.1"	99°56'48.4"
Khlong Khon Bay estuary	KK4	13°18'09.6"	99°59'50.6"

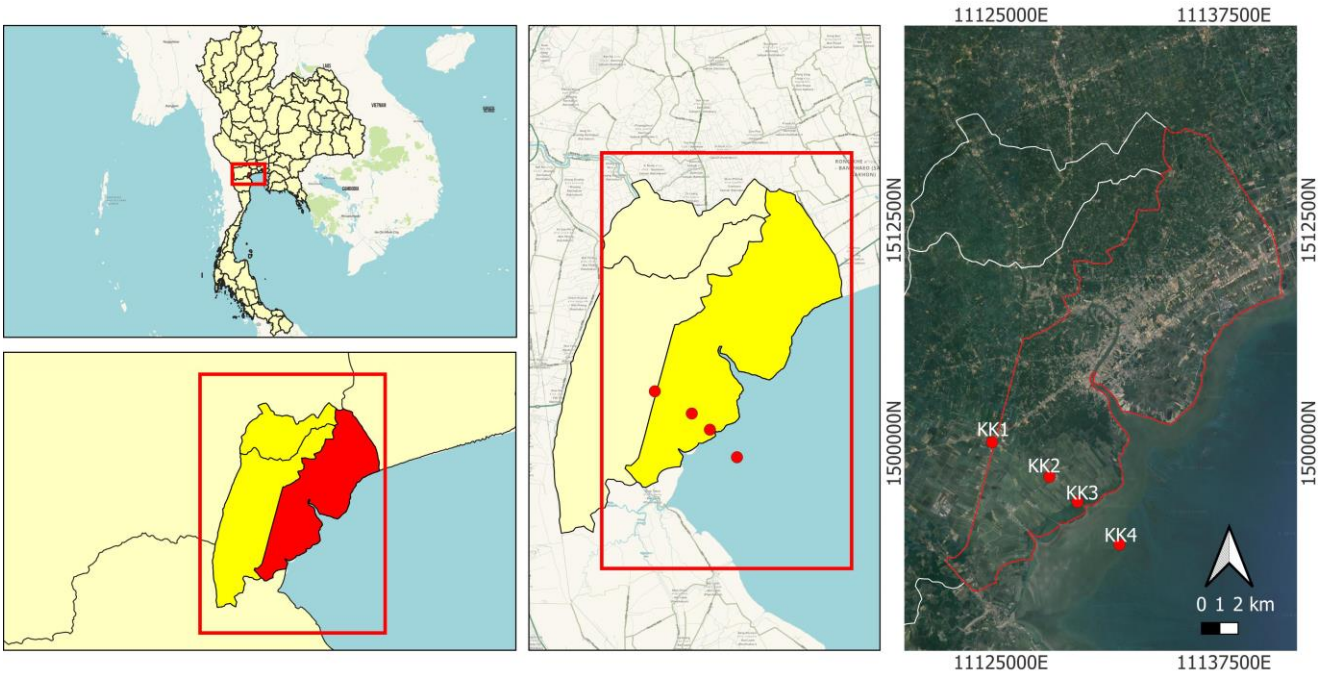

Figure 1. Map showing the location of the Don Hoi Lot Ramsar Site, indicating the following sampling sites: KK1 (13°21'10.0", 99°55'59.5"), KK2 (13°20'09.2", 99°57'43.5"), KK3 (13°18'44.1", 99°56'48.4"), KK4 (13°18'09.6", 99°59'50.6"), and the detected sites

Fish sampling

Fish samples were collected with the help of local fishermen by using two kinds of devices, the first of which was cast nets with a mesh size of 1.5 cm and 2.5 cm. Simple fish collection techniques were employed, using two people, one on each end of the cast net, walking in parallel through the water, with the cast net forming a U-shape behind them. At each sampling station, the trawling was repeated three times. The second kind of device was gill nets with a mesh size of ¾inch, 1.5 inches, and 2 inches, organized in a semicircular shape at each station. The sampling was conducted once per month per station from January to April 2023. The fish trapped in the net were collected in a tray filled with water, in which a few drops (1-2 mL) of clove oil were added to make the fish handling easier. Clove oil, a natural product, imparts no great harm considering the doses used and can be used as an effective anesthetic to immobilize fish (Javahery et al. 2012). Specimens were deposited at the Natural History Museum, National Science Museum, Thailand (THNHM). Afterward, photographs of the specimens were taken, and they were identified using the available field guide Hubbs and Lagler (1958). The systematic arrangement follows that of Carpenter et al. (1999) and Nelson (2006). For further identifications, 1-2 samples of each species were preserved in 10% formalin solution, whereas others were released back into the water. With proper tagging and labeling, the samples were brought to the laboratory of the Natural History Museum, National Science Museum, Thailand (THNHM), for further species-level identification.

Analysis of the diversity of the fish community

The species diversity index was calculated using the Shannon-Wiener diversity index, with the formula as follows:

$H' = \sum - (P_i * \ln P_i)$

$i = 1$

Where:

H' : the value of the Shannon-Wiener diversity index

S : the number of species

P_i : the proportion of the total sample belonging to the i^{th} species

$\ln P_i$: the natural logarithm of P_i

The evenness index, with values indicating the distribution of fish species at each site, was calculated using Pielou's index, with the formula as follows:

$J = H'/H'max$

Where:

E : the value of the evenness index (range 0-1); the closer to 1, the higher the evenness

H' : the Shannon-Wiener index

S : the total number of species

$H'max$: the maximum species diversity

The species richness index was calculated, using the Margalef index, with the formula as follows:

$R = (S-1)/\ln (n)$

Where:

R : richness index

n : the total number of fish found

S : the total number of fish species found

Environmental parameters

For this study, to analyze water quality in the estuary and canal, a range of physicochemical parameters were measured in situ using a YSI 556 (MPS) multi-probe system, i.e., temperature, pH, salinity, and transparency. Water samples from each site were collected in 1,000 mL sampling bottles and promptly stored in an icebox for physicochemical analysis. Subsequently, the water samples were transported to the laboratory of the Aquatic Resources Research Institute (ARRI) at Chulalongkorn University in Bangkok, Thailand, so that the inorganic nutrients in the water samples, including nitrite, nitrate, ammonia, phosphate, and silicate, could be analyzed by following the standard protocol provided by Parsons et al. (1984).

Data analysis

Data on fish numbers, fish species, Shannon diversity index (H′), species richness (S), and evenness index (E), collected from each sampling station in each month during the survey period at the Don Hoi Lot Ramsar site, were analyzed using one-way ANOVA (Tukey's honest significance test, P = 0.05) conducted using SPSS© (Simpson 1949; Shannon and Wiener 1963; Krebs 1985; Magurran 1988). Non-metric multidimensional scaling (NMDS), which was used for the analysis of fish species at the study sites, and principal component analysis (PCA), which was used for the analysis of physicochemical properties at the study sites, were performed by the PRIMER-e (Version 7.0.21) computer program. All the data sets of fish relative abundance found at each sampled site and in each season were transformed by using square root and creating a lower triangular resemblance matrix by using Bray-Curtis similarity. Analysis results were presented as a dendrogram with hierarchical clustering. Statistical analysis was performed with the aid of the SPSS version 20 computer software.

RESULTS AND DISCUSSION

A total of 1,389 fish specimens were collected, representing 36 species from 26 families. The most diverse families, which comprised three species, were Dorosomatidae (*Anodontostoma chacunda* Hamilton 1822, *Escualosa thoracata* Valenciennes 1847, and *Sardinella gibbose* Bleeker, 1849), Ariidae (*Arius maculatus* Thunberg 1792, *Arius venosus* Valenciennes 1840, and *Hexanematichthys sagor* Hamilton 1822*)*), and Carangidae (*Atropus armatus* Forsskål 1775, *Scomberoides commersonnianus* Lacepède 1801, and *Selaroides leptolepis* Cuvier 1833*)*). There were four fish families which were composed of two species, namely, Hemiramphidae (*Hyporhamphus limbatus* Valenciennes 1847 and *Hyporhamphus quoyi* Valenciennes 1847), Gerreidae (*Gerres erythrourus* Bloch 1791 and *Gerres filamentosus C*uvier 1829), Oxudercidae (*Boleophthalmus boddarti* Pallas 1770 and *Periophthalmodon schlosseri* Pallas 1770), and Cichlidae (*Oreochromis mossambicus* Peters 1852 and *Sarotherodon melanotheron* Rüppell 1852). Meanwhile, the remaining families were composed

of only one species. The most dominant species was black-chin tilapia (*S. melanotheron*), which exhibited the highest relative abundance (32.04%), followed by *Triacanthus nieuhofii* Bleeker 1852 (9.94%) and *O. mossambicus* (7.85%). Furthermore, seven species specific to the downstream site (KK3) also showed particular relative abundance, namely, *Brevitrygon heterura* Bleeker 1852 (0.36%), *Pisodonophis boro* Hamilton 1822 (0.36%), *Thryssa hamiltonii* Gray 1835 (0.50%), *Ilisha kampeni* Weber and de Beaufort 1913 (0.36%), *Anodontostoma chacunda* Hamilton 1822 (0.43%), *E. thoracata* (1.73%), and *S. gibbose* (1.51%). The highest abundance of fish species was observed in January (470 individuals), after which fish abundances decreased to totals of 354, 303 and 262 individuals of fish observed in February, March and April, respectively (Table 2).

Spatial and temporal variations in the fish community at the Don Hoi Lot Ramsar site are shown in Figure 2. Higher numbers of fish species (28-35 species) and higher fish abundances (84-173 individuals) were observed at the downstream station (KK3) and the estuary station (KK4). Approximate totals of 10-15 species and 57-84 fish individuals were found at the midstream station (KK2), whereas the lowest number of fish species (three species) and fish abundance (29-50 individuals) was recorded at the upstream station (KK1). Regarding temporal variation, the number of fish species did not change during sampling periods, but fish abundance slightly decreased from January to April at every sampling station. The most dominant species at the Don Hoi Lot Ramsar site was *S. melanotheron*, followed by *O. mossambicus*. In contrast, *T. nieuhofii* and *S. leptolepis* were recorded in higher proportions at the downstream station (KK3), while higher proportions of *T. nieuhofii* and *S. argus* could be found at the estuary station (KK4).

Similar to the number of fish species, diversity indices were higher in seaward areas, where the values of the Shannon diversity index were about 2.98 and 2.90, species richness values were 10.40 and 13.11, and evenness index values were 0.88 and 0.81 at estuary (KK4) and downstream (KK3) stations, respectively. On the contrary, the lowest fish diversity was observed at the upstream station (KK1), with the lowest Shannon diversity index (0.75), species richness (0.92), and evenness index (0.98) values recorded there (Table 3).

Table 2. Fish species distribution at the Don Hoi Lot Ramsar Site, Thailand

Family	Scientific name	Frequency	Relative abundance (%)	Study area			
				KK1	KK2	KK3	KK4
Dasyatidae	*Brevitrygon heterura*	5	0.36			●	
Ophichthidae	*Pisodonophis boro*	5	0.36			●	
Engraulidae	*Thryssa hamiltonii*	7	0.50			●	
Pristigasteridae	*Ilisha kampeni*	5	0.36			●	
Dorosomatidae	*Anodontostoma chacunda*	6	0.43			●	
	Escualosa thoracata	24	1.73			●	
	Sardinella gibbosa	21	1.51			●	
Ariidae	*Arius maculatus*	32	2.30		●	●	●
	Arius venosus	27	1.94		●	●	●
	Hexanematichthys sagor	18	1.01		●	●	●
Plotosidae	*Plotosus lineatus*	21	1.51			●	●
Belonidae	*Strongylura strongylura*	20	1.44		●	●	●
Hemiramphidae	*Hyporhamphus limbatus*	23	1.66		●	●	●
	Hyporhamphus quoyi	29	2.09		●	●	●
Zenarchopteridae	*Zenarchopterus ectuntio*	15	1.08		●	●	●
Ambassidae	*Ambassis nalua*	46	3.31		●	●	●
Latidae	*Lates calcalifer*	21	1.51	●	●		●
Lutjanidae	*Lutjanus russellii*	14	1.01			●	●
Terapontidae	*Terapon jarbua*	28	2.02		●	●	●
Gerreidae	*Gerres erythrourus*	23	1.66			●	●
	Gerres filamentosus	16	1.15			●	●
Carangidae	*Atropus armatus*	26	1.87		●	●	●
	Scomberoides commersonnianus	11	0.79			●	●
	Selaroides leptolepis	39	2.81			●	●
Scatophagidae	*Scatophagus argus*	63	4.54				●
Siganidae	*Siganus javus*	30	2.16			●	●
Scombridae	*Rastrelliger brachysoma*	26	1.87			●	●
Eleotridae	*Butis humeralis*	8	0.58		●	●	
Gobiidae	*Acentrogobius viridipunctatus*	13	0.94		●	●	
Oxudercidae	*Boleophthalmus boddarti*	25	1.80			●	●
	Periophthalmodon schlosseri	26	1.87			●	●
Soleidae	*Dagetichthys commersonnii*	11	0.79			●	●
Cynoglossidae	*Cynoglossus quadrilineatus*	17	1.22			●	●
Triacanthidae	*Triacanthus nieuhofii*	138	9.94			●	●
Cichlidae	*Oreochromis mossambicus*	109	7.85	●	●	●	●
	Sarotherodon melanotheron	445	32.04	●	●	●	●

From the observations of non-metric multidimensional scaling (NMDS), the fish in the study area could be clearly divided into three groups according to the sampling location: (i) the first cluster from the upstream site, which comprised only three species, namely, *Lates calcalifer* Bloch 1790, *O. mossambicus*, and *S. melanotheron*, (ii) the second cluster from the midstream site, where the dominant species was *S. melanotheron*, followed by *O. mossambicus* and *Ambassis nalua*, and (iii) the third cluster from the downstream and estuary stations, which showed high diversity and abundance (Figure 3).

The physicochemical factors were measured in-situ, with the average temperature ranging from 27.53-28.98°C. Similarly, the pH value ranged from 7.25-7.88, salinity ranged from 16.10-22.15 psu, transparency ranged from 40-53 cm, nitrate-nitrogen ranged from 13.45-20.19 μg-N/L, nitrite-nitrogen ranged from 0.90-1.08 μg-N/L, ammonia-nitrogen ranged from 3.21-4.01 μg-N/L, phosphate-phosphorus ranged from 3.25-7.71 μg-P/L, and silicate-silicon ranged from 120.78-219.38 μg-Si/L (Table 4).

Table 3. Summary of Shannon diversity and values of species richness and evenness indices from each site

Sites	Species diversity	Species richness	Evenness
KK1	0.75	0.92	0.68
KK2	1.85	5.72	0.68
KK3	2.90	13.11	0.81
KK4	2.98	10.40	0.88

Table 4. Ranges of physico-chemical parameters measured at the Don Hoi Lot Ramsar Site, Thailand

Environmental parameters	KK1	KK2	KK3	KK4
Temperature (°C)	27.53±0.97	27.60±1.20	28.78±2.55	28.98±2.73
pH	7.25±0.26	7.25±0.21	7.83±0.53	7.88±0.62
Salinity (ppt)	16.10±3.62	17.83±2.65	19.55±3.84	22.15±3.90
Transparency (cm)	40±8.77	46±5.25	44±12.44	53±10.91
Nitrate-nitrogen (μg-N/L)	15.00±8.86	20.19±13.56	14.89±6.86	13.45±9.21
Nitrite-nitrogen (μg-N/L)	1.08±0.18	0.99±0.21	0.78±0.27	0.90±0.46
Ammonia-nitrogen (μg-N/L)	3.21±3.71	3.25±3.93	3.46±4.20	4.01±4.99
Phosphate-phosphorus (μg-P/L)	3.25±1.37	4.24±2.89	4.87±2.60	7.71±1.96
Silicate-silicon (μg-Si/L)	120.78±18.64	156.02±34.16	174.20±49.11	219.38±42.99

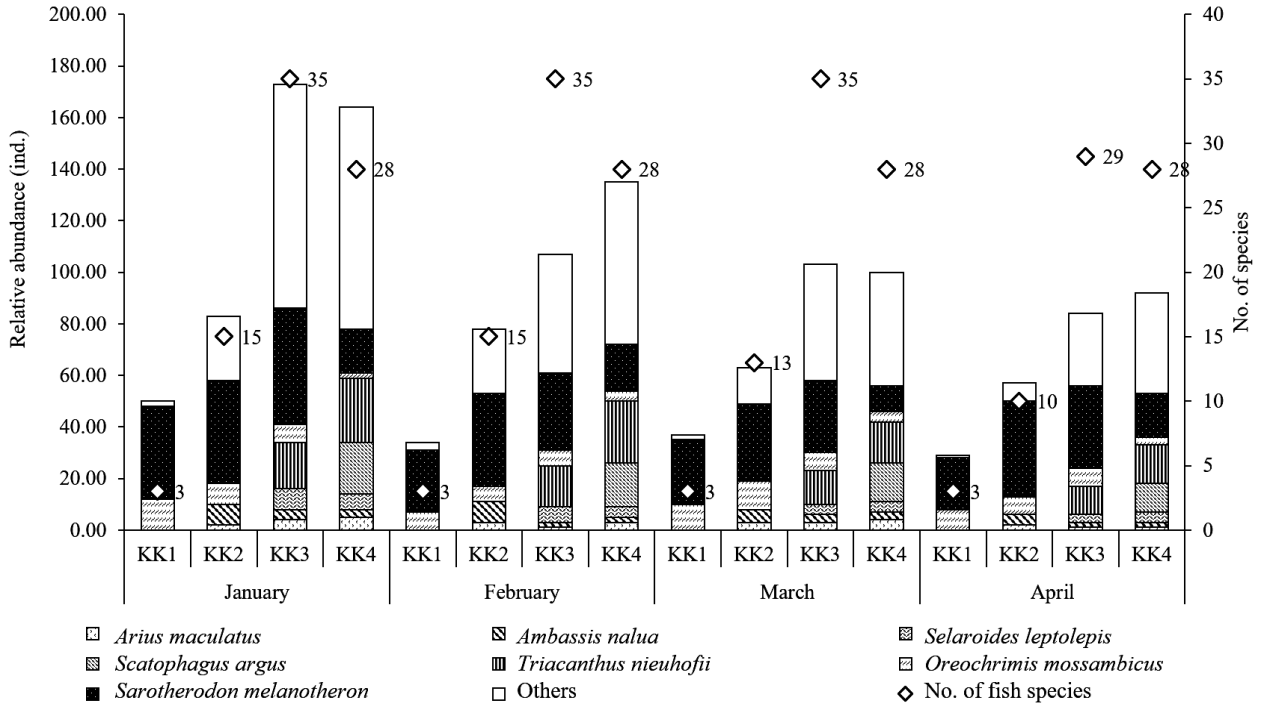

Figure 2. Spatial and temporal distribution of dominant fish species at the Don Hoi Lot Ramsar Site, Thailand from January to April, 2023. Relative abundance for dominant species is shown by bar graphs and the total number of fish species found at each station is indicated by diamond boxes and numbers

Results from the principal component analysis (PCA) reveal spatial (upstream and midstream vs. downstream and estuary) and temporal (January and February vs. March and April) variations, as shown in Figure 4. Spatial variations (PC2), in particular, higher concentrations of ammonia-nitrogen, nitrite-nitrogen and higher relative abundance of fish, were observed at upstream and midstream stations, while other physicochemical parameters were higher in downstream and estuarine environments. Regarding temporal variation (PC1), higher values of environmental parameters, except those of ammonia-nitrogen and nitrite-nitrogen, were observed mostly in March and April. This could imply that the fish community at the Don Hoi Lot Ramsar Site differed according to sampling location, with positive correlations shown between fish abundance and physicochemical parameters, including temperature, salinity, pH, transparency, silicate, phosphate and ammonia; however, negative correlations were shown between fish abundance and nitrite and nitrate concentrations.

Discussion

In the present study regarding the species diversity at the Don Hoi Lot Ramsar Site, the results showed 36 taxa from 26 families in the estuary and coastal areas. Most of these species are known to be tolerant of variations in salinity from freshwater to marine environments (Abidin et al. 2021). Similarly, Petsut et al. (2022) reported that a species diversity and population structure consisting of 37 fish species from 27 families had been found in the Khanom Estuary, Nakhon Si Thammarat Province, Southern Thailand. Naturally, the Khanom Estuary derives its influences from environmental factors such as coastal lines fluctuating due to strong waves. Moreover, a massive amount of freshwater run-off from the mainland flows to mix with seawater in the Khanom Estuary and the small brackish canal, leading to low salinity. Thus, this condition was one of the critical factors affecting the type and distribution of fish species existing in the estuary.

In this study, three major dominant species were observed at the Don Hoi Lot Ramsar site, and this observation was similar to those of several studies that reported the dominance of resident species in estuaries (Hossain et al. 2012). One of the sampled fish found in the present study was the dwarf whip ray *B. heterura*, which had been listed as an endangered species by the International Union for Conservation of Nature (IUCN). Two invasive species were found in this study: the mozambique tilapia *O. mossambicus*, and the black-chin tilapia *S. melanotheron*, likely escapees which probably came from company aquaculture (Noonin et al. 2022). The black-chin tilapia *S. melanotheron* has been found in higher proportions in terms of relative abundance compared to other species, especially at upstream (KK1) and midstream (KK2) stations. This introduction of invasive species from aquatic companies into natural ecosystems may have led to reduced diversity and resulted in the lower diversity index and species richness found in these areas. In contrast, the highest number of fish individuals and diversity index were observed at Klong Klon Bay estuary station (KK4) and the highest species richness was observed at the Mangrove Forest Conservation Center (KK3). This was due to relatively low levels of human activity and appropriate environmental conditions.

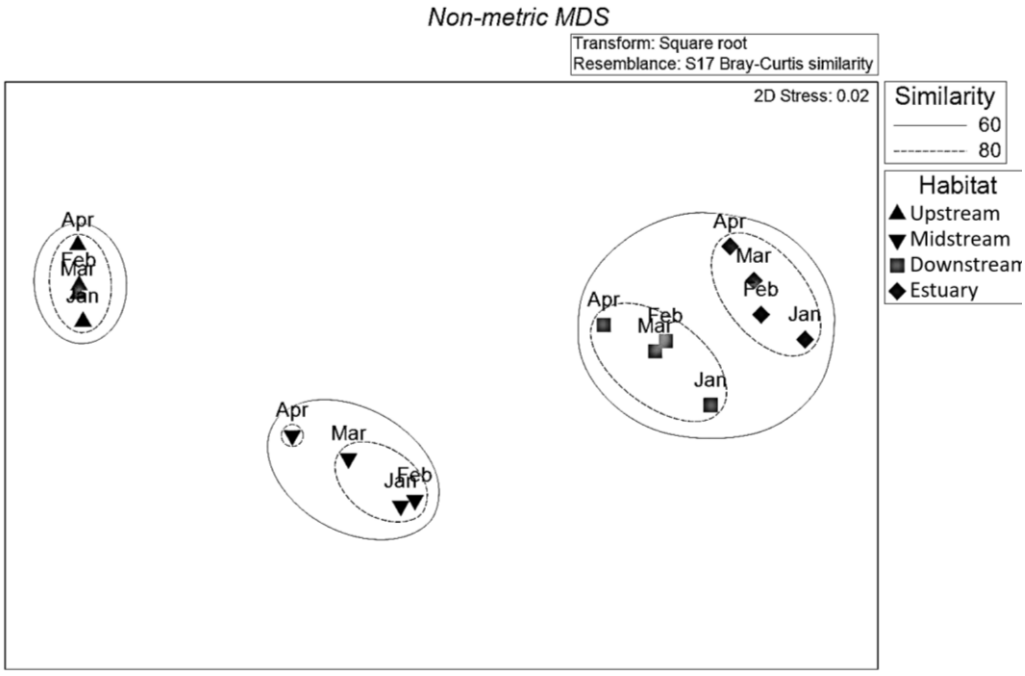

Figure 3. Non-metric multidimensional scaling (NMDS) plot from fish species and relative abundance illustrating four sampling locations, namely, upstream (triangle pointing up), midstream (triangle pointing down), downstream (square) and estuary (diamond) sampling sites, and the temporal relationships between January (Jan), February (Feb), March (Mar), and April (Apr)

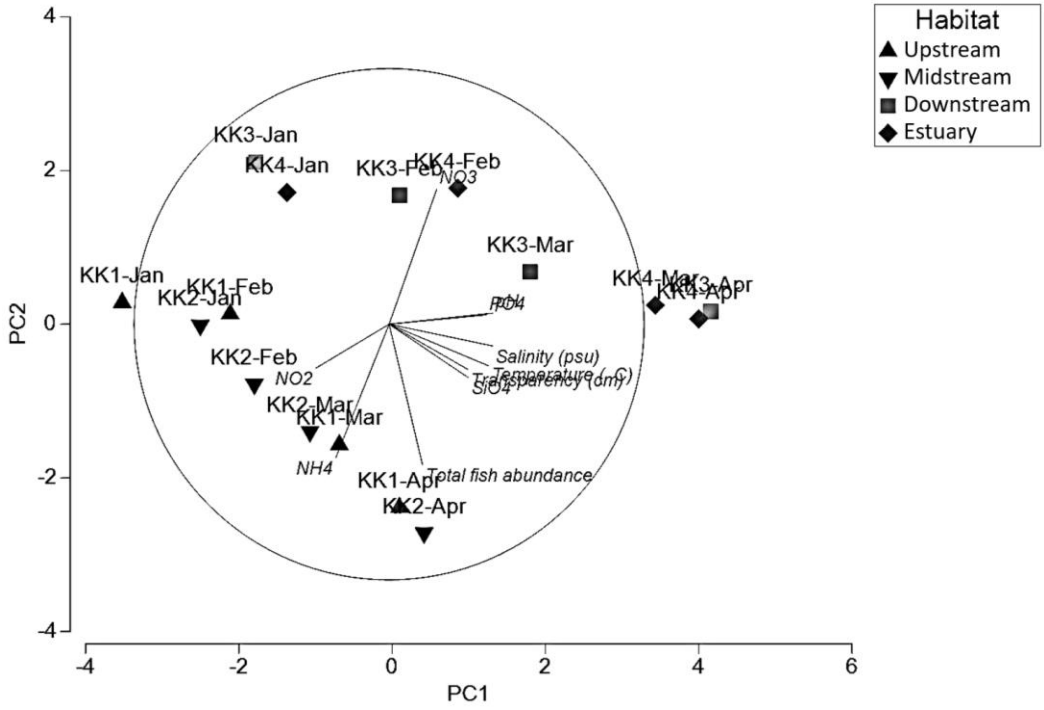

Figure 4. Principal component analysis (PCA) plot illustrating four sampling locations (points) at upstream (triangle pointing up), midstream (triangle pointing down), downstream (square) and estuary (diamond) sampling sites, and the temporal relationships between January (Jan), February (Feb), March (Mar), and April (Apr). Major components of environmental parameters which affected spatial and temporal distribution are indicated and labeled

On the other hand, the lowest number of individuals was observed at Saeng Tai Amphawa station (KK1) due to extreme human activities. At the upstream site, Saeng Tai Amphawa (KK1), three species were observed which were *L. calcalifer* from the family Latidae and *O. mossambicus* and *S.* melanotheron, which belong to the family Cichlidae; these two families are under the order Perciformes. A similar result was obtained from Manila Bay, near Manila City, specifically at Manila Ocean Park, Philippines, where the results showed that Cichlidae had been recorded, with *S. melanotheron* being the key species to the upper site (Ordoñez et al. 2015). The diversity index (0.75) and evenness index (0.678) were quite low at the Don Hoi Lot Ramsar Site, especially at the upstream site, Saeng Tai Amphawa (KK1), which was in contrast with the results from the Sungsang estuaries of South Sumatra, where higher diversity index (1.649-2.002) and evenness index (0.693-0.847) values have been recorded at the upstream stations of the rivers (Fauziyah et al. 2019). Saeng Tai Amphawa is located in an area more than 10 kilometers from the estuary. There is a lack of environmental complexity at this site, which otherwise could have been among the diversity-limiting factors, as mentioned by Ghimire and Koju (2021).

Similar to the upstream site (KK1), the midstream area of Ban Khlong Khon (KK2) was dominated by *O. mossambicus* and *S. melanotheron*. However, at this station, more brackish water fish species could be found and a total of 15 fish species from 10 families were recorded. With respect to distribution, these species had similar distributions to those found in a study of fish biodiversity in mangroves located on the Andaman coast in the south of Thailand (Petsut et al. 2017). Additionally, the distribution of fish in this region was limited by salinity, which is a crucial factor. Consequently, these species were hardly distributed in the downstream and estuary ecosystems.

The Mangrove Forest Conservation Center (KK3) serves as a downstream location that sustains a wide variety of fish communities, including vital endangered species such as the dwarf whip ray *B. heterura*, according to the IUCN. Similar to upstream and midstream stations, mangrove forests were also dominated by fish from the family Cichlidae; the location of these mangroves was characterized by small brackish water canals, muddy benthic substrates, and shallow coastal areas. Petsut et al. (2017) also noted that this family thrives in shallow coastal waters, rivers, and the transitional zones between freshwater and brackish water, as well as muddy habitats within estuary ecosystems.

The dominant species discovered in the present study belongs to the family Cichlidae, which exhibited the highest population numbers. These findings aligned with studies on fish population structures and fish larvae residing in mangrove ecosystems across various estuaries situated in both southeastern and southwestern parts of Thailand (Abidin et al. 2021), which likewise revealed that Cichlidae boasted the highest numbers in terms of both species count and abundance. This contrasts with the research conducted by Wahyudewantoro (2018), who

found that the Gobiidae family predominated in samples collected from mangrove estuaries with soft substrates in the Solomon Islands. It is suspected that species from these families either permanently or temporarily inhabit the waters of mangrove ecosystems.

The Klong Klon Bay estuary (KK4) also supports diverse fish communities. This site frequently offers an increased availability of food resources, resulting in greater biodiversity within the aquatic system, as observed by Ghimire and Koju (2021). Furthermore, the proximity of KK4 to the coastal areas of the Inner Gulf of Thailand has had an influence on its diversity, akin to the Khanom Estuary in Nakhon Si Thammarat Province, Southern Thailand, as highlighted by Petsut et al. (2022). Additionally, all the sampling sites provide heterogeneous habitats for various fish species.

Tudorance et al. (1975) reported that a diversity index that ranged from 1 to 3 could indicate suitable water conditions for aquatic animals to exist, but if less than 1, it meant that the natural water quality was not suitable. On the other hand, if this index is more than 3, it indicates that the water quality is optimal for the growth of aquatic animals. Similarly, in a recent study by Petsut et al. (2022), which surveyed fishes in the Khanom Estuary, Nakhon Si Thammarat Province, Southern Thailand, the fish diversity index was noted to range from 0.73 to 1.73 when the southwest monsoon-influenced study sites. Therefore, this data implied that the environmental condition of the Don Hoi Lot Ramsar site in the estuary on the inner-western coast of the Gulf of Thailand was still suitable for the existence of aquatic animals, especially downstream (KK3) and estuary (KK4) sites, where the diversity index values were nearly 3. Also, the evenness index was consistently and steadily low although the sites were affected by the southwest monsoon in July and the northeast monsoon in November. Furthermore, there are some fish species dominated more than other species in each collecting site, namely *O. mossambicus*, *S. melanotheron, A. nalua*, *S. argus*, and *T. nieuhofii*. These fish are considered an economic species for domestic consumption and high demand. This result indicates that the Don Hoi Lot Ramsar Site is still in good condition for fishery on the inner-western coast of the Gulf of Thailand.

Don Hoi Lot, listed as a Ramsar site under the Ramsar Convention, is the most distinct wetland ecosystem in Thailand's Mae Klong River. This deep mangrove area is located along the shoreline east of the Mae Klong River mouth (Chaisanguansuk et al. 2023) and stretches along this eastern shoreline (Pumijumnong 2014). The Don Hoi Lot Ramsar site is a hotspot for fishery on the western coast of the Gulf of Thailand, as previously mentioned. This area is surrounded by a blend of urban developments and aquaculture zones, making it a crucial hub for fisheries and the cultivation of clams, mussels, and giant tiger prawns (Department of Marine and Coastal Resources 2018). This can expose it to potential invasions by non-native species, whether intentional or accidental. These introduced species have the capacity to become invasive, leading to significant alterations in the ecosystem through mechanisms such as competition, predation, hybridization, disease transmission, and ecosystem modification. The consequences of invasive species can be far-reaching, encompassing the loss of recreational value, income, and damage to public health. Tropical wetland invaders come in various forms, some of which can be highly destructive (Pegg et al. 2021).

Within the Don Hoi Lot Ramsar Site in Thailand, the estuary area typically supports a community of brackish water and coastal marine fish. However, the findings in this survey have revealed a notable deviation from the norm, with black-chin tilapias, specifically *S. melanotheron*, emerging as the predominant species in this particular area. Guèye et al. (2020) found that the *S. melanotheron* species could successfully reproduce in this ecosystem, even at extremely high salinity (up to 130 psu), which raises concerns about its potential adverse impact on native fish populations. This phenomenon could be attributed to intensified competition for successful reproduction within the ecosystem and the potential effects of predation. Consequently, there is an urgent need to conduct a comprehensive assessment of the distribution and establishment of *S. melanotheron* within the Don Hoi Lot Ramsar Site. Numerous factors favor the rapid expansion of the black-chin cichlid population, especially in environments with abundant food resources, such as the ponds of aquaculture farms. Consequently, more study is necessary to examine the ecological and environmental effects.

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