# Native Japanese strain of the common carp (*Cyprinus carpio*): a precious natural heritage remaining in Lake Biwa

Kohji Mabuchi

Lake Biwa Branch Office, National Institute for Environmental Studies

Keywords: Lake Biwa, common carp, native Japanese strain, subspecies

## **ABSTRACT**

Wild common carp (Cyprinus carpio) are suffering worldwide because of the invasion of conspecific domesticated strains, and the native Japanese strain is no exception. A survey of mitochondrial (mt) DNA sequences from 11 localities in Japan revealed that roughly half of the haplotypes found at all 11 localities were those of introduced Eurasian strains, indicating that the native Japanese strain is suffering as a result of the invasion of Eurasian strains. However, an intensive mtDNA survey of the Lake Biwa population, the largest freshwater body in Japan, demonstrated that about 80% of the individuals in its deep off-shore waters (30-70 m depths) had native Japanese haplotypes. Moreover, a population genetic analysis of nuclear single nucleotide polymorphisms (SNPs) revealed that Lake Biwa harbored a relatively pure native population in its deep waters. Morphological analyses of the Lake Biwa and introduced Eurasian carp using genetic hybrid indices based on the SNPs showed that the typical native Japanese carp surviving in the lake differed from the introduced Eurasian carp in a body-shape trait, some internal features, and some meristic and morphometric characters used for carp taxonomy. A metadata comparison of the last characters between the native Japanese and native Eurasian carp indicated that the native Japanese carp deserves at least subspecific status. In addition, the native Japanese strain was located in a relatively basal position in the molecular phylogenetic tree of the common carp. Therefore, the native Japanese strain surviving in Lake Biwa has world conservation priority, to preserve genetic and taxonomic diversity of the species.

# 1. INTRODUCTION

Freshwater ecosystems are experiencing greater declines in biodiversity than those in the most affected terrestrial ecosystems, and invasion by exotic species is one of the five major threats to global freshwater biodiversity<sup>[1]</sup>. (The remaining four are overexploitation, water pollution, flow modification, and habitat destruction or degradation.) The extent and impact of such invasions, however, are probably underestimated because of the presence of cryptic invaders; i.e., the invasion of a community by a species (or a formerly allopatric population) that is morphologically similar to a resident species will likely be undetected. Molecular techniques are leading to breakthroughs in the detection of cryptic invaders, and in describing the genetic structure of alien and source populations<sup>[2]</sup>. For adequate protection of freshwater biodiversity, molecular analyses are now indispensable.

In this proceeding, I briefly review recent molecular studies of the Japanese common carp, *Cyprinus carpio* L.

1758 (sensu lato). The common carp is one of the most frequently cultured fish species worldwide. While it has now been introduced to all continents, except for Antarctica, anthropogenically, its natural range was originally restricted to temperate Eurasia, from Europe to East and South-east Asia. Although the origin of the Japanese common carp had long been controversial, a recent molecular study including individuals from various parts of its natural range revealed that both the native Japanese and alien Eurasian strains occur in Japanese freshwaters, such that there is an unexpected conservation problem with this very familiar fish. Subsequent studies demonstrated that Lake Biwa, the largest freshwater body in Japan, harbors a relatively pure native population in its deep waters, and that the typical native Japanese carp surviving in the lake is morphologically different from Eurasian subspecies.

By presenting case studies of the Japanese common carp, I hope to demonstrate the importance of

geographically wide-range molecular (and morphological) analyses to fully understand a local conservation problem.

# 2. MITOCHONDRIAL DNA SURVEY OF NATURAL POPULATIONS IN JAPAN

A large survey of mitochondrial DNA sequences (the complete D-loop region) from 11 localities in Japan was conducted<sup>[3]</sup>. From 166 individuals, 28 haplotypes were detected, fitting into six divergent clades. One of the six clades included 19 closely related haplotypes with moderate nucleotide differences, while the remaining five clades each included either a single haplotype or two almost identical haplotypes. Further phylogenetic analysis, together with the published Eurasian haplotypes, demonstrated that the five monotypic clades were sisters to various Eurasian lineages, whereas the 19 related haplotypes formed a monophyletic group distinct from the Eurasian clade. Given their monophyly and genetic diversity, the 19 related haplotypes were thought to originate from the Japanese native strain. Conversely, the nine haplotypes of the five monotypic clades were considered domesticated strains introduced from Eurasia, judging from their phylogenetic affinity to Eurasian lineages and unnaturally low genetic diversity.

Among the 166 individuals sequenced, almost half of the individuals in all 11 locations had Eurasian haplotypes, which indicated that the native populations were widely endangered in Japan by the introduction of domesticated Eurasian strains.

# 3. MITOCHONDRIAL DNA SURVEY OF LAKE BIWA POPULATIONS

An intensive mitochondrial DNA survey<sup>[4]</sup> was conducted to examine the populations in Lake Biwa, the largest freshwater body in Japan. Lake Biwa is an ancient lake harboring over ten endemic fish species/subspecies. The lake consists of the large deep "North Basin" and smaller shallow "South Basin". From 40 locations in the lake, 856 common carp were collected. For each specimen, the mitochondrial cytochrome b gene was genotyped (native Japanese or non-native Eurasian haplotypes) by PCR using

allele-specific primers<sup>[5]</sup>. Analysis of the haplotype frequencies showed that the native Japanese haplotype was significantly more frequent in deep (30–70 m) off-shore waters and waters along the steep northern coast of the North Basin (about 80% on average) than in shallow coastal waters in the South Basin and waters along the eastern coast of the North Basin (less than 50% on average).

These results suggested that the deep waters of the lake contain a relatively pure native population. To test this hypothesis, nuclear single nucleotide polymorphism (SNP) markers<sup>[6]</sup> were developed, and confirmed the hypothesis in a yet-to-be-published study. Other than in the deep off-shore water of Lake Biwa, no population has been found that matches the population of that habitat in the high frequency of native Japanese haplotypes.

# 4. MORPHOLOGICAL ANALYSIS USING THE GENETIC HYBRID INDEX

Morphological analyses of 183 specimens of Japanese common carp (171 from Lake Biwa and 12 from nursery ponds) were conducted<sup>[7]</sup>. To account for the effects of domesticated strains hybridization with Eurasian (introduced strains), the extent of hybridization (expressed by the hybrid index, HI, where 0 corresponds to the pure introduced strain and 1 to the pure native strain) was assessed for each specimen using nuclear SNP markers<sup>[6]</sup>. The relationship between the value of HI and various morphological traits was then evaluated using correlation or regression analysis. The traits that showed significantly positive or negative correlations with HI were considered to be those that distinguish the native and introduced strains. The results showed that the typical native Japanese carp has a more elongated body, more branched dorsal-fin rays, fewer and shorter gill rakers, a more developed pneumatic bulb, a more coiled pneumatic duct, a longer posterior swim bladder, and shorter intestine than the typical introduced strain.

To review the taxonomic status of the native Japanese strain, predicted trait values of taxonomically important meristic characters for the typical native Japanese strain were compared with the metadata values published by Baruš *et al.*<sup>[8]</sup> for the Eurasian subspecies. Baruš *et al.* classified the wild strain of the common carp into the following three subspecies based on morphology: *Cyprinus carpio carpio*, distributed in Europe and Central Asia; *C. c. haematopterus* Temminck & Schlegel 1846, found in East Asia; and *C. c. viridiviolaceus* Lacépède 1803, found in southern China and Vietnam. Of the three nominal subspecies, the typical native Japanese strain most closely resembled *C. c. viridiviolaceus*. However, the native strain differed from the southern China and Vietnam subspecies in having more lateral-line scales and slightly more branched dorsal-fin rays, which indicated that the native Japanese carp deserves at least subspecific status.

# 5. CONCLUSION

By including the published Eurasian haplotypes, molecular analysis of common carp from Japanese freshwaters first revealed the possibility that the native strain remains in Japan, although it is widely endangered by the introduction of domesticated Eurasian strains. Intensive mtDNA and nuclear SNP surveys of Lake Biwa populations demonstrated that the deep waters of the lake actually contain a relatively pure native population, which is exceptional among Japanese freshwaters. The morphological features of the typical native strain were reconstructed using the HI based on nuclear SNP markers, and a metadata comparison of the taxonomically important meristic characters with Eurasian subspecies indicated that the native Japanese strain deserves at least subspecific status. In the mtDNA phylogeny described above, however, the native Japanese strain was sister to the remaining lineages of the species. Therefore, we can conclude that the native Japanese strain surviving in Lake Biwa has world conservation priority, to preserve the genetic and taxonomic diversity of this species.

In recent publications, such as the online Catalog of Fishes<sup>[9]</sup>, the subspecies *Cyprinus carpio carpio* is elevated to species rank as *Cyprinus carpio* (*sensu stricto*),

which is restricted to the Black, Caspian, and Aral Sea basins, and the subspecies *C. c. haematopterus* and *C. c. viridiviolaceus* are listed as synonyms of *Cyprinus rubrofuscus* Lacépède 1803, found in eastern Asia. Based on these taxonomic ranks, the native Japanese strain deserves specific status.

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# Current status of and future research on biodiversity assessment and ecosystem conservation in Japanese lakes

Noriko Takamura and Shin-ichiro S. Matsuzaki

Lake Biwa Branch Office, Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Tsukuba

Keywords: Lake Kasumigaura, biodiversity, ecosystem service, long-term ecosystem research, sustainability

#### **ABSTRACT**

In Japanese lakes, about 28% of the freshwater fish species and 31% of the aquatic plant species have disappeared from historical to current (since 2000) time period <sup>[6, 7]</sup>. This decrease in the number of emerging species can be regarded as one important signal indicating the current state of biodiversity deterioration of Japanese lakes. Further, Matsuzaki & Kadoya (2015) <sup>[8]</sup> estimates of catch per unit effort (CPUE) of fishery resources revealed that the CPUEs declined in most lakes, and the functional group richness of exotic piscivores was the most important predictor of changes in the CPUE among several drivers. This suggests a substantial decline in ecosystem services. To mitigate further degradation of biodiversity and ecosystem services, and to continue to enjoy the benefits of freshwater areas and their adjacent ecosystems, we have started analyzing the relationships among multiple ecosystem services in the Lake Kasumigaura Basin. Our dataset includes biological monitoring of plankton and benthos in Lake Kasumigaura accumulated for about 40 years along with the water quality monitoring since the 1970s <sup>[10-13]</sup>. This research seeks to make use of such long-term data for evaluation and conservation of biodiversity and ecosystems, in addition to developing new monitoring methods and tools (e.g., environmental DNA).

# 1. INTRODUCTION

Vertebrate populations in freshwaters are more degraded than those on land and in the sea <sup>[1]</sup>, but the assessment of their biodiversity is limited owing to high numbers of species, their uneven distribution, and the difficulty of investigation <sup>[2]</sup>. Freshwater conservation activities might be more effective at the regional or basin scale.

In Japanese lakes, rapid eutrophication and water quality deterioration occurred during the 1960s and 1970s with increasing economic growth, along with the construction of weirs or gates and the lining of shorelines with concrete banks to develop water resources and control floods. Since the 1980s, invasive alien species have spread out and increased in abundance [3]. All of these anthropogenic factors have degraded the biodiversity of Japanese lakes. Biodiversity losses can cause declines of ecosystem services through the degradation of ecosystem functioning [4]. A high reported willingness to pay for the ecosystem services of lakes [5]

suggests that people recognize the importance of lakes. To maintain the health of lakes, therefore, we should continue pursuing their wise use, appropriate management, rehabilitation, and conservation.

In this presentation, we first show the current state and trends of lake biodiversity in Japan. Next, focusing on Lake Kasumigaura and its catchment, we describe research to identify links within the pelagic food chain using long-term biological monitoring data and to evaluate the ecosystem services of the catchment. Finally, we discuss perspectives for future studies to support lake conservation.

# 2. BIODIVERSITY ASSESSMENT OF JAPANESE LAKES

Broad-scale biodiversity assessment was advanced in the 2011–2015 research program (S9) sponsored by the Ministry of the Environment, Japan; N. Takamura led the research group for the assessment of freshwaters. We

compiled records of both strictly freshwater fish and aquatic vascular plants (floating-leaved and submerged macrophytes) in Japanese lakes (N=45 and 52, respectively), and revealed that about 28% of the freshwater fish species and 31% of the aquatic plant species have disappeared from historical to current (since 2000) time period <sup>[6, 7]</sup>. The main driver for the loss of fish was the richness of exotic piscivores. Those for the loss of aquatic plants were eutrophication and grass carp invasion. These changes in native species richness can be regarded important signals indicating the loss of biodiversity in Japanese lakes.

Matsuzaki and Kadoya <sup>[8]</sup> compiled long-term data on the annual catch, fishing effort, and fishing power of 23 Japanese lakes dating back to the 1950s, and showed using Bayesian state-space models that the catch per unit effort (CPUE) has declined in most lakes. The richness of exotic piscivores was the most important predictor of changes in the CPUE among several drivers, and it destabilized CPUE. The results clearly suggest that it would be highly desirable to prevent further invasions by exotic piscivores.

We prioritized lakes for species conservation by scoring- and complementarity-based approaches using compiled data on the fauna and flora of Japanese lakes [9].

# 3. LONG-TERM ECOLOGICAL RESEARCH IN LAKE KASUMIGAURA

Lake Kasumigaura is regarded as an important water resource for industry, agriculture, and drinking. Fisheries thrived in this lake until water resource development began in 1959, with gate construction downstream in 1959–1963 to prevent seawater inflow and the construction of concrete dikes around the whole shoreline during 1970–1995 to secure water resources. The use of the lake expanded during the 1960s, including for tap water in 1961 and for pen culture of carp in 1963. Overuse of the lake eventually caused massive cyanobacterial blooms and extensive carp mortality because of low levels of nighttime dissolved oxygen in the 1970s [3].

NIES began monthly monitoring in Lake Kasumigaura (Nishi-ura) in 1976. Our dataset covers plankton [10-13] and benthos, along with water quality. Since 1996, when the water level of the lake was raised by 30 cm, no additional strong anthropogenic alterations have occurred. However, maintaining good water quality still conflicts with pursuing high-level fisheries and agricultural production, so it is necessary to continue gathering data for wise use of this lake.

Recently, we quantified the strength and direction of the causal relationships among environmental variables, primary production, and the abundances of five functional zooplankton groups (large cladocerans, small cladocerans, rotifers, calanoids, and cyclopoids) in the lake from 1996 to 2015 by using convergent cross-mapping, a non-linear causality test [14]. Primary production was causally influenced only by NO<sub>3</sub>-N. Rotifers and cyclopoids were limited by primary production, and cyclopoids were further influenced by rotifers. In addition, the relative abundance of pond smelt—the most important commercial fish in the lake—was significantly and positively correlated with the abundance of rotifers and cyclopoids. Therefore, the between bottom-up linkages nutrients, production, and zooplankton might be key mechanisms supporting high planktivore abundance in Lake Kasumigaura.

# 4. EVALUATION OF ECOSYSTEM SERVICES IN THE LAKE KASUMIGAURA CATCHMENT

Catchment management is key to lake conservation. The NIES research team <sup>[15]</sup> (led by S.S. Matsuzaki) is evaluating ecosystem services of the catchment of Lake Kasumigaura for the period 2015 to 2019. We divided the basin into 50 subcatchments and clarified spatial synergies and tradeoffs in each subcatchment among fish species richness (an indicator of biodiversity) and 10 ecosystem services: two supporting (net primary production, habitat diversification), three provisioning (crops, rice, livestock), four regulating (water purification, climate regulation, carbon storage, flood regulation), and one cultural (spiritual and religious values). The fish fauna was evaluated by using environmental DNA.

Factor analysis showed that the two supporting and three of the four regulating services (climate regulation, carbon storage, flood regulation) showed spatial synergies, and two of the provisioning services (crops, livestock) showed a tradeoff relationship with a regulating service (water purification). Although we treated each service as equivalent, forested areas should be given priority because they have multiple ecosystem services; and countermeasures such as suppressing nutrient flows from fields into rivers would be worth investing in to alleviate the tradeoff relationship.

# 5. CONCLUSIONS AND PERSPECTIVES

Our studies reveal that biodiversity in Japanese lakes has clearly decreased since 2000, and therefore restoring it is a priority. The extirpation of exotic piscivores might be effective for conservation of native fish species. The high rate of loss of aquatic plant species should be addressed in lake restoration.

Ecosystem services vary by regions, so regional characteristics should be evaluated. Our research in the Lake Kasumigaura catchment began by visualizing the natural services of each subcatchment; this could provide a useful and easy-to-grasp tool for building consensus about methods for improving the overall services of a region.

Analyzing long-term monitoring data by using a newly developed test to distinguish causality allowed us to food-web the linkage controlling Kasumigaura. New monitoring techniques using environmental DNA, rapid species identification through DNA barcoding, and unmanned aerial vehicles have advanced. As comprehensive ecosystem monitoring is important for lake management, scientists should be involved in lake conservation, such by improving techniques for measurement, analysis, and evaluation.

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# Vertical Distribution of Phytoplankton and Their Relationship with Water Quality in Mae Kuang Reservoir, Chiang Mai, Thailand

Tatporn Khunpradit<sup>1,2\*</sup>, Pongpan Leelahakriangkri<sup>1,2</sup>, Phisanupakin Chaimongkol<sup>1,2</sup>,

Atinut joradol<sup>1,2</sup> and Rungnapa Tagun<sup>1,2</sup>

<sup>1</sup>Biology Department, Faculty of Science and Technology, Chiang Mai Rajabhat University

<sup>2</sup> Centre of Excellence of Biodiversity Research and Implementation for Community,

Chiang Mai Rajabhat Univesity

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## **ABSTRACT**

This study aims to investigate the vertical distribution of phytoplankton and their relationship with the water quality in Mae Kunag Reservoir, the large important irrigation and water supply in Chiang Mai-Lamphun Basin. The sampling was carried out from 2014-2015. Every five meters deep from surface were investigated at deepest point of the reservoir. The samples were collected in summer, rainy and cooldry seasons. One hundred and one species of phytoplankton in 37 genera and 6 divisions were found. The highest number of phytoplankton species were found in Division Chlorophyta (56 species) followed by Division Euglenophyta (17 species) Division Cyanophyta (11 species) Division Chrysophyta (6 species), Division Bacillariophyta (6 species) and Division Pyrrhophyta (5 species) respectively. The dominant species were *Cosmarium* sp, *Staurodesmus* sp., *Staurastrum limneticum*, *Pseudanabaena* sp. and *Trachelmonas acanthostoma*. The overall water quality of Mae Kuang Reservoir was classified to the level 2<sup>nd</sup> when compared to the standard surface water of Thailand. It means that the water was appropriated to using for household consumption by passing the standard treatment.

# 1. INTRODUCTION

The Mae Kuang Udomtara reservoir is situated in the Doi Saket district, Chiang Mai province, approximately 30 km North-east of Chiang Mai city. It was constructed in 1991 to irrigate 280 km² of agricultural area of three districts of Chiang Mai and Lamphun province. The reservoir also supplies drinking water to villages by the water line irrigated to the local and regional water works station in Chiang Mai. In addition, the reservoir is used for fishery and recreational activities. Therefore, the maintenance of an ecological balance and suitable water quality in the reservoir is extremely important. Continuous physical, chemical and biological monitoring of the water quality are necessary to meet required standards.

# 2. METHOD

The study site

The Mae Kuang reservoir was created in 1991 and more than 150,000 people lived in communities inundated by this reservoir. The Mae Kuang Reservoir has been short of water from many past years. Declining water yield from the watershed was reflected in low reservoir levels except the wet and flooding years in 2011

Physcio-chemical parameters studied

Secchi depth was measured with a black and white disk. pH and temperature were measured in the field using a pH meter. Conductivity of water was measured in the field by a conductivity meter (WTW Company). Chemical analyses of the water followed APHA, AWWA, and WPCF [4]. The quantity of dissolved oxygen in the individual samples was measured using the azide modification method (to avoid interference from high nitrate and iron concentrations). Alkalinity was measured in the laboratory by endpoint titration with an indicator (Methyl-orange). Nitrite nitrogen was measured by a colorimetric method using sulfanilamide, nitrate nitrogen by the cadmium reduction method, ammonium nitrogen by the phenate method, soluble reactive phosphorus and total phosphorus (after acid hydrolysis) by the ascorbic acid method and total iron by the atomic absorption method. For colorimetric detections by the spectrophotometer, "Generys 5" of Spectronic Instrument, USA was used.

# The samples collected

The samples were collected in the deepest point of Mae Kuang reservoir. At the deepest point, every 5 meter depth were collected from surface to bottom. Before sampling at each site, the sampling equipment (a net, bucket, and plastic jar) was washed to remove any organisms and other matter left from the previous site. Quantitative samples were collected by water sampler for total volume of 10 L. The 10 L of water collected was filtered slowly through a plankton net (mesh size of 10 µm) to avoid any overflow. When the water volume remaining in the net was about 100 mL, the water was transferred to a plastic jar (250 ml volume). The samples were immediately fixed in the field with Lugal's solution [5].

## Laboratories works

The phytoplankton species and details of larger species were examined on a microscope slide under a compound microscope at a magnification of  $100 - 400 \, x$ . All individuals collected were counted and identified to the species level of taxonomy.

## 3. RESULTS

The investigation of diversity and distribution of phytoplankton in Mae Kuang reservoir found that 101 species of phytoplankton in 37 genera and 6 divisions were found. The major group was Division Chlorophyta (56 species) followed by Division Euglenophyta (17 species) Division Cyanophyta (11 species) Division Chrysophyta (6 species), Division Bacillariophyta (6 species) and Division Pyrrhophyta (5 species)

respectively. The dominant species were desmid group as *Cosmarium* sp, *Staurodesmus* sp., *Staurastrum limneticum*, *Pseudanabaena* sp., and the euglenoid as *Trachlomonas acanthostoma* (Table 1). On the other hand, the evenness index investigation was revealed that not significantly difference through the sampling year (Table 2).

## 4. DISCUSSION

The study on distribution in depth distribution profiles of phytoplankton in Mae Kuang Reservoir were investigate from surface to deepest point in each sampling time. The distribution in each sampling time had a similar distribution pattern. The number of phytoplanktonn was higher on the epilimnion zone (surface and 5 meter depth.). Although, the phytoplankton could not have mobility but some of them usually had a gas vacuole in their cell and living along the epilimnion area [1]. This area also had a high light intensity and more nutrient than metalimnion and hypolimnion [2], therefore, it leads to have more distribution of growing factors.

The number of abundance indicated that, the density of phytoplankton will be decrease in deeper sampling. This was also strongly related to the diversity and evenness. Moreover, the results showed that, the distribution of phytoplankton in each sampled was significantly related to the changing of water condition in each seasons. In this work the high number of phytoplankton were found in the upper layer, epilimnion zone. However, the highest number of phytoplankton was not found in the surface but in the 5 meter deeper. The

Table 1 The dominant species of phytoplankton in different season and depth

Depth	winter	summer	Rainy
	Cosmarium pseudarctoum	Sturodesmus sp.	Sturodesmus sp.
0 meter	Sturastrum smithii	Cosmarium sp.	Staurastrum longbrachiatum
	Trachelomonas volvocina	Staurastrum longbrachiatum	Trachlomonas acanthostoma
	Pseudanabaena sp.	Staurastrum. longbrachiatum	Staurastrum longbrachiatum
5 meter	Cosmarium sp.	Staurastrum protectum	Stauodesmus curvatus
	Eudorina elegans	Tetraedron incus	Trachelomonas volvocinopsis
	Cosmarium sp.	Staurastrum longbrachiatum	Cosmarium sp.
10 meter	Pseudanabaena sp.	Staurastrum protectum	Sturodesmus sp.
	Staurastrum schmidle	Botryococcus braunii	Staurastrum paradoxum
			Cosmarium sp.
15 meter			Trachlomonas acanthostoma
			Peridinium sp.

Table 2 The diversity index, evenness index and number of species of phytoplankton in Mae Kuang Reservoir. (December 2014, April and June 2015)

Sampling time	Site	Number of species	<b>Evenness index</b>	<b>Diversity Index</b>
Dec-14	MK 1	31	0.642*	0.9325
(Winter)	MK 2	48**	0.3525	0.8603
	MK 3	35	0.4994	0.906*
Apr-15	MK 1	23	0.7211	0.6884
(Summer)	MK 2	17	0.678	0.8922
	MK 3	18	0.5944	0.8676
	MK 1	25	0.7262	0.9334**
Jun-15	MK 2	29	0.6245	0.929
(Rainy)	MK 3	21	0.7976**	0.929
	MK 4	16*	0.7483	0.8956

Remark: \*\*highest value, \*lowest value

strong sunlight and high temperature of surface was influenced factor in the water column [6]. Thus, the highest abundance of living organism was not in the surface but higher at the underneath layer. However, the photic zone was limiting factor to the primary producer activities in the standing water [7]. On the other hands, the distribution revealed that some of them were found in all conditions but many of them were specifically grown in the different sampling depth and seasons, that were directly related to their microhabitat and environment. From this relationship, we could be use a phytoplankton as a bio-monitor index to assess water quality, at lease the changing of annual water conditions [8]. Moreover, the dominant species of phytoplankton were used to find the relationship in both single parameters and overall water quality (including conductivity, alkalinity, nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus). However, the Multivariate Statistical of dominant species distribution and ecological data should study. The relation and calculation will lead to the biotic indices the including the species score. Each dominant species will have a different individual index that depends on the correlation level to the water quality [9].

# **5.CONCLUSION**

The study aims to investigate the vertical distribution of phytoplankton and their relationship with the water quality in Mae Kunag Reservoir was carried out from 2014-2015. The 101 species of phytoplankton in 37 genera and 6 divisions were found. The phytoplankton abundances found as a moderate compared to the other Thailand reservoirs and the major group was a

Chlorophyta. The water quality compared to the standard surface water of Thailand was classified to the level 2<sup>nd</sup>, it means that the water quality was appropriate to using for household consumption by passing the standard treatment.

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# Relationship between Phytoplankton Community Structure and Water Quality in the Lake Gold Coast, North Jakarta

Aliati Iswantari<sup>1</sup>, Inna Puspa Ayu<sup>1</sup>, Niken Tunjung Murti Pratiwi<sup>1</sup>, Sigid Hariyadi<sup>1</sup>, and Vina Nursyarah<sup>1</sup>

Bogor Agricultural University

Keywords: artificial lake, Lake Gold Coast, microalgae, nutrient, and water quality

## **ABSTRACT**

The input of organic materials into aquatic environment has potential to become nutrients that could be utilized by phytoplankton. Nutrient utilization by phytoplankton may affect plankton community structure in environment. This study aimed to determine the relationship between phytoplankton community structure and water quality in the Lake Gold Coast. This study was conducted from January to December 2016 in five observation sites. Physical water quality parameters (depth, transparency, turbidity, temperature, pH, salinity, and dissolved oxygen, chemical parameters (ammonia, nitrite, nitrate, and orthophosphate) and biological parameters (phytoplankton) was analyzed. Group of phytoplankton found were Bacillariophyceae, Chlorophyceae, Cyanophyceae, and Euglenophyceae. Bacillariophyceae has the highest abundance during observations. Based on water quality, there were three groups of site. This grouping was used to conduct the further analysis. PCA analysis result showed that phytoplankton abundance was in close position to orthophosphate. This was strengthened by Pearson correlation test that showed high correlation between abundance of phytoplankton with orthophosphate.

## 1. INTRODUCTION

Lake Gold Coast is an artificial ornamental lake located in Pantai Indah Kapuk, North Jakarta. Lake Gold Coast has shallow depth ranged from 0.32 to 0.45 m. Main function of this lake is as aesthetic additional value in luxurious residential area. It is also functioned as rain catchment to prevent flood and wastewater shelter of domestic activity surrounds the lake. However, this is not proper condition since wastewater contains organic matter that could decrease water quality. Based on previous research, Lake Gold Coast has already polluted by organic matter in medium level [1].

Organic matter has potency as source of nutrients that could be utilized by phytoplankton. Nutrient is one of variables controlling phytoplankton community structure and biomass<sup>[2,3]</sup>. Nutrients utilization of phytoplankton could influence plankton community structure in the lake. Based on that condition, this research was aimed to analyze the relationship between phytoplankton community structure and water quality in Lake Gold Coast.

## 2. METHOD

This research was conducted in Lake Gold Coast in twelve months (January to December 2016) with a month time interval. There were 5 sites that represent lake condition. Site 1 is close to Sewage Treatment Plant and Pump Station. Site 2 is located before Sewage Treatment

Plant. Site 3 is the deepest area of lake. Site 4 is area that receives input from Bukit Golf Mediterania interconnection. Site 5 is located in the corner of lake.

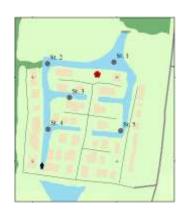


Fig. 1 Lake Gold Coast, North Jakarta

Physical and chemical parameter (depth, transparency, turbidity, temperature, pH, salinity, and dissolved oxygen) measurement was conducted *in situ*. Other chemical parameter such as BOD, COD, ammonia, nitrite, nitrate, and orthophosphate was analyzed in laboratory. Analysis was conducted based on standard method <sup>[4]</sup>. Biological parameter, phytoplankton, was sampled using plankton net with 30 μm mesh size and preserved by Lugol solution.

Plankton was observed and counted using Sedgewick Rafter Cell under compound microscope. Morphological identification was conducted based on identification book <sup>[5]</sup>. Abundance of phytoplankton was calculated using

standard method formula<sup>[4]</sup>. Data analysis was conducted using Canberra Index<sup>[6]</sup>, Diversity Index<sup>[7]</sup>, Principal Component Analysis (PCA)<sup>[8]</sup>, and Pearson correlation.

## 3. RESULTS

The highest taxa number of phytoplankton was found in January and September, and the lowest is December. The highest abundance of phytoplankton was found in June and the lowest was in December (Fig. 2).

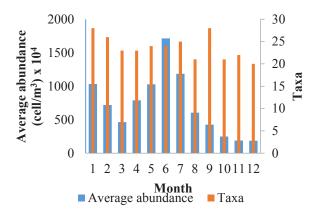


Fig. 2 Average abundance of phytoplankton

Chlorophyceae has the highest taxa proportion of and it was occured every month (Fig. 3), while the abundance proportion was fluctuated every month (Fig 4).

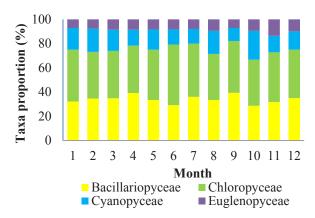


Fig. 3 Taxa proportion of phytoplankton

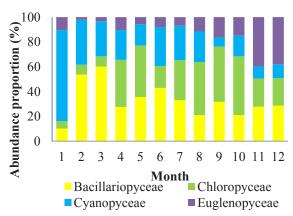


Fig. 4 Taxa proportion of phytoplankton

Based on water quality, there were three groups, Group I (Site 2 & 4), Group 2 (Site 1 & 3), and Group III (Site 5) (Fig. 5). Nutrient concentration was fluctuated during observation (Fig. 6).

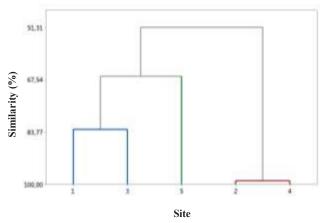


Fig. 5 Dendrogram of site grouping based on water quality

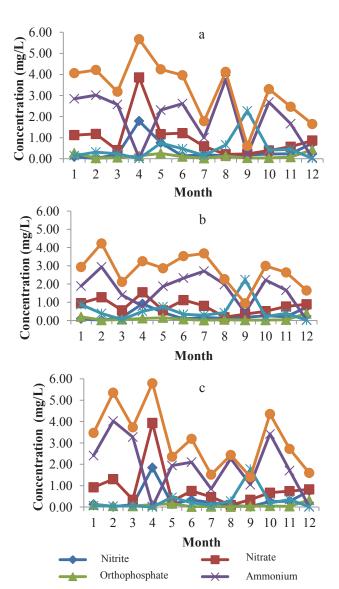


Fig. 6 Nutrient characteristic (nitrite, nitrate, orthophosphate, ammonium, free ammonia, DIN) (a) Group I (b) Group II (c) Group III

Relationship between phytoplankton abundance and water quality is illustrated in Fig. 7.

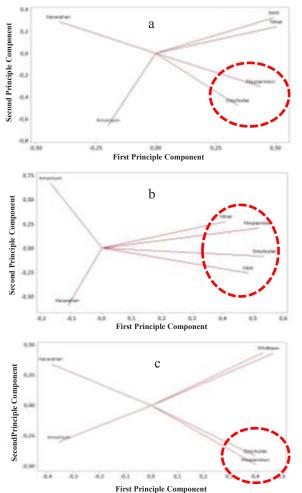


Fig. 7 Biplot phytoplankton abundance and water quality a) Group I, b) Group II, and c) Group III

## 4. **DISCUSSION**

Bacillariophyceae has the highest abundance and Chlorophyceae has the highest taxa proportion. The high abundance of Bacillariophyceae was presumed influenced by the high N:P ratio (58:1). Class Bacillariophyceae will have high abundance in N:P ratio condition = 20:1<sup>[9]</sup>.

Dendrogram in similarity level of 75% formed 3 groups based on water quality characteristics. Site 2 and 4, was formed into one group, presumably since it has the highest turbidity, Site 1 and 3 has the highest nitrite, nitrate, and orthophosphate, and site 5 has the lowest turbidity and the highest salinity.

Diversity and dominance index in 3 groups were relatively low, and similarity index was moderate. PCA biplot showed the relationship between phytoplankton and water quality. Nitrate, nitrite, and orthophosphate were close to phytoplankton. Correlation test result between phytoplankton and water quality in 3 groups showed that phytoplankton has positive correlation with

orthophosphate. This because of lower value of orthophosphate was followed by lower abundance of phytoplankton. Orthophosphate is inorganic nutrients which dissolved in water and could directly utilized by phytoplankton<sup>[10]</sup>.

Scendesmus, Oscillatoria, Microcystis, Navicula, Nitzschia, and Euglena are phytoplankton that usually found in waters polluted by organic matter. These genera were found in Lake Gold Coast. It supports the previous research that showed Lake Gold Coast has already polluted by organic matter. The main source of organic matter is domestic activity surround the lake. Therefore, management is needed to prevent water quality degradation of lake<sup>[11]</sup>.

## 5. CONCLUSION

Group of phytoplankton found in Lake Gold Coast were Bacillariophyceae, Chlorophyceae, Cyanophyceae, dan Euglenophyceae. The highest abundance was group Bacillariophyceae. The abundance of phytoplankton was influenced by orthophosphate.

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# Littoral Phytoplankton Community in Lake Lananao Along Marawi City, Philippines

Husna A. Dimapalao<sup>1</sup>, Camar P. Ameril<sup>1</sup>, Carimah M. Usman<sup>1</sup> and Norjemma Abdulgaffar<sup>1</sup>

<sup>1</sup> Mindanao State University-Marawi, Philippines

Keywords: Lake Lanao, phytoplankton community, Marawi City

# **ABSTRACT**

Being an effective biological indicator, phytoplankton diversity can determine productivity of and physicochemical status of any bodies of water. This study was performed to explore the diversity of phytoplankton in the littoral zone of Lake Lanao along Marawi city, LDS, Philippines. Freshwater samples were collected on the months of November 2016 and February 2017 following oblique towing using a planktonet with a mesh size of 23 microns. A total of 27 species, including one unidentified, representing 24 genera belonging to the divisions Bacillariophyta, Chlorophyta, Cyanophyta, Charophyta, and Dinophyta were recorded. The identified genera are as follows: *Anabaena, Ankistrodesmus, Coelosphaerum, Coscinudiscus, Cyclotella, Cymbella, Cylindrocystis, Diatoma, Dictyosphaerum, Eudorina, Golenkinia, Lyngbya, Melosira, Microcystis, Navicula, Nitzschia, Oedogonium, Pandorina, Peridinium, Rhizosolenia, Rhopalodia, Staurastrum, Synedra* and an unidentified genus. Result showed that the unidentified species was the most abundant with relative abundance of 71.58% and 64.27% and the Shannon's Index of Diversity was 0.99 and 1.06 for the November 2016 and Febraury 2017 samples, respectively.

#### 1. INTRODUCTION

Phytoplankton is an important primary producer and the base of the food chain in aquatic ecosystems<sup>[1]</sup>. It is highly sensitive to even slight fluctuations in water quality. Even slight disruptions result in disequilibrium of community structure and absence of some species from the system<sup>[2]</sup>. As a result, species composition of phytoplankton community is efficient bio-indicator for water quality. The last phytoplankton study in Lake Lanao that was published was by Lewis<sup>[3]</sup> in the year 1978. This study reassessed the current composition and diversity of phytoplankton in the chosen area. The result of the study can help assess the current ecological status of the Lake Lanao in Marawi City, Philippines, to somehow provide a point of reference for future related researches in the lake, to serve as a source of realization for the people dwelling near the lake of the importance and need to preserve the Lake.

#### 2. METHODS

This study has the research design of being purposive. Lake Lanao, the second largest lake in the Philippines, is geographically located in the province of Lanao del Sur, Central Mindanao<sup>[3]</sup>. The most populated and developed area then among all localities surrounding Lake Lanao was

Marawi City. The study was conducted along the littoral zone of Lake Lanao along Marawi City Three sub-sites were established to serve as replicates, with coordinates as follows, 07°59.714 N and 124°17.276 E, 07°59.626 N and 124°17.330 E, 07°59.614 N and 124°17.433 E.

Vertical towing was done using a planktonet with a mesh size of 23 microns. The collected water samples were strained to remove water and was replaced with equivalent amount of 5% formalin into a plastic bottle for fixation and immediately treated with Logul's iodine for staining and preservation. Each sampling container was sealed and labeled accordingly. This was done thrice for every substation.

Physico-Chemical such as temperature, Secchi disk transparency, pH level, conductivity, Dissolved Oxygen and the depth parameters were taken.

The phytoplankton samples were observed at the Department of Biology, College of Natural Sciences and Mathematics, Mindanao State University, Marawi City. An 1ml aliquot of the sample was examined drop by drop using an improvised counting chamber with a mounting area of 1.8x1.8 cm<sup>2</sup> and a depth of 0.5 mm using a Euromex I-scope microscope under 400x magnification. The phytoplankton was counted

following the right hand rule method with the aid of an advanced tally counter android application [4]. Distilled water was used to wash the improvised counting chambers before using it for the next counting to avoid retention of any plankton. The species observed were then photographed using an Iphone. In the identification of phytoplankton species, references include the works of (Bellinger & Sigee, 2010)<sup>[5]</sup>, (Botes, 2003)<sup>[6]</sup>, (Van Vuuren, Taylor, Van Ginkel, & Gerber, 2005)<sup>[7]</sup>.

The following formulas were used to compute the abundance and diversity of the Phytoplankton;

Abundance:

$$x = \frac{\text{total no. of organisms counted}}{\text{volume of sample (ml)}} \times \text{dilution volume} \times \frac{1}{AVWF}$$

Where: AVWF (Actual Volume of Water Filtered) = mouth area of plankton net × distance towed

Relative Abundance (%)

$$RA = \frac{\text{abundance of individual species}}{\text{total abundance of species present}} \times 100$$

Shannon's Index of Diversity

$$H' = -\sum (Pi \log Pi)$$

# 3. RESULTS

There were a total of 27 species of phytoplankton belonging to 24 genera, including one unidentified species. Table 1 shows the relative abundance of phytoplankton species for the two sampling periods.

The unidentified species showed a high value of relative abundance for both sampling periods. The least abundant species is the *Coscinudiscus Spp*. The calculated Shannon's Index of Diversity were 0.99 and 1.06, for the first and second sampling, respectively.

Relatively, the overall species diversity of Phytoplankton in Lake Lanao along Marawi city was quite low. The sampling sites contained also a large number of *Anabaen*a, a genus that is known to release neurotoxic substances which mean that the water is not potable. Furthermore, there is a huge amount of *Oedogonium* present in the area which may indicate algal blooms, this could explain the bluish-green look of the water in the area. Several other species that are also known to cause bloom can be observed in the area, such genera as *Peridinium* and *Staurastrum* that

accounts for a taste and odour problem for the drinking water supplies [8].

Table 1. Relative Abundance of Phytoplankton for the 1<sup>st</sup> and 2<sup>nd</sup> sampling.

SPECIES	CIES RELATIVE ABUNDANCE			
	1st Sampling	2nd Sampling		
Bacillariophyta	1	1 0		
Coscinudiscus sp.	0.01%	0.04%		
Cyclotella sp.	0.06%	0.04%		
<i>Cymbella</i> sp.	0.26%	0.08%		
Diatoma sp.	Not Observed	0.10%		
<i>Melosira</i> sp. 1	0.12%	0.02%		
Melosira sp. 2	0.51%	0.22%		
Navicula sp.	0.31%	0.05%		
Nitzschia sp. 1	0.73%	0.09%		
Nitzschia sp. 2	0.18%	2.70%		
Rhizosolenia sp.	0.09%	0.00%		
Rhopalodia sp.	0.06%	0.00%		
Synedra sp.	0.08%	2.14%		
Chlorophyta				
Ankistrodesmus sp	. 0.03%	Not observed		
Dictyosphaerium sj	p. 0.07%	0.20%		
Eudorina sp.	0.02%	0.02%		
<i>Golenkinia</i> sp.	0.15%	0.06%		
Oedogonium sp.	20.01%	26.39%		
Pandorina sp.	0.75%	0.18%		
Staurastrum gracil	le 0.17%	0.10%		
Cyanophyta	Cyanophyta			
<i>Anabaena</i> sp.	0.77%	0.42%		
Coelosphaerum sp.	0.13%	0.05%		
<i>Lyngbya</i> sp.	0.04%	0.01%		
Microcystis sp.	0.13%	0.36%		
Charophyta				
Cylindrocystis sp.	2.92%	1.04%		
Dinophyta				
Peridinium sp.	0.59%	0.13%		
Unidentified	,			
Unidentified sp.	71.58%	64.27%		

Table 2. Some Physico-Chemical Measures

Measure	Sampling 1	Sampling 2
Secchi Depth	3.45	3.18
Temp (°C)	27.10	25.10
Conductivity (µS/cm)	0.15	0.13
pH	8.88	7.49
Dissolved Oxygen (mg/l)	6.03	5.98

#### 4. DISCUSSION

Stressed environment are known to have lower number of species, with only one or two species having significantly greater individuals than the other species<sup>[9]</sup>. In this study, there was only two relatively very abundant species which is the Unidentified species and a chlorophyta *Oedogonium* species.

The Shannon's Index of Diversity of 0.99 and 1.06, for the first and second sampling, respectively, showed very little difference.

Some physico-chemical characteristics measured (Table 2). Temperature does not significantly different due to the relatively uniform temperature that prevails all throughout the year in tropical countries. Water communities in the tropics do not exhibit extreme seasonal fluctuation as it does in the land. Change due occur but with fluctuations<sup>[10]</sup>.Suspended including organic matter can make the water turbid and also traps light penetrating the surface, also the phytoplankton trap more light in their cells thereby contributing to more turbid water. Thus turbidity an increase in phytoplankton abundance<sup>[11]</sup>. Specific conductivity can be utilized as a rapid measurement of dissolved solids, conductivity value decreases diversity of the phytoplankton [12]. This seems to coincide with the pattern of the study. There was a Shannon index of 0.99 in the first sampling in which the conductivity value is 0.15 and then 1.06 with the decreased conductivity value of 0.13 in the 2<sup>nd</sup> sampling, diversity of phytoplankton increased.

# 5. CONCLUSION

In this study, there was only two relatively very abundant species which is the Unidentified species and a chlorophyta *Oedogonium* species. The total Shannon's Index of diversity of all of the observed phytoplankton is 0.97 for the littoral zone of November 2016 and 1.06 for the same zone of January 2017, the values were relatively not far

from each other, mean that the species number in the site is poor.

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# The typology and taxonomic diversity of the Abkhazia

# Republic lakes and rivers (Caucasus)

Nafisa Mingazova<sup>1</sup>, Dina Mingazova<sup>2</sup>, Roman Dbar<sup>3</sup>, Venera Ivanova<sup>1</sup>, Irshat Shigapov<sup>1</sup>, Olga Palagushkina<sup>1</sup>, Olga Derevenskaya<sup>1</sup>, Elvira Nabeeva<sup>1</sup>, Elina Kilmamatova<sup>1</sup>, Nail Nazarov<sup>1</sup>, Lyuba Pavlova<sup>1</sup>, Aigul Galiullina<sup>1</sup>, Rinat Mingaliev<sup>1</sup>, Julia Mayatina<sup>1</sup>, Julia Badretdinova<sup>1</sup>, Fairouza Valiullina<sup>1</sup>

<sup>1</sup>Kazan (Volga region) Federal University, Russia, <sup>2</sup> Melbourne Royal University of Technology, Australia, <sup>3</sup> Institute of Ecology of the Academy of Sciences, Abkhazia Republic

Keywords: lakes, rivers, Caucasus, Abkhazia, phytoplankton, zooplankton, zoobenthos, typology, biodiversity

#### **ABSTRACT**

The Republic of Abkhazia (the Western Caucasus) has a significant number of water objects - plains and mountain lakes and rivers. Many of them are associated with karst processes and caves. According to the inventory, 28 flat lakes and at least 70 lakes of mountain origin are identified. Lakes are very different on genesis, area, depth, water regime, mineralization and composition of hydrobionts. The largest lakes in the plain are lakes Inkit (40 ha), Skurcha (140 ha), Bibisiri (76 ha), Sukhumskoe (27 ha) and a lake near Ochamchira city (74 ha). The most famous mountain lakes are Lake Big Ritsa (127 ha) and Small Ritsa (10 ha) of the National Ritsinsky Park, as well as Amtkel Lake (58 ha) and Mzi Lake (15 ha). Researches of the Kazan Federal University (Russia) and the Institute of Ecology of the Academy of Sciences of Abkhazia in 2007-2017 revealed the typology and biodiversity of river and lakes, rare and endemic species. Thus the diversity of types of water bodies causes a high biodiversity of lakes and rivers of Abkhazia.

# 1. INTRODUCTION

Abkhazia is located in the northwestern part of Transcaucasia between the rivers Psou and Ingur, in the south-west it is washed by the Black Sea (Fig. 1). The coast, more than 210 km long, is not very indented, there are often wide pebble beaches. The sea spaces, subtropical vegetation, turbulent rivers and mountain peaks of the Greater Caucasus give Abkhazia exceptional picturesqueness. The fame of Abkhazia is attached to mountain ranges, numerous caves, clear rivers and mountain lakes.



Fig. 2 Map of the Republic of Abkhazia

The number of water bodies in Abkhazia is not known from the literature. Mountain rivers of Abkhazia have dozens of tributaries and hundreds of streams, the total amount is very difficult to establish.

Up to now, no more than 10 large lakes have been described and investigated with respect to the lakes (Big Ritsa, Small Ritsa, Blue, Skurcha, Inkit, etc.). Mostly these are studies from the 2000s of employees of the Kazan (Volga region) Federal University and of the Institute of Ecology of the Abkhazia Academy of Sciences<sup>[1]</sup>. Taking into account that fresh and salt lakes are found among lakes, small and large in size, with different water regime, the problem of lakes typification with the use of different classifications arises<sup>[2]</sup>.

The purpose of the research is to identify various types of lakes in Abkhazia and their biodiversity.

# 2. METHOD

More than 120 water bodies of Abkhazia (including 15 lakes, 80 rivers, as well as streams, waterfalls and cave lakes) were surveyed during the Russian-Abkhazian expeditions of 2007-2017 for the purpose of hydrological, hydrochemical, hydrobiological and ichthyological studies. Studies were conducted by traditional methods <sup>[3]</sup>. The physic-chemical and hydrological measurements, water sampling for chemical and hydrobiological analyzes and ichthyological fishing was made during field investigations. The water samples were analyzed in the Abkhaz State Center for Environmental Monitoring. Hydrobiological and ichthyological samples were analyzed in Kazan Federal University.

The Google Earth, SAS.Planet and QGis programs were used to determine the number of water bodies and hydrographic parameters.

#### 3. RESULTS

# Number and types of water bodies

The territory of Abkhazia is characterized by a well-developed river network, including a large number of watercourses belonging to the Black Sea basin. The hydrographic network of Abkhazia is represented by a large number of watercourses and lakes, which is a consequence of excessive moistening of the territory. Many rivers originate from glaciers of the Main Caucasian Range and flow along the plain to the Black Sea. Large deviations in the terrain are determined by the rapid flow of rivers and the large coefficient of tortuosity of their riverbed. According to research conducted in the 1970s, 4049 watercourses with a total length of 8,256 km were identified for 26 main rivers of Abkhazia, taking into account their river basins [4].

According to the analysis of maps, space images and field studies, we identified 61 rivers and 6 streams flowing into the Black Sea, with a total length of more than 1100 km. The typology of rivers flowing into the Black Sea is shown in Table 1.

Table 1 Typology of the Abkhazia rivers

1.	Length of the rivers, flowing into the sea, km		
	More 100	2 (River Bzyb, Kodor)	
	50-70	4 (River Kelasur, Galidzga, Okoum,	
		Mokva)	
	10-50	22	
	3-10	23	
	1-2	10	
	Less 1,0	6 (streams)	
2.	Nature of the current		
	Plain	Mountain-plain	
	29	32 (3 with an underground channel	
		- Reprua, Psyrtsha, Maanikvara)	

Many of them have a well-developed river network with dozens and hundreds of tributaries (permanent and temporary). Thus, the mountain river Bzyb, flowing from a height of 2310 m above sea level, has a length of 113.8 km, a coefficient of tortuosity of 1.39; the river has 284 tributaries. The total number of all rivers and tributaries of Abkhazia is estimated previously at 4977 objects, including temporary watercourses; the total length is probably about 10 thousand km.

Based on the results of the study of maps and space images, 28 lakes of Abkhazia located on the plain and at least 70 lakes of mountain origin have been identified. The largest lakes in the plain are Lake Inkit (40 ha), Skurcha (140 ha), Bibisiri (76 ha), Sukhumskoe (27 ha) and the lake east of Ochamchira (74 ha). The total area of lowland lakes is about 440 ha. The largest mountain lake is Lake Big Ritsa, with an area of 127 ha. Tectonic lakes predominate among the mountain lakes.

# Hydrobiological characteristics

Phytoplankton. Phytoplankton of Abkhazia water

objects is very diverse. Over the years of research there were identified 256 species of 8 departments with a predominance of diatoms. The brackish Skurcha Lake researchers found 119 species (59 diatoms), Inkit Lake - 98 (32), Big Ritsa Lake - 90 (44), Blue Lake - 40 (27) of 7-8 divisions. In rivers, the number of species is less: Kodor River - 35 species (27), Auadhara River - 31 (20) of 4 divisions; in small rivers and streams met 5-20 species. The caves found 40 species of 7 divisions.

**Zooplankton.** Zooplankton of water objects of Abkhazia is represented by 46 species (22 rotifers, 24 - crustaceans). The greatest number of species is found in lakes; rivers and lakes in caves are characterized by extremely low species abundance of zooplankton. Quantitative indicators of zooplankton are low. 27 species of zooplankton were identified in fresh lakes, in saline - 22. The greatest number of species was found in samples from the freshwater lake Mzy - 22 species, in other lakes there are 3-9 species. Among salt lakes, the most species were found in the Lake Scurcha (20).

The highest values of zooplankton abundance were recorded in freshwater lakes (up to 260 000 ex./m3 in Lake Big Ritsa) and in the salt Lake Inkit (14 000 ex./m3). In cave lakes and salt lakes, the abundance of zooplankton was extremely low. The biomass of zooplankton in most cases did not exceed 0.4 g/m3, while water objects tentatively belonged to the α-oligotrophic type. The highest biomass of zooplankton was recorded in freshwater lakes. Vertically, zooplankton is distributed unevenly. In the Lake Big Ritsa the highest density of zooplankton was observed in the epi- and metalimnion, and biomass in the epilimnion (0-4 m). In the lakes Small Ritsa and Amtkel the greatest density was in the hypolimnion. In the salt Lake Scurcha zooplankton is concentrated in the surface layers of water, which is associated with a deficit of oxygen (25-30%) in the water column and the presence of hydrogen sulphide.

**Zoobenthos.** In the species composition of the zoobenthos of the water bodies of Abkhazia 287 taxa (species) were identified, belonging to 3 types, 6 classes, 25 orders, 103 families and 187 genera. The occurrence of species in water bodies varies greatly. The greatest number of species is recorded in the littoral of lakes and rivers with a calm current. 88 species (32% of all known species) were identified in the water bodies of the Kodorosko-Skurchi system (Lake Skurcha, the Kodor River), which belong to 3 types, 6 classes, 19 orders, 48 families and 64 genera (Table 2).

Table 2 Taxonomic diversity of the zoobenthos

Water object	Types /	Orders /	Genera /
	Classes	Families	Species
River Codor	1/1	4/16	19/21
Lake Akuna	3/4	9/10	10/11
Lake Scurcha	3/6	13/29	40/55
Total	3/6	19/48	64/88

The variety of types of water bodies leads to

high biological diversity, rare species and endemic species are encountered. In the cave waters there are crustaceans Diacyclops bicuspidatus (Copepoda), endemic gammarus Niphargus alasonius, endemic freshwater shrimp Troglocaris anophthalamus (white in the absence of illumination). Crustaceans (amphipods), living in the mountain-plain rivers, provide a fodder base for ichthyofauna. In Abkhazia, there is an old fish farm for the reproduction of trout on the river Mchishta. This river has an underground channel, comes out from under the rock with a lot of suspensions, and here in mass numbers live the amphipods, which feed on fish.

Ichthyofauna. Ichthyofauna of water bodies of Abkhazia is represented by 23 species of fish for estuaries of rivers and 26 species for lakes. Most diverse the Cypriniformes order (13 species). The background species are Alburnoides bipunctatus and Gambusia affinis, often occur Alburnus charusini, Leucaspius delineatus and Phoxinus phoxinus. According to the confinement to the salinity regime in estuarine river areas and on lakes, freshwater species predominate (57% and 54%). By type of food, most species are identified as benthophags (52% in rivers and 46% in lakes). By the ratio of faunal groups, most species belong to the Ponto-Caspian freshwater complex. According to the indices of the species diversity, the highest values were obtained for the rivers Gudou, Pshap, Gumista, Bzyb, Mchishta, Maanikvara and Lake Skurcha. The most similar in species composition of ichthyocenosis are the Maanikvara, Kodor and Bzyb rivers, and lakes Akuna and Bebesiri (the coefficient of generality was 0.33).

## 4. DISCUSSION

Types of water bodies in Abkhazia are very diverse. Water objects are represented by mountain fresh-water karst and tectonic lakes; plains brackish-water lakes (lagoon); mountain and plain freshwater rivers, freshwater lakes and streams in karst caves, brackish streams. There are rivers with a double riverbed (surface and underground), disappearing in the rocks. The river Reprua is the shortest river in the world, its length on the surface is only 18 m, at the same time its main stream flows from the largest cave of the world - the cave Voron'a-Krubera about 2 km high. Many mountain lakes of Abkhazia are unique, they are the standards of clean waters of the planet. Of particular interest here are the cave lakes and their inhabitants.

A variety of types causes a variety of habitat conditions. In terms of chemical composition, the water bodies of Abkhazia are very diverse. Here, there are ultra-fresh waters with very little mineralization (Lake Bolshaya Ritsa, mountain lakes). Mountain rivers and mountain-plains predominantly belong to fresh water with a small mineralization. Plain rivers are characterized by waters of high mineralization. Plain lakes are brackish or saline.

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Ritsa, many mountain lakes). Mountain rivers and mountain-plains predominantly belong to fresh water with a small mineralization. Plain rivers are characterized by waters of high mineralization. Plain lakes are brackish or saline.

Of the great number of water bodies of Abkhazia (more than 5000), we studied 120 rivers, lakes and streams that correspond to certain types, including 40 major rivers in estuaries and the largest lakes. Based on these studies, a high biological and taxonomic diversity of rivers and lakes of Abkhazia was revealed: 256 species of microscopic algae, 46 - of zooplankton, 287 - of zoobenthos and 26 species of ichthyofauna. Many species are rare.

The rivers and lakes of Abkhazia are also experiencing anthropogenic impact, especially in cities in post-war conditions. Urban rivers are polluted by sewage. Plain small lakes and rivers are very vulnerable to anthropogenic impact, especially grazing and watering on them. To preserve the water bodies of Abkhazia, it is necessary to observe the regime of water protection zones, prohibit the grazing and watering of livestock on them, and constantly monitoring their condition. Mountain lakes of Ritsinsky National Park are actively used for recreational purposes, for them it is necessary to reduce the recreational load.

## 5. CONCLUSION

The Republic of Abkhazia is well provided with water resources, this is a strategic resource for the development of the country. The high diversity of types of water bodies in Abkhazia (the Caucasus) generates biological diversity, the presence of rare species. It is necessary to take measures to preserve the prosperous ecological state of rivers.

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# **O1-5**

# DNA Barcoding Reveal The Current Status Unevaluated Species Of *Rasbora Sp* (Cyprinidae) From Beratan Lake, Bali

Gde Raka Angga Kartika<sup>1</sup> and Pande Gde Sasmita Juliyantoro<sup>1</sup>
<sup>1</sup>Faculty Of Marine Science And Fisheries, Udayana University, Bali

Keywords: Beratan Lake, Dna Barcoding, Rasbora sp,

## **ABSTRACT**

One of the fish species that is a living resource found in Beratan lake is *Rasbora sp. Rasbora sp* contained in Beratan lake has unique characteristics that have not been much studied yet either morphometric or genetic. DNA barcoding can be used to determine the status of *Rasbora sp* contained in Beratan lake. From the results of the research, it is known that *Rasbora sp* species in Beratan lake is a complex species that cannot be clearly identified because the species is identical to the species *Rasbora lateristriata* and *Rasbora baliensis* based on barcoding DNA. The grouping of rasbora species based on their living areas also occurs due to geographic isolation such as waters, thus affecting the genetic diversity of *Rasbora sp* into low. In addition, data on conservation status, population trends and trade status of *Rasbora lateristriata* and *Rasbora baliensis* species are not available adequately. This data is important for the management of the species on *Rasbora sp* found in Beratan lake.

## 1. INTRODUCTION

One type of fish that is a biological resource in Beratan lake is *Rasbora sp*. This fish is an endemic species that can only be found in Beratan lake. This fish is alive and active in surface water and included in fish group active during the day (diurnal). Their living is by colony and never aloof, their habitat is commonly found living in clear water, where it is not too swift. These fish are often located near plants water<sup>[26]</sup>. To know the specific characteristics of this fish and its taxonomy, only done by Identification method, based on the morphological character of *Rasbora sp*<sup>[6]</sup>.

Molecular identification with the deoxyribonucleic acid (DNA) barcoding technique can be used in the identification of an organism from species to subspecies accurately to a variety of species that are difficult to distinguish morphologically<sup>[35]</sup>. This technique is widely developed to identify species, because it is relatively easy to do comparison to other techniques<sup>[38]</sup>. DNA has an important role in taxonomy to identify and define species<sup>[34]</sup>. Mitochondrial DNA markers (mt DNA) is one of getting more attention, because of their potential to study species evolution, maternal inheritance and high mutation rates<sup>[2]</sup>.COI gene of mitochondrial genome is a standard gene that is often used as a marker gene in animal identification<sup>[13]</sup>. COI gene is a COI Gene with two advantages. First, the universal primers of this gene are so solid that they are able to recognize the 5 'end of most animal groups. Second, COI gene has the highest molecular evolution compared to other mitochondrial genes, thus having low intraspecific variation, but interspecific high

divergence between adjacent taxa<sup>[15]</sup>.

A combination of phylogenetic systems with conservation genetics, provides an important framework for understanding diversity<sup>[11]</sup> and predicts the extent of fish exploitation<sup>[32]</sup>. The efficiency of this method depends on the degree of genetic divergence between species and intra-species level identification<sup>[37]</sup>. Therefore, barcode DNA is important for identifying fish species in conservation and biodiversity survey efforts, based on the diversity of sequences produced<sup>[14][24]</sup>. DNA barcoding also has an advantage on the higher accuracy of the results in identifying animal or fish species when compared with morphological observation identification technique. This has been successfully done on various types of marine and freshwater organisms<sup>[19][22][27][30]</sup>.

Currently there is a decrease in the population of *Rasbora sp* in Beratan lake. This is caused by several factors such as habitat destruction and changes in lake waters quality, overfishing, and introduction of other fish species from outside Beratan lake such as the introduction of Zebra fish<sup>[31]</sup>. Understanding the existing fish species in a region is the first step in conserving aquatic biodiversity and promoting a sustainable fishery concept. The goal of the study is to use a DNA barcode approach to identifying The Current status of *Rasbora sp* based on mitochondrial DNA (COI)

# 2. METHOD

A total of 48 *Rasbora sp* specimens were collected in the Beratan lake in june - july 2017. fish caught using gill nets and fish traps (bubu) captured in waters of Beratan lake

Observations were made on several key factors the fish's body. Based on the modification of Jordan<sup>[12]</sup> there are 15 morphometric characters observed in fish, among others: total length (TL), standart length (SL), forked length (FL), Pre dorsal length (PDL), Pre pectoral length (PPL), Pre pelvic length (PVL), pre anal length (PAL), eye diameters (ED), dorsal length (DL), Pectoral length (PL), Pelvic length (PLL), anal length (AL), caudal length (CL), Body depth (BD), head length (HL). Morphometrics characteristic compared with data from <a href="https://www.fishbase.org">www.fishbase.org</a> [7]. and analysed the similarity by PAST 3.1 software based on bray curtis index.

For barcoding analysis, 1 gram of fish muscle tissue was taken using sterile scissors to avoid the contamination. The tissue is placed on a 2 ml tube and preserved using 96% ethanol until the dna extraction process. The dna samples extraction was performed by using chelex method<sup>[36]</sup>

Samples were amplified at COI (Cythochrom Oxydase Sub Unit I) locus using PCR (Polymerase Chain Reaction) method<sup>[28]</sup> Mixed reagents used in the PCR process for each sample and the PCR process is carried out with temperature optimization. The successfully amplified product was then sent to the UC Berkeley sequencing facility for the sequence read from the sample. The sequencing method used is the Sanger method

The basic sequence of DNA sequencing results is verified by the similarity to the COI skuen present in the gene bank (http://blast.ncbi.nlm.nih.-gov) using the Basic Local Alignment Search Tool (BLAST) method<sup>[25]</sup> (NCBI). The sequencing results were then aligned using Clustal W to determine whether the homologous sequence results with others. Phylogenetic trees were made using MEGA 6 software, with neighbor-joining based on Kimura2 Parameters method and bootstrap 1000 replication<sup>[29]</sup>. Phylogenetic trees were used to see the kinship of *rasbora sp*.

A review of the conservation status and population trend of each species was determined using IUCN (International Union for Conservation of Nature and Natural Resources) [10]. The trade status of identified species was determined using CITES (Convention on International Trade in Endangered Species).

# 3. RESULTS AND DISCUSSION

# 3.1 Morphometric analysis

Measurement results of morphometric characters on *Rasbora sp* contained in the Beratan lake are made in percentage of head length and standard length compared with the morphometric character of two species of rasbora species found at www.fishbase.org that is *Rasbora baliensis* and *Rasbora lateristriata*.

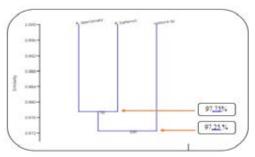


Fig 1. Dendogram Similarity Of Rasbora Sp With Rasbora Lateristriata And Rasbora baliensis Based On Morphometric Characters

The morphometric character values are then grouped based on similarity of morphometric characters using UPGMA clustering analysis in PAST 3.1 software based on Curtis bray index. From the grouping results based on the similarity of morphometric characteristics, it is found that Rasbora sp in the Beratan lake forming a group of isolated and non-clumped species to one species, both in Rasbora lateristriata species and Rasbora baliensis. From the analysis results obtained that morphometric character, Rasbora sp in Beratan lake has 97.02% similarity level to Rasbora lateristriata and 97.4% to Rasbora baliensis. For Rasbora baliensis and Rasbora *lateristriata* themselves have a similarity of 97.7% (figure 1). although clustering of Rasbora sp in Beratan lake is not clustered to Rasbora lateristriata or Rasbora baliensis, but the morfometric value of Rasbora sp in Beratan lake is relatively closer to Rasbora baliensis.

# 3.2 Barcoding Analysis

Application PCR gene COI by using JGLCO Primer and JGHCO primer successfully performed well. Phylogenic analysis and genetic distances were performed using COI gene scuens from Rasbora sp samples in Beratan lake and some sequences of data from GenBank [3][17][19]. Genetic distance analysis was performed using the neighbour joining tree method based on Kimura 2 Parameter (K2P) Analysis with bootstrapping 1000x replication. The phylogenetic tree is a method to determine the rate of evolution and kinship of a species. The phylogeny tree shows that the Rasbora sp squen in the Beratan lake forms a large clade with the Rasbora lateristriata and Rasbora baliensis scuen found on Bali Island with a bootstrap value of 56%. While the *Rasbora* lateristriata and Rasbora baliensis squen form a separate clade according to the area in which the sample was found, including clade Rasbora lateristriata from Eastern Java, clade Rasbora baliensis from eastern Java clade Rasbora lateristriata from central java and Rasbora agyrotaenia.

The mean genetic distance between *Rasbora sp* scores in the lake was associated with the *Rasbora lateristriata* and *Rasbora baliensis* scores found in the bank gene is 2.41%.

This value indicates that from the length of 650 bp squence there are 16 different nucleotides between the squences. Genetic distance is one indication of an individual with another individual having close or distant kinship relationship

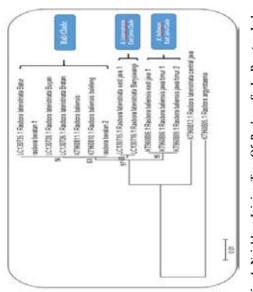


Fig. 4. Neighbor-Joining Tree Of Rasbora Sp In Beratan Lake 3ased On Kimura 2 Parameter With Bootstrap1000 Replication

. The result of the genetic distance analysis found that the closest genetic distance is known between the squamous species of *Rasbora sp* in the Beratan lake with *Rasbora baliensis* KT960811.1, *Rasbora lateristriata* Batur LC130735.1, *Rasbora lateristriata* Buyan LC130728 and *Rasbora lateristriata* Bratan LC130726.1 with a value of 0%. This is consistent statement that genetic distance is based on COI gene sequences with values more than 0.02 is classified into different species<sup>[37]</sup>.

The value of haplotype diversity (Hd) of *Rasbora sp* scores found in Beratan lake is found to be 0, this means that the value of haplotype diversity *Rasbora sp* in Beratan lake is in very low. When compared to some other freshwater fish species such as *catla catla*, *Pelteobagrus fulvidraco* and *Channa striata*, this Hd value *Rasbora sp* in Beratan lake is the lowest <sup>[4] [8] [20]</sup>. The results of this analysis indicate that the classification of a species based on genetics can occur due to the influence of isolation from each geographical state. In addition, geographic isolation in waters in a particular region will also affect the genetic diversity of the species living in it. Isolation of the geographic region will cause the species to have a smaller migration opportunity which results in a low chance of crossing this species with other species or strains outside the region<sup>[1]</sup>.

# 3.3 Status Conservation, Population Trends and Trade Status

The status of Conservation and Population trends by IUCN as well as trading status by CITES. Based on the IUCN category the species of asboras studied are categorized as vulnerable

and not evaluated. Population trends of both species of rasboras are also unknown. In addition, all the species identified in this study have not been evaluated by CITES to know its status. Lack of data contained in the IUCN and CITES will certainly have implications for fish management in Beratan lake especially for species *Rasbora sp* considering this fish spesies is one of the species that are targeted by fishermen catching fish in Beratan lake. Basic information on the genetic variations resulting from this study will be useful for devising an effective strategy for the conservation and rehabilitation of this endangered species of rasbora sp.

## 4. CONCLUSION

Morphometric analysis, phylogenetic trees and genetic distance from all samples of *Rasbora sp* in Beratan lake indicate that this species is still a very complex species to be clearly identified both morphologically and genetically whether the species can be grouped into species *Rasbora laterisetriata* or *Rasbora baliensis*.

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# Development of Specific Markers for Monitoring Distribution of Spotted Barb (*Barbodes binotatus*) using eDNA Analysis

Sekar Larashati<sup>1</sup>, Maria Holzmann<sup>2</sup> and Jan Pawlowski<sup>2</sup>

<sup>1</sup>Research Center for Limnology-LIPI, Indonesia, <sup>2</sup>Department of Genetics and Evolution, University of Geneva, Switzerland

Keywords: eDNA, spotted barb, specific primer design, fish distribution

#### **ABSTRACT**

Indonesia has numerous lakes and rivers inhabited by many native and endemic fishes. It has been reported that many of Indonesian native fishes are declining because of overexploitation, habitat degradation, and pollution. Better management and conservation of Indonesian fish resources are needed for the sustainable utilization of the fishes. However, long term monitoring using traditional methods is time consuming and laborious. Currently, the environmental DNA (eDNA) approaches have been proposed as alternative tools to detect and monitor species in aquatic ecosystems. Primer design and test is an important step when targeting a particular species. The study aimed to develop specific primers for monitoring distribution of spotted barb in of Lake Maninjau and Lake Toba using eDNA approach. In this study, the design primers were tested in silico and in vitro. Our designed primer seemed to be specific for *B. binotatus* from Lake Maninjau and Lake Toba. However, further test should be conducted to increase the specificity of the primers. Moreover, newly designed primers will be developed from another segment of mitochondrial DNA.

## 1. INTRODUCTION

Indonesia has numerous lakes and rivers inhabited by many native and endemic fishes. The total number of freshwater fishes in Indonesia is approximately 1218, including 630 endemic species <sup>1</sup>. According to published reports, some of Indonesian native fishes are threatened to become extinct, due to overfishing and other human activities such as habitat degradation and water pollution. Some population of consumed native fishes that live in the lakes in Sumatera such as Lake Toba and Maninjau and the rivers surround are declining in the wild and becoming rare in the local market <sup>2</sup>.

Management and conservation efforts which include biota and habitat management are important to achieve sustainable utilization of fish resources. Long-term monitoring is required to asses fish distribution and abundance. However, the conventional methods for such monitoring are costly and labour intensive <sup>3–5</sup>. Moreover, the conventional monitoring requires people with taxonomic knowledge that can identify fish morphology and the sampling procedure can be invasive on species or ecosystem <sup>6</sup>.

Currently, analysis of environmental DNA (eDNA) can be used to monitor fish species by detecting their DNA in water samples. Fishes discard slimes, scales, epidermal cells, and faeces, which contains DNA that remains preserved in water for a certain time <sup>7</sup>. Many studies on fish have conducted eDNA analysis in the river and lake <sup>8–10</sup>. These studies show that the eDNA has higher detection power and lower cost compared to the conventional methods and therefore it allows more effective population management program.

A barcode sequence which is specific to the species should be determined for fish identification and recognizing eDNA signal from the water sample. Primer design and test is an important step when targeting a particular species. The primers can be designed by eye or using a software. The designed primers should be tested for their specificity bioinformatically or in silico and in vitro <sup>6</sup>. The in silico test is conducted by subjecting the designed primers to a software and see if the primers bind only to the target or also to non-target <sup>6</sup>. The in vitro test is performed by testing the primers against the extracted DNA of fish tissue <sup>6</sup>.

#### 2. METHOD

Spotted barb was selected as the target fish because the fish is economically valuable for the local people in West and North Sumatra, nevertheless spotted barb population in Lake Maninjau and Lake Toba is declining. Moreover, spotted barb lives in clear water, their presence and absence may relate to environmental changes due to

anthropogenic activities. Tissue samples of Barbodes binotatus were obtained from two lakes (Lake Maninjau in West Sumatra and Lake Toba in North Sumatra) and the rivers surrounding them, and preserved in 96% ethanol. The specimens were photographed, labelled and fixated in 4% formaldehyde followed by preservation in 70% ethanol for morphological identification. The morphological characterization was conducted in the Research Center for Biology-Zoology Department LIPI. DNA extraction was conducted from the muscle tissue or fin following the procedure for purification of mammalian and rodentia (GeneJet Thermo Fisher Scientific). Total DNA concentration of different samples was measured by Thermo Scientific NanoDropTMOne. A 650 bp barcode region of Cytochrome Oxidase 1 (CO1) was amplified following 11,12 and performed using C1000 Thermal Cycler (Biorad). The primers used are Fish F1: TCAACCAACCACAAAGACATTGGCAC and Fish R1: TAGACTTCTGGGTGGCCAAAGAATCA (reverse)<sup>12,13</sup>.

The PCR products were run on 1.5% agarose gel for 20 minutes and then visualized using Gel Doc imager (Bio-Rad). The amplicons were bidirectionally sequenced in 1st BASE Pte 1td, Malaysia. The sequencing results and sequences obtained from Genbank were aligned using SeaView to develop specific primers for B. binotatus. The specific primers were tested in silico by submitting the specific primers in Primer Blast NCBI and in vitro by PCR using templates from target and nontarget species. The annealing temperature for the specific primers was adjusted using Tm calculator (ThermoFisher scientific). The non-target templates were species which are closely related and those potentially lived in the study field. The target samples from Lake Maninjau and Lake Toba were coded with SU and P, respectively. Non target samples were Barbodes gonionotus (TA1), Barbonymus schwanenfeldii (TK1), Barbonymus balleroides (LA1), Tor sp. (GA1), Osteochilus waandersii (L1-5), and Neolissochilus sp.(I1). Positive result of the specific primers was indicated by visible DNA band in a particular size.

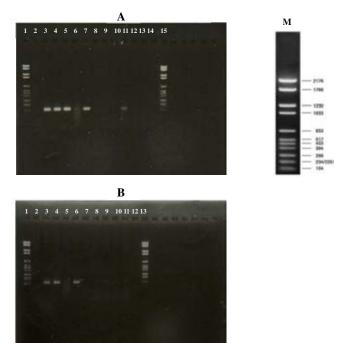
## 3. RESULTS AND DISCUSSION

The combination of designed specific primers (Table 1) amplified 147 nt COI segment of *B. binotatus*. The *in silico* test showed that the primers combination of 150F.1 and 297R may hit one species of non-target fish with some mismatches pair, two mismatches in the forward region and one mismatch in the reverse region.

Table 1 Designed specific primers

No.	Name of	Sequence (5'-3')
	primers	
1	150F.1	GCCGCTAATGATTGGGGCYC
	(forward)	
2	297R	GGGCCAGGTTACCCGCG
	(reverse)	

We continued the test to amplify the target and non-target sequences using primers 150F.1-297R with annealing temperature of 58°C. A visible band was detected in non-target fish (*Osteochilus waandersii*, well no. 11) (Fig 4A). Similar result was shown when the test was repeated with annealing temperature increased by 60°C (Figure is not shown).



**Fig 1.** Amplification of target and non target gene by primer pair 150F.1-297R. A: 1= marker, 2= negative control (PCR reagents without DNA template), 3= SU1, 4= SU2, 5= SU4, 6= P7, 7= P10, 8= TA1, 9= TK1, 10= LA1, 11= GA1, 12= L1, 13= I1, 14= Negative control, 15= marker. B: 1= Marker, 2= negative control, 3=SU1, 4= P10, 5= P8, 6= P14, 7= L1, 8= L2, 9= L3, 10= L4, 11= L5,12= negative control, 13= marker.

There should be no visible band detected in *O. waandersii* template because *O. waandersii* and *B. binotatus* are not closely related. A contamination might happen during sample preparation before amplification in the PCR machine. Therefore, we repeated the test and included all individual samples of *O. waandersii*. The

result showed visible bands in the target templates and none in samples L1 (the one used previously in Fig 4A) and L2. However, three samples of *O. waandersii* (L3-L5) exhibited very faint bands. It was posible that the faint bands are artifacts from the primer dimers.

Target templates of P7 (Fig 4A) and P8 (Fig 4B) did not show any detected DNA bands. When analyzed using phylogenetic tree, P7 and P8 were not in the same group with other target samples (figure will be shown during the conference). That could explain why the two samples were not amplified. Further analysis should be conducted for the two samples whether there was a contamination during the pre PCR step or misidentification in the morphology.

## 4. CONCLUSION

Our designed primer seemed to be specific for *B. binotatus* from Lake Maninjau and Lake Toba. However, further test should be conducted to increase the specificity of the primers, such as optimizing the annealing temperature and to include the positive control which is samples of *B. binotatus* DNA template from Java. Furthermore, newly designed primers will be developed from another segment of mitochondrial gene such as cytochrome b.

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# Predicting climate change impact on zooplankton community structure based on thermally polluted lakes

Marcin Dziuba<sup>1</sup>, Lukasz Wejnerowski<sup>1</sup>, Witold Szczuciński<sup>1</sup> and Slawek Cerbin<sup>1</sup>

Adam Mickiewicz University, Poznan, Poland

Keywords: biodiversity, COI, Daphnia, global warming, heated lakes

## **ABSTRACT**

Thermal pollution of freshwater basins is an increasing problem related to power plants activity. Change in thermal regime of lakes can have far-reaching negative consequences as a source of environmental disturbance, posing a risk of local extinctions and supporting establishment of invasive species. On the other hand, heated lakes and reservoirs can be used as a large scale experiment for testing global warming related predictions. In order to address the question, how increased temperature will impact genetic diversity and structure of local community, we used a system of five lakes heated by lignite combusting power plant in Poland. Due to warm water discharge these lakes are ca. 3-4°C warmer than non-heated control lakes nearby. We checked how five decades of temperature elevation affected genetic diversity of *Daphnia* community, and compared genetic structure of *Daphnia* community inhabiting heated lakes with control lakes community. Furthermore, we genotyped resting eggs extracted from sediments, produced by *Daphnia* in heated lake before warm water discharge and afterwards, to check how the community changed through the time after temperature elevation. Collected data provide an evidence for significant impact of thermal regime on structure and genetic diversity of local *Daphnia* community. Adaptation to climate warming will likely induce such changes in lake communities worldwide, as they might be inevitable for community functioning in new environmental conditions.

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# 1. INTRODUCTION

Currently, one of the most concerning issues in ecology and environmental protection is global warming. According to Intergovernmental Panel on Climate Change the average Earth's temperature is now ca. 0.8°C higher comparing to the beginning of XX century, and it is expected to be even 3-4°C warmer<sup>1</sup>. Such changes in environmental conditions are expected to cause drastic alterations in biocenoses all over the world, including shifts in phenology, migrations, biological invasions and extinctions<sup>2-4</sup>. However, current approaches in studying responses of organisms to future climate change are limited, as they either rely on investigation of habitats in different

climate zones, historical records covering only about 0.5°C difference, or experimental surveys of one up to several species with relatively short-term temperature elevation. Therefore, the question of how species will respond to global warming remains open.

Here, we use lakes heated by power plant and warmed up in significant rate as a simulation of climate change scenario. We investigated genetic structure of *Daphnia* community in heated lakes and compared it to the control (non-heated) lakes population, to check whether increase in temperature triggers any significant change in *Daphnia* community structure and biodiversity of heated lakes.

## 2. METHODS

Five heated (Goslawskie, Patnowskie, Lichenskie, Mikorzynskie and Slesinskie) and four control lakes (Goplo, Skulskie, Skulska Wies, Budzislawskie) were investigated for 3 years (2014-2016) in monthly intervals. Daphnia samples were collected and physical-chemical parameters (temperature, oxygen concentration, TDS, conductivity, pH, secchi disc visibility) were monitored at the same time. Daphnia were collected with plankton net (100µm) in lakes deepest part, and preserved in ethanol 96%. DNA of some of the collected Daphnia was isolated using DNeasy Blood & Tissue Kit (Qiagen) and daphnids from heated and control lakes were genotyped on Cytochrome C oxidase subunit I (COI) barcode. Obtained sequences were analyzed by constructing phylogenetic tree using Neighbor-Joining method. For reference, we used several sequences of COI of Daphnia galeata (G.O. Sars, 1864), D. longispina (O.F. Müller, 1776) and D. cucullata (G.O. Sars, 1862) published in NCBI GenBank. Furthermore, we obtained sediments from heated Mikorzynskie lake, conducted the dating with Cs137 isotope and isolated resting eggs of Daphnia from layers representing past Daphnia community in the lake. We counted and measured ephippia (structure covering resting eggs) and genotyped resting eggs using 16 microsatellite markers. We further analyzed genetic data with DAPC analysis.-

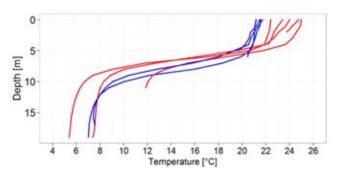


Fig. 1 Temperature of heated (red lines) and control (blue lines) lakes measured every meter from the surface to 20m depth. Lines represent summer average for 3 years when measurements were conducted.

## 3. RESULTS

The temperature measurements revealed strong difference in thermal conditions between heated and control lakes, especially in epilimnion. Heated lakes are 1-4°C warmer either in entire water column or in epilimnion zone, depending on whether they are stratified or not (Fig. 1). Such rate of temperature increase corresponds to what is

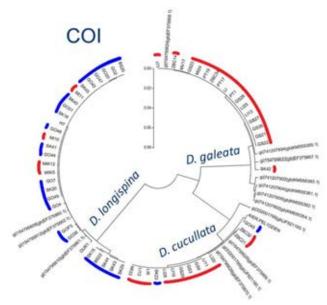


Fig. 2 Phylogenetic tree of COI sequences, constructed with Neighbor-Joining method. Red lines indicate clones from heated lakes, blue lines indicate clones from control lakes. Species name indicated on each branch is based on reference genotypes from NCBI GenBank.

expected in next 100 years by IPCC<sup>1</sup>.

Phylogenetic tree showed significant difference in community structure of zooplankton. In heated lakes we detected mostly *Daphnia galeata and D. cucullata*, whereas in control lakes there was mainly *D. longispina* (Fig. 2).

Fossil data also showed significant alteration in heated lake after warm water discharge.

# 4. DISCUSSION

Clear difference in epilimnion temperature corresponds with what is expected as a result of climate change<sup>2</sup>. Therefore, we assume the system of heated lakes is a good model for investigation of climate change in freshwater ecosystems. The rate of temperature difference is similar to what scientists use in artificial experiments <sup>5-7</sup>, but the size of the experiment in case of heated lakes is spanned onto entire ecosystem. Moreover, the duration of temperature increase is way longer than what any experiment reached so far, and selection conditions for organisms are more natural.

Genetic analyses of *Daphnia* community indicate that temperature increase triggered selection on *Daphnia* and caused shifts in species composition. It is known, that *D. galeata* is more pronounced on the south of the Alps, whereas *D. longispina* (former *D. hyalina*) occurs more frequently in northern Europe<sup>8</sup>.

Daphnia from heated lakes are also significantly larger and cope way better in presence of filamentous

cyanobacteria<sup>9</sup>, which indicates, that thermal selection and change in community composition of Daphnia in heated lakes was adaptive. It has former been confirmed, that long-term exposition to elevated temperature triggers selection and adaptation to elevated temperature<sup>6,7,10</sup>. It is also known, that such adaptation requires former change structure<sup>5,11,12</sup>. population/community genetic Moreover, it has been observed that due to change in thermal regime local communities might undergo significant change in species structure<sup>13</sup>. Therefore we claim that European lakes undergoing global warming might become dominated by Daphnia galeata, which takes advantage over D. longispina in warmer environment.

#### 5. CONCLUSION

Our study confirms significant impact of temperature increase on structure of freshwater communities. We conclude, that further temperature elevation will cause shifts in European *Daphnia* communities towards domination of *D. galeata* and small *D. cucullata*, whereas occurrence *D. longispina* might be limited. Alterations in community structure due to change in temperature regime are also confirmed by fossil data.

Climate change is a phenomenon that currently acquires a lot of attention. Ecological hazard that temperature increase is expected to pose is investigated with various approaches, but all of them have some important limitations. Here we show that aquatic ecosystems altered by power plants might be useful climate-change simulation in entire ecosystem. The temperature increase in investigated heated lakes corresponds to climate change forecasts and the character of response of *Daphnia* community to higher temperature looks reliable in comparison to former experimental studies.

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# **O1-8**

# Zooplankton abundance and biomass size spectra in shallow urban lakes: Analysis using laser optical plankton counter

Reliana Lumban Toruan<sup>1,2</sup>, Anas Ghadouani<sup>1</sup> and Elke Reichwaldt<sup>3</sup>

<sup>1</sup>University of Western Australia, <sup>2</sup>Research Centre for Limnology, Indonesian Institute of Sciences, <sup>3</sup>Department of Water and Environmental Regulation, Western Australia

Keywords: Zooplankton size spectra, urban lakes, Laser Optical Plankton Counter, NBSS

#### **ABSTRACT**

A laser optical plankton counter was used to examine zooplankton size structure in seven urban lakes across the Swan Coastal Plain (SCP), Western Australia. Based on the zooplankton abundance and biomass of equivalent spherical diameter ESD in 32 bins for every 0.1 mm between 0.3 and 2.5 mm, zooplankton normalised biomass size spectra (nbss) were constructed to display community structure. Nbss parameters slope and intercepts were used to classify the zooplankton community size spectra. Zooplankton size distribution varies across the lakes. Based the LOPC measurement, small-sized zooplankton (<1.0 mm ESD) was dominant in most lakes which contributed to >50% of the total abundance. However, the contribution of large-sized zooplankton (1 – 2.5 mm ESD) toward total biomass was significant in Lake Yangebup, Little Rush, Bibra and Herdsman Lake. Small-sized zooplankton (0.3-1.0 mm ESD) biomass, on the other hand, was evident in Lake Monger, Joondalup and Yonderup. A Bray-Curtis Analysis using the nbss parameters classified the zooplankton communities into three groups. A group with a lower slope was observed only for Lake Yonderup (slope= -0.471) which indicates the dominance of small-sized zooplankton. Lower slope was caused by the dominant of small copepod (0.5-1.0 mm ESD) which contribute to >80% of the total biomass. Group 2 and group 3 have a steeper slope closer to the theoretical value of -1 which indicate a significant contribution of large-sized zooplankton toward total biomass. >50% of the total biomass was constructed by larger-sized zooplankton (1.0-2.5mm ESD) for this group.

# 1. INTRODUCTION

Zooplankton are key ecological indicator in aquatic ecosystems because of their quick response to environmental changes such as land-use, water quality, nutrient concentration `[1], and hydrological regimes [2, 3]. They are also key elements in the aquatic food webs and trophic structure as they respond to phytoplankton community and fish predation [4]. Planktonic communities may include a variety of taxonomic and functional group. Zooplankton abundance and biomass, for example, often scale with body size, with smaller-sized generally more abundant than larger size and biomass generally increased with size ([5].

A shift in the size structure of zooplankton community may indicate the changes in the functioning of the lake. As such, the size structure of zooplankton community can be used to display community structure and trophic interaction as a response to both bottom-up and top-down factors [6]. Normalised biomass size spectra (nbss) is a measure of total abundance or biomass in logarithmically equal of each size classes. Nbss approach has been widely used to describe planktonic community structure in marine and lakes ecosystem while the nbss parameters (slope and intercepts of the nbss) are used to classify zooplankton community and their response to environmental variables [7, 8].

Studies on zooplankton size structure in urban lakes are rare [9]. In particular, little attention has been paid to the zooplankton community structure in lakes within the Swan Coastal Plains (SCP) areas in Western Australia, although the ecological importance of the habitats is recognised [10]. The SCP is an important bioregion in Western Australia

situated at the foot of the Darling Ranges facing the Indian Ocean in the South-West of Western Australia with two main waterways, the Swan and the Canning Rivers. The wetlands of the SCP support a diverse range of terrestrial and aquatic biota. However, growing urban, agricultural and industrial development over the past century has significantly reduced the number of wetlands that once existed on the SCP. Less than 25% of the remaining wetland continues to face direct threats due to urbanisation and indirect threats through land clearing and nutrient enrichment and pollutant contamination.

This study aimed to examine the spatial distribution of the zooplankton size spectra (abundance and biomass) in seven urban lakes across the Swan Coastal Plain. A laser optical plankton counter was used to extract zooplankton abundance and biomass data and size spectra information. Normalised biomass size spectra approach was used to display zooplankton community structure, and nbss parameters (slope and intercept) were used to classify the zooplankton size spectra.

# 2. METHOD

Zooplankton samples were collected from six lakes across the Swan Coastal Plain, Western Australia by horizontal tow using a 56 um mesh net with a mouth of 25 cm diameter from a 3-meter distance at a constant speed of  $\sim 0.5 \, \text{m.s}^{-1}$ . Size composition was classified into five size fractions,  $0.3-0.5 \, \text{mm}$ ,  $0.5-0.75 \, \text{mm}$ ,  $0.75-1.0 \, \text{mm}$ ,  $1.0-1.5 \, \text{mm}$ , and  $1.5-2.5 \, \text{mm}$  Equivalent Spherical Diameter (ESD). Although the LOPC measures zooplankton from  $0.1 \, \text{to } 3.5 \, \text{mm}$  ESD, we analysed abundance and biomass only

for zooplankton size range between 0.3 and 2.5 mm ESD. We used these size range as we found that air bubbles in the lab circulator LOPC prevented accurate counts for particle size >0.3mm and extremely low density for zooplankton larger than 0.25mm ESD. The zooplankton within size range 0.3 – 2.5 mm includes small copepod and larger cladocerans, but may not include microzooplankton, such as copepod nauplii and rotifers, and larger-sized zooplankton such as *Mysis* and *Leptodora* [11].

The size spectrum of zooplankton display a measure of total abundance or biomass in the logarithmically equal interval of body size [12]. The normalised biomass size spectrum (nbss) plots the normalised biomass of the zooplankton community against the log-biomass of the zooplankton in each size classes. The slopes and intercepts of the nbss regression lines are then compared between lakes using one-way ANOVA test for multiple comparisons followed by applying Fisher's LSD post hoc test to identify differences within each pair of slopes. The nbss was organised for zooplankton within the range of 0.3-2.5 mm ESD.

# 3. RESULT

The <0.5 mm size fraction was composed of small cladocerans such as juvenile cladocerans and ceriodaphnia, and copepodite of both calanoid and cyclopoid groups. The >0.5mm was mainly composed of large cladocerans and adult calanoid. Large cladocerans were represented by *Daphnia carinata* and *Daphnia lumholtzhi*. The small-size fractions (0.15–0.3 mm ESD) were mainly composed of rotifers and small copepodid and nauplii and were excluded from the analysis due to coincident count by the LOPC.

Zooplankton size distribution shows similarity for all sampling site where the abundance peak was observed for the 0.3–0.5 mm size classes. The abundance of the 0.3–0.5 mm size fraction represented, on average, 40–80% of the total zooplankton abundance Larger-sized zooplankton (>1.0mm ESD fraction) represented, on average, 1–12% of the total zooplankton abundance.

Zooplankton biomass ranged from 2.26 mg.L<sup>-1</sup> (Lake Joondalup) to 16.20 mg.L<sup>-1</sup> (Lake Yangebup). Larger-sized zooplankton (size 0.10–0.25mm ESD) represented a significant contribution toward total biomass in Lake Yangebup, Little Rush, Bibra and Herdsman. Size 0.10–0.25mm ESD accounted for > 50% of the total biomass in these lakes (Fig.1). The figure was slightly different for Lake Monger, Joondalup and Yonderup where larger-sized contributed less than 25% to the total biomass.

The normalised biomass size spectra (nbss) parameters (slope and intercept) showed significant linearity in all sampling sites (One-way ANOVA, p<0.05, r2 = 0.63 to 0.84) except for Lake Yonderup which indicates an increased biomass with size. The nbss for lake Yonderup showed weak linearity ( $r^2 = 0.30$ ) with higher intercept and lower slope which corresponded to a lower proportion of large-sized zooplankton towards biomass. The slope value in all sampling sites combined ranged from -0.47 to -1.02 with the intercept ranged from 1.6 to 3.22.

Cluster analysis based on nbss parameter classified, the lakes into three groups (Figure 2). Group 1 represents Lake Yonderup; group 2 represents Lake Joondalup, Bibra,

Monger and Lake Little Rush; group 3 represents Lake Yangebup and Lake Herdsman. Group 1 represented lake within low urban density area. Lake Yonderup situated in the national park with relatively low urban density. Group 2 and 3, on the other hand, are situated within the highly urbanised area, thus higher urban stressor (e.g. nutrient loading).

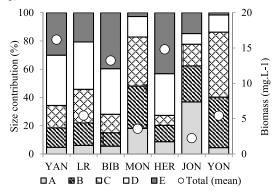


Fig. 1 Mean total biomass and the relative contribution of each size classes toward total biomass in all sampling sites (A=0.3–0.5mm ESD; B=0.5–0.75mm; C=0.75–1.0mm; D=1.0–1.5mm; E=1.5–2.5mm ESD)

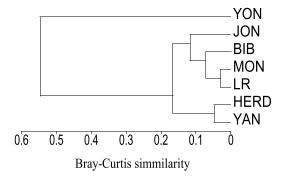


Fig. 2 Bray-Curtis cluster analysis by nbss slopes and intercepts

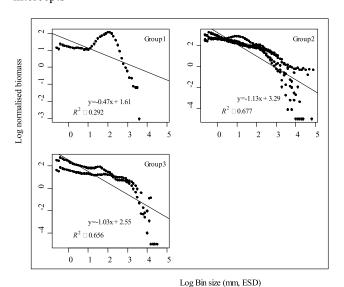


Fig. 3 Normalised biomass size spectra in different lakes. Group 1, Yonderup Lake; Group 2, Lake Joondalup, Bibra, Monger and Little Rush; Group 3, Herdsman and Yangebup.

#### 4. DISCUSSION

Zooplankton nbss variation can be used to display zooplankton community structure in urban lakes. A relatively flat slope (more negative) reflect the higher contribution of larger-sized organisms toward biomass and the increased biomass with size. The intercept of the nbss reflects the abundance of organisms and the ecosystem productivity. Higher intercept correspondence with higher organisms abundance and productivity [13]. Zooplankton size spectra in the SCP lakes and wetlands showed distinct spatial patterns. Group 1 was observed only in a lake in the area of the north of Perth Metropolitan areas located in the national park and characterised by low productivity and relatively low nutrient input. Group 2 and 3 are urban lakes situated in highly urbanised areas characterised by high nutrient content.

The slope of the nbss for group 2 and 3 (-1.03 and -1.13) were within the similar result with the previous study on zooplankton size spectra analysis in a freshwater ecosystems. Sprules and Munawar [14] found the slope of -0.90 to -1.03 for inland water while Gaedke [15] revealed nbss slope of -0.94 to -1.16 for Lake Constance.

Higher intercept value in group 2 and 3 indicating higher zooplankton abundance and productivity there. This was corresponded with a higher values of Chlorophyll a (35.56 to 53 mg.L<sup>-1</sup> during summer). The high proportion of the small-size fraction and its contribution toward total biomass in Lake Yonderup indicate that the zooplankton community in this lakes is characterised by small-sized zooplankton.

Zooplankton BSS variation in different lakes indicates that the different bottom-up and top-down factors could regulate the zooplankton differently. Eutrophic water could support high phytoplankton production and may interfere with zooplankton filter feeding ability which coincides with the absence of large filter feeding zooplankton such as Daphnia [16]. High concentration of surface Chlo-a in Lake Monger, Yangebup, Little Rush, and Lake Bibra were observed in field investigation and by the study of Sinang, et al. [17]. High nutrient input can support high phytoplankton production and abundance and even induce phytoplankton bloom and affect the size structure of zooplankton.

## 5. CONCLUSION

From this analysis, we can display zooplankton community structure based on the composition of each size classes and the corresponding biomass. Through the LOPC analysis, the zooplankton size spectra in urban lakes in The Swan Coastal Plain were separated into 3 groups. One group has a lower slope (-0.47) which caused by the dominant of small-sized contribution towards total biomass. Two groups have a steeper slope (-1.03 and -1.13) which were close to the theoretical value (-1) caused by the dominance of large-sized zooplankton contribution towards total biomass.

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# Zooplankton in Assessing of the Water Quality in Urban Areas

Olga Derevenskaya, Nafisa Mingazova Kazan Federal University, Russia

Keywords: zooplankton, urban territories, water ecosystem, water quality

# **ABSTRACT**

The results of studying the zooplankton communities of various water bodies located in the urbanized areas of the Middle Volga Region (Russia) are presented. The aim of the research was to assess biodiversity, characterize the structure of zooplankton communities and identify the factors that have the greatest impact on zooplankton. The studied reservoirs were divided into 4 groups: lakes located in floodplains of rivers and urban forest parks; small shallow-water lakes in residential micro-districts; ponds located on rivers flowing through the city and careers on rivers; small shallow-water lakes where rehabilitation measures were carried out. As a result of the studies, 180 species of zooplankton were identified. The greatest number of species was in lakes located in parks and in residential areas. It has been established that in urbanized areas, pollution and eutrophication processes have the greatest negative impact on zooplankton communities. Negative correlations of quantitative indices of taxonomic groups of zooplankton with water content of copper, iron, manganese, lead, zinc, permanganate oxidation were revealed.

## 1. INTRODUCTION

The ecological consequences of economic anthropogenic activity are most pronounced in urbanized areas. Anthropogenic impact on water objects of urban areas is very diverse. Small lakes and ponds are a special type of aquatic ecosystem, differ in many respects from large systems such as reservoirs and large lakes. As habitats of hydrobionts, they are more susceptible to weather and various anthropogenic factors, including those related to the nature of land use. A distinctive feature of lakes located in urbanized areas is their involvement in the urban environment. Due to the peculiarities of the hydrological characteristics, such as low depth, absence of stratification, an unpredictable regime of mixing and rapid response to changes in environmental conditions are typical for small lakes<sup>[1]</sup>. However, they play a vital role in preserving the biodiversity of aquatic organisms within highly transformed urban areas. They often maintain a higher biodiversity of aquatic invertebrates than, for example, in rivers<sup>[2, 3]</sup>. In this sense, the importance of small lakes in urban areas has not been adequately studied, since they can have high local species richness, contain rare and endemic species not found in other lakes and rivers, and also contribute to regional differences, due to their high variability over time and space<sup>[4]</sup>. These features of urban lakes are important to know when developing a strategy for managing water resources in cities. In addition, such studies are necessary to understand the impact of anthropogenic impact of various species on aquatic ecosystems in general and on the state of aquatic communities in particular. Small shallow lakes can act as models for studying the main anthropogenic stressors on communities of aquatic organisms and, in particular, on zooplankton<sup>[4]</sup>.

The aim of the research is to assess biodiversity, characterize the structure of zooplankton communities, identify the factors that have the greatest impact on zooplankton of small lakes in urban areas.

## 2. METHOD

Studies were carried out on 61 different water bodies located in the cities: Kazan, Ulyanovsk, Bugulma, Almetyevsk (Russia).

All the studied water bodies we divided into 4 groups.

- 1) Lakes located in the forest park zone of Kazan, in the floodplains of the Volga, Kazanka and Sviyaga rivers (11 lakes). Lakes are little transformed in comparison with other water bodies studied, their characteristics are close to natural ones.
- 2) Small shallow-water lakes in Kazan, located in residential micro-districts with different population density. The catchment area of these lakes is heavily transformed, usually asphalted and built up. Most lakes are man-made derivatives. They were formed as a result of unloading of high-lying groundwater and accumulation of surface runoff in pits or in depressions in the territories of former wetland complexes (40 lakes).
- 3) Ponds located on the rivers flowing through the cities of Bugulma and Almetyevsk, as well as quarries formed after the extraction of sand in the channel of the river.

Sviyaga in the city of Ulyanovsk (8 reservoirs).

4) Small shallow-water lakes, where rehabilitation measures were carried out (2 lakes). As a consequence, in one of the lakes the hydrological regime and the type of water changed (Lake Lebyazhie, Kazan), and the other was artificially restored at the site of the reservoir previously covered for building (Lake Chishmale, Kazan). Zooplankton of lakes was studied during the period from 1992 to 2016. The frequency of sampling at water bodies was different. On 2-3 lakes from each selected group (except for ponds) studies were carried out during 1-3 vegetative periods. The remaining water bodies were surveyed 1-3 times in June-August. Most of the small lakes in Kazan were studied in the course of work on the inventory of water bodies carried out in 2007 [5].

In shallow-water lakes, samples were taken by straining 50 liters of water through the Apshtein network (mesh size - 100 microns). From ponds and quarries, samples were taken in the central, deepest part by pulling the Jedi net from the bottom to the surface. Cameral processing included the determination of species composition of zooplankton, calculation of abundance and biomass in accordance with generally accepted hydrobiological methods<sup>[6]</sup>. In total, more than 280 quantitative samples of zooplankton were selected and processed during the study period.

# 3. RESULTS

Morphometric parameters of the studied reservoirs vary. The surface area of the lakes was from 0.07 to 80 ha, the average depth from 0.12 to 4 m, the maximum depth from 0.6 to 10.1 m (Table 1). The smallest in terms of area and depth of water bodies were groups of lakes in residential areas and restored lakes. The average depths of water bodies in these groups are less than 1 m, and the maximum depths are less than 2.5 m, the area - up to 2 hectares.

The physical and chemical characteristics of the water of the studied water bodies varied in a rather wide range. Transparency of water in most cases was low and in terms of the magnitude of the trophic state index (ITS) the water bodies are eutrophic. PH values characterize the environment in the lakes as neutral. The oxygen content in the surface layers of water usually exceeded 100%, but in the near-bottom, oxygen deficiency was often observed, even in lakes with shallow depths. A fairly high content of ammonium ions was noted in water bodies. High content of nitrites (on the average  $0.63 \pm 0.48$  mg / l) and phosphates  $(0.58 \pm 0.05$  mg / l) was noted in ponds.

180 species of zooplankton have been identified in the

study of water bodies of urban areas, of them Rotifera - 83 (46%), Cladocera - 54 (30%), Copepoda - 43 (24%). In general, the small lakes of urbanized areas are characterized by rather high species richness.

Most often in the lakes there were *Chydorus sphaericus* (O.F.Muller, 1785) (82% of lakes), *Keratella quadrata* (Muller, 1786) (59%), *Mesocyclops leuckarti* (Claus, 1857) (57%), *Euchlanis dilatata* Ehrenberg, 1832 (44%), *Bosmina (B.) longirostris* (O.F.Muller, 1785) (44%), *Lecane (s.str.) luna* (Muller, 1776), *Simocephalus vetulus* (O.F.Muller, 1776) and *Eucyclops serrulatus* (Fischer, 1851) (in 39%).

If we consider the species richness of zooplankton separately for the selected types of water bodies, then the largest number of species (132), as expected, was found in the little transformed lakes located in the parks. In the samples from these water bodies, the greatest number of species (15.6  $\pm$  1.1) was encountered. Fewer species (118) were found in anthropogenically derived lakes located in residential areas, but samples from these lakes were poor, compared to other groups of water bodies, an average of 9.1  $\pm$  0.6 species.

The quantitative indicators of zooplankton in small water bodies of urbanized areas were relatively low. The largest average summer abundance (285.26  $\pm$  98.71 thousand ind / m³) were in lakes located in parks. The highest values of biomass (1.47  $\pm$  0.31 g / m³) were in lakes located in residential areas. The lowest values of quantitative indicators were in the restored lakes and in ponds.

Rotifers prevailed in ponds in abundance, in other water bodies - copepods. According to biomass rotifers prevailed in ponds on rivers, Cladocera - in lakes located in parks and dwellings in massifs and Copepoda - in restored lakes<sup>[7]</sup>.

Rotifers prevailed in ponds in numbers, in other water bodies - copepods.

# 4. DISCUSSION

Biodiversity is one of the most important indicators characterizing the sustainability of ecosystems. Reducing the species richness of plants and animals is observed with pollution, which is especially characteristic of aquatic ecosystems located in the territories of large cities. Our research has shown that the water bodies of urbanized areas, despite the low quality of water in them, are characterized by a rather high species richness (180 species). The variety of types of water bodies and biotopes within them supports the diversity of zooplankton. Shallow-water lakes, as a rule, overgrow with macrophytes, creating additional shelters for zooplankton. Thus, a high diversity of zooplankton

species is maintained within a small area.

The greatest species of wealth was the lake of natural origin, located in parks and floodplains of rivers, as well as small lakes in residential areas. In the latter, the number of species in each individual water body could be low, but the composition of species is different. There are no species found in all water bodies and only three species - *C.sphaericus*, *K. quadrata* and *M. leuckarti* are found in more than 50% of the investigated lakes.

Studies have revealed low quantitative indicators of zooplankton, which, in our opinion, is a consequence of the impact on zooplankton of pollutants and other unfavorable factors. This also explains the high proportion of cyclops in the juvenile stages.

The indicators characterizing the structure of the zooplankton communities differ for lakes of different types. The average individual mass of the zooplankter (w) was small, in the restored lakes it corresponded to the polytrophic, in the other reservoirs it was highly trophic. The ratio of the number of eutrophic species to oligotrophic (E / O) characterizes the studied water bodies as eutrophic<sup>[8]</sup>. The same species are resistant to the effects of adverse environmental factors and are most often found also in contaminated lakes.

The calculation of the Pearson correlation coefficients revealed significant (p <0.05) negative dependences of the quantitative indicators of zooplankton-the total abundance, abundance and biomass of many Rotifera and some Cladocera and Copepoda with copper, iron, manganese, lead, zinc in water, permanganate oxidizability. This proves that the increased content of pollutants in water reduces the quantitative indicators of zooplankton and changes the structure of communities.

During the multivariate analysis performed by the main component method, it was found that the quantitative indicators of zooplankton are negatively correlated with the indices of the integrated water quality assessment (TSI, water pollution index and average ranking index). This suggests that in water bodies of urban areas the greatest impact on the development of zooplankton communities is caused by water pollution, as well as the parallel process of eutrophication.

# CONCLUSION

Studies have shown that small reservoirs of urbanized areas have a fairly high species richness of zooplankton. The quantitative indicators of zooplankton in small water bodies of urbanized areas were relatively low. Due to pollution and eutrophication, structural characteristics of zooplankton communities change, quantitative indicators (numbers and biomass) are reduced.

Negative correlations of quantitative indices of zooplankton with copper, iron, manganese, lead, zinc, and permanganate oxidation in water have been revealed. To a lesser extent, the effect of eutrophication and pollution is manifested in lakes with conditions close to natural (lakes located in parks) and in larger ones in transformed lakes and ponds.

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# 01-10

# Zooplankton communities in Lake Nasser under current flood regime before the implications of Grand Ethiopian Renaissance Dam (GERD) construction.

Mahmoud H Hegab<sup>1</sup>\*, Nehad Khalifa<sup>1</sup>, Walid Aly<sup>2</sup>

<sup>1</sup> Freshwater and Lakes Division, National Institute of Oceanography and Fisheries, Egypt

<sup>2</sup> Fisheries Division, National Institute of Oceanography and Fisheries, Egypt

\* Corresponding author: hegabmh@yahoo.com

Keywords: Zooplankton, Flood, Lake Nasser

# **ABSTRACT**

Lake Nasser is a subtropical monomictic, mesotrophic lake and it is the main Nile water reservoir in Egypt. The Nile flood comes once a year in late August and has a direct effect on the Lake ecosystem. This study investigates and documents the effect of Nile flood (the current regime) on zooplankton communities in Lake Nasser as a large artificial water body before filling of Grand Ethiopian Renaissance Dam (GERD). Zooplankton sampling was performed during flood (August 2016), post-flood (December 2016) and preflood (May 2017). Five sectors were selected as sampling locations covering the whole lake. zooplankton populations at the studied area composed of four main groups, *Copepoda*, *Cladocera*, *Rotifera* and *Protozoa* in addition to *meroplankton*. The standing crop of the total zooplankton was higher during flood season with an average density of 117803 Org. m<sup>-3</sup>, while it decreased to more than the half during post-flood and pre-flood seasons with averages densities of 31602 and 31828, respectively. The study concluded that, flood is an important factor that affects zooplankton composition and density in Lake Nasser and any future change in flood regime will have its impact on zooplankton communities in Lake Nasser.

## 1. INTRODUCTION

Lake Nasser is a subtropical monomictic, mesotrophic lake [1] which is the second largest human-made lake in the world after Lake Volta (Ghana); it was formed due to the construction of Aswan High Dam for the purpose of flood control and water storage. The Nile flood comes once a year in late August originating from the Ethiopian highlands continuing to late November [2]. The ecosystem of Lake Nasser mainly affected by the yearly flood of the Nile, which in turn may affect abundance and compositions of organisms like phytoplankton and zooplanktons and others [3].

The Grand Ethiopian Renaissance Dam (GERD), which is being constructed on the Blue Nile at the Ethiopian border with Sudan, would have a direct effect on the net annual discharge downstream. The negative hydrological impacts of GERD will increase by increasing the height of its spillway dam and increasing the storage capacity may affect the strategic storage for the reservoirs in Sudan and Egypt [4]. Moreover, the filling period of GERD reservoir will be accompanied by an impact on Egypt and reservoir impounding of this size over 6 years will cause annually decrease inevitably Nile flow to Lake Nasser by about 12 BCM, which represents approximately 23% of Egypt's

annual share of Nile water. The adverse impacts on Egypt during and after filling of GERD reservoir will increase especially during periods of low flood, [5].

Thus, this study investigates and documents the effect of Nile flood (the current regime) on zooplankton communities in Lake Nasser before filling of GERD to represent a reference point for any future impact on zooplankton communities as result of flood regime change.

#### 2. METHOD

# **Site Description**

Lake Nasser has an area of about 5248 km², a mean depth of 21.5 – 25.5 m (maximum 90 m) and the maximum width is about 60 km, the average width is 8 km. Lake Nasser extends between 22° 31′ to 23° 45′N and 31° 30′ to 33° 15′E and filled during late 1970s [6].

# Sampling program

Sampling was performed during flood (August 2016), post-flood (December 2016) and pre-flood (May 2017). Five sectors were selected as sampling locations covering the whole lake (Fig.1). At each sector, water samples were collected from three sites (eastern bank, middle of the lake and western bank). The average densities of zooplankton at the three sites were expressed as the density of

zooplankton at each sector.

Samples were vertically hauled from upper 5 meters by plankton net 55  $\mu$  mesh size, 30 cm diameter and 80 cm length. The collected samples were transferred to a labeled clean bottle and immediately fixed with 4 % formaldehyde. Three subsamples were examined under a binocular research microscope with magnification varied from 100X to 400X. Zooplankton species were identified according to many publications and taxonomic references. Zooplankton population density was expressed as the number of individuals per cubic meter.

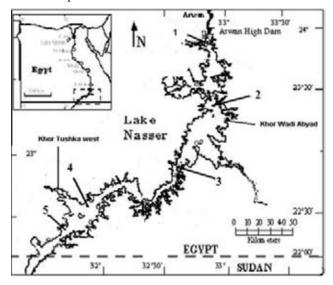


Fig. 1 Map of Lake Nasser showing the selected sectors at the main channel

## Data analysis

The diversity indices including species richness, evenness and Shannon-Wiener index were calculated using Primer 7 software. Spatial variations of zooplankton data were represented on study area using ArcGIS 10.5 software.

## 3. RESULTS

# Distribution of total zooplankton

The standing crop of the total zooplankton was higher during flood season with an average density of 117803 Org. m<sup>-3</sup>, while it decreased to more than the half during postflood and pre-flood seasons with averages densities of 31602 and 31828, respectively (Fig.2). The zooplankton populations at the studied area composed of four main groups *Copepoda*, *Cladocera*, *Rotifera* and *Protozoa* in addition to *meroplankton*.

Figures 3, 4 and 5 show that, *Copepoda* was the dominant zooplankton group at all sectors along the main channel of the lake during the three seasons except sector 1 during post-flood season and sector 5 during pre-flood season, where *Rotifera* and *Cladocera* were the dominants, respectively.

Copepoda was represented by three adult species; Thermodiaptomus galebi, Mesocyclops ogunnus and Thermocyclops neglectus, in addition to larval stages. Nauplius larvae consisted the main bulk of Copepoda with averages densities of 48167, 11395 and 8196 Org. m<sup>-3</sup> during flood, post-flood and pre-flood, respectively. Thermocyclops neglectus was the dominant adult copepod species with averages densities of 6886, 1377 Org. m<sup>-3</sup> during flood and post-flood, while Thermodiaptomus galebi was the most abundant during pre-flood with an average of 4698 Org. m<sup>-3</sup>.

Twenty-one rotifer species (16 during flood, 14 during post-flood and 4 during pre-flood seasons) were dominated by *Keratella tropica* during flood season while, *Keratella cochlearis* was the dominant one during post-flood and pre-flood seasons.

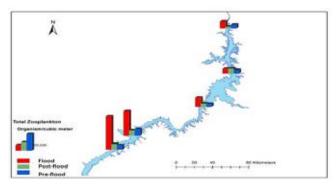


Fig. 2 The distribution of total zooplankton along the main channel of Lake Nasser during the three seasons.

## **Zooplankton composition**

Cladocera was represented by 6 species during the study period. Diaphanosoma mongolianum was the most abundant species during flood and post-flood with averages densities of 15376, 943 Org. m<sup>-3</sup>, respectively. During pre-flood season, Ceriodaphnia dubia was the dominant cladoceran species with an average density of 6792 Org. m<sup>-3</sup>.

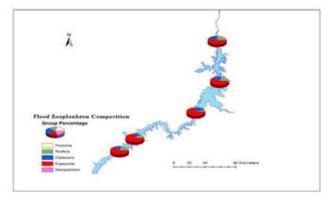


Fig.3 zooplankton composition along the main channel of Lake Nasser during flood season.

Generally, the highest species number (34 species) was recorded during pre-flood season, while the lowest species number (18 species) recorded during post-flood season. The richness index was nearly equal (2.82 and 2.89) during pre-flood and flood seasons, but it decreased to 1.64 during post-flood season. However, the evenness index (0.74) was higher during post-flood season compared to that reported during pre-flood and flood seasons. The shanone values reported during this study were 1.98, 2.07 and 2.13 during pre-flood, flood and post-flood seasons respectively.

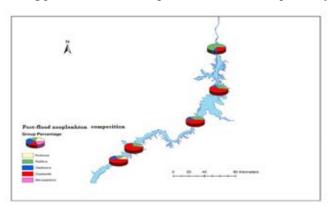


Fig.4. zooplankton composition along the main channel of Lake Nasser during post-flood season.

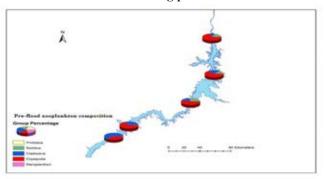


Fig.5. zooplankton composition along the main channel of Lake Nasser during pre-flood season.

# 4. DISCUSSION

In the current study, the increasing of zooplankton during flood season is associated to increasing of larval stages of *Copepoda* which feed mainly on phytoplankton [7]. The highest density of phytoplankton in the lake was recorded during autumn season [8], where the flood samples of the present study were collected during late of August (autumn season). Moreover, the total zooplankton density was increased at southern part of the lake especially during flood season which agrees with previous studies [1, 9], which concludes that the southern part (upstream) is richer in zooplankton than the northern one (downstream).

Generally, the changes of zooplankton density in Lake Nasser always accompanied by the fluctuation of phytoplankton abundance [10, 11]. *Copepoda* was the most dominant group during the three seasons. This is due

to the abundance of nauplius larvae which mainly feed on phytoplankton [7, 12]. The dominate adult copepods, *T. galebi, T. neglectus* and *M. ogunnus* are a cosmopolitan species which show typical limnetic habit and avoiding grossly polluted waters. The increasing of the rotifer percentage at sector 1 during post-flood season and cladoceran percentage at sector 5 during pre-flood season is accompanied with the decreasing of copepod percentage during the same time and at these sectors.

# 5. CONCLUSION

Flood is an important factor that affects zooplankton composition and density in Lake Nasser. Many studies were carried out on Lake Nasser from different aspects due to its importance for the Egyptian people. However, more future studies should be conducted to monitoring the expected changes in the lake ecosystem by the construction of the Renaissance Dam in Ethiopia.

## **AKNOWLEDGEMENT**

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# Status of some Wetlands from South Western Maharashtra-India with special reference to Floristic Diversity, Ecosystem benefits, Threats and Management strategies

C.B. Salunkhe<sup>1</sup>, S.V. Raskar<sup>1</sup>, S.M. Moholkar<sup>2</sup> and S.U. Shinde<sup>3</sup>

<sup>1</sup>Krishna Mahavidyalaya, Shivnagar, Rethare (Bk.) - 415108, M.S., India <sup>2</sup>Raja Shripatrao Bhagwantrao Mahavidyalaya, Aundh - 415510, M.S., India <sup>3</sup>Shankarrao Mohite Mahavidyalaya, Akluj - 413101, M.S., India chandrakantsalunkhe62@gmail.com

Keywords: Wetlands, South Western Maharashtra-India, Floristic Diversity, Ecosystem benefits, Management strategies

#### **ABSTRACT**

India has a wealth of wetland ecosystems that support unique habitats with large diversity of biota representing almost all taxonomic groups. As per the National Wetland Atlas 2011, India has about 757.06 thousand wetlands with a total wetland area of 15.3 m ha, accounting for nearly 4.7% of the total geographical area of the country<sup>[20]</sup>. Out of these, only 26 wetlands have been designated as Ramsar Sites<sup>[21]</sup>. Upon recognizing the ecological significance of the wetland habitats, an extensive and intensive survey was undertaken during 2012-2016 to document floristic diversity of 20 wetlands from South Western Maharashtra. The floristic diversity survey revealed that about 263 plant species belonging to 210 genera of 63 families inhabiting in the study area. Of these 263 species, 191 are dicotyledons belonging to 162 genera of 51 families and 72 species are monocotyledons belonging to 48 genera of 12 families. To highlight the environmental and geographical significance mapping of these 20 wetlands have been completed with the help of GPS. This paper reviews the wetland wealth of South Western Maharashtra, India in terms of floristic diversity, their geographic distribution and ecosystem benefits. The paper also discusses threats, priority area and management strategies for conservation of these fragile ecosystems.

#### 1. INTRODUCTION

The Convention on Wetlands of International Importance especially as Waterfowl Habitat was adopted at Ramsar in 1971 (www.ramsar.org). The Convention was focused at the fundamental ecological functions of wetlands as regulators of water regimes and the value of wetlands in economic, cultural, scientific, and recreational terms. Wetlands play an important role in maintaining ecological balance through their biotic and abiotic components. These are important

ecosystems and forms life support systems for flora, fauna and fisheries. India is having 58.4 million ha area covered by wetlands<sup>[11]</sup>. These wetlands plays significant role in protecting and conserving biological resources occurred in them. Extensive studies on wetlands have been carried out in different geographic areas. These include ecosystem studies, food and feeding habits of birds and population studies.

The biodiversity value and high biological productivity of wetlands are well recognized[14]. Biswas and Calder[1] described 115 species including lower groups for the wetlands of British India and Burma. Subramanyam<sup>[2]</sup> has made major contribution to the wealth of aquatic plants of India by providing key for identification of aquatic flowering plants. CDK Cook et.al.[3] has made taxonomic overview of fresh water aquatic plants. The Indian subcontinent harbors about 470 aquatic species of flowering plants[7]. CDK Cook[12] in his book has recorded 485 species of aquatic and semiaquatic flowering plants with key for identification, description and illustration. Karthikeyan et.al. [5] have contributed to the aquatic plant diversity of India. The total numbers of aquatic plant species exceed 1200 and they provide a valuable source of foo, especially to waterfowl[18]. A directory of Asian wetland<sup>[6]</sup>, Indian Wetlands, Handbook of Wetland Management[7] are the important sources of information on wetlands of national importance.

This study is an attempt to identify the composition of plant species diversity present in 20 wetlands from Satara, Solapur, Kolhapur and Ratnagiri districts of South Western Maharashtra-India. Identifying the plant species composition of these wetland habitats will provide better understanding of the ecosystem and its functioning and will offer a better perceptive regarding the stability of ecosystem.

#### 2. METHOD

During 2012-2016, an extensive and intensive survey throughout the study area was undertaken periodically. All the collected specimens were identified and processed as per the conventional methods of drying, poisoning, mounting and labelling<sup>[4]</sup>, <sup>[8]</sup>. The collected specimens were identified by referring various floras<sup>[9]</sup>, <sup>[10]</sup>, <sup>[13]</sup>, <sup>[16]</sup>, <sup>[17]</sup>, <sup>[19]</sup> The identified specimens were then confirmed with

the help of authentic specimens available in SUK and BSI Herbaria. The nomenclature of plants has been adapted and updated applying ICBN<sup>[15]</sup> and referring to latest taxonomic literature from online nomenclatural database (www.theplantlist.org). The processed herbarium specimens were deposited in the herbarium at Post Graduate Center of Botany, Krishna Mahavidyalaya, Rethare Bk., M.S. India. Mapping of 20 selected wetlands has been completed with latitude, longitude and altitude with the GPS (Garmin model e-trex). Imageries are developed with the help of Garmin software and internet.

#### 3. RESULTS

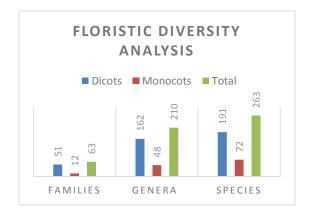
In the present work, a total of 263 plant species, belonging to 210 genera and 63 families of dicots and monocots have been collected and documented. Dicotyledons are represented by 191 species belonging to 162 genera and 51 families while monocotyledons are with 72 species belonging to 48 genera and 12 families.

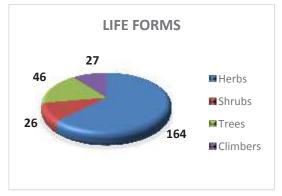
The 263 reported species includes 164 herbs, 26 shrubs, 46 trees and 27 climbers. The family Poaceae is dominant with maximum number of species (42) followed by Asteraceae (21 species), Fabaceae (19 species), Cyperarceae (14 species) and Euphorbiaceae (13 species). Twenty four families were represented each by single species.



Fig.1. Map showing the location of Mayni Bird habitat, Maharashtra

Regarding the life forms, about 164 species are Herbs followed by trees (46), lianas (27) and shrubs (26). *Cyathocline manilaliana* an endemic threatened plant were recorded form the area.





#### 4. DISCUSSION

Wetlands provide numerous products and services to humanity like multiple uses of water services, flood control, carbon sequestration, biodiversity conservation and fisheries. They provide a refuge for migratory birds. Mayni and Yeralwadi wetlands are the best bird habitats.

Urbanization influences major threat to wetlands. Pollution, poor management, demands for water, agricultural runoffs, wastewater discharges are the severe threats.

Separate legal provisions for protection of water quality and protection of biodiversity of wetlands are the urgent need to save these fragile ecosystems. Besides national legislation a majority of wetlands are ignored in the policy process. It is essential to identify ecologically important and sensitive wetlands which greatly help in making conservation strategies. Regular water monitoring plan is essential to prevent them from getting polluted. It helps in maintaining their biota, hydrology and ecological integrity.

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# 01-12

# Fisherman's Willingness to Pay for Sustainable Ecosystem Management of Jaffna Lagoon

<sup>1</sup>Sooriyakumar Krishnapillai, <sup>2</sup>Sarujan Sathiyamoorthy, <sup>3</sup>Sivakumar Sivashankar <sup>1,2,3</sup>Department of Agricultural Economics, University of Jaffna, Sri Lanka

Keywords: Choice Modeling, Lagoon, Sustainable Ecosystem Management, Willingness to Pay

#### ABSTRACT

The economy of the fishing community along the Jaffna lagoon is heavily dependent on fishing activities, but overfishing and overexploitation of lagoon ecosystem is a significant challenge for the Sri Lankan government to support sustainable lagoon ecosystem. The purpose of this study is to analyze the fisherman choices, preferences and willingness to pay for different sustainable lagoon ecosystem management practices. Choice modeling approach was used to estimate the willingness to pay different lagoon ecosystem management. Along the lagoon, 65 fishermen from four densely populated town fishing communities and 53 fishermen from two less densely populated village fishing communities are randomly selected for this survey. Conditional logit model was estimated for the selection of choices of fishing communities. Results show that fishermen from town communities are willing to pay for less for increase the number of mangroves and develop the tourist facilities when compared to village fishing communities as there are high competitions for land along the lagoon shore in townfor fishing activities, anchoring fishing boats and housing. As fishing is the major source of income for both communities, they are not willing to support for banning inappropriate fishing gears. Establishing fishing harbour facilities, properly planned multistory housing units, increasing the awareness of negative impact of inappropriate fishing gears and developing alternative sources of income for fishermenwould increase their support for the sustainable lagoon ecosystem management.

#### 1. INTRODUCTION

Coastal lagoons support a range of natural services that are highly valued by society including fisheries, storm protection, controlling soil erosion and flood, climate regulation, ecotourism, anchorage, recreation etc (Gonenc and Wolflin, 2005). Coastal lagoons are highly productive ecosystems. They contribute to the overall productivity of coastal waters by supporting a variety of habitats, including salt marshes, seagrasses, and mangroves. They also provide essential habitat for many fish and shellfish species. Because of high nutrient availability, coastal lagoons can foster high rates of primary production, thereby supporting high rates of secondary production compared to other aquatic ecosystems (Nixon, 1995). Protection and sustainable use of coastal ecosystem is a major concern of many international institutions. Jaffna lagoon is the largest lagoon system in the country. The economy of the fishing community along this lagoon is heavily dependent on fishing activities, but overfishing and overexploitation of lagoon ecosystem is a significant challenge for the Sri Lankan government to support sustainable lagoon ecosystem (Katupotha, J. Silva, E.I.L. 2013).

Although various programs have been launched to control overfishing and overexploitation of lagoon ecosystem, fishermen are still hesitant to implement mitigation practices due to economic and environmental uncertainty. Therefore, the government should design a cost effective policy to adopt the needs of fishermen and promote sustainable lagoon ecosystem. The purpose of this study is to analyze the fisherman choices, preferences and willingness to pay for different sustainable lagoon ecosystem management practices.

#### 2. METHODOLOGY

Choice modeling is increasingly being formulated in a random utility framework, which allows measurement of the values of non-market goods and services. The Utility function (U) is a function of an observable component (indirect utility function) and unobservable error component.

$$U = V + \varepsilon \tag{1}$$

Where V is the indirect utility function and  $\varepsilon$  is the stochastic error term. We assume that the indirect utility is a linear form,

$$V_i = \beta_i X_{ki} + \alpha y = \beta_1 + \beta_2 x_{2i} + \beta_3 x_{3i} + \dots + \beta_k x_{ki} + \alpha_i y_i$$
 (2)

Where  $X_{ki}(=\{x_1, x_2, \dots, x_k\})$  is a vector of k attributes associated with alternative i,  $\beta$  is a coefficient vector,  $y_i$ is income for a respondent choosing the alternative i bundle, and  $\alpha$  is the coefficient vector of income. If the stochastic error term is logistically Gumbel distributed (Type I extreme value distributed), the choice probability for alternative i is given by,

$$\Pr(i) = \frac{\exp(\rho V_i)}{\sum_{j \in C}^{J} \exp(\rho V_i)}$$
 (3)

Where  $\rho$ a positive scale parameter and C is the choice set for an individual. For convenience we generally make the assumption  $\rho = 1.$ To estimate the welfare impacts, i.e., willingness to pay, for a change from the status quo state of the world to the chosen state, the following formula is used:

$$V_i(X_i, y) + \varepsilon_i = V_i(X_i, y - CV) + \varepsilon_i$$
 (4)

 $V_i(X_i, y) + \varepsilon_i = V_j(X_j, y - CV) + \varepsilon_j$  (4) Where  $V_i$  and  $V_j$  represent utility before and after the change and CV is compensating variation, the amount of money that makes the respondent indifferent between

the status quo and the proposed scenario. Conditional logit model can be applied to estimate the welfare measure in equation (4). Equation (4) can be restated as:

 $\beta_i X_{ki} + \alpha_i y + \varepsilon_i = \beta_j X_{kj} + \alpha_j (y - CV) + \varepsilon_j$  (5)  $\alpha_i$  and  $\alpha_j$  are assumed to be equal if marginal utility of income for a respondent is constant. In conditional logit model, coefficient of k attributes across the all alternatives are the same, and  $\beta_i = \beta_j$ ; only the attribute levels differ across the alternatives, Under this condition, welfare change is estimated by the following:

 $CV = -\frac{1}{\alpha} \left[ \left( \beta (X_{ki} - X_{kj}) + (\varepsilon_i - \varepsilon_j) \right] \right]$  (6) The conditional logit model (equation 6) is used to estimate welfare changes in lagoon ecosystemmanagement, since the impact of the attributes of lagoon ecosystem management is assumed to remain the same across all choice alternatives. In the choice questions, respondents were asked to select an option they favoured the most out of the three alternatives provided. Each option contains the three attributes and the cost to the household with various levels of attribute combinations. Respondents were asked to answer similar types of choice questions sets multiple times. There are two levels for fishing method attributes, three levels for number of mangroves attributes, two levels for tourism attributes and six levels in the cost to household. Here we assume interaction effects between attributes insignificant.Unrealistic options were excluded. 21 choices were selected. "No change" in the current attributes levels (Option C) was included in the each choice set. Along the lagoon, 65 fishermen from four densely populated town fishing communities and 53 fishermen from two less densely populated villagefishing communities are randomly selected for this survey. Conditional logit model was estimated for the selection of choices.

Table 1. Definitions of selected lagoon ecosystem managementattributes

Attributes	levels	Definitions		
Fishing Method	Banning inappropriate Fishing gears (BIFG)	Implementing full ban on inappropriate fishing gears		
	No change	No change in the current Fishing gears		
Number of Mangroves	Big increase (BIM)	50% increase in number of mangroves		
	Small increase (SIM)	20% increase in number of mangroves		
	No change	Maintain current number of mangroves		
Tourism	Improving tourist Facilities (ITF)	Developing tourist spots with boatingfacilities		
Cost to Household 0, 50,100,200,400,800		Annual payment to regional office		

#### 3. RESULTS

Table 2: Choice Modeling and Willingness to Pay

Attributes level	Coefficent		Village (WTP)	Town (WTP)
BIFG	083 (0.1	.08)		
SIM	.182* (0.0	42)	444	
BIM	.330* (0.0	00)	804	335
ITF	.592* (0.0	000)	1442	557
Cost	0008* (0.0	000)		
Town*BIFG	.037 (0.5	89)		
Town*SIM	145 (0.2	21)		
Town*BIM	192* (0.0	55)		
Town*ITF	363* (0.0	00)		
No of Observations	3,540			
Likelihood Ratio ( $x^2$ )	241.08			

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The results of choice modeling and willingness to pay are shown in Table 2. Big increase in number of mangroves and improving tourist facilities variables are significant at 5% level and Banning inappropriate fishing gears is not significant at 5% level in both fishing communities. Coefficient of cost is negative as expected and significant at 5% level. Results show that village fishermen are willing to pay Rs 804 and Rs 1442 per year and town fishermen are willing to pay Rs 335 and Rs 557 per year for big increase in number of and developing mangroves tourist facilities respectively.

#### 4. DISCUSSION

Coefficient of big increase in number of mangroves and improving tourist facilities show relatively large magnitudes in village fishing communities. As there are high competitions for land along the lagoon shore in town for fishing activities, anchoring boats and housing, fishermen from town communities are willing to pay for less for increase the number of mangroves and develop the tourist facilities when compared to village fishing communities. Since fishing is the major source of income for both communities, they are not willing to support for banning inappropriate fishing gears. Their low education level is also another reason to understand the impact of inappropriate fishing gears on fish growth in the lagoon. As village fishing communities have an additional source of income from coconut plantation, they are less sensitive to the cost of choice of lagoon ecosystem management compared to fishermen from town.

#### 5. CONCLUSION

Establishing fishing harbour facilities to accommodate more fishing boats and properly planned multistory housing units to make available land for planting mangroves, developing tourist spots and increasing the awareness of negative impact of inappropriate fishing gears for the sustainability of fishing stocks and developing alternative sources of income for fishermenwould increase their support for the sustainable lagoon ecosystem management.

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<sup>\*-</sup> significant at 5% level, P value in parenthesis

Can scientific evidence to depict the ecosystem services effectively resolve the conflict between the protected area managers and the local dependent community? Nalabana bird sanctuary; Chilika Lake a wetland of international importance, India; a case study.

A.K. Pattnaik<sup>1</sup>, S.K.Mohanty<sup>2</sup>, Saibal Parida<sup>2</sup>, Sibani Patnaik<sup>2</sup>

<sup>1</sup>Wetlands International South Asia, <sup>2</sup>Wetland Research & Training Center, Chilika Development Authority

Key Words: ecosystem services, lake ecosystem, protected area, scientific evidence, spawning, nursery

#### **ABSTRACT**

Chilika Lake is the most significant coastal wetland along the east coast of India and a Ramsar site. The highly productive lake eco-system with its rich fishery resources sustains the livelihood of more than 0.2 million fishers. Chilka is a vital wintering ground for the migratory bird of Asiatic subcontinent. Nalabana island within the lake, spread over 15.53 sq. Km, designated as a bird sanctuary in 1973. Nalabana was known for highly productive capture fishing ground till 1973. The ban on fishing clamped after notification led to conflict between the local fishers and the protected area managers. Interestingly, after the designation of protected area the fishers have been getting a good harvest from the trap they lay outside the sanctuary boundary. However, the ecosystem services, contributed by the sanctuary quietly has been gone unnoticed by the local fishers. For the ecosystem service paradigm to be relevant to resourcedependent communities, it is essential to generate awareness and their perception of it. The study carried out during July 2011 to June 2014, by the authors, attempted to establish through scientific evidence, the ecosystem services provided by the sanctuary in the form of potential nursery and spawning ground for fish. The outcome of the study conclusively demonstrated that sanctuary is serving as feeding and nursery for 45 spawning of 11 resident species, contributing significantly to the lake fishery. The outcome of the study changed the perception of local fishers about the sanctuary and ecosystem services it provides, also in resolving longstanding conflict.

#### 1. INTRODUCTION

Nalabana bird sanctuary spread over 15.53 km<sup>2</sup>, within Chilika lake (Fig-1) is a protected area designated as a sanctuary in the year 1973 under Wild Life (Protection) Act since from 1973. Fishing activities have been prohibited in the sanctuary since from 1973. Historically, Nalabana was the largest traditional Jano fishery ground, a form of traditional capture fishery. The local fishers practice this traditional fishery until it became a protected area in 1973. Fishers have been objecting to such prohibition within the sanctuary area, leading to a conflicting situation between the protected area managers and the local fishers. More than 0.4 million migratory bird congregate in the sanctuary during winter every year. The droppings of these birds in the form of guano triggers the abundant growth of phytoplankton and zooplankton.

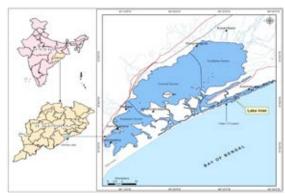


Fig-1 Chilika Lake along the east coast of India and the Nalabana Sanctuary the study area located in the central sector.

This attracts many commercial finfish for feeding and spawning. Interestingly after the ban on fishing within the sanctuary, now the fishers lay their box-net traps outside the sanctuary at proximity to sanctuary boundary and get a bumper harvest.

The ecosystem services sanctuary provides, apparently remained unnoticed by the local communities. For sustainable management of the sanctuary, the awareness and perception of local fishers about the ecosystem services is crucial. This necessitated the present study to establish the flow of ecosystem services from the sanctuary as a potential nursery and spawning ground for finfish and shellfish based on scientific the local fishermen. The objective of the study has been to generate awareness about the supporting and regulatory ecosystem services, from the sanctuary in the form of spawning, feeding and nursery ground for important fish species. Also, to change the understanding of local fishers, about, how the undisturbed sanctuary area is the lake fishery, benefiting the fishermen in the long run. The study was initiated in July 2011 for three years. The outcome of the study demonstrated convincingly to the local fishers, how the undisturbed conservation area is serving as a sanctuary for fish, maintaining the ecosystem resilience and flow of ecosystem services.

#### 2. METHOD

The study site (Fig 2) is within Nalabana sanctuary area of 15.53 km<sup>2</sup> with core zone of 6.72 km<sup>2</sup> and buffer zone of 8.81 km<sup>2</sup> in Chilika lake. For the study, the sanctuary was divided into four sectors by using GPS (Garmin-Model:72H). In each sector, one sampling station (N-1, N-2, N-3 & N-4) has been fixed. Four control stations outside the sanctuary area (C-1, C-2, C-3 & C-4) are set as the control for comparison. Regular sampling carried out in 4 stations within Sanctuary and four control stations at an interval of 30 days. During each campaign, the water quality, macrobenthos, phytoplankton, zooplankton, macrophytes, postlarvae of finfish and shellfish and spawners and juveniles were studied. The sieved material collected in the glass bucket tied to the tail end of the tow net was collected in the rectangular enamel trays. The post-fry were collected after removing the debris, and the materials were segregated into fry and post-larvae of different species. The segregated specimen was measured, photographed and preserved in plastic bottles for transport to the laboratory for further investigation.

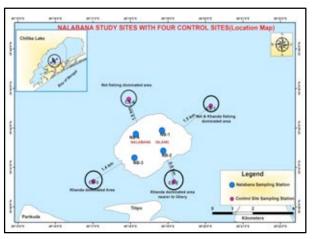


Fig-2 The study site (Nalabana sanctuary in Chilika lake) with four stations inside Nalabana sanctuary area and four control stations outside sanctuary area

For the sampling of adult and juvenile fishes, a nylon dragnet of thirty feet long and three feet height with 15mm mesh size was used. Spawners, particularly, females with ripe ovary and juvenile fishes were collected from dragnet and were segregated. The females were dissected to expose mature ovaries and preserved in 10% buffered formalin. Developing fish eggs collected from each sampling station were studied under a microscope for identification.

# 3. RESULT

The study results for ecological parameters, i.e. water quality variables, phytoplankton and zooplankton abundance, macro-zoobenthos status, and nutrient status, macrophyte biomass and diversity etc.) indicated that the overall ecological conditions of Nalabana sanctuary are very conducive for productive fish habitat. Nalabana sanctuary as a potential fish habitat is rich in available natural food elements, i.e. zooplankton, benthic organisms and general bottom biota, benthic filamentous algae, organic detritus etc. Macroalgae and organic detritus are known to be the preferred food by mullets are abundantly found in the sanctuary. The abundant seagrass meadows of Halophila ovalis, provides the best shelter for juveniles of mud crab and shrimp which were abundantly found in the sanctuary area, testify the study area as a potential nursery ground for fish and shellfish.

The average values for zooplankton population density in Nalabana sanctuary area (25647± 18623.08 no/m<sup>3</sup>) was not significantly higher (p<0.068) than the control stations (20833 $\pm$ 14931.19 no/m<sup>3</sup>) as obtained from ANOVA test. The average zooplankton density for Nalabana sanctuary core area was also found to be higher than the average values for the central sector of the Chilika lake (22020 no/m<sup>3</sup>) and main lake area (16959 no/m<sup>3</sup>) during the study period. The zooplankton diversity of Nalabana sanctuary core area was more or less the same as the central sector of the lake. The main zooplankton groups were copepods, amphipods, cladocera, gastropod veligers, bivalve veligers, copepod nauplii, cirripede nauplii and hydrozoans. The zooplankton diversity was dominated by copepods at both Nalabana sanctuary area and control stations.

The gut content of 10 commercially important species revealed that the food elements encountered matched with the available food materials in the sanctuary. The gut content of above species has been, gastropods, bivalves, polychaetes, amphipods, filamentous algae, diatoms, organic detritus and seagrass leaves.

# 4. DISCUSSION

The outcome of the study carried out for three years confirmed that 11 resident fish species of Chilika lake breed in Nalabana sanctuary area. Survey on juvenile fish abundance in sanctuary area indicated that more than 45 species of juveniles of finfish and shellfish species are frequenting sanctuary area for feeding purposes throughout the year. The pooled average data of the operation of tow net hauls of 10 minutes' duration to study the early juvenile fish and prawn frequenting for feeding purpose (nursery ground) are recorded and analysed. The study confirmed that 45 species of finfish and shellfish (prawns) are utilizing Nalabana area as a potential feeding/nursery ground.

Variation in the abundance of different species studied from tow net collections is recorded. In general, food elements traced from the guts of 10 economic fishes i.e. M.gulio, E. suratensis, N.nasus, M. cephalus, L.macrolepis, G.setifer, H.limbatus, R. sarba, E.tetradactylum & Arius arius, matched with the available natural food materials from Sanctuary. Above findings indicated that the juvenile fishes are attracted to the study area because of abundant availability of food materials. No evidence of spawning of finfish and shellfish and potential nursery ground for juvenile fishes and shellfishes from control stations (outside sanctuary area) could be gathered during the study period in respect of the species recorded from the sanctuary.

#### 5. CONCLUSION

Awareness about the ecosystem services flowing from the sanctuary was low. The fishers were ignorant about the regulating and supporting services provided by sanctuary. This has resulted in a conflicting situation. The present study with scientific evidence demonstrated that Nalabana sanctuary significantly contributes to lake fishery. The rich seagrass meadows provide an excellent nursery ground for the finfish and shellfish. The scientific evidence from this participatory research resolved the long-standing conflict between the fishers and the protected area managers.

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# 01-14

# 霞ヶ浦の生態系サービスの経済評価と評価手法の課題

北村 立実<sup>1</sup>, 松崎 慎一郎<sup>2</sup>, 久保 雄広<sup>2</sup>, 山野 博哉<sup>2</sup>, 西 浩司<sup>3</sup>, 幸福 智<sup>3</sup>, 菊地 心<sup>3</sup>, 吉村 奈緒子<sup>3</sup>, 松本 俊一<sup>1</sup>, 福島 武彦<sup>1</sup> <sup>1</sup>茨城県霞ケ浦環境科学センター, <sup>2</sup>国立環境研究所, <sup>3</sup>いであ株式会社

キーワード: 生態系サービス、代替法、コンジョイント分析

#### 抄録

霞ヶ浦の多様な生態系サービスについて整理し、代替法とコンジョイント分析を用いて経済評価を行った。その結果、代替法では供給サービスと調整サービスが大きく、その中でも洪水調節が最も大きかった。一方、基盤サービスは算出できなかった。また、コンジョイント分析では水質改善に最も重きをおいていることが明らかとなった。評価の課題として、水質汚濁と生態系のトレードオフを考慮した価値の算出の必要性や、供給サービスと文化的サービスのダブルカウント等が考えられた。また、今回評価した項目以外にも評価できる項目があった可能性があり、今後はこれらの課題の解決を図りながら、霞ヶ浦がどうあるべきなのか、多様なステークホルダーが議論する為の基礎資料として利用したい。

#### 1. はじめに

霞ヶ浦は茨城県の南部に位置する湖であり、湖面積 は 220km<sup>2</sup>と日本で 2 番目に大きい湖である(図 1)。現 在, 霞ヶ浦流域には約96万人が生活している。また, 霞ヶ浦周辺は水田やハス田,畑地が広く分布し,鹿島 臨海工業地帯もあることから農業や工業も盛んである。 これらの生活や経済活動の多くは霞ヶ浦の水が用いら れている。さらに、 霞ヶ浦湖内ではワカサギ等の漁獲や コイ等の養殖など水産業も盛んであり、佃煮の生産や 帆引き船の操業等の伝統を残すとともに観光資源とし ても利用されている。また、 霞ヶ浦はコンクリート護岸に 覆われ,常陸川水門によって水位が操作され,農業や 工業などの利水の他に,大雨時の洪水を調整する治水 の役割も担っている。このように、多くの人々は霞ヶ浦か ら多様な恩恵(生態系サービス)を受けている。今後も 人々が霞ヶ浦の生態系サービスを持続的に利用してい くためにはどのようなサービスをどのくらい受けているの かを把握し、湖沼・流域管理に結びつける必要がある。

2016 年に環境省は生物多様性及び生態系サービスの総合評価(JBO2)を実施し、生態系サービスの内容や変化に与える要因等についてまとめている<sup>[1]</sup>。また、2010 年に生物多様性条約の第 10 回締約国会議において「生態系と生物多様性の経済学(The Economics of Ecosystems and Biodiversity, TEEB)」報

告書が公表されており、そこでは適切な管理のためには計測し定量化できる指標が必要であることが記載されている<sup>[2]</sup>。これらのことから、霞ヶ浦の生態系サービスを分かりやすく認識するためにサービスの内容を整理し、経済的な価値(貨幣価値)に置き換え可視化することが重要である。

そこで、本研究では JBO2 に準じて霞ヶ浦の生態系サービスの内容を把握し、経済学的手法を用いて生態系サービスの経済的な価値を評価するとともに、評価手法の課題について検討した。

#### 2. 評価対象および評価手法

評価対象は霞ヶ浦(湖沼部分)のみとし、生態系サー

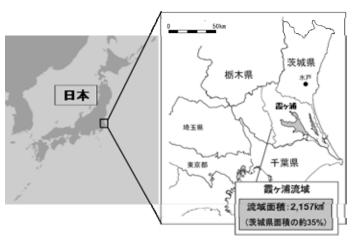


図1 霞ヶ浦とその流域

ビスの経済評価については現在の価値を評価すること とした。評価項目及び評価指標については JBO2 で用 いられている項目や指標を参考にし、供給サービス、調 整サービス、文化的サービス、基盤サービスの4つに分 類し、さらに中項目、小項目に細分化し評価指標を設 定した(表 1)。経済評価手法は、代替法および選択型 実験(コンジョイント分析)とした。

# 2-1. 代替法

評価する年代は現在とし、直近の既存の統計資料等から、実際に市場に流通しているものについては市場価格で代替し、それ以外の指標については主に代替となる原単位を設定し、物量をかけることで算出した。

#### 2-2. コンジョイント分析

コンジョイント分析はウェブアンケート調査によって全

表1 霞ヶ浦の生態系サービスの評価項目及び指標

項目	中項目	小項目	指標			
	食糧・	水産物 (漁業)	漁穫量			
	原材料	水産物	淡水真珠			
供給		(養殖)	コイ, その他魚類			
			農業用水			
	水供給	取水量	工業用水			
			水道用水			
調整		地下水	地下水涵養量			
	水の調整	涵養量	地下水個食里			
		水質浄化	脱窒量			
	気候の調整	潜熱効果	蒸発散量			
	自然災害	洪水調節	治水容量			
	の防護	<b>供</b> // 响即	111小台里			
	宗教・祭り	水神の数				
	<del>数</del> 杏	霞ヶ浦を利用した体験学習、霞ヶ				
	数字	霞ヶ浦を利用	した体験学習, 霞ヶ			
	教育	霞ヶ浦を利用 浦を題材とした				
	教育 景観,観光・		に環境教育			
		浦を題材とした	定環境教育			
	景観,観光・	浦を題材とした	た環境教育 帆引き船利用者数			
文化	景観、観光・レクリエーシ	浦を題材とした レクリエーション利用者	に環境教育 帆引き船利用者数 釣り利用者数			
文化	景観、観光・レクリエーシ	浦を題材とした レクリエーション 利用者 数	<ul><li>環境教育</li><li>帆引き船利用者数</li><li>釣り利用者数</li><li>水遊び人数</li></ul>			
文化	景観、観光・レクリエーション	浦を題材とした レクリエーション利用者 数 伝統的建造	<ul><li>環境教育</li><li>帆引き船利用者数</li><li>釣り利用者数</li><li>水遊び人数</li><li>妙岐の鼻地区カモ</li></ul>			
文化	景観,観光・ レクリエーション G統芸能・	浦を題材とした レクリエーション利用者 数 伝統的建造物	で環境教育 帆引き船利用者数 釣り利用者数 水遊び人数 妙岐の鼻地区カモ ノハシ・ヨシ群落面			
文化	景観、観光・レクリエーション	浦を題材とした レクリエーション利用者数 伝統的建造物 (茅葺屋根の原材料)	ご環境教育 帆引き船利用者数 釣り利用者数 水遊び人数 妙岐の鼻地区カモノハシ・ヨシ群落面 積(茅葺に利用される群落) 佃煮生産量(わかさ			
文化	景観,観光・ レクリエーション G統芸能・	浦を題材とした レクリエーション利用者数 伝統的建造物 (茅葺屋根の原材料) 伝統的水産	で環境教育 帆引き船利用者数 釣り利用者数 水遊び人数 妙岐の鼻地区カモノハシ・ヨシ群落面 積(茅葺に利用される群落) 佃煮生産量(わかさぎ・はぜ・アミ・えび・			
文化	景観,観光・ レクリエーション G統芸能・	浦を題材とした レクリエーション利用者数 伝統的建造物 (茅葺屋根の原材料)	で環境教育 帆引き船利用者数 釣り利用者数 水遊び人数 妙岐の鼻地区カモノハシ・ヨシ群落面 積(茅葺に利用される群落) 佃煮生産量(わかさ			

国の 20 歳以上の成人を対象に実施し、有効サンプル 数は 1,181 データであった。アンケートの内容として、ま ず霞ヶ浦の仮想的状況として以下の説明を行った。

『ここでは仮に、今後、対策をしないと、2040年頃 には様々な霞ヶ浦の恵みを受け取れなくなる恐れが あるとします。しかし、霞ヶ浦を現在より良い水環 境にするための対策を実施することで、現在の恵み を維持、またはより多くの霞ヶ浦の恵みを受け取れ るようになるとお考え下さい。そこで、 霞ヶ浦の水 環境をより良くするための基金を設置して、広く一 般の方から寄付をつのり、お金をかけていくつかの 対策を実施することにしました。つまり、あなたが 今回この基金に募金をすることで、2040年において も望ましい霞ヶ浦の恵みを維持し、守っていくこと ができるとします。ここでは、先の説明で述べた「水 質」,「全国的に希少とされている魚類」,「漁獲量」, 「湖岸植生帯」の4つの要素で霞ヶ浦の状態を考え ることにします。その上で,以下に示す各要素につ いての将来生じうるレベルをご覧ください。』

この説明文に続き、各要素のレベルを示した選択肢を各アンケート票の中で7問提示し、回答者には各質問につき1個ずつ最も望ましい対策を選択させた。この結果から4つの要素を各生態系サービスとして設定し、1単位変化することによる一人当たりの支払意思額を算出した。そして、その支払意思額に湖沼の現状から最も良い状態に改善した場合の改善量と調査対象とした人口をかけることで各生態系サービスの価値として算出した。設定した単位や湖沼の現状と最も良い状態については表2のとおりである。

表 2 単位及び湖沼の現状と最も良い状態

項目	単位及び湖沼の状態				
供給	漁獲量が 1t 増えること。				
	<現状:900 t, 最も良い状態:17,500 t>				
調整	湖岸植生帯が1%増えること。				
	<現状:13.2%, 最も良い状態:90.0%>				
文化	CODが 1 mg/L 下がること。				
	<現状:8 mg/L, 最も良い状態:5 mg/L>				
基盤	稀少な種(魚類)が1種増加すること。				
	<現状:3 種, 最も良い状態:6 種>				

ただし、現状は2018年とし、改善を目指す年は2040年 としたことから、得られた金額を22で除することで1年当 たりの金額とした。

#### 3. 経済評価結果及び評価の課題

代替法では供給サービスが329億円/年,調整サービスが751億円/年,文化的サービスは2億円/年と見積もられた。基盤サービスは評価することができなかった。項目としては洪水調節が最も大きかった。コンジョイント分析では供給サービスが0.09億円/年,調整サービスが22億円/年,文化的サービスは279億円/年,基盤サービスは165億円/年と見積もられた。文化的サービス(水質改善)が最も大きくなり,水質改善に重きをおいていることが明らかとなった。

本研究において霞ヶ浦の生態系サービスの経済評価が明らかとなったが、評価方法についての課題も明らかとなった。課題について以下にまとめた。

- (1)水質浄化(脱窒量)の価値の算出方法は,単位除去量当たりの下水処理場の建設費と維持管理費を原単位として,それに実際の脱窒量をかけることによって算出した。脱窒は微生物の代謝によって起きるが,硝酸濃度が高いと脱窒量が増えることが報告されている<sup>[3]</sup>。つまり,霞ヶ浦の窒素濃度が高い(汚濁が進む)ほど水質浄化の価値が高くなり,霞ヶ浦の価値も高くなるという霞ヶ浦の価値の評価として矛盾してしまう。窒素濃度が高いと別の価値が減少する等のトレードオフの関係を検討する必要がある。
- (2) 水道用水の価値の算出方法は、水道用水の単価から水処理として利用される活性炭の使用単価を引いた値を原単位とし、実際の取水量をかけることで算出した。しかし、水道用水の水処理は活性炭のみならず、微生物による分解やオゾン処理がされている浄水場もあり、より詳細に検討する必要がある。
- (3)漁獲と伝統水産工芸品の佃煮がダブルカウントの可能性がある。供給サービスと文化的サービスがダブルカウントになりやすい。
- (4)生物や植物の種の多様性(基盤サービス)等は市場で取引されていないため代替法では評価することができない。
- (5)コンジョイント分析では特定の行為を各生態系サー

ビスとして設定したが,他のサービスと関連する可能性がある。

#### 4. 結論

本研究によって霞ヶ浦の生態系サービスの経済評価 について明らかにするとともに評価手法の課題を整理した。詳細は以下のとおりである。

- ・代替法では供給サービスや調整サービスで大きく、その中で洪水調節が最も大きかった。コンジョイント分析では文化的サービス(水質改善)や基盤サービスが大きく、特に水質改善に重きをおいていることが明らかとなった。
- ・経済評価の課題として、水質汚濁と生態系のトレード オフを考慮した価値の算出の必要性や供給サービスと 文化的サービスのダブルカウントなどが明らかとなった。

本研究によって霞ヶ浦の生態系サービスの経済的な価値が明らかとなったが、課題も多かった。また、今回評価した項目以外にも評価できる項目があった可能性がある。今後はこれらの課題の解決を図りながら、霞ヶ浦がどうあるべきなのか、いろいろな当事者が議論する為の基礎資料として利用したい。

#### 謝辞

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# Terminalia arjuna as a Riparian Species to the Protection along the Lake, Tank and Catchment Areas in Sri Lanka

D.L.Y. Amarasinghe<sup>1</sup>, D.A.B.N. Amarasekera<sup>2</sup>, T. Sivananthawerl<sup>3</sup> and K.T. Premakantha<sup>4</sup>

<sup>1</sup> Department of crop science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.93yasodha@gmail.com

<sup>2</sup> Department of crop science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

<sup>3</sup> Department of Crop Science, University of Peradeniya, Peradeniya, Sri Lanka

<sup>4</sup> Regional Deputy Conservator of Forests Office (North Western province/Western Province) Palawatha, Battaramulla, Sri Lanka

Key Words: Riparian species, Terminalia arjuna, Diameter at breast height

#### **ABSTRACT**

Terminalia arjuna is a characteristic component of dry riparian forests in Sri Lanka which helps to control erosion, acts as a natural bio filter and protects the wet land areas. The current, field-based approaches for estimation of volume in tree species are laborious and expensive process due to destructive harvesting of more trees. Therefore, to protect the riparian species, we require accurate, flexible and valid common equation to do predictions of volume estimation. This research mainly focuses on evaluation of volume of *Terminalia arjuna* trees in order to make suitable management decisions regarding biodiversity and biological resource services in riparian areas.

For the current study, the secondary data was taken from Moragahakanda area in Sri Lanka. 400 observations for *Terminalia arjuna* were considered for constructing final volume estimation. All possible combinations of linear equations were developed with the relationship of total volume (V) with diameter at breast height (DBH), Total height (Ht) and basal area (BA). The most appropriate model was selected considering the higher adjusted coefficient of determination (Adj R²), low root mean square error (RMSE) and other parameters used for model adequacy checking such as Durbin-Watson statistics (Dw), Anderson-Darling normality test etc. Overall result indicates that *Terminalia arjuna* is a higher value timber species which needed to introduce to conserve riparian areas and timber volume can be estimated without uprooting the trees by using the below equation.

 $ln(V) = -1.50 + 0.572 ln(D) + 1.06 ln(H) + 1.11BA^2$ 

#### INTRODUCTION

Natural rivers associated with riparian habitats are considered as one of the most diverse, dynamic and complex ecosystems in the world. The river margin communities are highly influenced by the changes in hydrology and flow patterns. As a result, riparian communities are considered as good indicators of the environmental changes caused by long-term river flow regulations. One of the most important functions of riparian vegetation is its ability to control erosion, acts as a natural biofilter that helps reduce pollution in surface runoff and shallow groundwater<sup>[1]</sup>.

Terminalia arjuna is well-known as Kumbuk in Sinhala, Arjun tree or tropical almond in English and Marudha marum in Tamil. This tree is a water loving plant and it occurs naturally along the rivers, banks of streams and seasonally dry water resources at low elevation and mostly available in dry tropical riparian forests in, Sri Lanka, Bangladesh and South and central India<sup>[2]</sup>. This species can be identified by its superficial, shallow root system spreads radially along stream banks. Thus, *Terminalia Arjuna* is classified as "Luxury Grade" by Sri Lanka Timber Corporation.

Stem volume is one of the vital variable measurement in tree management. Volume can be easily measured using variables such as diameter and total

height of the tree<sup>[4]</sup>. Volume equations shoud be the acceptably, accurate, easy, cheap and effective tool for estimating the individual tree volume which was developed for management purposes<sup>[3;7]</sup>. But it is difficult to measure due to laborious, destructive and time consuming. If we uproot the valuable riparian tree species, it will adversely affect for the riparian eco system.

Therefore, the present study was conducted with the objectives of constructing a volume prediction models for *Terminalia arjuna* using multiple linear regressions technique and it leads to making suitable management decisions of *Terminalia arjuna* with their dbh, total height and tree volume. In addition, timber valuation<sup>[5]</sup> can be done without uprooting trees by using this equation. Such information can be useful in implementation of conservation practices to restore riparian plant communities along the river banks in the future.

#### **METHODOLOGY**

This study was conducted in sites across the area of Moragahakanda which govern by State Forest Department in Sri Lanka in North Central Province. This

area was located in Dry Zone of Sri Lanka. Less than 1750m annual rainfall with elevation range between 151m -175m can be expected from this area. The mean annual temperature fluctuated between 26.5 °C to 28.5 °C while RH varied between 64.7% and 87.6%. Reddish brown earths (nonlateritic loamy soils) is the major soil type and the other important soil type is the Alluvials that occur along the lower courses of rivers<sup>[6]</sup>.

Secondary data (Table 1) of *Terminalia arjuna* were collected from inventories (Enumeration and Way

Side Deport Registers) of State Timber Corporation in Moragahakanda. Merchantable height, individual tree diameter (over bark) at breast height (1.3m above ground: DBH) were recorded as before felling the trees and number of logs, mid girth and log lengths of each log were recorded as just after felling the trees. Merchantable height was defined as the height to the first live branch of tree species.

Table 1. Statistical summary for diameter at breast height (DBH) and merchantable height (Ht), basal area (BA) and merchantable volume (V) of sample trees

Variables	Mean	Min.	Max.	SD	Variables	Mean	Min.	Max.	SD
DBH (m)	0.88	0.20	2.94	0.55	Height (m)	12.45	1.20	50.20	7.50
Basal area (m²)	0.36	0.017	2.30	0.28	volume (m³)	5.14	0.08	27.08	5.05

(Source: STC, 2016)

Field measurements were entered and tabulated. The volume (V) (m3) of each individual stem was calculated by dividing it into sections of different length (L) (m) and measured the mid diameter (D) (m). Finally, Huber's formula was used to calculate volume of each log (Rawat *et al.*, 2003).  $V = \frac{\pi D^2 L}{4}$ . By using above information, volume for each log were calculated starting at the base and ending at the top of the tree (cumulative values) and then added to get the total merchantable volume *Terminalia arjuna* separately.

Below procedure was used in testing the overall significance of the regression equation. The regression coefficients should be significant at 95% confidence limit (p<0.05) and check out the multicollinearity effect of variables with Variance Inflation Factor (VIF<10). The coefficient of determination (R<sup>2</sup>) measures the proportion of variation in the dependent variable that is explained by the behavior of the independent variables. In order for the model to be accepted, the adjusted R<sup>2</sup> value must be high i.e >50%[8]. The each model was selected by examining p-values of Anderson-Darling statistic (the data is normal at p-value > 0.05) to determine the normality of residuals and Durbin-Watson statistic (Dw) which assists to detect autocorrelation in the residuals. Finally the best models were validated by using residual plots (predicted versus residuals), correlation between measured versus predicted volumes (r), MAPE (Mean Absolute Percentage Error) values and RMSE. The Root Mean Square Error (RMSE) must be relatively samll for the model to be acceptable for volume estimation<sup>[9]</sup>.

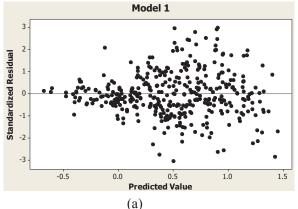
# RESULTS AND DISCUSSION

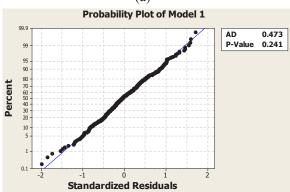
Various linear regression volume equations were developed for *Terminalia arjuna* (Kumbuk) hence the models were assessed and validated to ensure their adequacy for the prediction of merchantable volume of tree species.

The best fitted two models were selected by consideration of the higher adjusted coefficient of determination (R<sup>2</sup><sub>adj</sub>). Insignificant variables were not selected and developed models contained variables with significant parameters and significance of the regression coefficients (p<0.05) at 5% level. The calculated variance inflation factor (VIF) is less than 10 implied about absence of multicollinearity. Durbin-Watson (DW) statistic for the resulted equation around 2. Anderson-darlin normality test show in Figure 1 (c) and normal distribution of residuals due to p value is more than 0.05. Some tree modeling studies observed that, not normal distribution of residuals and it was explained as an inevitable phenomenon for forest population due to the nature of the growth process<sup>[11;12;13]</sup>. The models could be validated by the residual plots, correlation between predicted versus measured values (r), MAPE values and RMSE. Root Mean Square Error (RMSE), MAPE, and correlation between measured versus predicted were used to determine the accuracy of the developed models and RMSE, MAPE values were very low for developed equations. This was eleborated by the report of<sup>[9]</sup> who explained that RMSE estimation is a common measure of model fitting in regression models and low values indicating better fit. Simillar parameters were used by<sup>[5]</sup>and<sup>[10]</sup> to select the best fitted models to estimate the wood volume. Standard residuals of the fitted models for Terminalia arjuna was plotted against predicted values in Figure 1(a). Except few points, almost majority of the values were in between -2 to +2 and referring to that there are only very few unusual data points. The residual plots of predicted versus residuals was elaborated the best volume model performances and it shows the random distribution or no pattern of the residuals. The predicted values of *Terminalia arjuna* was plotted against measured volume and Figure 1(b). According to that, the higher correlation coefficient was recorded for all develop models. It suggested that the developed model had a good fit for tree species.

 $R^2$ DW **Developed equations** Model Adi P value of **RMSE MAPE**  $\mathbb{R}^2$ Anderson-Darlin No % **Normality Test**  $\ln V = -1.50 + 0.572 \ln (D) + 1.06 \ln (H) + 1.1 (BA)^2$ 90.3% 1.931 90.3% 0.256 0.100 0.26  $\ln V = -0.87 + 1.04 \ln (D) + 0.908 \ln (Ht)$ 83.1% 83.1% 1.768 0.085 0.180 1.63

Table 2. Developed equation and the evaluation criteria for *Terminalia arjuna* (kumbuk)





Model 1
Y= 0.0524 + 0.9253 X

R-Sq 88.2%
R-Sq(adj) 88.2%
r 0.939
P-Value 0.000

(b)

Figure 1 (a) The plot of Predicted versus Residual value (b) The plot of Measured versus Predicted volume (c)The probability plot (Anderson-Darlin Normality test) of *Terminalia arjuna* for best fitted model 1

#### CONCLUSION

Model 1 is the best fitted developed model for volume estimation for *Terminalia arjuna* (Kumbuk) and that equation can be applied with relative ease under applications

 $In(V) = -1.50 + 0.572 \ln(D) + 1.06 \ln(H) + 1.11BA^{2}$ 

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(c)

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