



# DNA barcoding for fish species identification and diversity assessment in the Mae Tam reservoir, Thailand

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## Abstract

The purposes of this research were to identify fish species using DNA barcodes or partial sequences of cytochrome b (Cytb) and to assess the diversity of fish in the Mae Tam reservoir, Phayao province, Thailand. Fish samples were collected 3 times, during the winter, summer, and rainy seasons, from 2 sampling sites using gillnets with 3 mesh sizes (30, 50, and 70 mm). A total of 34 representative samples were classified into 12 species, 7 families and 6 orders by morphological- and DNA barcoding-based identifications. However, one cichlid species, *Cichlasoma trimaculatum*, could only be identified using DNA barcoding. Family Cyprinidae had the greatest diversity, 50.00%. The diversity, richness and evenness indices ranged from 0.43–0.65, 0.64–1.46, and 0.27–0.40, respectively, indicating that fish diversity at both sampling sites was relatively low. A comparison of the catch per unit effort (CPUE) with 3 different mesh sizes found that the 50 mm mesh size was the best ( $474.80 \pm 171.56$  g/100 m<sup>2</sup>/night), followed by the 70 mm ( $417.41 \pm 176.24$  g/100 m<sup>2</sup>/night) and 30 mm mesh sizes ( $327.88 \pm 115.60$  g/100 m<sup>2</sup>/night). These results indicate that DNA barcoding is a powerful tool for species identification. Our data can be used for planning the sustainable management of fisheries resources in the Mae Tam reservoir.

**Keywords:** DNA barcoding, Cytochrome b gene, Species identification, Fish diversity, Mae Tam reservoir

## Introduction

Biological diversity is the variability among living organisms from all sources, including diversity within species, between species, and within ecosystems (United Nations, 1992). Aquatic ecosystems consist of communities of several living organisms in the water, such as fishes, shrimps and mollusks. Fishes were

the first vertebrates to evolve and are also the most diverse and numerous vertebrates on Earth. Over one-half of the world's living vertebrate species (32,000 out of 60,000) are fishes (Nelson et al., 2016). According to a report on the diversity of freshwater fish in Thailand in 1997, there were 570 species from 56 families, of which at least 50 were unprecedented and unidentified species (Vidthayanon et al., 1997). In 2017, at least 858 species

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in 81 families were listed, up to 70 species of which were from unidentified taxa, while at least 137 species were threatened (Vidthayanon, 2017). Human activities often affect biodiversity, causing degradation or loss (United Nations, 1992), including of genetic diversity (Nguyen et al., 2022). Sustainable development is a process for resource management and sustainable utilization. However, the correct identification of species is required to assess and understand the biodiversity of an ecosystem and is the first step in biological research (Kürzel et al., 2022).

Generally, the species identification of fishes is based on morphology, such as body shape, numbers of fin rays, and scales in the lateral line (Nelson et al., 2016). However, several fish species have very similar appearances and are difficult to correctly identify. Even one species in different developmental stages or fishery products may also cause errors in the identification of species. DNA-based identification called “DNA barcoding” is utilized because it is an accurate and rapid technique for nonexpert taxonomists. DNA barcoding is the partial sequencing of a mitochondrial DNA gene. Mitochondrial DNA evolves faster than nuclear DNA, producing a higher degree of sequence variation (Stoneking & Soodyall, 1996), which is suitable for distinguishing fish species. Several genes have been used as DNA barcodes. The cytochrome b (Cytb) gene has been effectively utilized to identify diverse vertebrate animals (Parson et al., 2000), including fishes such as chub and spotted mackerels (Sezaki et al., 2001), family Goodeidae (Doadrio & Domínguez, 2004), four *Thunnus* tuna species (Lin et al., 2005), *Acipenser gueldenstaedtii*, *Acipenser stellatus* and *Huso huso* (Çiftci et al., 2013).

Mae Tam reservoir is located in Mueang district, Phayao province, Thailand. It has the longest reservoir ridge in northern Thailand, approximately 1,800 meters long. Its capacity is 37 million cubic meters (MaeKa Subdistrict Municipality, 2023). This reservoir is one of the Projects Initiated by His Majesty King Bhumibol Adulyadej that the Royal Irrigation Department considered construction in 1981 to supply water to farmers in the Muang and Dok Kham Tai districts for use in agriculture. It is also a source of fish breeding for the communities around the reservoir. In a study of the diversity of fish species in the Mae Tam reservoir, a total of 8 families and 15 species were found (Soontornprasit & Sangsrichan, 2014). The environment of the reservoir has changed over time, which along with the advent of more efficient fishing gear, may affect the species, diversity and quantity of fish caught.

The aims of this research were to identify fish species using

DNA barcodes of the partial Cytb gene and to assess the diversity of fishes in the Mae Tam reservoir, Thailand. The fish samples were collected using gillnets with 3 mesh sizes at 2 sampling sites in 3 seasons. Fish diversity was assessed through diversity, richness, and evenness indices. In addition, the catch per unit effort (CPUE) was calculated for measurement the relative abundance of fish species. The obtained data can be used as basic information for planning the conservation and management of aquatic animal resources in the Mae Tam reservoir for sustainable utilization.

## Materials and Methods

### Sample collection

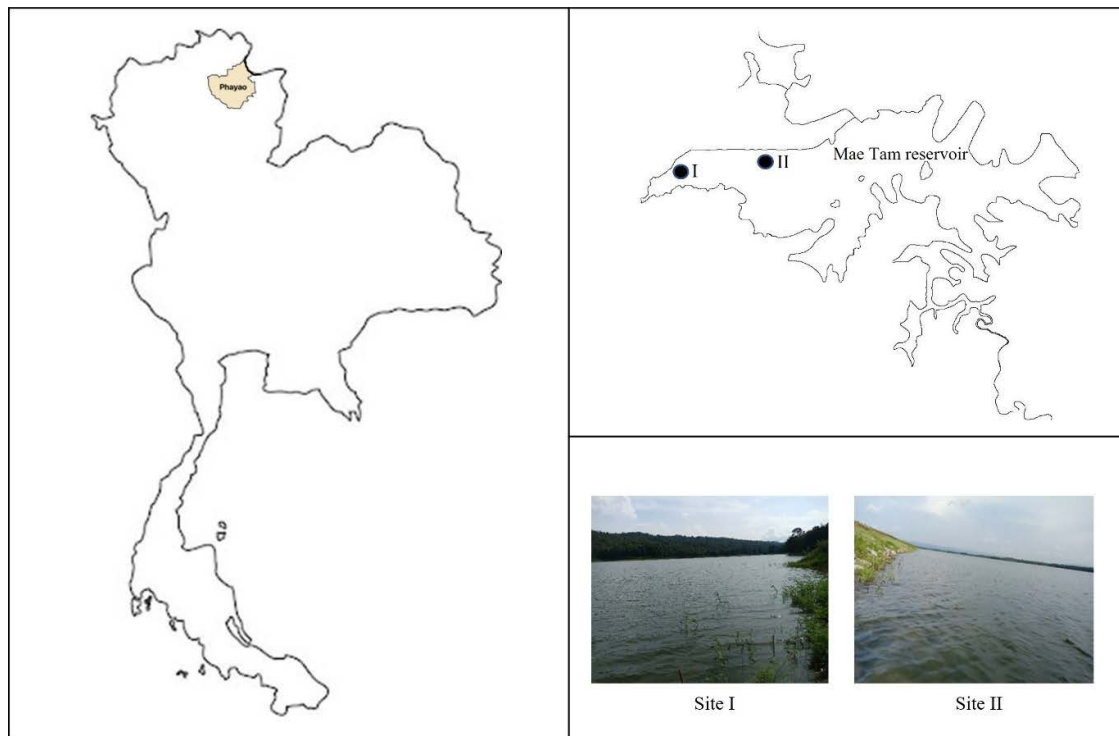
Fish samples were collected using gillnets with one of three different mesh sizes, 30, 50, and 70 mm. The seasons of sampling were winter (February 2018), summer (May 2018), and the rainy season (September 2018). Two study sites were selected: site I (19°00'13.3"N 99°56'26.5"E) was far from any human activities, while site II (19°00'15.3"N 99°56'55.2"E) was near the road on the ridge of the reservoir, which has many human activities, such as fishing, feeding the fishes and picnicking (Fig. 1). Gillnets were laid in the evening and were gathered in the morning of the next day, after a duration of laying of 12 hours. Total fish of each mesh size gillnet were photographed, counted, and weighed.

Similar-looking fish were grouped together. Species classification based on morphology was performed according to the fish taxonomy of Nelson et al. (2016). The representative fish were stored in absolute ethanol at room temperature until DNA extraction.

### Identification of fish species using DNA barcodes

#### DNA extraction

Because some fish samples could not be identified at the species level and for confirmation of the species that could be morphologically identified, DNA barcoding was utilized. The fish fin of each representative sample was extracted for genomic DNA isolation using 20 mg/mL protease, followed by the standard phenol-chloroform extraction method (Sambrook & Russell, 2001). The quality of extracted DNA was determined using 1.2% agarose gel electrophoresis, immersion in Novel Juice (Bio-Helix, New Taipei City, Taiwan) and exposure to UV light. The good-quality DNA was stored at -20 °C.



**Fig. 1. Two study sites of fish species in the Mae Tam reservoir, Phayao province, Thailand.**

### **Cytb amplification and sequencing**

The partial fragments of the *Cytb* gene were amplified by polymerase chain reaction (PCR) using primers L14735: 5'-AAAAACCACCGTTGTTATTCAACTA-3' and H15149AD: 5'-GCICCTCARAATGAYATTTGTCCTCA-3' (Burgener, 1997). The 25  $\mu$ L PCR mixture consisted of 2.5  $\mu$ L of 10x *Taq* buffer, 1 mL of 50 mM  $MgCl_2$ , 2  $\mu$ L of 2.5 mM dNTPs, 0.3  $\mu$ L of 1 U *Taq* DNA polymerase (Bio-Helix), 2  $\mu$ L of each 10  $\mu$ M primer and 1  $\mu$ L of extracted DNA (100 ng/ $\mu$ L). The thermal cycling program was 35 cycles of denaturation at 94  $^{\circ}C$  for 40 s, annealing at 50  $^{\circ}C$  for 80 s and extension at 72  $^{\circ}C$  for 80 s (Sotelo et al., 2001). The PCR products were identified using 1.2% agarose gel electrophoresis.

The visible PCR products were purified using the HiYield™ Gel/PCR DNA Fragments Extraction kit (RBC Bioscience, New Taipei City, Taiwan) according to the manufacturer's instructions. The purified PCR product was sequenced at Macrogen (Seoul, Korea).

### **Sequence analysis**

All nucleotide sequences were determined the stop codons,

insertions and deletions by eye using the Chromas program (Technelysium, Queensland, Australia) and ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). The scientific name of each sample was determined by comparing its sequence with references in the GenBank database using the Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). As a basic criterion for species identification, a sampled sequence had to match a top hit of a reference sequence with more than 98% similarity (Ward et al., 2009). All sequences have been deposited in GenBank (accession numbers OQ561413-OQ561446).

The identified fish species were classified according to the fish taxonomic system of Nelson et al. (2016). The conservation status of each fish species was found on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species version 2022-2 (<https://www.iucnredlist.org>) as of February 2023.

For evolutionary relationship analysis, a phylogenetic tree of translated amino acid sequences was constructed by the neighbor-joining method (Saitou & Nei, 1987) using the program MEGA11 (Tamura et al., 2021) with 1,000 bootstrap

replications. The Cytb sequence of *Macrobrachium rosenbergii* (accession no. KY865098) was used as an outgroup species sequence. The intraspecies distances were also calculated using MEGA11 (Tamura et al., 2021).

### Measurements of fish diversity and catch per unit effort

Fish diversity was measured by the indices diversity, richness and evenness, which were calculated using the following formulas:

$$\text{Shannon-Weiner diversity index } H = -\sum (P_i \log P_i)$$

where  $H$  = diversity index, and  $P_i$  = number of individuals of species  $i$ /total number of sample (Shannon & Weaver, 1949);

$$\text{Richness index } R = (S - 1) / \ln(n)$$

where  $R$  = richness index,  $S$  = number of different species in the sample, and  $n$  = total number of individual species in the sample (Margalef, 1968); and

$$\text{Evenness index } E = H / \ln S$$

where  $E$  = evenness index,  $H$  = Shannon-Weiner diversity index, and  $S$  = number of different species in the sample (Pielou, 1966).

CPUE was calculated according to the formula of Swingle (1950):

$$\text{CPUE} = \frac{\text{Total weight of fish sample (g)}}{\text{Total net area (100 m}^2) \times \text{Fishing period (night)}}$$

Moreover, the frequency of occurrence of each fish species at

each sampling site and season was calculated following Kolding (1989).

### Statistical analysis

All data are presented as mean  $\pm$  SD. For comparisons between the two sampling sites, analysis of variance (ANOVA) with the independent samples  $t$ -test was used. One-way ANOVA was used to consider seasonality and CPUE. Statistical significance was set at  $p < 0.05$ .

## Results and Discussion

### Morphological-based identification of fish species

A total of 34 representative fish samples were morphologically identified to 12 species, 11 genera, 7 families and 6 orders according to Nelson et al. (2016). The most abundant order was Cypriniformes (50.00%), followed by Gobiiformes (16.67%) (Fig. 2A). Cyprinidae was the most abundant family (50.00%) (Fig. 2B). Cypriniformes is the most diverse fish order in Southeast Asia (Nelson et al., 2016), including in Thailand. Moreover, family Cyprinidae is the most common and the largest family of freshwater fish, followed by family Gobiidae (Nelson et al., 2016). As for their IUCN status, all species were assessed as least concern except *Systemus orphoides*, which was not evaluated. One cichlid fish could not be identified at the species level from morphology due to the inexperience of the researchers. Thus, molecular-based identification was necessary in this study to specifically identify this species and confirm the identities of the remaining species.

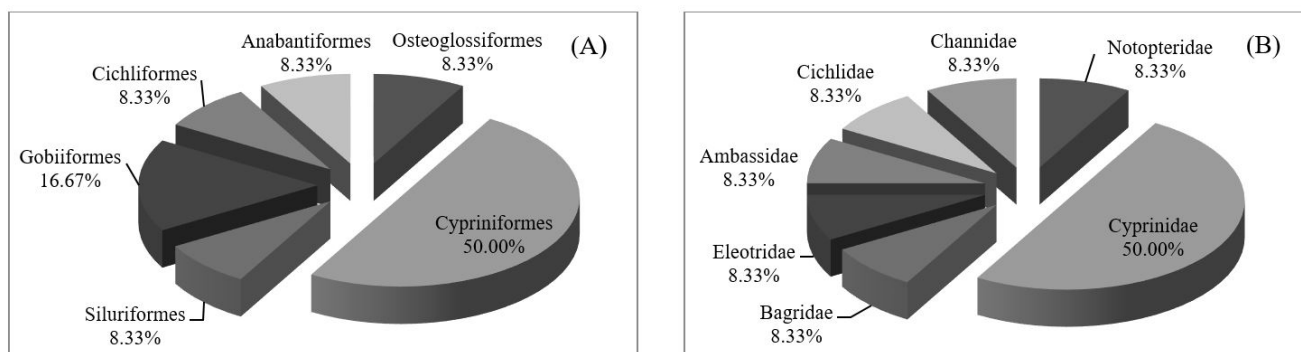


Fig. 2. Percentages of fish species by order (A) and family (B) collected in the Mae Tam reservoir, Phayao province, Thailand.

**DNA barcoding-based identification of fish species**

A molecular-based identification technique, DNA barcoding is one tool that has gained attention and is widely used for identification of organism species. The Cytb gene fragments could be successfully amplified in all fish samples. From comparison with reference sequences in the database, all samples could be identified at the species level with 98.97%–100% identity (Table 1) according to the criteria of Ward et al. (2009). The average length of the Cytb gene was 390 bp, ranging from 381 to 396 bp, and we found no stop codons, insertions, or deletions. Therefore, these nucleotide sequences were deemed normal.

We picked twelve species to classify with both DNA barcoding and morphology. One cichlid fish could not be identified to the species level by morphology but could be identified by DNA barcoding: *Cichlasoma trimaculatum*, which had 99.74%–100% identity. This exotic cichlid is an alien species that was introduced into Thailand for ornamental purpose (Silarudee & Kongchum, 2008). Because of its predacious nature (Daniel et al., 2020), the infestation of *C. trimaculatum* into the natural waters would cause many impacts. *C. trimaculatum*'s

habit of eating smaller fishes creates a hazard to the biodiversity of native fishes (Daniel et al., 2020). Many natural waters were infested with this invasive species, including Madambakkam Lake, India (Daniel et al., 2020), Lake Buluan, Philippines (Noquilla, 2021), and Lake Mahalona, Indonesia (Nasution et al., 2022).

One main advantage of DNA barcodes, including of the Cytb gene, is that the low intraspecies distances provided enable easier distinguishing of one species from others (Kartavtsev et al., 2007; Tobe et al., 2010). Ward et al. (2005) mentioned that the successful identification of species depended on the nucleotide sequences being more similar than those of different species. Generally, the mitochondrial Cytb gene of fishes presented intraspecific differences of less than 1%, while interspecific differences were higher than 10% (Li et al., 2018; Shen et al., 2013). In the present study, the low intraspecies distances ranged from 0.0% to 0.3% (Table 1), as in several fishes including, six flatfish species ( $0.46 \pm 0.19\%$ ) (Kartavtsev et al., 2007), *A. gueldenstaedtii* (0.42%), *A. stellatus* (0.32%) and *H. huso* (0.17%) (Çiftci et al., 2013). From the relationship

**Table 1. Fish species identification in the GenBank database, number of representative samples, intraspecies distance, length of the Cytb gene and accession numbers**

Order/family	Scientific name	GenBank		No. of samples	IUCN status	Intra-species distances (%)	Length of Cytb (bp)	Accession no.
		Species	Identity (%)					
Osteoglossiformes								
Notopteridae	<i>Notopterus notopterus</i>	<i>N. notopterus</i>	99.24	2	LC	0.0	396	OQ561413 - OQ561414
Cypriniformes								
Cyprinidae	<i>Barbonymus gonionotus</i>	<i>B. gonionotus</i>	99.24–99.75	5	LC	0.3	393	OQ561415 - OQ561419
	<i>Cyclocheilichthys armatus</i>	<i>C. armatus</i>	99.22	5	LC	0.0	387	OQ561420 - OQ561424
	<i>Hampala dispar</i>	<i>H. dispar</i>	99.74	3	LC	0.0	387	OQ561425 - OQ561427
	<i>Hampala macrolepidota</i>	<i>H. macrolepidota</i>	99.75–100	5	LC	0.2	393	OQ561428 - OQ561432
	<i>Hypsibarbus vernayi</i>	<i>H. vernayi</i>	98.98	1	LC	-	393	OQ561433
	<i>Systemus orphoides</i>	<i>S. orphoides</i>	98.97	1	NE	-	390	OQ561434
Siluriformes								
Bagridae	<i>Hemibagrus spilopterus</i>	<i>H. spilopterus</i>	98.97	3	LC	0.0	387	OQ561435 - OQ561437
Gobiiformes								
Eleotridae	<i>Oxyeleotris marmorata</i>	<i>O. marmorata</i>	100	1	LC	-	387	OQ561438
Ambassidae	<i>Parambassis ranga</i>	<i>P. ranga</i>	100	5	LC	0.0	393	OQ561439 - OQ561443
Cichliformes								
Cichlidae	<i>Cichlasoma trimaculatum</i>	<i>C. trimaculatum</i>	99.74–100	2	LC	0.3	381	OQ561444 - OQ561445
Anabantiformes								
Channidae	<i>Channa micropeltes</i>	<i>C. micropeltes</i>	100	1	LC	-	387	OQ561446

Cytb, cytochrome b; IUCN, International Union for Conservation of Nature; LC, least concern; NE, not evaluated.

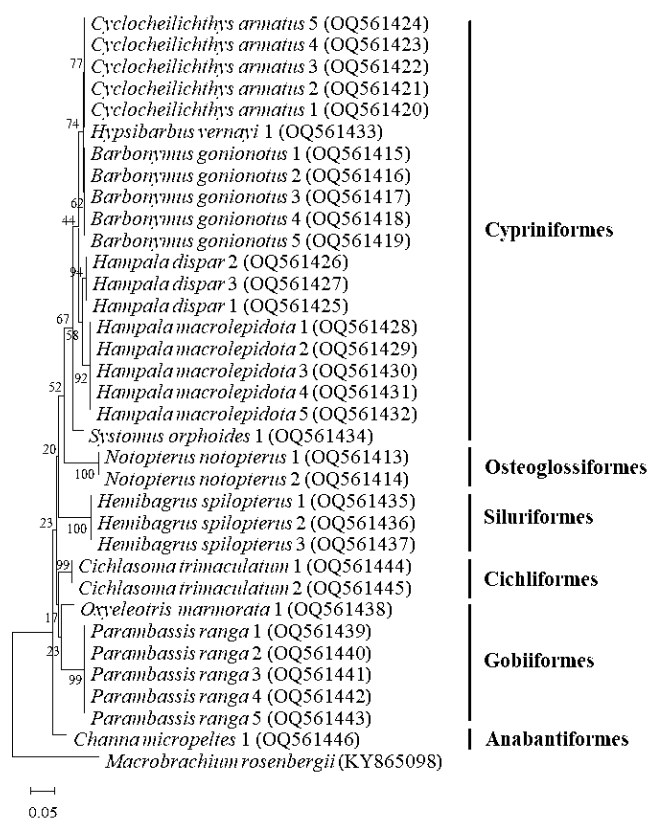
analysis, the phylogenetic tree of Cytb gene also showed that each given fish species was clustered together with itself (Fig. 3). In addition, 12 fish species were correctly grouped according to the taxonomic system. The number of sequences of fish deposited in the GenBank database is rapidly increasing, and invalid or incorrectly identified species may be submitted, depending on personal experience. The low values of divergence and phylogenetic tree of the Cytb gene could detect the errors in numerous sequences submitted in GenBank (Li et al., 2018).

Although DNA barcoding is a useful tool for non-fish taxonomists to identify fish species. However, collaboration with fish taxonomists to confirm DNA barcoding results is necessary for correct fish species identification (Panprommin & Manosri, 2022). A database with a large amount of accurate data is useful for identifying fish species in the investigation of species diversity and in several kinds of study, including studies on different developmental stages and on the fish meat from fishery products. Therefore, the correct identification of an unknown sample depends on the availability of the sequences in the database (Parson et al., 2000).

### Fish species diversity

A total of 371 individuals were collected from site I and site II over three seasons (Table 2). They were identified to 12 species by both morphology and DNA barcoding. From the comparison of a previous study, 15 fish species were collected monthly between January and June 2011 at 3 sampling sites using gillnets with 4 mesh sizes including 25, 40, 70, and 100 mm (Soontornprasit & Sangsrichan, 2014). Among these species, 8 species were collected in both studies. Seven species were found only in the previous study, while 4 species were found only in this study. Several factors may be affected different of fish species such as mesh sizes of gillnets, period and location of sampling (Keawkhiew et al., 2013; Soontornprasit et al., 2019b). Nowadays, the areas around this reservoir are increasingly being utilized for buildings and agricultural areas, thus, domestic wastes and the use of agricultural drugs and chemicals may be caused the water pollution. The environmental disturbances may have seriously destroyed the fish habitat and led to the decline of fish species diversity (Guo et al., 2018).

At site I, a total of 273 individuals (73.58%) were identified to 5 species, while 98 individuals (26.42%) were identified to 12 species from site II. This may be because site II is an easily accessible area and people tend to feed the fish regularly, which raised the number of fish species at site II relative to site



**Fig. 3. NJ phylogenetic tree based on Cytb amino acid sequences of 12 fish species from 34 representative samples.** The numbers on the branches represent bootstrap values (1,000 replicates). The bar at the bottom indicates 5% amino acid sequence divergence. *Macrobrachium rosenbergii* (KY865098) was used as an outgroup species. NJ, neighbor joining; Cytb, cytochrome b.

I. Fish feeding is not only food intake directly to the fish but also increases nutrients to the water environment (Yang et al., 2021). These increased nutrients affect the diversity of the fish. Henderson & Crampton (1997) demonstrated that fish diversity in nutrient-rich habitat (108 species) was higher than nutrient-poor habitat (68 species). However, the number of individuals at site I was greater than that at site II because site I is a quiet area and has little human activity. In addition, the area of site I has several shelters, such as aquatic plants, grass, and roots. A large number of shelters contributes to the abundance of fish, as they are suitable for habitation, refuge and foraging (Chamason & Janekitkarn, 2005).

We collected 236 (63.61%) and 74 (19.95%) in winter and summer, respectively, which were identified to 7 species. We

**Table 2. Frequency of occurrence of each fish species according to the season for each sampling site**

Species	Site I			Site II			F-value (%)
	Winter	Summer	Rainy	Winter	Summer	Rainy	
<i>Notopterus notopterus</i>	-	-	-	+ (2)	-	-	16.7
<i>Barbonymus gonionotus</i>	+ (18)	+ (4)	+ (19)	+ (11)	+ (12)	+ (3)	100
<i>Cyclocheilichthys armatus</i>	-	+ (16)	-	-	+ (7)	-	33.3
<i>Hampala dispar</i>	-	-	-	-	-	+ (3)	16.7
<i>Hampala macrolepidota</i>	+ (15)	+ (12)	+ (26)	+ (23)	+ (6)	+ (1)	100
<i>Hypsibarbus vernayi</i>	-	-	-	-	+ (1)	-	16.7
<i>Syomus orphoides</i>	-	-	-	+ (1)	-	-	16.7
<i>Hemibagrus spilopterus</i>	-	+ (5)	-	-	+ (4)	-	33.3
<i>Oxyeleotris marmorata</i>	-	-	-	+ (1)	-	-	16.7
<i>Parambassis ranga</i>	+ (144)	+ (6)	+ (8)	+ (20)	-	-	66.7
<i>Cichlasoma trimaculatum</i>	-	-	-	-	+ (1)	+ (1)	33.3
<i>Channa micropeltes</i>	-	-	-	+ (1)	-	-	16.7
Total species	3 (177)	5 (43)	3 (53)	7 (59)	6 (31)	4 (8)	
(no. of individuals)	5 (273)			12 (98)			

+, present; -, not present.

collected the fewest individuals in the rainy season, 61 (16.44%), and identified them to 5 species (Table 2). In winter and summer, these sites have less water, so more fish individuals could be collected than in the rainy season, when there is higher water content. The different water levels in each season are an important factor for the abundance and productivity of fish (Chamason & Janekitkarn, 2005).

The independent samples *t*-test detected differences in various indices between the two sampling sites. The Shannon-Weiner diversity index ( $0.60 \pm 0.04$ ) and richness index ( $1.46 \pm 0.01$ ) at site II were higher ( $p < 0.05$ ) than those at site I ( $0.45 \pm 0.27$  and  $0.64 \pm 0.47$ , respectively), while the evenness index presented no significant difference ( $p > 0.05$ ) between the two sites (Table 3). Comparing the indices between seasons, the diversity and richness indices were the highest ( $p < 0.05$ ) ( $0.65 \pm 0.01$  and  $1.26 \pm 0.28$ , respectively) in summer, while the evenness index was the highest ( $p < 0.05$ ;  $0.40 \pm 0.00$ ) in the

rainy season. However, the average diversity indices were lower than 1, indicating low diversity (Ulfah et al., 2019). Ecosystems are abundant at low levels and may be contaminated by heavy pollution (Wilhm & Dorris, 1968), corresponding to the Margalef richness index, which represents diversity in biological populations by comparing organism communities and identifies rich or poor ecosystems by their species numbers (Malvandi et al., 2021). The evenness index ranges from 0 to 1 (Krebs, 1989). The evenness indices of this study ranged from 0.27 to 0.40, indicating that the distribution of the number of individuals of each species is not similar between sampling sites and seasons, so there is a tendency for one species to dominate (Krebs, 1989; Ulfah et al., 2019).

In this study, the CPUE was calculated for 2 sampling sites and 3 seasons (Table 4). The CPUE at site I ( $472.63 \pm 94.66$  g/100 m<sup>2</sup>/night) was higher ( $p < 0.05$ ) than that at site II ( $340.76 \pm 153.34$  g/100 m<sup>2</sup>/night). Because site I was an area that was

**Table 3. Diversity, richness and evenness indices between the two sampling sites and three seasons**

Index	Site		Season		
	I	II	Winter	Summer	Rainy
Diversity index	$0.45 \pm 0.27^b$	$0.60 \pm 0.04^a$	$0.43 \pm 0.23^b$	$0.65 \pm 0.01^a$	$0.50 \pm 0.08^c$
Richness index	$0.64 \pm 0.47^b$	$1.46 \pm 0.01^a$	$0.93 \pm 0.76^c$	$1.26 \pm 0.28^a$	$0.97 \pm 0.66^b$
Evenness index	$0.35 \pm 0.11^a$	$0.35 \pm 0.04^a$	$0.27 \pm 0.04^c$	$0.38 \pm 0.03^b$	$0.40 \pm 0.00^a$

<sup>a-c</sup> Different letters in each row indicate significant differences among sites and seasons at  $p < 0.05$  level.

**Table 4. Catch per unit effort (g/100 m<sup>2</sup>/night) in the two sampling sites and three seasons**

Mesh size gillnets (mm)	Site		Season			Mean ± SD
	I	II	Winter	Summer	Rainy	
30	405.25	250.52	492.08	268.63	222.94	327.88 ± 115.60 <sup>c</sup>
50	431.78	517.81	485.26	230.95	708.17	474.80 ± 171.56 <sup>a</sup>
70	580.85	253.96	484.29	204.94	562.99	417.41 ± 176.24 <sup>b</sup>
Mean ± SD	472.63 ± 94.66 <sup>a</sup>	340.76 ± 153.34 <sup>b</sup>	487.21 ± 4.24 <sup>a</sup>	234.84 ± 32.02 <sup>b</sup>	498.04 ± 249.05 <sup>a</sup>	

<sup>a,b</sup> Different letters indicate significant differences among sites and seasons at  $p < 0.05$  level.

difficult to access and was not disturbed by human activities, the fishes have a chance to grow and gain more weight. Moreover, the CPUE in the rainy season ( $498.04 \pm 249.05$  g/100 m<sup>2</sup>/night) and winter ( $487.21 \pm 4.24$  g/100 m<sup>2</sup>/night) were greater ( $p < 0.05$ ) than the CPUE in summer ( $234.84 \pm 32.02$  g/100 m<sup>2</sup>/night). Guo et al. (2018) demonstrated that CPUE in the wet season was higher than CPUE in the dry season. However, the average CPUE values of this study were lower than 500 g/100 m<sup>2</sup>/night, indicated that this reservoir is low abundance based on the standard of CPUE for gillnet of Srirachoenham et al. (2015). Generally, the CPUE is an index for assessment the relative abundance of fish species (Rayan et al., 2020). Two fish species could be collected at both sampling sites and in every season, *Barbonymus gonionotus* and *Hampala macrolepidota*, 100% F (Table 2). These species are economic fish that could be caught in large numbers, 67 (18.06%) and 83 (22.37%) individuals, respectively, especially in the winter and rainy seasons. In addition, since both these species spawn in the rainy season (Tuncharoen et al., 2018), the weight of the fishes was also high, which affected the CPUE. The glassy fish (*Parambassis ranga*) was the most dominant species in this study, with 178 (47.98%) individuals. This genus is a small fish in Thailand that is mainly distributed in several natural waters in Phayao province, including Kwan Phayao (Soontornprasit et al., 2019a) and the Nong Leng Sai Wetland (Soontornprasit et al., 2019b; Tuncharoen et al., 2020).

The CPUE using gillnets with 3 mesh sizes, including 30, 50, and 70 mm, showed that gillnets with a 50 mm mesh size had the highest average ( $p < 0.05$ ), which was  $474.80 \pm 171.56$  g/100 m<sup>2</sup>/night (Table 4), followed by gillnets with 70 and 30 mm, with averages of  $417.41 \pm 176.24$  and  $327.88 \pm 115.60$  g/100 m<sup>2</sup>/night, respectively. This indicated that gillnets with 50 mm mesh sizes were the most efficient at catching the fishes in the Mae Tam reservoir. The fish caught were usually medium to large in size, letting the small fish escape from the gillnets to continue to grow and reproduce.

In conclusion, twelve fish species could be identified using both morphological and DNA barcode approaches. However, one cichlid species could be specified to the species level using only the DNA barcode of the Cytb gene, although not a fish taxonomist. The various diversity indices indicated that fish diversity at both sampling sites in the Mae Tam reservoir was relatively low. However, study of other sampling sites should be undertaken for a more accurate and comprehensive assessment.

#### Competing interests

No potential conflict of interest relevant to this article was reported.

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#### Availability of data and materials

Not applicable.

#### Ethics approval and consent to participate

This study protocol was approved for animal ethics by the Laboratory Animal Research Center, University of Phayao (UP-AE56-01-04-0007).

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## References

- Burgener M. Molecular species differentiation of fish and mammals [Ph.D. dissertation]. Bern: University of Bern; 1997.
- Chamason O, Janekitkarn S. Distribution of fishes in Pasak Jo-lasid reservoir, Lop Buri province. In: Proceedings of 43rd Kasetsart University Annual Conference: Fisheries, Natural Resources and Environmental Economics; 2005; Bangkok, Thailand.
- Çiftci Y, Eroğlu O, Firidin S. Mitochondrial cytochrome b sequence variation in three sturgeon species (*A. stellatus* Pal-las, 1771, *A. gueldenstaedtii* Brandt, 1833, *H. huso* Linnaeus, 1758) from the Black Sea coasts of Turkey. *Turk J Fish Aquat Sci.* 2013;13:291-303.
- Daniel N, Praveenraj J, Suresh E, Karal Marx K, Felix S. Report on the occurrence of invasive alien fish, *Cichlasoma trimaculatum* (Günther, 1867) at freshwater lake of Chennai. *J Entomol Zool Stud.* 2020;8:2418-20.
- Doadrio I, Domínguez O. Phylogenetic relationships within the fish family Goodeidae based on cytochrome b sequence data. *Mol Phylogenet Evol.* 2004;31:416-30.
- Guo Q, Liu X, Ao X, Qin J, Wu X, Ouyang S. Fish diversity in the middle and lower reaches of the Ganjiang river of China: threats and conservation. *PLOS ONE.* 2018;13:e0205116.
- Henderson PA, Crampton WGR. A comparison of fish diversity and abundance between nutrient-rich and nutrient-poor lakes in the Upper Amazon. *J Trop Ecol.* 1997;13:175-98.
- Kartavtsev YP, Park TJ, Vinnikov KA, Ivankov VN, Sharina SN, Lee JS. Cytochrome b (*Cyt-b*) gene sequence analysis in six flatfish species (Teleostei, Pleuronectidae), with phylogenetic and taxonomic insights. *Mar Biol.* 2007;152:757-73.
- Keawkhiew P, Keawtip S, Seetakoses P, Montien-art B. Biodiversity of fish in Maesaw creek at the Initiative Highland Agricultural Development Station Project, Ban Santisuk, Khunkual subdistrict, Phong district, Phayao province. *J Fish Tech Res.* 2013;7:70-81.
- Kolding J. The fish resources of Lake Turkana and their environment [Ph.D. dissertation]. Bergen: University of Bergen; 1989.
- Krebs CJ. Ecological methodology. New York, NY: Harper & Row; 1989.
- Kürzel K, Kaiser S, Lörz AN, Rossel S, Paulus E, Peters J, et al. Correct species identification and its implications for conservation using Haploniscidae (Crustacea, Isopoda) in Icelandic Waters as a proxy. *Front Mar Sci.* 2022;8:795196.
- Li X, Shen X, Chen X, Xiang D, Murphy RW, Shen Y. Detection of potential problematic *Cytb* gene sequences of fishes in GenBank. *Front Genet.* 2018;9:30.
- Lin WF, Shiau CY, Hwang DF. Identification of four *Thunnus* tuna species using mitochondrial cytochrome b gene sequence and PCR-RFLP analysis. *J Food Drug Anal.* 2005;13:382-7.
- MaeKa Subdistrict Municipality. Mae Tam reservoir [Internet]. 2023 [cited 2023 Sep 11]. <https://online.fliphtml5.com/ghu-we/rzme/#p=2>
- Malvandi H, Moghanizade R, Abdoli A. The use of biological indices and diversity indices to evaluate water quality of rivers in Mashhad, Iran. *Biologia.* 2021;76:959-71.
- Margalef R. Perspectives in ecological theory. Chicago, IL: University of Chicago Press; 1968.
- Nasution SH, Akhdiana I, Muchlis AM, Cinnawara HT. Size distribution and length-weight relationship of invasive species flowerhorn (*Cichlasoma trimaculatum* (Günther, 1867) in Lake Mahalona, South Sulawesi. *IOP Conf Ser Earth Environ Sci.* 2022;1062:012002.
- Nelson JS, Grande TC, Wilson MVH. Fishes of the world. 5th ed. Hoboken, NJ: John Wiley & Sons; 2016.
- Nguyen HV, Nguyen MT, Vo ND, Phan NTT, Hoang QT. Genetic diversity of spotted scat (*Scatophagus argus*) in Vietnam based on COI genes. *Fish Aquat Sci.* 2022;25:637-47.
- Noquilla GM. On the occurrence and successful establishment of *Cichlasoma trimaculatum* (Günther, 1867), an invasive species of cichlid, in Lake Buluan, Philippines. *Uttar Pradesh J Zool.* 2021;42:1095-9.
- Panprommin D, Manosri R. DNA barcoding as an approach for species traceability and labeling accuracy of fish fillet products in Thailand. *Food Control.* 2022;136:108895.
- Parson W, Pegoraro K, Niederstätter H, Föger M, Steinlechner M. Species identification by means of the cytochrome b gene. *Int J Legal Med.* 2000;114:23-8.
- Pielou EC. Species-diversity and pattern-diversity in the study of ecological succession. *J Theor Biol.* 1966;10:370-83.
- Rayan S, Chartchumni B, Kaewdonree S, Rayan W. Fish species composition and catch per unit effort in Nong Han wetland, Sakon Nakhon province, Thailand. *Songklanakarin J Sci Technol.* 2020;42:795-801.

- Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* 1987;4:406-25.
- Sambrook J, Russell DW. *Molecular cloning: a laboratory manual.* 3rd ed. Long Island, NY: Cold Spring Harbor Laboratory Press; 2001.
- Sezaki K, Kuboshima Y, Mitani I, Fukui A, Watabe S. Identification of chub and spotted mackerels with mitochondrial cytochrome b gene and its application to respective pelagic eggs fixed with formalin. *Nihon Suisan Gakkaishi.* 2001;67:17-22.
- Shannon CE, Weaver W. *The mathematical theory of communication.* Urbana, IL: The University of Illinois Press; 1949.
- Shen YY, Chen X, Murphy RW. Assessing DNA barcoding as a tool for species identification and data quality control. *PLOS ONE.* 2013;8:e57125.
- Silarudee S, Kongchum P. Masculinization of flowerhorn by immersion in androgens. *Silpakorn U Science & Tech J.* 2008;2:26-32.
- Soontornprasit K, Panprommin D, Khungboon L. Fish communities structure and catch efficiency from gillnets in the Kwan Phayao, Phayao province. *Khon Kaen Agr J.* 2019a;47:655-66.
- Soontornprasit K, Sangsrichan T. Efficiency and selectivity of gillnets in Mae Tam reservoir, Phayao province. In: *Proceedings of Phayao Research Conference; 2014; Phayao, Thailand.*
- Soontornprasit K, Wongkumpilat K, Thammawanta H, Sudrakam S, Sansupa I. Fish communities structure and catch efficiency from gillnets in the Nong Leng Sai Wetland, Phayao province. *Khon Kaen Agr J.* 2019b;47:1183-94.
- Sotelo CG, Calo-Mata P, Chapela MJ, Pérez-Martín RI, Rehbein H, Hold GL, et al. Identification of flatfish (Pleuronectiforme) species using DNA-based techniques. *J Agric Food Chem.* 2001;49:4562-9.
- Sricharoendham B, Boonthongchuay J, Poomikong P. Spatio-temporal variations in fish community of the Chao Phraya river between 2008 and 2014. *Thai Fish Gazette (Thailand).* 2015;68:201-23.
- Stoneking M, Soodyall H. Human evolution and the mitochondrial genome. *Curr Opin Genet Dev.* 1996;6:731-6.
- Swingle HS. *Relationships and dynamics of balanced and unbalanced fish populations.* Alabama: Agricultural Experiment Station of the Alabama Polytechnic Institute Auburn; 1950. Report No.: 274.
- Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 2021;38:3022-7.
- Tobe SS, Kitchener AC, Linacre AMT. Reconstructing mammalian phylogenies: a detailed comparison of the cytochrome B and cytochrome oxidase subunit I mitochondrial genes. *PLOS ONE.* 2010;5:e14156.
- Tuncharoen S, Panprommin D, Soonthornprasit K, Pitakpol S. Spawning season of fishes in Kwan Phayao, Phayao province. *Khon Kaen Agr J.* 2018;46:147-54.
- Tuncharoen S, Uppapong S, Soontornprasit K. Diversity of fishes in Nong Leng Sai Wetland, Phayao province. *Khon Kaen Agr J.* 2020;48:283-92.
- Ulfah M, Fajri SN, Nasir M, Hamsah K, Purnawan S. Diversity, evenness and dominance index reef fish in Krueng Raya Water, Aceh Besar. *IOP Conf Ser Earth Environ Sci.* 2019;348:012074.
- United Nations. *Convention on biological diversity [Internet].* 1992 [cited 2023 Feb 9]. <https://www.cbd.int/doc/legal/cbd-en.pdf>
- Vidthayanon C. *Checklist of freshwater fishes in Thailand.* Bangkok: Office of Natural Resources and Environmental Policy and Planning; 2017.
- Vidthayanon C, Karnasuta J, Nabhitabhata J. *Diversity of freshwater fishes in Thailand.* Bangkok: Office of Environmental Policy and Planning; 1997.
- Ward RD, Hanner R, Hebert PDN. The campaign to DNA barcode all fishes, FISH-BOL. *J Fish Biol.* 2009;74:329-56.
- Ward RD, Zemplak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci.* 2005;360:1847-57.
- Wilhm JL, Dorris TC. Biological parameters for water quality criteria. *BioScience.* 1968;18:477-81.
- Yang Z, Huang S, Kong W, Yu H, Li F, Khatoun Z, et al. Effect of different fish feeds on water quality and growth of crucian carp (*Carassius carassius*) in the presence and absence of prometryn. *Ecotoxicol Environ Saf.* 2021;227:112914.