Plankton Community Structure in Saguling Dam

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ABSTRACT

Saguling Dam is a semi natural man-made ecosystem which is located at Bandung District, West Java. For the time being, the characteristic of Saguling Dam is changing because of human activity, such as floating net cage (FNC) activity. FNCs on Saguling Dam increase organic waste level at Saguling Dam. This causes plankton communities to change around the FNCs on Saguling Dam. Plankton communities can be used as an aquatic biologic parameter. Aspects that were inspected are the composition, abundance, and diversity of plankton species. This research's goal is to compare plankton communities at 3 different state of FNC: dense, distant, and vacant; at three different lake depths: 0, 3, and 6 meter(s) below the surface. Plankton communities on each point of point sample is tested using Sorensen's index. This research's conclusion is that the diversity index at Saguling Lake is at medium level; the highest plankton abundance is at 3 meters depth, and the highest predominant genus for phytoplankton is *Trachelomonas* and for zooplankton is *Paradileptus*. Sorensen's index results show that there are similiarities of plankton communities at several point samples.

1. INTRODUCTION

Saguling Dam is a semi-natural man-made ecosystem which is located at Bandung District, West Java. The construction of Saguling Dam is done by stemming the flow of Citarum River. The condition of Saguling Dam is constantly changing over time, both naturally and based on human activities in the riparian (DAS) mainly due to the activity of floating net cages (FNC)^[1]. The existence of FNC increases waste content of Saguling Dam, which took shape in the form of organic waste dominated by nitrogen and phosphorous compounds that derived from fish feed [2].

All accumulated organic waste can affect the community of plankton living in the Saguling Dam. These wastes can increase the content of nitrogen and phosphorus compounds in the waters that affects the abundance and plankton community structure [6]. Therefore, this study was conducted to determine the effect of FNC on plankton community structure as the biological parameter of waters in Saguling Dam.

2. METHOD

Study Area

The research took place at the Saguling Dam, Bandung District, West Java. In the Citarum River cascade dam system, Saguling Dam is located in the most upstream area compared to the other two Dams, namely Cirata Dam and Jatiluhur Dam. Geographically, Saguling Dam is located at 107°21'58.42" E and 6°54'46.3" S ^[3]. At the study area, we sampled a total of 9 sites in the three different types of FNC: dense, distant,and vacant. Description of research area is shown in **Fig. 1**.



Fig. 1 Map of Saguling Dam^[3]

Activities Description

Data sampling was done on November 14th, 2017 at three different types of FNC: dense, distant, and vacant. Data analysis was done at Laboratory of SITH ITB on 15 - 30 November 2017.

Plankton Sampling & Water Sampling and Physical-Chemistry Parameter

Plankton sampling was done by towing method using water sampler then filtering water using plankton nets. Sampling was conducted in 3 sites with three different lake depths: 0, 3, and 6 meter(s) below the surface. The samples were poured into the film bottle and preserved later identification. Dissolved oxygen (DO), temperature, pH, ammonium, nitrite, nitrate, and orthophosphate were measured as physical-chemistry parameter of water.

Determining the Abundance, Diversity, and Dominance of the Plankton

1 mL of water sample containing plankton from each location and each depth was dropped into Sedgewick rafter, then observed under microscope. Plankton abundance was determined by the following equation that were developed by APHA^[4],Plankton diversity index was determined by the formula of Shannon-Wiener index^[5].Plankton dominance index was determined by the formula of Simpson index

^[6].The similarity of planktons in the two locations was determined with the formula of Sorensen Similarity Index (Is) ^[10].Further analysis was done by using multivariate analysis with MVSP software.

3. RESULTS

Plankton Community Analysis

Fig. 2 showed that the highest total phytoplankton abundance was found on distant stage of FNC region in 0 m depth (74.875 cell/mL).



Fig. 2 Total phytoplankton abundance in three FNC stations

Total abundance of zooplankton in Saguling Dam is shown on **Fig. 3**.



Fig. 2 Total phytoplankton abundance in three FNC stations

The highest total abundance of zooplankton (11 cells/mL) was found at distant stage of FNC in the depth of 3 meters. *Biological indexes*

Phytoplankton's biological indexes that could be analyzed from the results are shown on **Table 1**. Zooplankton's biological indexes that could be analyzed from the results are shown on **Table 2**.

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Cathan had	HC.	5	D	E	Hŕ.	5	0	E.	H	5	D	T.
0	1.92	21.00	0.28	0.63	2.25	22.00	0.14	0.73	2.48	21.00	0.13	0.81
1	2.91	33.00	0.08	0.83	2.08	17.00	0.16	0.74	2.98	36.00	0.08	0.83
6	2.68	23.00	0.08	0.85	2.71	21.00	0.08	0.89	2.10	24.00	9.07	0.66
Т	able	e 2 Si	ımm	ary	of zo	opla	nkto	n bi	ologi	ical i	ndex	
Depth and		High Det	isity FNC		- J	Moderate 0	ensity FN	¢	Non-FNC			

Table 1 Summary of phytoplankton biological index

Similarity Coefficient

The points of observation which has the most similar phytoplankton are the high density FNC at 3 m depth with 6 m depth. Its similarity coefficient (Is) value is 0.691. The points of observation which has the most similar zooplankton is the dense FNC in 3 m depth with the vacant FNC in 3 m depth with 0.667 as its similarity coefficient (Is) value.

Waters Physical and Chemical Condition Analysis

The water temperature at Saguling Dam decreases along with usage of the water for FNC. Water temperature in Saguling Dam was approximately at 30° C. Temperature was declining at 0 m – 3 m, and at 3 m – 6 m. Water pH in

Saguling Dam was approximately at 6.5 - 7.3. The DO level at Saguling Dam was approximately at 2.5 - 5 ppm. The area with the highest DO concentration was distant FNC area. The highest concentration of ammonium was at 6 m depth in the non-FNC area and lowest ammonium concentration was at 0 m depth in the dense FNC area, and highest nitrate concentrations were found in non-FNC areas and the nitrite concentrations were not stable.

4. DISCUSSION

Plankton Community Analysis

The depth of 0 m in the lake ecosystem belongs to the epilimnion layer or 'mixed layer'. This layer gets the best light intensity especially during the daytime. Phytoplankton tends to move on the water surface (horizontal movement) to get enough light to support the process of photosynthesis. If supported with adequate nutrients such as in the distant type of FNC region, the phytoplankton will have a high abundance in the epilimnion layer ^{[7][8]}.

The highest total abundance of zooplankton (11 cells/mL) was found in Station 2 in the depth of 3 meters. This is due to the vertical migration pattern of zooplankton; in daytime, zooplanktons tend to stay at the epilimnion layer of the lake (0-5 meters deep) and will move towards hypolimnion layer (5-10 meters deep) at nighttime ^[9].

Biological Indexes

The three stations generally have moderate diversity. This explains that Saguling Dam's phytoplankton community structure has enough productivity, balanced ecosystem, and currently under moderate ecological pressure ^[10]. This is in line with the fact that the Saguling Dam is the location of many human activities (e.g. fisheries and aquaculture, also recreation) - this may be the cause of the 'ecological pressure' mentioned before ^[11]. Index of phytoplankton evenness in all three stations indicates that all phytoplankton are distributed quite evenly until almost evenly [12]. Therefore, it can be concluded that the stability of a community in different spots and depth of sampling is moderate ^[12], due to no existence of a species that dominates the others ^[13] (equal proportions of individual numbers). There are no dominance existed - dominance exists if the value of the index of dominance is close to $1^{[15]}$.

In all three stations, zooplankton community generally have low to moderate diversity. This is caused by the fact that the Saguling Dam is the location of many anthropogenic activities – the same thing that affects phytoplankton's diversity ^[11]. On the other hand, zooplankton's index of evenness is similar with phytoplankton's. On stations with greatly even distribution of individuals, the index of evenness will be greater – and vice versa ^[13]. There was one spot with exactly only one species of zooplankton (High density FNC in 6 meters deep); therefore the evenness index of this spot cannot be calculated. There are also some sampling spots with dominance of one species at each spots: high density FNC in 6 meters deep and moderate density FNC in 3 meters deep. In high density FNC 6 meters deep, the dominance was due to the existence of only one species of zooplankton; and in moderate density FNC 3 meters deep, the dominance was of the species *Brachionus* sp.

The relative abundance of species is used to determine the predominant phytoplankton species. It was found that the genus of predominant phytoplankton in dense FNC station at 6 m, 3 m and 0 m depth respectively are Trachelomonas (13,776%), Chroococcus (21,505%), and Trachelomonas (50%). Trachelomonas are quite common in freshwater environments, because euglenids are commonly found in water habitats containing both high and low soluble minerals with varying pH and light levels, especially in swamps and wetlands that have a lot of organic matter ^[16]. In moderate density FNC station, the predominant genus that was found at 6 m, 3 m and 0 m depth respectively are *Dictvosphaerium* (15.574%),Cryptomonas (21,939%) and Euglena (22,286%). In non-FNC stations, the predominant genus at 6 m, 3 m and 0 m depth respectively are Trachelomonas (19,412%), Trachelomonas (19,412%) and Cryptomonas (29,6%). Overall, the predominant genus on this station is Trachelomonas that were found at a depth of 6 m and 3 m. This fact is possible because at this point in this station the nutrient content is high enough. Trachelomonas is a cosmopolitan organism that could become a good indicator to identify moderately contaminated waters ^[30].

The relative abundance of species is used to determine the predominant zooplankton species. The predominant zooplankton genus that was found in the Saguling Reservoir area in dense FNC station at 6 m and 3 m could not be concluded – the genus that was predominant at 0 m depth is Paradileptus (45.455%). In distant FNC station, the predominant zooplankton genus at 6 m, 3 m and 0 m depth respectively are Paradileptus (50%), Brachionus (93.023%), Anuraeopsis (28,571%). At vacant-FNC station, the predominant zooplankton genus at 6 m, 3 m and 0 m depth respectively are Glochidium (60%), Askenasia (54.167%) and Cestum (28.571%). Paradileptus is the genus that has the highest frequency of appearance, which appeared at 2 out of 9 observation points. Paradileptus can be identified with the existence of cilia that contained on part or all of the body surface^[31].

Similarity Coefficient

Combination with other observation points made the Sorensen's similarity coefficient score lower: below 0,5. This phenomenon indicated that the environmental condition and difference of depth will represent different species too, because every species in nature develops different adaptation mechanisms and tolerance towards their habitat^[14].

5. CONCLUSION

The index of diversity in the three stations in Saguling Dam is at medium level. The highest abundance of plankton is at 3 m depth. The predominant genus of the Saguling Dam is *Trachelomonas* for phytoplankton and *Paradileptus* for zooplankton. Sorensen's index result show that are similarities of plankton communities at the dense FNC at 3m with 6m depth, distant FNC station with non-FNC station at 0 m depth, distant FNC station at 3 m depth with 6 m depth, dense FNC station at 3 and 6 m depth with distant FNC station and vacant FNC station at 0 m depth, and finally at those points above with vacant FNC station at 6 m depth.

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Microbial competition for acetate: an important factor to mitigate methane emission from *Sasa*-invaded wetland soils

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Keywords: Sasa-invaded wetland soil; stable isotope probing; acetate; uncultured microorganism

ABSTRACT

Acetate is the most important precursor to CH₄ during anaerobic degradation of organic matters. Wetland soils are a significant source of atmospheric CH₄ and their invasion by Sasa (bamboo) species results in the decrease of the CH₄ emission. However, the microbial interspecies competition for acetate is largely unknown. Here, we identified actively acetate-assimilating microorganisms by ultra-high-sensitivity rRNA-stable isotope probing with ¹³C-labeled acetate. The Sasa-invaded soil slurry was amended with ¹³Cacetate at a concentration of 5 mM and afterwards incubated anaerobically in the dark at 25°C for 2 weeks. A gradual degradation of the acetate amended was accompanied with the production of CH₄ and CO₂ during the incubation. RNA was extracted from the slurries at week 2 and density-separated by isopycnic centrifugation according to molecular weight. Microbial communities in density fractions of rRNA were comprehensively screened by high-throughput Illumina sequencing. Methanosaeta spp. responsible for aceticlastic methanogenesis were significantly labeled with the acetate ¹³C. Notably, 34 different species of Bacteria were identified as the active ¹³C-acetate assimilators. One of them was closely related to the dehalo-respiring Dehalogenimonas alkenigignens with acetate-oxidizing activity. The others were phylogenetically novel, exhibiting quite low sequence similarities (i.e., 77.8%-92.5%) of 16S rRNA genes from cultured relatives. The result of this study demonstrated for the first time that hitherto unidentified bacterial species as well as a known dehalogenating bacterium significantly assimilated acetate, thereby being competitive with aceticlastic methanogens in the Sasa-invaded wetland soils.

1. INTRODUCTION

Acetate is an important precursor to CH₄ during anaerobic degradation of organic matters, and it is mostly utilized by aceticlastic methanogens^[1]. Wetland soils are a significant source of atmospheric CH₄ and their invasion by Sasa (bamboo) species results in the decrease of the CH₄ emission (Table 1)^[2]. The potential competitors of aceticlastic methanogens are microorganisms catalyzing anaerobic acetate oxidation. However, this reaction is highly endergonic (ΔG° =+105 kJ mol⁻¹). The diversity and ecology of the acetate-oxidizing microorganisms are largely unknown.

Table 1 Environmental	characteristics in	the study	site ^[2]
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Gas flux $(g-C m^{-2} y^{-1})$	Sasa-invaded wetland (Vegetation type: Sasa palmata)	Wetland (Vegetation type: <i>Sphagum</i> spp.)
CH ₄	$6.6~\pm~6.2$	13.2 ± 3.5
CO ₂	$223.8~\pm~3.5$	5.8 ± 31.1

Recently, we have developed ultra-high-sensitivity

stable isotope probing of rRNA (rRNA-SIP) to thoroughly characterize the metabolic function of uncultured microorganisms in natural environments ^[3]. The objective of this study was to identify actively acetate-assimilating microorganisms in anoxic *Sasa*-invaded wetland soil by ultra-high-sensitivity rRNA-SIP with ¹³C-labeled acetate.

2. METHOD

A *Sasa*-invaded wetland soil was collected in Bibai, Hokkaodo, Japan. Soil slurry was prepared by mixing the soil with sterilized water at a ratio of 1:4 (v/v). Aliquots (20ml) of the homogenized slurry were transferred anaerobically into 50-ml serum vials. The head space of each sample vial was flushed with N₂. Four soils were produced: (i) a non-autoclaved soil amended with ¹³Clabeled acetate to a final concentration of 5 mM (¹³C treatment), (ii) a non-autoclaved soil with amended with unlabeled acetate to a final concentration of 5 mM (unlabeled treatment), (iii) a non-autoclaved soil with addition of sterilized water in place of acetate (no acetate addition), and (iv) an autoclaved soil amended with unlabeled acetate to a final concentration of 5 mM (heatkilled treatment). All treatments were run in triplicate with static incubation for 4 weeks at 25 °C in the dark.

Samples of the head space gas, slurry water and solidphase soil were taken at week 0, 1, 2, and 4 from each vial. Total CH₄ and CO₂ in the head space gas were analyzed by dual-detection gas chromatography (GC) with both thermal conductivity and flame ionization detectors as described previously ^[4]. The carbon isotopic composition of gaseous CH₄ and CO₂ were measured with GCcombustion-isotope ratio mass spectrometry ^[5]. The concentration of acetate from the slurry water was determined by high-pressure liquid chromatography ^[4].

RNA was extracted from the slurries at week 2 and density-separated by isopycnic centrifugation (128,000 g for >60 hours at 20°C ^[3]). Gradients of density-separated RNAs were fractionated in triplicate. The heaviest (1H), second-heaviest (2H), and light (L) fractions of RNA with buoyant density of 1.796–1.798, 1.788–1.791 and 1.769–1.772 g ml⁻¹, respectively, were subjected to RT-PCR. Microbial communities in density fractions of RNA were comprehensively screened by high-throughput Illumina sequencing of V4 region of 16S rRNA transcripts. The sequences in each sample were characterized phylogenetically using the QIIME software ^[6].

DNA was extracted from the slurries at week 0 of the ¹³C treatment and was used as PCR template for PCR amplification of the V4 region of 16S rRNA genes. Then, Illumina sequencing was conducted.

3. RESULT AND DISCUSSION

Under both the ¹³C and the unlabeled treatments, biogeochemical activities were almost same. With respect to the slurry water of the both treatment samples, the acetate concentration decreased to 1.44-1.64 mM at week 2 and was completely depleted at the end of the incubation. On the other hand, the acetate concentration kept constant in the heat-killed treatment. In the gaseous phase of the two treatments, CH₄ and CO₂ were produced after the beginning of the incubation. The concentrations of CH₄ and CO₂ increased by 0.778-0.784 mM and 1.986-2.106 mM, respectively, at week 2. The production of CH₄ and CO₂ in the control treatment (no acetate addition) was less than those in the ¹³C and unlabeled treatments. These data indicated that acetate was converted to CH₄ and CO₂ by soil microbial communities. The fate of ¹³C-labeled acetate was traced by monitoring the ¹³C atom percentage of the gaseous products CH₄ and CO₂ over the incubation time. ¹³CH₄ and ¹³CO₂ were produced from the start of the incubation and increased by 1.14 mM and 0.74 mM at week 2, respectively.

We phylogenetically characterized the 1H, 2H, and L

fractions of RNA from the ¹³C and unlabeled treatments by high-throughput Illumina sequencing. The total number of 16S rRNA molecules analyzed in 18 density fractions was around 3.1 million, corresponding to an average of 173,000 sequences per library. Phylum and class level phylogenetic analysis indicated that no significant changes with the increase in BDs were observed in either treatment. Nevertheless, high-resolution operational taxonomic unit (OTU)-level survey identified 36 microbial OTUs exhibiting significantly higher relative abundances in the ¹³C treatment in the than unlabeled treatment. Methanosaeta for spp. responsible aceticlastic methanogenesis were significantly labeled with the acetate ¹³C. Notably, 34 different species of Bacteria were identified as the active ¹³C-acetate assimilators. One of them was closely related to the dehalo-respiring Dehalogenimonas alkenigignens with acetate-oxidizing activity. The others were phylogenetically novel, exhibiting quite low sequence similarities (i.e., 77.8%-92.5%) of 16S rRNA genes from cultured relatives. As a result of analyzing the relative abundances of the 36 ¹³Cincorporating OTUs at week 0, the identified acetate oxidizer OTUs were low-abundance (< 0.15% of the total).

4. CONCLUSION

This study demonstrated for the first time that hitherto unidentified low-abundance bacterial species as well as a known dehalogenating bacterium significantly assimilated acetate, thereby being highly competitive with aceticlastic methanogens in the *Sasa*-invaded wetland soils.

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P1-3

A New Hypothesis for the Declining Cause of the Japanese Eel.

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Keywords: Japanese Eel, Declining Cause, L. Kasumigaura, The Tone R., Water gates, Water resource Development.

ABSTRACT

A hypothesis to explain the cause of the decline in Japanese eel *Anguilla japonica* is proposed ,based on the analysis of catch trends and information on environments downstream of the Tone River. It was evident that the Tone River used to contribute more than 80% of the total spawning. Spawning migration was inhibited by a water gate constructed in 1963, and by the complete closure in 1975. Based on this, a hypothesis was proposed, stating that the Japanese eel decline was caused by the inhibition of spawning and upstream migration of eels into lakes, which were used as habitats, due to the water resource development projects. Support for this hypothesis was obtained by examining the parent-child relationship and the influences of the project on catches in areas other than the gate.

1. INTRODUCTION

The catch of Japanese eel, one of the most important fishery resources in Japan, has declined greatly since around 1970. The causes have been studied focusing on the three hypotheses: excessive fishing, regime shift of the ocean [1,2], and the environmental destruction of rivers and lakes [3], but the exact cause has not yet been determined. .This study attempted to obtain a new hypothesis for the causes of the resource decline in the Japanese eel A. japonica by studying fishery information downstream of the Tone R., the greatest fishing ground and once shared about 30% of the eel catch and 80% of glass eels. First, a hypothesis on the decline cause was suggested by examining the characteristics of temporal changes in catches in two different sections of the Tone River system and in other areas. The hypothesis was examined using the parent-child relationship and the influences of development projects on catches in areas other than the Tone Rover system..

2. METHOD

The location downstream of the Tone River is shown in Fig. 1. The Tone River is 322-km long, has a drainage area of 16,840 km². Lake Kasumigaura is the second largest shallow eutrophic lake in Japan with a drainage area of 2,157 km².



Fig.1. Location of douwnstreem of the Tone R.

Approximately 1000 fishermen fish for eel, pond smelt, gobies, and shrimp so on in the lake, which was originally brackish with indirect connections to the Pacific Ocean via the Hitachitone and Tone Rivers. In 1963, the Hitachigawa gate was constructed at the outlet of the lake to accelerate water resource utilization The construction of the gate started in 1959 and was completed in 1963. The gate was occasionally closed from 1964 to 1974, and has been completely closed to all but downward flow since 1975. Since then, the water in the lake has been maintained 0.3-m higher than before the water resource was supplied, in accordance with the development plan, from 1996.

Catch data. The data are from "The Annual report of catch statistics on fishery and aquiculture" (Ministry of Agriculture, Forestry and Fisheries..In "The Annual report of catch statistics on fishery and aquiculture", catches are recorded as "eel" or "seed." Here, "eel" represents individuals weighing over 150 g, which are edible, and "seed" represents glass eel.

3. RESULTS

The changes in catches and the ratio of the Tone R. system to the total catch The catches are shown in Fig. 2 and the ratio of the Tone R. system to the total catch in Fig. 3. The proportion of the Tone River system catch to the total eel catch fluctuated around 30% until 1975, began to decline in 1976. The ratio of total glass eel was 84% in 1962, but declined since 1970.

Relationship between the eel catch in Lake Kasumigaura and the total glass eel catch. The relationship between the eel catch in Lake Kasumigaura and the total glass eel catch is shown in Fig. 4. There was a positive correlation between both. The relationship is given by the following equation:

$$Y_{GW} = 0.69 X_{EK} + 13 \ (R^2 = 0.92)$$

This suggests that Lake Kasumigaura may provide a large proportion of parents for spawning.

4. DISCUSSION

Characteristics of the Tone River system. The ratio of the Tone River system catch to the total glass eel catch exceeded 70% before the construction of the gates..In addition, the total glass eel catch is proportional to the eel catch in Lake Kasumigaura. Therefore, the main decline cause may be attributed to the use of Lake Kasumigaura for water resource development.

A new hypothesis. There may be another more likely reason than conventional ones. Thus, the hypothesis states that the decline was caused by the Kasumigaura Development Project.

Verification of the hypothesis. Evidence to support this is needed to verify the following two examinations.

A. Use of the Hitachigawa Gate caused the decrease in the eel catch in areas other than the Tone River system.

B. The correlation exists between the parent of the Tone River system and the total glass eel catch, and no correlation between eel in other areas and the total glass eel catch.

Examination A. The effect of the gates on catches in other areas could be caused by the decline in glass eel recruitment, because recruitment is dependent on a common origin of spawning [4], which is largely influenced by the eel resource of the Tone River system. Therefore, the catches in other areas must have declined in 1963 (completion of the gate), 1975 (start of complete closure of the gate), and 1996 (start of water resource administration). Fig. 5 shows the temporal changes in the total eel catch in other areas except for the Tone River system. The eel catch decreased drastically in 1970, 1982, and 2003. The times 7 years exceed these fallings agree with these.. Two of those 7 years are for spawning and transportation of larvae to the Japanese archipelago, and for growing to parents. Hence, there is evidence to support examination A.

Examination B The second examination aims to confirm that a correlation exists between parent fish of the Tone River system and glass eel recruitment. The relationship between the eel catch in the Tone River system and the total glass eel catch 2-years later across the whole country was examined in Fig.6 (left). In Fig. 6 the open circles represent the catches in 1964–1970, during the construction of the Tone River Mouth Gate. Hence, the correlation was estimated using logistic regression, with the exception of data from the construction period of the gate. This is given by the following equation (R^2 =0.89):

$$Y = Y_{\text{max}} / (1 + exp(aX + b))$$
 (1)

where *Y*, Y_{max} , *X*, *a*, and *b* represent the glass eel catch 2-years later, the maximum of *Y*, the eel catch in the Tone River system, and coefficients. The values *X*, *a*, and *b* are 247, -0.0060, and 3.53, respectively. This relationship confirms that the parent fish in the Tone River system result in larvae recruitment across the whole country. Fig.6 (right) shows the relationship between the glass eel

catch in the whole country 2-years later and the eel catch in other areas except the Tone River system. There is no clear correlation between the both. This shows that the other areas have little effect on the recruitment of glass eel. However, a detailed analysis revealed that a weak correlation exists when the catch is below 1250 t.

This relationship can be also expressed by equation (1), and Y_{max} , *a*, and *b* are 24, -0.002, and 1.24, respectively. The relationship between the eel catch and the glass eel catch corresponds to the parent-child relationship of the Japanese eel, and can be estimated using a logistic curve. The Y_{max} values and the coefficients correspond to the carrying capacity, and the population growth rate. The carrying capacities of glass eel in the Tone River system and the other areas were estimated to be 247 and 24 t. Hence, the effect of the other areas will be essentially less. Therefore, it can be concluded that the new hypothesis mentioned above may be effective.

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レンコン品識別法開発とポリフェノール含量の多様性

Development of a method for lotus variety identification and variations in polyphenol contents

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キーワード: 生物利用, 農業, 食糧生産, レンコン, DNA マーカー

抄録

ハス (*Nelumbo nucifera*)の肥大した根茎はレンコンと呼ばれ野菜の一つとして消費される. レンコン生産の ために栽培されるハスの品種群もレンコンと称される。霞ヶ浦周辺はレンコン生産が非常に盛んな地域であり, 日本の生産量の約半分を出荷している。レンコンは主に根茎で増殖するため、品種の識別が困難であることか ら、DNA マーカーによる品種識別法の開発を行った。一塩基多型を HRM 法によって識別したところ、レン コン品種群は 6 個のマーカーで2 8 の遺伝子型に識別された。また、レンコンの食品としての機能性向上を目 的として、東京大学が保有する観賞用ハス(花蓮)のコレクションにおいて根茎のポリフェノール含量を調査 した。節と節間を凍結乾燥した後、ポリフェノールを抽出し、フォーリンチオカルト法で濃度を測定したとこ ろ、花蓮はレンコン品種群と比較して多様性が高く,有望な育種素材であることが明らかとなった。

1. はじめに

ハス のうち地下茎が肥大する品種群はレンコンとして 食用に栽培されており、観賞用である花ハスと区別され る。レンコンの生産出荷額は茨城県が1位であり、霞ヶ浦 周辺で栽培されている。日本の各産地ではそれぞれ特 徴のあるレンコン品種群が栽培されているが、ハスは地 下茎で増殖するため、他の品種の混入、種子からの実 生に由来する個体の混入を把握することが困難であり、 品種の維持管理が容易ではない。そのため、レンコンで もDNAマーカーを開発し、品種識別法の開発や類縁関 係の解明を行う必要がある。そこで本研究では、近年、 報告されたハスゲノムの概要塩基配列情報(Ming et al. 2013^[1], Wang et al. 2013^[2])を活用し、ddRAD-seq 解析 を行って得られた一塩基多型(SNP)情報に基づく DNA マーカーを作製し、栽培されているレンコン品種におけ る品種識別法を開発した。以上に加え、花ハスのポリフ ェノール含量を調べることにより高ポリフェノール含量を 持つレンコンの育種材料の探索も行った。

2. 方法

ハスゲノム概要配列から SNP を含む塩基配列を得て BLAST 検索し、ゲノム内で複数コピー存在しないものを 選抜した。次に、Primer3を用い PCR 産物のサイズが 80 bp から 110 bp となるよう SNP を挟むプライマー対を設計 した。プライマーの塩基配列は再度 BLAST 検索を行い、 標的配列がユニークであるものを用いた。実験にはレン コン 42 系統、花ハス 2 系統、キバナハス (*N. lutea*) 1 系 統を用いた。遺伝子型の決定は、LightCycler 96 システ ム(ロッシュ)を用い、High Resolution Melt (HRM)解析に よって行った。得られた各 DNA マーカーの遺伝子型を もとにデータマトリックスを作成し、ソフトウエア Past 3.18 (Hammer 1999-2017)を用いて UPGMA 法によるクラスタ 一解析を行った。

ポリフェノール含量の測定に関しては、東京大学大学 院農学生命科学研究科 附属生態調和農学機構で維 持・栽培されている花ハス54品種の節と節間をそれぞ れさいの目状に切り出したのち、凍結乾燥させ粉状にし た。その後 80%メタノールを用いてポリフェノール抽出を 行い、これを試料とした。ポリフェノールの測定はフォー リンチオカルト法を用い、100, 200, 300, 400, 600, 800 ppmクロロゲン酸を標準とし、750nm での吸光度をマイク ロプレートリーダー (FlexStation 3 Microplate Reader, Molecular Device Japan Co., Ltd.)を用いて行った。

3. 結果

ddRAD-seq 解析で検出された 4901 箇所の SNP のう ちレンコンの系統間で多型のあった 1248 箇所を調査し た。作製した 351 個の DNA マーカーのうち 191 個の DNA マーカーで多型識別が可能であった。開発された DNA マーカーのうち遺伝子型の識別が良好であった 6 マーカーを栽培されている45系統に用いたところ、28の 遺伝子型に識別することができた。ハスの系統はクラス ター解析で、花ハスとレンコンに分かれたあと、レンコン 内で4つのクラスターに分類された。茨城県および新潟 県で栽培される系統群と主に西日本で栽培されるレンコ ンの系統群が異なるクラスターとして分類された。54系 統の花ハスで節間部で生重量当たりのポリフェノール含 量を測定したところ、最も少ないものは62.91 mg/100 gfw (漁山紅蓮)で最も多いものは735.58 mg/100 gfw (紅ガ ニ) であった。また、節でも同様に測定したところ 1458.72-2929.55 mg/100 gfw の範囲でポリフェノールが 含まれていた。

4. 考察

調査した 10 マーカーでは品種名が異なるにもかかわ らず遺伝子型が同一、または極めて類似したグループ が 6 つあった。逆に、遺伝子型が異なるにも関わらず、 同一の品種名を持つ例もあった。このような品種名と遺 伝子型の不一致は本研究で開発された品種識別法を 適用することにより解消することが可能である。ポリフェノ ール含量については、今回測定した花ハスコレクション の結果はレンコン品種群(節間部 41.0-131.0 mg/100 gfw、 節部 793.1-1866.0 mg/100 gfw)と比較して多様性が高 く、より濃度の高いポリフェノールを含む系統が存在する ことが明らかとなった。

5. 結論

本研究の結果、少数の DNA マーカーセットで既存レ ンコン品種の多くを識別可能であることが実証された。ま た、花ハスはレンコン品種群と比較してポリフェノールを 多く含む系統が存在し、有望な育種素材であることが明 らかとなった。

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Water quality and fisheries of the Bui Reservoir, Ghana: Five years after impoundment

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Keywords: Limnology, dams and reservoirs, fisheries, ecology, biology, water quality

ABSTRACT

Ghana has derived many useful benefits from reservoirs including hydroelectric power generation, potable water supply, fisheries, irrigation, transportation and raw water supply for domestic, industrial and agricultural use. The Bui Dam is the third to be created in the Volta River System for hydroelectric power generation after the Akosombo and Kpong dams and has a surface area of 444 km². The study is the first assessment of the water quality and fisheries of the Bui Reservoir five years after impoundment. Field visits were undertaken between October and December 2016. Profiles of pH, temperature, dissolved oxygen, chlorophyll-a and turbidity were measured in situ using the YSI EXO II Sonde. Total Nitrogen and Total Phosphorus were determined using the Hach digestive methods. The fishery assessment was undertaken using a battery of multifilament and monofilament gill nets of laterally stretched mesh sizes of 12.5 mm -40.0 mm and 38.1 mm - 177.8 mm respectively. The water quality profile assessment showed that the reservoir was thermally stratified. Mean temperature difference between surface and bottom water was 4.5° C. The water quality in the epilimnion was distinctly varied from that in the hypolimnion in relation to the physical parameters and chlorophyll-a but the nutrients showed minimal variation in concentrations between the surface and bottom water. The fisheries assessment revealed a decline in fish species composition by 57 % and a shift from riverine to lacustrine species as well as a shift in trophic dominance from piscivores to benthic omnivores when compared to the pre-impoundment results.

1. INTRODUCTION

The construction of dams for hydroelectric power and irrigation tends to alter the ecosystem from riverine to lacustrine conditions leading to major changes in water quantity, quality and flows, nutrient inputs and balances as well as fish habitats and fish assemblage [1,2]. Due to their storage, dams are believed to change river discharges to a much greater extent than any adjustment expected from global climate change [3].

The Bui Dam is the third to be created in the Volta River System for hydroelectric power generation after those at Akosombo and Kpong in Ghana, West Africa. The dam is built on the River Black Volta at the Bui Gorge and has a surface area of 444 km² at full supply level with capacity to generate 400 Megawatts of electricity. The dam was impounded in June 2011 and commissioned for operation in December 2013. Although the dam has been in existence for about five years, very little information exists on its water quality status and impacts the modification in habitat may have had on the fisheries of the dam. The study assessed the water quality and changes in fisheries of the reservoir. The fish species composition data were compared with results obtained from the preimpoundment study carried just before impoundment.

2. METHOD

Seven sites were selected on the Bui Reservoir (Fig 1) for water quality assessment in the post-rainy season between October 2016 to December 2016. Two water samples were collected per site: 1m below the surface and 1m from above the bottom of the reservoir using a Van Dorn water sampler.

Depth profiles of water temperature, pH, dissolved oxygen (DO), conductivity, turbidity and Chlorophyll-a (Chl-a), and Total Dissolved Solids (TDS) were measured in situ using the YSI EXO II Sonde. Total Nitrogen (TN) and Total Phosphate (TP) were determined using the Hach persulphate digestion and Molybdovanadate with Acid Persulfate Digestion Methods respectively.

Sampling for fish assessment was undertaken at sites 1, 2 and 3 (Fig 1) which were selected to coincide with sites for the pre-impoundment study. Multifilament and monofilament gill nets of laterally stretched mesh sizes of 12mm - 40.0 mm and 38.1 mm - 152.4 mm were used in sampling fish. The nets were set at dusk and retrieved at dawn the following day at each station.

The catch at the end of each sampling session was identified with the keys of [4, 5,6], sorted into various species, and counted. Each individual fish was weighed to the nearest 0.1 gramme (g) using a measuring scale while standard length and total length were measured to the nearest 1.0 millimetre (mm) using a measuring board. The catches of local fishermen were also observed.



Fig 1: Sampling sites on the Bui Reservoir

3. RESULTS

The depth of water at the sampling sites ranged from 33.8 m to 86.0 m bringing the mean depth of the reservoir to about 50m. Results from the water quality profiles showed the dam was thermally stratified and the water quality in the epilimnion was distinctly varied from that in the hypolimnion in relation to the physical parameters. Surface water temperatures ranged from 29.1 °C to 31.4 °C whiles of the bottom temperature ranged from 25.3 °C to 28.8 °C with an average difference between surface and bottom water of about 4.5 °C (Fig 2). The pH was slightly more alkaline at the surface than at the bottom with values ranging from 7.48 - 7.92 and 6.40 to 7.0 respectively (Fig 2). DO concentrations ranged from 7.93 to 8.78 mg/l at the surface water and declined rapidly with depth till about 20 to 30 m depth, after which the water becomes completely anoxic (Fig 3 and Fig 4). Chlorophyll-a concentrations were typically less than 5 mg/l at all the monitoring sites.

Minimal variations were observed in nutrient concentrations. TN concentrations ranged from 0.56-0.87 mg/l at the surface and 0.82 to 0.87 mg/l in the bottom water (Fig 5). Whiles TP ranged from 0.07 to 0.14 at the surface and 0.07 to 0.09 mg/l at the bottom.



Fig 2: Mean water temperatures at the sites



Fig 3: Dissolved oxygen profile at one of the sites



Fig 4: Mean dissolved oxygen at the sites



Fig 5: Total Nitrogen concentrations at the sampling sites

Thirty-Six (36) fish species belonging to 23 genera and 10 families based on a total of 356 fish samples were recorded in the study area. The major families by numbers and weight are presented in Fig 6 and Fig 7. Cichlidae were dominant by number and Cyprinidae dominant by weight

(Fig 6 and 7). The trophic composition was made up of benthic omnivores (42.12 %) semi pelagic omnivores (25.84 %), piscivores (24.15 %) and aufwuch-detritus and herbivores (7.58 %) based on number and by weight was semi pelagic omnivores (37.61 %) piscivores (29.15 %), benthic omnivores (25.52 %) and aufwuch-detritus & herbivores (6.30 %).



Fig 6: Fish species composition by number



Fig 7: Fish species composition by weight

4. DISCUSSION

Tropical lakes are typically warm monomictic [7]. Water density however responds very well to changes in temperature thus a high temperature results in adequate density difference between the top and bottom water of lakes to maintain seasonal stratification as observed in the Bui Reservoir. Mixing could, however, be expected at a later period as only few lakes are persistently stratified by temperature gradients alone [8]. DO levels in a lake is an important indicator of overall lake health. Although DO concentrations were high at the surface, the levels declined rapidly with depth and this could be attributed to decomposition of submerged vegetation. Per the Chl-a levels, of which they were mostly less than 5 μ g/l but greater than 3 μ g/l, the reservoir water may be described as mesotrophic [9].

The reservoir exhibited very low fish species diversity, very good species richness and very low species evenness indicating a generally weak ecological stability. Compared to pre-impoundment fishery studies, there was a general decline in the number of fish species by about 57 %. The abundance of Tilapia, a member of the *Cichlidae* family has increase from 1 % before impoundment to 45.09 % and 24.55 % based on number and weight respectively after impoundment. The presence of Cichlids (*A. guntheri* and *H. fasciatus*) as well as Claroteids (*C. nigrodigitatus* and *C. auratus*) at the levels of importance recorded is an indication that the two families are becoming very important in the Bui Reservoir as it occurred in Lake Volta and Kpong Reservoirs. The abundance of *A. guntheri* could be attributed to its preference for calmer habitats with submerged cover such as tree roots and fallen branches.

5. CONCLUSION

Water in the Bui reservoir is good quality at the surface but anoxic at the bottom. The number of fish species have declined and there is a shift in fish species composition from typically riverine species to lacustrine species.

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Comparison of Environmental Condition using Biological Indicators in Yatsu Tidal Flat locating in Tokyo Inner Bay

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Keywords: Yatsu Tidal Flat, Environmental conditions, Green tide, Biological index, Water quality

ABSTRACT

From the water quality of the Yatsu Tidal Flat (Funadamari/ Sankaku Tidal Flat), we aimed at to consider to about environment transition of the characteristics of water quality and pollution load factor of Yatsu tidal flat from the results of the water quality. And from the appearance situation of benthos organisms and attached diatoms. Characteristics of the water quality of the Yatsu Tidal Flat Characteristics of the water quality of the Yatsu Tidal Flat were estimated as being polluted progressing because high COD value in Fundamari and Daisankaku ② and ③were high. This is thought to be caused by weak flow than other site. T-P of Shosankaku is high because domestic wastewater was flowing in there. As water quality analysts, COD of Sankaku tidal flats(2.4mg/l) was almost stmilar to that of Tokyo Bay (2.7mg/l). From the results of benthic organisms overall the Funadamari is often evaluated as somewhat dirty in the degree of environmental preservation II, and Sankaku Tidal Flat is evaluated as somewhat clean in the degree of environmental preservation III. The results of DAIpo show that the value of DAIpo decreased in the Funadamari ④ from spring to autumn, but the value of DAIpo rose due to the increase in the number of saproxenous in other points. The Funadamari which is the most inner part of the Yatsu Tidal Flat shows the past figure of Yatsu Tidal Flat and the Sankaku Tidal Flat near Tokyo Bay shows the future of Yatsu Tidal Flat.

1. INTRODUCTION

Yatsu Tidal Flat (Narashino city, Chiba prefecture) is connects with Tokyo Bay by the two canals of the Yatsu River and the Takase River and closedness water area by changes due to the filled of the tide. In recent years, irregular growth Ulva sp. become a problem.

Water quality is evaluated using pH, BOD, SS, DO etc. of the environmental standard value set by the Ministry of the Environment, Japan. Water quality is evaluated by instantaneous value at quality is sampling At the same time, advantages of biologically to investigate water quality can know in the chemical analysis, cannot judge long-term change of the water environment ,by observing creatures living there can know the degree of the ecological pollution tendency of mentioned

In this research, we aimed at to consider to about environment transition of the characteristics of water quality and pollution load factor of Yatsu tidal flat from the results of the water quality . And from the appearance situation of benthos organisms and attached diatoms.

2. METHOD

2.1 Survey site

The survey sites were set to 10 places in total including the Funadamari ① to ④,Daisankaku 1) to 3 and Shosankaku 1), 2 as shown in

Fig.1. 2.2 Survey period On Fiscal Year 2016 – 2017, we conducted in spring,

2.3 Field survey

At each site, water samples were collected using a heyroht water sampling device and poly bin, and NO₂-N, NO₃-N, PO₄-P, and COD were measured by pack test. As the observation parameters, temperature, water temperature, pН, DO. transparency, weather, water depth, flow rate were



Fig. 1 Survey site

measured. Benthic organisms (higher - level consumer) were collected together with sediments four times using an Eckman-purge bottom sampler machine or a quadrat frame of 15 cm × 15 cm and a scop. Sediment was also collected to measure IL, but because there are points where only gravel and shells are collected, only seven site were collected. Adhered diatom (producer) was collected from the surface of shellfish or the like using a $5 \text{ cm} \times 5 \text{ cm}$ quadrate frame and a brush. **2.4 Analysis parameters**

The sampled water was brought back to the laboratory immediately and were analyzed for pH, DO, COD, Chl.a, Cl⁻, NO₂-N, NO₃-N, T-N, PO₄-P and T-P.

2.5 Evaluation method by benthic

From the harvested benthic organisms, according to the method of Kyutokenshi (Table 1), scores were assigned to the four criteria of crustacean ratio, ignition loss, dominant indicator organism in total number of occurrence types ,and we calculated the total points of them.

The evaluation was divided into 5 stages, and the total points were fitted to the score table of the evaluation point evaluation classification (Table 2), and the environment condition was evaluated. The point where sediment can not be collected was evaluated by applying it to another scoring table (Table 2) excluding score by ignition loss. Table 3 shows the classification with provisional expressions each evaluation.

2.6 Evaluation method by diatom

Adherent diatom was collected from the surface of shellfish using a $5 \text{ cm} \times 5 \text{ cm}$ quadrate frame and a brush. DAlpo (Diatom Assemblage Index to Organic Water Pollution), which is an organic pollution index based on adherent diatom assemblage, classified, identified and counted and The river comprehensive evaluation RPId (River Pollution Index based on DCI) was calculated.

$$DAIpo = 50 + ((A - B)/2) \cdots (1)$$

- A : The sum of the relative frequencies of all the saproxenous that appeared at that point.B : The sum of the relative frequencies of all
- saprophilous that appeared at that point.

$$RPId = A/L \cdots (2)$$

- A : The area of the part surrounded by the vertical axis and the line connecting the plotted points. Extension of flow path of investigated river.
- L:

3. RESULTS AND DISCUSSION

The water quality date of COD,Chl.a,Cl⁻, NO₂-N, NO₃-N, NH₄-N, T-N, PO₄-P in T-P in spring, summer, autumn and winter are shown in Table 5 toTable 8.

Evaluation by the benthic organism at each point in spring, summer, autumn and winter are shown in Fig.2. Evaluation by the attached diatoms at each point in spring, summer, autumn

and winter are shown in Fig. 3. From spring to autumn, Funadamari and Daisankaku (2), (3) were polluted. At any point, there are fewer flows than other point and it is thought is easy to accumulate organic matter. The result of DAIpo value of Funadamari ④decreases from spring to autumn. The result of the benthic organism was less species and Environmental preservation degree was lowered. The bottom mud of Funadamari is sludge.

Therefore, it is understood that it is an environment where living things are difficult to live. As a cause, it was suggested that the flow of wastewater from Funadamari ④ may have flowed in. On the other hand, Shosankaku of Sankaku Tidal Flat is connected to Tokyo Bay, and the value of COD was the closest that of to Tokyo Bay. There is no flow in Funadamari, and nutrient salts accumulate and are polluted, but In Shosankaku and Yatsu River, it is thought that it became near the water quality of Tokyo Bay by being mixed with the purification action such as shell and *Ulva* .sp and water of other sites. However, the value of T-P was high.

DAIpo has increased overall value to Funadamari (4), and the water quality has been improved.

As a result of the benthic organisms, environment al preservation degree is higher than Funadamari except for Daisankaku ③, and is considered to be inhabit than Funadamari. From these outcomes, an environment Funadamari which is the most inner part of Yatsu Tidal Flat is thought to represents the appearance of the past of Yatsu

Table 1 Evaluation by Kyutokenshi Method

1	Number of occurrence types in benthic organisms	More than 30 species	$20 \sim 30$ species	$10 \sim 19$ species	Less than 10 species	No biological
	Scores	4	3	2	1	0
2	Percentage of Crustaceans in ①	More than 20%	Less than 10~ 20%	Less than 5~ 10%	Less than 5%	0%
	Scores	4	3	2	1	0
3	Loss on ignition of sediment	Less than 2	Less than $2{\sim}5$	Less than $5\sim 10$	Less than $10 \sim 15$	More than 15
	Scores	4 3		2	1	0
	Dominant pointer biology		A	в	С	D
		Organisms oth	er than B and C	Lumbrineris	raraprionospi	No biological
				longiforia	sp.(typeA)	
				Raeta	Theora lata	
				rostralis		
				Prionospio	Sigambra	
				pulchra	hanaokai	
	Evaluation by dominant species of the top three species	Dominant spec three species C	ties of the top other than B and	Not classified as A, C and D rank	More than two species of C	No biological
	Rank		A	в	С	D
	Scores		3	2	1	0
	※ 1 ∶ If the total	number of occu	rrences types is l	ess than four, the	grade is 1 rega	rdless of the ratio.
	※ 2 ∶ If the total	number of occu	rrences types is l	ess than two spec	ies, the rank C.	

Table 2 Evaluation division by Kyutokenshi Method

Evaluation classification	Score	Evaluation classification	Score
Environmental conservation degree IV	$14 \sim$	Environmental conservation degree N	$12 \sim$
Environmental conservation degree III	$10 \sim 13$	Environmental conservation degree III	$9 \sim 11$
Environmental conservation degree II	$6 \sim 9$	Environmental conservation degree II	6~8
Environmental conservation degree I	$3\sim 5$	Environmental conservation degree I	$3\sim 5$
Environmental conservation degree0	$0 \sim 2$	Environmental conservation degree0	$0 \sim 2$

(considering IL)

(not considering IL)

Table 3Evaluation Expression by Kyutokenshi Method

Evaluation classification	Interim(Expression)			
Environmental conservation	d Clean			
Environmental conservation	l Slightly clean			
Environmental conservation	l Slightly polluted			
Environmental conservation	l Polluted			
Environmental conservation	lExtremely polluted			

Table 4 Relationship of DAIpo, BOD and pollution level

DAIpo	BOD	Pollution level
$100 \sim 85$	$0 \sim 0.0625$	Extremely poor water area
$85 \sim 70$	$0.0625 \sim 1.25$	βpoor water area
$70 \sim 50$	$1.25 \sim 2.5$	αpoor water area
50~30	$2.5 \sim 5.0$	ßmedium toxic water area
30~15	5.0~10.0	amedium toxic water area
15~0	10<	Extremely toxic water area

Table 5 Water quality(spring)

Spring	Unit symbol	Funadamari(1)	Funadamari(2)	Funadamari(3)	Funadamari(4)	Daisankaku(1)	Daisankaku(2)	Daisankaku(3)	Shosankaku(1)	Shosankaku(1)
COD	mg/l	5.2	6.4	14.8	16	6.0	5.2	5.6	6.8	6.4
T-N	mg/l	10.9	6.7	7.0	7.6	4.1	2.1	2.9	3.9	3.9
NH4-N	mg/l	0.40	0.50	0.50	0.30	0.29	0.35	0.30	0.40	0.33
NO2-N	mg/l	0.005	0.0097	0.006	0.000	0.0377	0.0175	0.0187	0.0155	0.0349
NO3-N	mg/l	2.2	2.8	2.1	1.1	2.0	1.3	1.5	1.1	1.4
T-P	mg/l	3.33	3.25	1.10	5.53	1.09	2.32	1.80	6.90	6.30
PO4-P	mg/l	0.97	0.96	0.88	0.30	0.30	0.63	0.49	1.88	1.72
CI-	mg/l	9749.6	4077.1	5318	10104.1	5140.7	5052.1	4697.5	2481.7	2942.6
Chl.a	µg/l	3.0	3.0	1.8	2.8	2.5	2.5	2.7	3.0	4.6
SS	mg/l	21.05	18.10	0.05	0.25	22.10	38.25	56.45	27.90	13.25

Table 6 Water quality(summer)

Summer	Unit symbol	Funadamari(1)	Funadamari(2)	Funadamari(3)	Funadamari(4)	Daisankaku(1)	Daisankaku(2)	Daisankaku(3)	Shosankaku(1)	Shosankaku()
COD	mg/l	6.0	10.4	8.8	7.2	4.0	8.4	8.4	4.4	4.4
T-N	mg/l	7.9	5.8	10.6	6.5	4.9	3.8	4.5	3.6	3.7
NH4-N	mg/l	0.19	0.30	0.40	0.21	0.36	0.24	0.32	0.36	0.34
NO2-N	mg/l	0.0533	0.1396	0.035	0.0825	0.0167	0.0083	0.0101	0.01	0.0094
NO3-N	mg/l	2.5	1.5	3.1	1.6	1.0	0.8	0.9	1.3	1.2
T-P	mg/l	0.36	1.66	0.40	0.41	2.02	1.43	1.43	1.15	2.05
P04-P	mg/l	0.93	0.92	1.58	1.46	1.87	1.94	2.05	2.24	2.00
CI-	mg/l	10281.4	18081.0	12408.6	17017.4	16414.7	15953.9	19499.2	18612.8	17584.7
Chl.a	µg/l	0.9	0.9	1.6	1.5	15.2	3.6	7.3	5.2	5.9
SS	mg/l	37.75	45.55	25.45	21.70	4.10	24.00	16.35	4.25	12.55

Tidal Flat, and Sankaku Tidal Flat near Tokyo Bay represents the figure of the future of Yatsu Tidal Flat.

4. CONCLUSION

1) Characteristics of the water quality of the Yatsu Tidal Flat were estimated as being polluted progressing because high COD value in Funadamari and Daisankaku ② and ③ were high. This is thought to be caused by weak flow than other site. T-P of Shosankaku is high because domestic wastewater was flowing in there.

2) As water quality analysts, COD of Sankaku tidal flats(2.4mg/l) was almost stmilar to that of Tokyo Bay (2.7mg/l).

3) From the results of benthic organisms, the Funadamari is often evaluated as somewhat dirty in the degree of environmental preservation II, and Sankaku Tidal Flat is evaluated as somewhat clean in the degree of environmental preservation III.

4) The results of DAIpo show that the value of DAIpo decreased in the Funadamari ④ from spring to autumn, but the value of DAIpo rose due to the increase in the number of saproxenous in other points.

5) The Funadamari which is the most inner part of the Yatsu Tidal Flat shows the past figure of Yatsu Tidal Flat and the Sankaku Tidal Flat near Tokyo Bay shows the future of Yatsu Tidal Flat.

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Table 7 Water quality(autumn)

Autumn	Unit symbol	Funadamari①	Funadamari(2)	Funadamari(3)	Funadamari④	Daisankaku(1)	Daisankaku(2)	Daisankaku ③	Shosankaku(1)	Shosankaku (1)
COD	mg/l	4.4	5.2	4.8	3.2	4.0	6.4	8.4	4.0	3.6
T-N	mg/l	10.3	8.3	9.1	4.3	5.7	6.1	3.9	4.1	5.0
NH4-N	mg/l	0.65	0.57	0.64	0.20	0.31	0.19	0.15	0.16	0.24
NO2-N	mg/l	0.0778	0.055	0.0609	0.0261	0.0351	0.0266	0.0194	0.0353	0.0296
NO3-N	mg/l	3.8	2.9	3.1	1.6	1.1	1.5	1.4	1.6	1.6
T-P	mg/l	3.32	3.68	2.44	0.60	0.48	0.15	0.19	1.51	1.98
P04-P	mg/l	1.81	0.96	1.61	0.22	0.48	0.15	0.19	1.51	1.98
C⊢	mg/l	9040.5	10547.3	10104.1	18258.3	12231.3	11699.5	14003.9	14358.5	16485.6
Chl.a	µg/l	3.2	4.3	4.6	6.5	2.1	3.7	3.8	1.6	1.3
SS	mg/l	21.05	18.10	0.05	0.25	22.10	38.25	56.45	27.90	13.25

Table 8 Water quality(winter)

Winter	Unit symbol	Funadamari(1)	Funadamari(2)	Funadamari(3)	Funadamari④	Daisankaku (1)	Daisankaku(2)	Daisankaku ③	Shosankaku(1)	Shosankaku(1)
COD	mg/l	3.2	4.4	4.4	3.2	4.4	4.0	5.6	2.4	3.2
T-N	mg/l	12.9	15.4	6.4	4.1	11.0	13.7	4.9	4.7	5.3
NH4-N	mg/l	0.58	0.37	0.32	0.19	0.44	0.51	0.16	0.16	0.16
NO2-N	mg/l	0.0474	0.0376	0.0321	0.028	0.0293	0.0318	0.0275	0.0287	0.0457
NO3-N	mg/l	3.8	2.3	1.7	1.7	3.0	3.4	1.3	2.5	1.6
T-P	mg/l	0.36	0.52	0.28	0.26	0.09	0.09	0.48	0.39	0.31
P04-P	mg/l	1.50	1.67	1.59	1.59	1.37	1.32	0.03	0.03	0.03
CI-	mg/l	6972.4	5790.7	8804.2	14358.5	16840.2	16072.0	16485.6	16131.1	16840.2
Chla	µg/l	7.7	5.6	4.3	7.4	20.3	11.2	3.2	1.6	3.2
SS	mg/l	24.20	23.05	18.00	23.25	54.95	57.15	74.55	41.85	36.90



Fig. 2 Evaluation by benthic organism (Kyutokenshi Method)



Fig. 3 Evaluation by adhered diatom (DAIpo)

Diversity Models of Plankton in Gold Coast Ornamental Lake, Pantai Indah Kapuk, North Jakarta

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Keywords: abundances models, plankton, Gold Coast Ornamental Lake, water quality

ABSTRACT

Plankton diversity can be calculated through a model of species abundance. This study aimed to describe the structure of plankton communities using various models of diversity and coherence of the water quality. The study was conducted from July to December 2015, using the method of APHA, 2012. Based on water quality data, the five stations are grouped into three zones. Zone 1 consists of Station 1, Zone 2 consists of stations 2, 3, and 5, Zone 3 consists of Station 4. The difference in the water quality at each station causes differences plankton diversity. The results showed that the model of geometric and broken stick was not suitable for use in all zones. Log normal model was appropriate using in Zone 1 for data phytoplankton and zooplankton all zones to the data. Log series model was an appropriate model to describe the plankton communities throughout the zone. This model describes the waters were disturbed and their dominance.

1. INTRODUCTION

Gold Coast Ornamental Lake is located in Pantai Indah Kapuk Residence, North Jakarta. The waters is relatively small with total area about 7359 m2 and 1 m in depth. It has unique net-shape, The small and shallow condition lead a dynamic physical and chemical condition that infkuence the appearance of plankton community. The combination of relationship between water quality and plankton community could show the role of plankton as bioindicator of lake water condition [1].

Density and richness (number of genera) can be used as factors in calculating diversity index. Diversity index can be determined with three approached, i.e. index of richness, index based on spesies/genera relative abundance, and model of spesies/genera abundance [2].

This study emphesized on the third approach that usualy used to describe the community structure [3], especially plankton community. The model of spesies/genera abundance consist of several models, such as geometric, log series, log normal, and broken stick model [2]. The research was aimed to describe plankton community structure in Gold Coast Ornamental Lake, based on several diversity models.

2. METHOD

The research was done in six month, from July to December 2015, with monthly sampling periods at five sites. Station 1 was close to sewage treatment plant and pump station (outlet). Station 2 set at the north western part, before the Sewage Treatment Plant. Station 3 was inside of 'net' curve of waters. Station 4 was in southern part, close to inlet that receive input from next cluster residence, Bukit Golf Mediterania. Station 5 was at south eastern part of waters.

Water quality was observed and measured in situ and in laboratory, based on water quality analysis reference [4]. Identification of plankton was based on several references ([5], [6], [7], [8], [9]). The density of plankton was counted using SRC (Sedgewick Rafter Counting Chamber) [4].

Pre-analysis was done to set spatial cluster of station, based on water quality information, using clustering analysis of Canberra Index value [10]. Furthermore, for each clustered stations, there were calculation of plankton diversity with spesies/genera abundance model tah consist of geometric, log series, log normal, and broken stick models ([11]; [2]). Chi square test was used to fit the model in describing plankton Community structure in Gold Coast Ornamental Lake.

3. RESULTS

Water quality that illustrated by temperatur, is relatively warm (31,22-32,30 °C); high pH (8,73-8,87), existance of salinity (3,45-4,40 ‰), and high dissolved oxygen (9,42-13,23 mg/L). The condition of dissolved oxygen expressed supersaturation condition. Based on water quality, there were clustered Three groups of stations.



Figure 1. Abundance and number of taxa

Geometric Model Log series Model Log normal Model Broken stick Model Zone χ2 χ2 χ2 χ2 χ2 χ2 χ2 χ2 table table calculatio calculatio table table calculation calculation n n Phytoplankton Zone 1 341454,6 36,4 21,2 28,9 19,3 26,3 5804,9 28,9 27,6 Zone 2 22,6 25,3 25,0 13406,6 27,6 263613,1 36,4 Zone 3 379934,6 32,7 26,6 28,9 27,8 26,3 8191,6 28,9 Zooplankton Zone 1 9,5 9,5 12,6 36,8 3,5 12,6 4,2 17,4 Zone 2 468,3 11,1 11,6 12,6 11,4 15,5 31,6 12,6 Zone 3 476,9 9,5 10,3 19,7 11,9 16,9 108,5 19,7

Fable	2 The co	mparation	of	x2 calculation an	ıd	χ2	table of	each	zone
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The comparation value between χ^2 calculation (χ^2 c)

and χ^2 table (χ^2 t) (Table 2) shows that values of Zone 3 are higher than other zones; related to high density of

plankton. The values of $\chi 2c$ for geometric model were

higher than other models. The value of $\chi 2c$ for log

series model were lower than χ 2t. There were a

consistance pattern of $\chi 2t$ of each model of all zones.

Table 3 The result of fit test for models toward data of phytoplankton and zooplankton

		Uji kes	euaian model	
Zone	geometric Model	log series	Log normal	broken stick
	geometric Model	Model	Model	Model
Phytoplankton				
Zone 1	Not suit	suit	suit	Not suit

Zone 2	Not suit	suit	Not suit	Not suit
Zone 3	Not suit	suit	Not suit	Not suit
Zooplankton				
Zone 1	Not suit	suit	suit	Not suit
Zone 2	Not suit	suit	suit	Not suit
Zone 3	Not suit	suit	suit	Not suit

4. DISCUSSION

In those water quality condition, there were found 27 genus from five classes of phytoplankton and seven taxa from two groups of zooplankton (Figure 1). In detail, the richness and density of plankton of each station were varied. The highest density was found at Zone 3 (2044 x106 cell/m3) and 1177 x103 individu/m3, for phytoplankton and zooplankton, respectively. The highest number of genera was found in Zone 2 with 26 genera and Zone 1 with five genera, for phytoplankton and zooplankton, respectively. The fit test for models is shown in Table 3. If the value of χ^{2}_{c} is lower than χ^{2}_{t} , it means that the model is fit, vise versa. It is shown that log series model is the most suitable model for all zone of Gold Coast Ornamental Lake, while log normal model suits in Zone 1 for phytoplankton data, and in Zone 1, 2, and 3 for zooplankton data. Furthermore, geometric and broken stick model were not suit for all zones.

5. CONCLUSION

The most suitable diversity model for phytoplankton and zooplankton data in Gold Coast Ornamental Lake was log serie model

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Antifungal Activity of Actinomycetes Isolated from Surface Sediments of Lake Lanao against *Candida albicans* and *Aspergillus niger*

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Keywords: Actinomycetes, Antifungal activity, Lake Lanao

ABSTRACT

Antibiotic resistance is not only a national problem but a global dilemma. There are many reasons why this type of resistance exists including inappropriate use of antibiotics. The search for novel antimicrobial compound is still a priority goal in drug discovery. Thus, actinomycetes from lake sediments are being explored. This study was conducted to determine if the isolated actinomycetes could exhibit antifungal activity against two test microorganisms namely Candida albicans and Aspergillus niger. A total of 34 actinomycetes was isolated from the western part of Lake Lanao specifically in the municipalities of Wato-Balindong, Tugaya and Bacolod-Kalawi, Lanao del Sur. Antifungal screening using Filter Paper-Disc Diffusion Method revealed that out of the 34 actinomycete isolates, only eight (23.53%) have inhibitory effect against Candida albicans with the mean zone of inhibition greater than the positive control. These actinomycetes, with their corresponding mean values, were A3 (26.39 mm), A9 (21.83 mm), A11 (22.66 mm), A18 (26.63 mm), A21 (24.74 mm), A25 (22.76 mm), A31 (14.76 mm) and A32 (16.88 mm). Whereas, only two (5.88%) of the actinomycete isolates inhibited the growth of Aspergillus niger with mean zone of inhibition greater than the positive control. These potential actinomycetes, with their corresponding mean values, were A11 (23.83 mm) and A31 (30.13 mm). Generally, isolates were found to be more effective against Candida albicans. Thus, this study concludes that Lake Lanao harbors potential actinomycetes that could be a source for the search of new and effective drugs against pathogenic fungi.

1. INTRODUCTION

Some bacteria, fungi, virus and other pathogenic microorganisms are resistant to their own antibiotics nowadays. As stated by Prabakarana et al., (2011), bacteria lived on earth for several billions of years. During this long period of time, they encountered range of naturally occurring antibiotics to which by now, they combated these and became resistant by the drugs synthesized today. Supported by the study of Spadari et al., (2013), Candida albicans, a pathogenic fungus which causes candidiasis is found to be resistant to its antibiotic (Fluconazole) by Disc-Diffusion method. Antimicrobial resistance is not just a national issue but a global problem and so, finding new and effective drugs is major challenge to the field of medicine. One possible candidate in the search of new and effective drugs is from the actinomycetes. Some actinomycetes form branching filaments that look like the branching hyphae formed by fungi and so, it was erroneously classified as fungi before. Actinomycetes are actually bacteria under the phylum, Actinobacteria. Under this phylum, actinomycetes tend to produce secondary metabolites; many of which have been successfully isolated and turned into useful drugs. It was in the fifties and sixties that the antibiotics discovered were mainly isolated from Streptomyces. Primarily, Streptomyces were active against bacteria and fungi (Prabakarana and Raja, 2011). The purpose of this study is to screen the 34 actinomycetes isolated from the surface sediments of Lake Lanao specifically from Wato Balindong, Tugaya and Bacolod- Kalawi for its antifungal activity against *Candida albicans* and *Aspergillus niger*.

2. METHOD

Thirty-four actinomycete isolates obtained from Lake Lanao were cultured in nutrient broth for 48 hours

and tested against the two test fungi, *Candida albicans* and *Aspergillus niger*. Inoculation of test fungi were done following the agar over-lay technique. The antifungal assay was done using filter paper disc diffusion method. Filter paper discs were impregnated with the 48 hour-old broth culture of the actinomycete isolates were then placed each on the surface of the pre-labeled plates. Petri plates were then incubated at 28°C for 7 days. The mean for every seven-day observation was calculated in three replicates of each isolate. Distilled water and nystatin were used as negative and positive control, respectively. One-way ANOVA and DMRT were used to analyze the zone of inhibition effect of the isolates against the two test-fungi.

3. RESULTS

Candida albicans

Out of the 34 actinomycete isolates that were tested for antagonistic screening, only eight isolates (23.53%) were found to inhibit the growth of *Candida albicans*. Among the actinomycete isolates, A18 has the highest mean zone of inhibition of 26.63mm other isolates ranges from 14.76 to 26.39 mm (Figure 1).

There is a statistically significant difference in the mean zones of inhibition among the actinomycete isolates (p value of <0.001). Duncan's Multiple Range Test results showed that the inhibitory effect of A3 and A 18 are comparable and were significantly different from the other bioactive isolates (Fig. 1). The mean values of the zone of inhibition of the positive control were less than of the bioactive isolates. The mean value of the actinomycete isolate, A3 (26.39mm) was greater than the positive control (13.89mm) and so with the other bioactive isolates, A9 (21.83mm>13.57mm), A11 (22.66mm>13.14mm), A18 (26.63mm>14.40mm), A21 (24.74mm>14.12mm), A25 (22.76mm>12.52mm), A31 (14.76mm>14.29mm) and A32 (16.88mm>13.63mm).



Figure 1. Antifungal activity of the bioactive actinomycete isolates vs. positive control (Nystatin) against *Candida albicans*.

Aspergillus niger

Out of the 34 isolates, there were only two (5.88%) bioactive isolates that shows inhibitory effect against *A. niger*. These bioactive actinomycete isolates were A11 and A31 only. Results showed that the zone of inhibition between the bioactive isolates, A31 has the highest mean value of zone of inhibition of 30.13mm followed by A11, with a mean value of 23.83mm (Figure 2). Results also shows that there is a statistically significant difference in the mean zone of inhibition between the actinomycete isolates (p<0.001).

Moreover, from the raw data, the mean values of the zone of inhibition of the positive control were less than of the bioactive isolates. The mean value of the actinomycete isolate, A11 (23.83mm) was greater than the positive control (10.14mm). Likewise, A31, with a mean value of 30.13mm was greater than the mean value of the positive control (8.86mm).



Figure 2. Antifungal activity of the bioactive actinomycete isolates vs. positive control (Nystatin) against *Aspergillus niger*.

4. **DISCUSSION**

Results showed that Lake Lanao has bioactive actinomycetes with antifungal activity. Unlike *Candida albicans*, the growth of *Aspergillus niger* is more difficult to inhibit (Das *et al.*, 2014). Thus, it correlates with the results that only two actinomycetes isolates (A11 and A31) inhibited the growth of *A. niger*. This is similar to the findings of Jiang and Xu (1996), in which only 118 (6.78%) out of 1741 actinomycete isolates inhibited the growth of *A. niger*. These 118 bioactive actinomycetes were isolated from Yunnan Middle Plateau lakes that are said to be clear and not as densely polluted by human activities. Supporting this study, the active actinomycetes

that inhibited the growth of *A. niger* with a percentage value of 5.88% were isolated from the lake sediments that are not densely polluted by the local residences of Wato-Balindong.

Antifungal activity the bioactive of actinomycetes may be described either having a fungistatic effect or fungicidal effect. Fungistatic effect explains the effect of inhibiting the growth of the fungi that may result to thinning of growth of the certain fungi. Fungicidal effect on the other hand, totally eliminates or kills the fungi. In the study of Bonjar et al., 2005, it was determined that clear zones were indicative of fungicidal effect and the zones bearing only mycelial growth had fungistatic effect. Bacteria are known to produce substances with fungistatic and fungicidal effects (Barbosa et al., 2017) just as actinomycetes produced in various studies. All of the bioactive actinomycetes that inhibited the growth of C. albicans have fungistatic effects as shown in Figure 3. We observed thinning of the growth of the test fungi as seen in the surroundings of the growth of the actinomycete isolate. Though fungistatic effects do not totally inhibit the growth of the test fungi, fungistatic effects still weakened the growth of the said fungi. Welsch (1942), showed that the independence of bacteriolytic and bacteriostatic properties is highly improbable or unlikely. As for inhibiting the growth of A. *niger*, the two bioactive actinomycete isolates clearly showed fungicidal effects as shown in Figure 4.



Figure 3. Zones of inhibition of the bioactive actinomycete isolates A3 and A32 with its – (negative control) and + (positive control) against *Candida albicans* at 28°C after 7 days.



Figure 3. Zones of inhibition of the bioactive actinomycete isolates A11 and A31 with its – (negative control) and + (positive control) against *Aspergillus niger* at 28°C after 7 days

5. CONCLUSION

Findings on this research conclude that the western part of Lake Lanao has bioactive actinomycete isolates. Thus, the lake is a potential ecosystem for antagonistic actinomycetes which deserves bioprospecting or to search for substances that are produced by these bacteria that may be of helpful to medicinal research in the future. Considering the above-mentioned results, eight isolates, A3, A9, A11, A8, A21, A25, A31 and A32 exhibited antifungal activity against Candida albicans and only two isolates, A11 and A31 exhibited antifungal activity against Aspergillus niger. These bioactive actinomycete isolates have the potential to contribute in the search for new and effective drugs.

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Environmental Impact Risk Assessment of Alien Species using Microcosm System

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ABSTRACT

From the biota (structural parameter) and the P/R ratio (functional parameter), utilizing the microcosm, which is a microbial ecosystem capable of constructing the basic ecological structure of general lakes, *Daphnia (Moina macrocopa,* the top-level predator species in the food chain) and bacteria (*Escherichia coli,* the lowest-level prey species in the food chain) as foreign species invading from outside the lake ecosystem and conducted ecological risk assessment. When introducing the top-level predator species in the food chain into the microcosm, there would be a risk that existing top-level predators would increase due to invasion of foreign organisms that would become higher predator. When introducing the lowest-level prey species in the food chain, it was evaluated that there would be no major impact on existing ecosystems. The influence of alien species on existing ecosystem depends on which trophic stage the alien species are located, and the influence on existing ecosystem is shown to be stronger in high-level predator species in food chain.

1. INTRODUCTION

One of the factors that have a serious effect on ecosystems and biodiversity is invasion of alien species into existing ecosystems such as microbial pesticides, ballast water, abandonment of pets, etc. Some these alien species which cause damage and destruction of existing wildlife due to genetic disturbance and disruption of balance of food chain, which causes damage to primary industry, it has become a problem in recent years.

In this study, we used the microbial ecosystem (microcosm) that can construct the fundamental ecological structure of general lakes, and from the biota (structural parameters) and P/R ratio (functional parameters), *Moina macrocopa* (the top-level predator species in the food chain) and bacteria (*Excherichia coli*, the lowest-level prey species in the food chain) were evaluated as foreign species invading from outside the lake ecosystem, and the purpose was to evaluate the ecological risk impact.

2. EXPERIMENTAL PROCEDURE

2.1 microcosm

Microcosm is a simulated ecosystem that cut out a part of natural ecosystem, and it consists of basic components of ecosystem such as decomposer, producer, predator and their biological interaction, material circulation, energy flow. It is a microbial ecosystem that contains elements and is capable of constructing a stable ecosystem. In the Gnotobiotic type microcosm used in this study, the constitutive species is completely known, two producers of green algae Chlorella sp., Scenedesmus sp., one bluegreen algae Tolypothrix sp., one protozoan ciliate Cyclidium glaucoma (primary consumer), two metazoan rotiferas Lecane sp., Philodina erythrophthalma, one metazoan oligochaetes Aeolosoma hemprichi (top consumer), four kinds of bacteria (Bacillus cereus, Pseudomonas putida, Acinetobacter sp., coryneform bacteria) as decomposer, consisting of eleven species in total. It has been shown that this microcosm system (Ntype) has a high correlation with the natural ecosystem.

2.2 Culture method

200ml of TP (Taub's basal medium + polypeptone) medium was poured into a 300ml Erlenmeyer flask, and 10ml of subcultured microcosm seed was added, and cultivation was carried out for 30 days under a static condition of a temperature of 25°C and an illuminance of 2,400 lux (light/dark cycle 12hr./12hr.).

2.3 Introduced organisms

As a transferred organism (alien species), *Moina macrocopa* (the top-level predator in the food chain) and *Escherichia coli* HB101/pBR325 (the lowest-level prey in the food chain) which were not constructed in microcosm were supplied.

Moina macrocopa is filtration feeding minute crustaceans belonging to *Daphnia* with a physical condition of 0.6-1.2 mm and is usually parthenogenetic only in female, but males appear due to deterioration of the environment due to water pollution and the like to form resisting egg. Also useful as organisms are living baits for ornamental fishes, usefulness of toxicity testing of chemicals, attention as a biomanipulation introduced species, and so on. In this study, focusing on the competitive relationship with *Aeolosoma hemprichi* which is the top predator in the microcosm, *Moina macrocopa* is added at 1, 5 and 10 times the introduction amount of *Aeolosoma hemprichi* individuals, and the structure of the ecosystem and its effect on function.

Escherichia coli is gram-negative bacteria and belongs to facultative anaerobic bacteria and is one of the major species of bacteria present in the environment. This bacteria is also an intestinal bacterium and inhabits the large intestine in the digestive tract of warm-blooded animals (birds, mammals), especially humans. Its size is usually 0.4-0.7 μ m on the short axis and 2.0-4.0 μ m on the long axis, but the long axis is shortened, and some are nearly spherical. It is one of the model organisms as a representative of bacteria, and it is used as a material in various research such as genetic engineering, and it is also used for the production of chemical substances by

incorporating genes. In this study, we focused on the competitive relationship with the four species of bacteria which are the lowest species in the microcosmic food chain. *Escherichia coli* HB101/pBR325 was added at 1, 10 and 100 times the number of indigenous bacteria in the microcosm, and the influence on the structure and function of the ecosystem was examined.

2.4 Evaluation method

Evaluation items were two, number of individuals (structural parameter) and DO (functional parameter). The number of individuals was observed on days 0, 2, 4, 7, 14, 16, 18, 20, 23 and 30 from the start of cultivation using an optical microscope, and the ratio of the number of organisms on the 30th day to the control system evaluated from N_{30} . DO was continuously measured from the 16th day, and the P/R ratio was calculated from the production amount (P) and the respiration amount (R) and evaluated.

3. RESULTS AND DISCUSSION

3.1 Impact of top-level predator introduction

3.1.1 Evaluation by individual number (structural parameter)

In any of the addition systems, the producers *Chlorella* sp. and *Tolypothrix* sp. were decreased from immediately after introduction of Moina macrocopa. The cause was thought to be because the predation pressure increased by Moina macrocopa invasion and the latter could not grow sufficiently due to the swimming behavior of Moina macrocopa. In predators, Lecane sp. and Cyclidium glaucoma showed a decreasing tendency, and in the 10 times addition system, extinction was confirmed on the 18th day immediately after addition. This is thought because the competitive relationship of consumers in the microcosm became severe due to the predation pressure of Moina macrocopa and the difference in superiority or inferiority of predation ability occurred. In addition, Moina macrocopa added as a foreign organism in all additive systems converged on the 30th day by the time of measurement. In the 10 times addition system assuming the invasion of a larger amount of alien species, the number of individuals of Aeolosoma hemprichi, which is the top indigenous predator in the microcosm system, increased dramatically on the 30th day. This is because the existence of metabolites generated by the remains of Moina macrocopa and molting is a physical obstacle to filtering feeding zooplankton in predation behavior and only Aeolosoma hemprichi which is opening feeding intake advantageously can prey green algae Chlorella sp.. **3.1.2** Evaluation by DO (functional parameter)

From the P/R ratio, which is the ratio of the production and consumption of dissolved oxygen, the consumption activity of dissolved oxygen (DO) increases as the addition amount of *Moina macrocopa* increases, and immediately after the start of continuous measurement in the 10 times addition system After the DO value attenuated for about 70 hours, it stabilized. For this reason, introduction of ten times amount determined that the ecosystem was affected by the variation of the structural type of the structural parameter and the attenuation of the DO value of the functional parameter, and the top category of the food chain (higher predator) invaded in the microcosm the



Fig.1 Microcosm (microbial ecosystem model)



Fig.2 Population succession in microcosm (control system)



Fig.3 *Moina macrocopa* (the top-level predator species in the food chain) addition system



Fig.4 *Escherichia coli* (the lowest-level prey species in the food chain) addition system.

maximum no effect concentration (NOEC) was estimated to bebetween 5 and 10 times the existing top-level predator. Furthermore, since the *Lecane* sp. is drastically decreased in the 5 times addition system from the structural parameter, the m-NOEC (microcosm maximum effect free



concentration) is considered to be at a relatively low concentration among 5 to 10 times.

3.2 Impact of lowest-level prey introduction

3.2.1 Evaluation by individual number (structural parameters)

Escherichia coli rapidly decreased at 1, 10, or 100 times inoculation concentrations in viable counts by the selective media method after microcosm inoculation, and the number of small animal groups (in particular, it is a primary consumer, protozoan ciliate *Cyclidium glaucoma*) in the microcosm increased. Escherichia coli has been shown to be a suitable food source for microcosmconstituting micro animal group such as protozoan ciliates glaucoma and including Cyclidium Philodina erythrophthalma and Aeolosoma hemprichi in preypredator interaction test, it was considered to be dominated by these predatory actions. In N₃₀ comparison, no significant difference was observed between Escherichia *coli*-added system and non-added system (control system). **3.2.2** Evaluation by DO (functional parameter)

In the P/R ratio, which is the ratio of the production and consumption of dissolved oxygen, the consumption activity of dissolved oxygen (DO) increased as the amount of *Escherichia coli* added increased, but eventually the non-additive system (control system) converge same level, And the P/R ratio was also stabilized at about 1. For this reason, the introduction of *Escherichia coli* judged that there is no influence on the ecosystem from the variation of the structural type of the structural parameter, and even if the lowest species of the food chain invades microcosm, it was evaluated that there was no major impact on the ecosystem.

4. CONCLUSIONS

1) In the case of the top-level predator species in the food

chain (*Moina macrocopa*) was introduced into the microcosm, two predators were confirmed to extinct in the 10 times addition system. There was a risk that existing top level predators would increase due to invasion of alien species that would become higher predator. The possibility of collapse of the system was indicated.

2) In the case of introducing the lowest-level prey species in the food chain (*Escherichia coli*) was introduced into the microcosm, both the structure and function parameters converged to the same extent as the control system (nonadditive system) and the system recovered, and it was evaluated that there would be no big impact on existing ecosystems.

3) The influence of alien species on existing ecosystem depended on which trophic stage the alien species are located, and the influence on the existing ecosystem was shown to be stronger in high-level predator species more than low-level prey species in its food chain.

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ヨコエビ類を用いた久慈川流域水質環境の生物学的評価

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キーワード:ヨコエビ類,指標生物,侵入外来生物,生態系管理

抄録

久慈川流域で水質とヨコエビ類の生息を調査した.少し汚れている中・下流域で、ヨコエビ類が5地点で採取された.この結果、ヨコエビ類はきれいな水の指標生物になりにくいことがわかった.そこで、ヨコエビ類の種の同定を行った結果、4地点で侵入外来生物のフロリダマミズヨコエビ(*Crangonyx floridanus* Bousfield, 1963)が確認された. 残りの1地点では、関東地方の低地の河川、湖沼に生息する在来のアゴトゲヨコエビ(*Jesogammarus (J.) spinopalpus* Morino, 1985)であることが分かった.上流域には、関東・北陸・東北地方の山間部の渓流に生息する在来のヒメヨコ エビ(*Jesogammarus (J.) paucisetulosus* Morino, 1984)を確認した.ヨコエビ類の種の同定を行うことで、久慈川流域の 水質環境の生物学的評価が可能になった.今後、生態系管理の指標生物として利用できる可能性も期待できる.

1. はじめに

河川の水質環境評価の指標生物として用いられてい るヨコエビ類は、きれいな水に生息するとされている.し かし、1989年に千葉県我孫子市と茨城県取手市の県境 にある古利根沼から利根川に流出する小河川で,侵入 外来生物の北米原産のフロリダマミズヨコエビ (Crangonyx floridanus Bousfield 1963) が採取れた. そ の後,全国各地でフロリダマミズヨコエビが報告され,特 に在来のヨコエビがほとんど生息しないやや汚濁の進ん だ水域や水温が高い下流域で採取されている(多摩川, 鶴見川等)[1]. これらの結果はヨコエビ類が従来の指標 生物になりにくいことを示している. そこで福島県南部と 茨城県北部及び栃木県東部に流域を持つ久慈川のヨコ エビ類の生息に関する報告を調べたが見つけられなか った.これらの背景から久慈川流域の水質とヨコエビ類 調査を一定の期間に行って,生態系管理の指標生物と して、利用できる可能性も視野に入れ、ヨコエビ類を用 いた久慈川流域水質環境の生物学的評価を試みた.

2. 方法

1)水質調査

(1)調査期間と調査地点

水質調査は 2017 年 12 月 2 日~2018 年 3 月 18 日 に行った。調査地点(図1)は以下(川・用水路:地点,地 名,標高)のとおりである.

a.下流域:標高 2~50m

久慈川:①,落合,2m. ②,辰ノロ,25m. 用水路:町 下,19m.

b. 中流域: 標高 100~200m

久慈川:③,大子,100m. ④,米山下,200m. 川上 川:⑥,中塚,200m. 八溝川:⑧,川山,115m. 矢祭 川:⑨,牧野,144m.

c. 上流域: 標高 300~600m

久慈川:⑤,戸中,400m. 那倉川:⑦,川辺,400m.

(2)測定項目と方法

気温・水温はアルコール温度計で測定した. 化学的 酸素消費量:COD, アンモニウム態窒素:NH4⁺-N, 亜硝 酸態窒素:NO2-N, 硝酸態窒素:NO3-N, りん酸態りん: PO4³-Pは, 水質調査セット(TZ-RW,(株)共立理化学研究 所)を用いて測定した. pHはコンパクトpHメーター(ツイ ン:B-211:ホリバ(株)),透視度は透視度計(TO-100: ケニス(株))で測定した.

3)ヨコエビの生息調査と種の同定

(1)調査期間と調査地点

生息調査は 2017 年 12 月 2 日~2018 年 3 月 11 日に 行った.調査地点(図1)は以下(川・用水路:地点,地名, 標高)のとおりである.

a.下流域:標高 2~50m

久慈川:1,落合,2m. 7,辰ノ口,25m. 10,西金,50m. 山田川:3,上河合町,4m. 浅川:5,松栄町,12m. 枇 杷川:8,山方,36m. 用水路:2,下河合町,7m. 4,小 島町,7m. 6,下町,19m. 9,舟生,45m.



図1. 久慈川流域の水質とヨコエビ類の調査地点

b. 中流域: 標高 100~200m

久慈川:11,大子,100m. 21,江戸塚,150m. 34,米 山下,200m. 押川:12,中居,200m. 玉川:13,岩花, 111m. 八溝川:18,宮本,200m. 茗荷川:20,歯朶 平,200m. 中川:22,小田川,155m. 小田川:23,仲 町,200m. 川上川:35,中塚,200m. 渡瀬川:36,赤 坂,200m. 用水路:14,田中,134m. 15,仲野,140m. 33,米山下,200m.

c. 上流域: 標高 300~600m

久慈川:30,戸中,600m.31,戸中,400m.32,大岩 平,300m. 押川:16,如来,300m. 八溝川:17,磯 神,300m. 茗荷川:19,上茗荷,300m. 小田川:24, 明神,350m. 川上川:25,前田,300m. 片貝川:26, 片貝,600m. 那倉川:27,川辺,400m. 29,鳩の宮, 600m. 江竜田川:37,江竜田,400m. 渡瀬川:38, 丸谷地,600m. 用水路:28,小田井田 530m.

(2)ヨコエビ類の採取

各調査地点で主に落葉の蓄積や水草があるところに タモ網を入れた.採取したヨコエビ類は消毒用エタノー ルに入れて固定した.

(3)種の同定

ヨコエビ類は富川^[2]などに従い,種の同定を行った.

3. 結果

1)水質調査

調査期間の気温は4~14°C,水温は2~8°Cであった. 下流域の地点①,②,⑩,中流域の地点③,④,⑥,⑧,⑨の COD 値は2~4mg/L で少し汚れた水であった.上流域 の地点⑤,⑦の COD 値はほぼ 0mg/L できれいな水であ った.下流域の地点②の NO₂-N 値は0.02mg/L であった が,他の地点は 0.005mg/L で最低値あった.下流域の 地点②, ⑪,上流域の地点⑦の NO₃-N 値は,それぞれ 1 mg/L, 5mg/L, 0.5mg/L であったが,他の地点は 0.2mg/L で最低値であった.全調査地点の NH₄⁺-N 値と PO₄³-P 値は,それぞれ最低値の 0.2mg/L, 0.02mg/L, pH 値はpH6.9~7.3,透視度は 100 度以上であった.

2)ヨコエビ類の生息調査

下流域の地点3でフロリダマミズヨコエビ(図2,A),地 点6にアゴトゲヨコエビ(図2,B)が採取された.中流域 の地点21,33,34でフロリダマミズヨコエビ(図2,A)が採 取された.上流域の地点24,25,26,27,2829,37,38 でヒメヨコエビ(図2,C)が採取された.その他の地点で は採取されなかった.

4. 考察

久慈川流域の水質とヨコエビ類の生息を調査した結 果、少し汚れている中・下流域(地点 3,6,21,33,34)でも ヨコエビ類が採取された.この結果、ヨコエビ類は、きれ いな水の指標生物になりにくいことがわかった.ヨコエビ 類の種の同定の結果、4地点で侵入外来生物のフロリダ マミズヨコエビであることが分かった.残りの1地点6で



図2. 久慈川流域で採取されたヨコエビ類

関東地方の低地の河川,湖沼に生息する在来のアゴト ゲヨコエビ^[3]であった.その地点の水質はきれいな水と は言えない水であった.上流域には関東・北陸・東北地 方の山間部の渓流に生息する在来のヒメヨコエビ^[4]であ った.以上の結果,ヨコエビ類の種の同定を行うことによ り久慈川流域水質環境の生物学的評価が可能になっ た.今後,生態系管理の指標生物として利用できる可能 性も期待できる.

5. 結論

ヨコエビ類を用いた久慈川流域水質環境の生物学的 評価を行った結果,ヨコエビ類の種の同定を行うことで、 久慈川流域の水質環境の生物学的評価が可能であるこ とが示唆された.

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Distribution of Phytoplankton and Water Quality in Chiang Mai Moat, Thailand

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Keywords: Biodiversity, Chiang Mai Moat, Phytoplankton, Water Quality

ABSTRACT

The distribution of phytoplankton and the water quality of the Chiang Mai Moat, Thailand were investigated over a period of 4 months (September-December 2017). Samples were collected from 4 sites within the Chiang Mai Moat. The distribution of phytoplankton was found to be of the highest abundance at the Chang Phueak Gate site with thirty-nine species found, while the most abundant species were *Chlamydomonas* sp., *Trachelomonas* sp. and *Microcystis* sp. Thirty-four species were identified at the Suan Dok Gate site and the most abundant species were *Lepocinclis* sp. and *Trachelomonas* sp. Thirty-two species were identified at the Tha Phae Gate site and the most abundant species were identified at the Chiang Mai Gate site and the most abundant species were identified at the Chiang Mai Gate site and the most abundant species were *Pseudanabaena* sp., and *Peridinium* sp., respectively. In addition, the trophic status of the Chiang Mai Moat was classified as meso-eutrophic status.

1. INTRODUCTION

The Chiang Mai Moat was built in 1839. It has a square shape and surrounds the old city of Chiang Mai, which is located in Chiang Mai Province. It was originally built to protect the city from the Burmese army. At present, the Chiang Mai Moat is considered a famous tourist attraction of the city and is associated with both the Loy Kratong and Songkran Festivals. It is particularly important in the Songkran Festival (Thai New Year's festival) when people pour or splash moat water to one another as part of the cleansing ritual to welcome the New Year. However, around the moat there are many markets and a thriving community, which have affected the water quality in the moat. Moreover, phytoplankton is one of organisms that are sensitive to water quality because each species can grow in different types of water quality. For this reason, phytoplankton can be used to monitor water quality [1]. Our present investigation focuses on the distribution of phytoplankton and water quality in the Chiang Mai Moat by examining samples collected from four sampling sites.

2. METHOD

The samples were collected from 4 sites,

namely Tha Phae Gate, Chiang Mai Gate, Suan Dok Gate and Chang Phueak Gate, within the Chiang Mai Moat (Figure 1). The collection period involved the months of September, October, November and December in 2017. The physical and chemical factors of the water including pH, conductivity, DO, BOD₅, nitrate nitrogen, ammonium nitrogen and Orthophosphates values were measured according to the methods described by APHA, AWWA and WEF [2]. The trophic status of the water was classified using the Applied Algal Research Laboratory-Physical and Chemical Score (AARL-PC Score) [3].



Fig. 1 Aerial photograph of the Chiang Mai Moat and 4 sampling sites (O).

Phytoplankton samples were collected by filtering 10 liters of water with a 10 μ m mesh size plankton net in the field. The samples were then preserved by Lugol's solution. The samples were identified and counted according to relevant resource references [4-6]. The abundance of the phytoplankton species found was also determined for calculation of the diversity index using the Shannon method [7]

3. RESULTS

A total of sixty-eight species of phytoplankton were found in the Chiang Mai Moat. Thirty-two species were identified at the Tha Phae Gate site and the most abundant species (more than 10% of relative abundance) were *Aulacoseira granalata*, *Pediastrum biradiatum* and *Merismopedia* sp. Twenty-five species were identified at the Chiang Mai Gate site and the most abundant species were *Pseudanabaena* sp., and *Peridinium* sp. Thirty-four species were identified at the Suan Dok Gate site and the most abundant species were *Lepocinclis* sp. and *Trachelomonas* sp. Thirty-nine species were identified at the Chang Phueak Gate site and the most abundant species were *Chlamydomonas* sp., *Trachelomonas* sp. and *Microcystis* sp.

The diversity index ranged from 0.58-2.87 and the evenness ranged from 0.16-0.87. At the Suan Dok Gate site during the month of October, a total of 22 species were present. This was where the highest diversity index of 2.87 (evenness 0.80) was recorded. The trophic status of the Chiang Mai Moat was classified as meso-eutrophic status. The maximum and minimum values of the physico-chemical factors, trophic level, Total number of species, Diversity index, Eveness and Richness of each sampling site are shown in Table 1.

4. **DISCUSSION**

The distribution of phytoplankton and water quality revealed differences at each sampling site due to the different activities taking place around the moat. The most dominant species of each sampling site was similar to what was reported by Bicudo et al. [8], Solórzano et al. [9], Wołowski and Grabowska [10] and Komárek and Jankovská [11]. The species that are typically found in water bodies that are rich in organic matter and nutrients could be used to indicate the meso-eutrophic species. This was relevant to determining the trophic level of the Chiang Mai Moat according to the AARL PC score. In addition, Microcystis sp. were found to be in high abundance at the Chang Phueak Gate site and this species could produce Microcystins, which are a group of hepatotoxins that can actively affect fish, birds and mammals [12]. Therefore, water bodies should be considered in the investigation of water quality.

	Diversity index, Diverness and idenness in Chang Fur Wout between September to December 2017.					
Physico-chemical factors	Tha Phae Gate	Chiang Mai Gate	Suan Dok Gate	Chang Phueak Gate		
pH	7.01-7.52	6.93-8.15	7.05-7.41	6.87-7.59		
Conductivity (µs/cm ⁻¹)	212.5-299.1	194.3-234.3	203.5-228.9	198.9-241.3		
DO (mg/L)	5.4-7.0	4.4-7.4	5.6-6.8	4.4-6.4		
BOD ₅ (mg/L)	5.2-7.6	5.4-7.8	4.8-10.8	3.2-6.8		
Nitrate nitrogen (mg/L)	0.6-1.7	0.2-1.9	0.4-4.7	0.7-4.0		
Ammonium nitrogen	0.07-0.50	0.13-0.42	0.82-1.93	0.73-1.52		
Orthophosphates (mg/L)	0.07-0.58	0.05-0.31	0.07-0.23	0.21-0.57		
Trophic level*	Meso-eutrophic	Meso-eutrophic	Meso-eutrophic	Meso-eutrophic		
Total number of species	32	25	34	39		
Diversity index	0.58 - 1.97	0.73 - 2.37	2.16 - 2.87	2.13 - 2.80		
Evenness	0.16-0.71	0.42-0.76	0.68-0.87	0.36-0.68		
Richness	10-19	4-18	10-22	13-24		

 Table 1 Minimum and maximum values of the physico-chemical factors, trophic level, Total number of species,

 Diversity index, Evenness and Richness in Chiang Mai Moat between September to December 2017.

Note: * Trophic level was calculated by conductivity, DO, BOD₅, nitrate nitrogen, ammonium nitrogen and Orthophosphates

5. CONCLUSION

Sixty-eight species of phytoplankton were collected from the Chiang Mai Moat. The distribution of phytoplankton revealed differences at each sampling site. The dominant species are commonly found in the mesoeutrophic level of lentic ecosystems and could be used to indicator species. In addition, the water quality of the Chiang Mai Moat was classified as meso-eutrophic status.

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Biodiversity Assessment in and around the Museum Lake in Govt. Botanical

Garden and Museum, Thiruvananthapuram India

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Keywords: Biodiversity, Museum Lake, Avifauna, Ecosystem Health, India

ABSTRACT

Lakes perform several hydrological, biological, biogeochemical and other ecological functions both at ecosystem and landscape level with high aesthetic values. They significantly influence the microclimate and thereby influence the biodiversity in that aquatic ecosystem. An ecological survey carried out on the riparian flora and fauna around the Museum Lake recorded sixty bird species, thirty seven butterflies, twelve Odonates, forty six varieties of plants, five fish species, six varieties of reptiles and two varieties of amphibians. As urbanization is making depletion of the natural habitats, this *ex situ* conserved area well nourished with exotic and indigenous floras and perennial water body inside provides shelter for diverse fauna. The survey results will helps in highlighting the importance of this inland lake ecosystem and suggesting recommendations for the better conservation and management of the aquatic ecosystem as a whole.

1. INTRODUCTION

Freshwaters come under the lentic ecosystems are fundamental for life on Earth, and yet freshwater biodiversity remains poorly documented, understood, and protected (Ajayan et al., 2018). Lakes forms a significant source of precious water, it provides valuable habitats to a variety of flora and fauna. They influence the microclimate, improve the aesthetic beauty of the landscape and extend many recreational opportunities.

The restoration, conservation and management of lakes require a thorough understanding of all the components of that ecosystem. Ecological monitoring and assessment provides baseline information about the condition of the aquatic systems. A lake in an urban ecosystem forms an integral part of it performing significant environmental, social and economic functions ranging from as a source of drinking water, recharging groundwater, and acting as sponges to control flooding, supporting biodiversity and providing livelihoods. While cities are facing challenges of unplanned rapid urbanization, these water bodies plays an important role in maintaining environmental sustainability irrespective of whether they are natural or artificial wetlands.

2. METHOD

Thiruvananthapuram Government Botanical Garden and Zoo is one of the oldest of its kind in India which is located at the heart of the temple city (08°30' N, 076°57'E). It is well known for its vivid collection of plants and variety of animals in collection. The tropical trees, water body and its associated shores serve as a ground for many faunal species. It is a perennial water body but an artificial one with 159 year old since from its construction in 1859, has never lost its water level even in the scorching summers. The waterbody complete with an island, lush vegetation and the consequent abundance of winged visitors - has never failed to arrest the attention of visitors. This is a source of water for the Zoo Animals.

Ecological survey of the birds, butterflies, dragonflies, riparian vegetation, reptiles and amphibians associated with the water body has been carried out during February 2013 to October 2016. Field studies were carried out during the early morning hours from 6.30 am to 9.30 am and in the evenings from 4.30pm to 5.30 pm. Data were collected by direct observations, random walks and opportunistic observations and photographed in the study area. The field guides used for biodiversity assessment is

given in Table.1

Table.1 Field guides	for the ecological	survey
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Avifauna	(Ali and Ripley, 2007; Grimmet et al.,		
	2015 and Kazmierczak, 2000).		
Flora	Gamble, 1986		
Ichthyofauna	Jayaram, 1999		
Odonates	Kunte (2000) and Kehimkar (2008),		
	Kiran and Raju (2013)		
Amphibians and	Palot (2015) and Das (2015)		
reptiles			

3. RESULTS

During the study period a total of 60 bird species belonging to 33 families and 14 orders were recorded around the Museum Lake. Among them 51 of the birds recorded were included in the Schedule I and IV of the Wildlife Protection Act (WPA) 1972. 53 bird species in them are resident breeders, 14 of them are winter migrants. Little Grebe (Tachybaptus ruficollis), Black-crowned Night-heron (Nycticorax nycticorax), Lesser Whistling-duck (Dendrocygna Indian javanica), Pond-heron (Ardeola gravii), Cattle Egret (Bubulcus ibis), Intermediate Egret (Mesophoyx intermedia), Little Egret (Egretta garzetta), Little Cormorant (Phalacrocorax niger), Indian Cormorant (Phalacrocorax fuscicollis) are the waterbirds recorded from Museum Lake. A good but varying population of Cormorant type species and the Oriental Darter (Anhinga melanogaster) a bird enlisted in the Red Data Book of IUCN as a RET species, the Museum Lake within the zoo provides a potential habitat for the bird. Sighting of Asian Openbill Stork (Anastomus oscitans) is significant, which shows the availability of necessary resources from the water body.

A total of 37 butterfly species belonging to five families were observed from the riparian zone. One of them is endemic to the Western Ghats and three species are protected under various schedules of the Indian Wildlife (Protection) Act, 1972. Family Nymphalidae (brush-footed butterflies) dominated the butterfly fauna 15 species followed by Papilionidae (swallow-tails) 9 species, Lycaenidae (blues) 7 species, Pieridae (whites and yellows) 3 species and Hesperiidae (skippers) 2 species. Malabar Rose (*Pachliopta pandiyana*) endemic to Western Ghats was also observed from this area. Among the butterfly species Danaid Eggfly is protected under schedule - I of Indian Wildlife Protection Act 1972. Grey Count and Gram Blue are included under Schedule - II. Twelve varieties of dragonflies and damselflies were recorded from the study period. Long Legged Marsh Glider (*Trithemis pallidinervis*) was very common. The life cycle of odonates are closely associated with water bodies (Kannagi *et al.*, 2016) and their occurrence can be taken as an indicator of a healthy ecosystem and for quality of water (Emiliyamma *et al.*, 2005).

The fishes in the Museum Lake has been introduced and only five varieties of fishes - *Oreochromis mossambica* (Mozambique Tilapia), *Ctenopharyngodan idella* (Grass Carp), *Catla catla* (Catla), *Labeo rohita* (Rohu) and *Xiphophorous maculates* (Southern Platyfish) were observed. The fish species which was listed as some of the "100 of the world's worst invasive alien species" (Lowe *et al.*, 2000) - Egyptian Mouthbreeder or Tilapia was recorded from here. In conservation areas invasive fishes are a notable stressor for the ecosystem (Russell, 2011). Since the primary aim of zoos and conservation parks are protection and conservation, stocking of these invasive fishes in water bodies are to be prevented (Raghavan *et al.*, 2016).

A number of rare and exotic floras are associated with the lake and are kept as part of conservation and beautification in mind. The bamboo varieties serve as host plants for *Telicota ancilla (Dark Palm Dart)*. Seasonal flowering varieties like *Pongamia pinnata, Pteocarpus marsupium* and *Peltophorum peltocarpum* are attracting these nectar feeding butterfly groups. The rain tree- *Samanea saman* serves as the host plant for Common Grass Yellow (*Eurema hecabe hecabe*). *Mangifera indica* and *Barringtonia acutangula* along the riparian zone forms the host plant for Monkey Puzzle (*Rathinda amor*) variety of butterfly. The host plants for Common Cerulean and Lemon Emigrant was *Butea monosperma*.

From the ecological survey, six varieties of reptiles and two varieties of amphibians have been documented from the Museum Lake. The Indian Rat Snake (*Ptyas mucosa*) and Checkered Keelback (*Xenochrophis piscator*) are listed in the Schedule II under the Wildlife Protection Act of India (1972) and Travancore Kukri Snake (*Oligadon travancoricus*) endemic to Western Ghats and Travancore Wolfsnake (*Lycadon travancoricus*) listed in the Schedule IV of the WPA are the reptile varieties sighted around the Museum Lake.

4. DISCUSSION

The dense growth of different species of trees and water bodies and the resources provided for various exhibited animals offer diverse habitat and favorable conditions which are being used by the native and urban population of fauna. These flora and fauna are significant in maintaining the balance of this ecosystem. A serious issue of mismanagement in fish stocking with invasive carps and tilapias and southern platy fish was noticed during the study. The high density population of grass carps and tilapias can alter the water quality of the lake.

5. CONCLUSION

The associated flora and fauna of the Museum Lake confirms it as a diversified ecosystem. These flora and fauna are significant in maintaining the balance of this ecosystem. Regular monitoring of the biodiversity associated with this area and scientific management and conservation measures will help from degradation. This study stresses on the need for conservation of this aquatic ecosystem and a right management measure as the need of the time. The survey also points out towards the meaningful role in educational, entertainment, scientific and conservation activities of Thiruvananthapuram Zoo.

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Diversity of Fishes in Laguna de Cagayan Lake, Philippines: Status and Conservation Needs

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Keywords: species composition, abundance, IUCN, Red List status, diversity indices

ABSTRACT

Assessment of ichthyofauna and habitat stability of Laguna de Cagayan Lake is a pioneer study to document diversity and conservation towards sustainable ecosystem services. The study was conducted to characterize species composition, abundance, IUCN Red List status, and diversity of fishes caught in the lake from September, 2017 to March, 2018. Species composition revealed 1,714 individuals belonging to 14 species from 9 families. The dominant species *Trichopodus pectoralis* consisted 41.94% and the least 0.06% of the population is *Leiopotherapon plumbeus*, Family Osphronemidae dominated the catch followed by Cichlidae and Anguillidae *.Cyprinus carpio* VU is the only Red List species. But *Anguilla celebesensis*, *A. bicolor pacifica* and *Clarias macrocephalus* are NT fishes. Diversity T-test showed significant differences (P<0.05) on fish abundance between two sites and species richness of Luga, Margalef Index d= 1.77 is higher than Bangalao d= 1.34. Shannon-Weiner index H=1.59 revealed that the lake is fairly stable. Simpson's Reciprocal Index showed 1/D= 3.34- 4.29 means there are 3-4 commonly caught species and suggest good result of the study. Pielou's Evenness (J=0.69) observed more equal distribution of individual. Furthermore, Sorensen's Index Cs=2.67 showed more than half of the fishes identified in both sites are of the same species. Results of this study could serve as a baseline data in coordination with concerned line agencies to formulate sound-community based management strategy.

1. INTRODUCTION

The Philippine archipelago belongs to the world's richest mega diverse countries and one of the biodiversity hotspots worldwide with high diversity of endemic species significantly altered by human activities. The province of Cagayan (capital: Tuguegarao City, 17⁰ 37'N 121⁰43'E) lies in the northern part of Region II. Cagayan is endowed with rich biodiversity. In the recently conducted Philippine Biodiversity Conservation Setting, the region has been one of the priority conservation sites and identified as Key Biodiversity Areas.

Species richness and relative species abundance describe key elements of biodiversity. Diversity is maximum when all species that make up the community are equally abundant or have similar population sizes ^[1]. Diversity indices are commonly related to studies on species composition and distribution to evaluate stability of aquatic ecosystems.

Documentation of species composition and abundance of fishes at Laguna de Cagayan (LDC) Lake is important because this freshwater ecosystem has never been scientifically assessed. There is no detailed investigation on species diversity to date thus, this study was conducted. Proper documentation of the area is needed for policy formulation and proper management of the ecosystem.

This study generally aimed to assess the diversity and abundance of fishes along Laguna de Cagayan Lake. Specifically aimed to determine the species composition and evenness, Red List conservation status, and diversity indices of fishes caught.

2. METHODS

2.1 Study Area

Laguna de Cagayan Lake is bounded in the north by Bangalao Elementary School, in the south by Namunit hills and caves, in the west by a wide landmass uninhabited and the east by rice fields towards the Sierra Madre Mountains. LDC Lake has rich ecosystem diversity and natural scenic attractions promote the famous annual "National Eco-tourism Festival". Laguna De Cagayan (LDC), the town's premiere lake also known as Bangalao Lake is one of the most productive inland waters having diverse aquatic flora and fauna. Various aquatic vegetation's like the emergent native bakong grass, Hanguana malayana surrounds the lake abundantly, which is widely used in making expensive barongs, bags, and wall decors. The lake has an estimated area of 200 ha. located at Brgy. Luga, Sta. Teresita, Cagayan North. Three sampling points each were used in site 1-Sitio Bangalao and site 2- Barangay Luga.

2.2 Data Gathering and Analysis

Fishing practices used passive gears like fish corral in shallow portion. Gill net and hook and line were used at depths of 5-10 m. Specimen were collected at random from the catch of three fishermen/ cooperators each from the different fishing gears. Only two fishermen have skiff boat. Sampling was done thrice a month in the landing sites between 6:00 - 8:00 am from September 2017-March 2018. Fishes were counted and weighed fresh and classified to the lowest possible taxon. Some were preserved in ice packed cooler and brought to COFMS-Aparri laboratory for further identification.

Classified species were further verified at FishBase 2018^[2]. The conservation status of fishes classified were cross referenced with IUCN Red List of Threatened Species 2018. Species richness was determined by the number of freshwater fishes classified and abundance the number of individuals per species. Five indices were used to estimate the various measures of diversity using the formula as follows: (a) Margalef's diversity index d=S-1/logeN where S is the number of species and *logeN* is the natural logarithm of all individuals found. (b) Shannon-Wiener Index - H'= Σ - (Pi * ln Pi) where Pi is the fraction of the entire population made up of species i and ln is the natural logarithm to determine how diverse the ecosystem in terms of species composition. (c) Simpson's-Reciprocal Index- 1/D=1/ $\Sigma (n/N)^2$ where n is the number of entities belonging to the ith type and N is the total number of entities in the data set. (d) Pielou's Measure of Evenness - J' = H'/ln(S) where H' is the Shannon-Weiner index value and S is the number of species. (e) Sorensen's Similarity Index - CS=2j/a+b where j is the number of species common to a given pair of locations and b are the number of species occurring in either of the two locations. T-test was used to analyze diversity indices between sites using the SPSS version 19 program.

3. RESULTS

Table 1 presents a total of 1,714 individuals belonging to 14 species. The most dominant species is *Trichopodus pectoralis* 719 individuals (41.94%), *Oreochromis niloticus* 420 (24.05%) and *Channa striata* 121 individuals (7.06%). Least species are *Leiopotherapon plumbeus*, a rare fish in the lake and *Acanthopagrus latus*, a transient species from Buguey Lagoon, a nearby brackishwater water.

Table 1. Species Composition, Abundance and IUCNRed List Status

Species	Pcs	%	Status
Anguilla celebesensis	94	5.47	NT
A. bicolor pacifica	25	1.46	NT
A. marmorata	2	0.16	LC
Clarias macrocephalus	9	0.53	NT
C. batrachus	95	5.54	LC
Cyprinus carpio	19	1.10	VU
Ctenopharyngodon idella	80	4.66	NE
Trichogaster trichopterus	117	6.83	LC
Trichopodus pectoralis	719	41.94	LC
Oreochromis niloticus	420	24.50	NE
Channa striata	121	7.06	LC
Anabas testudineus	11	0.63	DD
Acanthopagrus latus	1	0.06	DD
Leiopotherapon plumbeus	1	0.06	NE

Diversity Indices of Fishes

Margalef's diversity index showed that Luga (d=1.77) is significantly richer than Bangalao (d=1.34) together with fish abundance. Significant difference revealed that Bangalao (1/D=4.29) has more number of common fishes caught than Luga (1/D=3.34). This

implies that in every fishing activity, there are 3 - 4 commonly species caught. Pielou's evenness values range from J'- 0.60 to 0.77 both near to 1, the distribution of fish assemblage described as high evenness Furthermore, Sorensen's Similarity index showed that more than half of the species caught in both sites (Cs=2.67) are of the same species.

 Table 2. Diversity Indices of Fishes Caught

Diversity Indices	Sitio Bangalao	Brgy. Luga	Pooled
Number of Species	8	14	14
Fish Abundance	186 ^a	1,528 ^b	1,714
Margalef's Diversity Index (d)	1.34 ª	1.77 ^b	1.56
Shannon-Weiner Index (H)	1.60 ^a	1.58 ^a	1.59
Simpson's-Reciprocal Index (1/D)	4.29 ^a	3.34 ^b	3.82
Pielou's Measure of Evenness (J')	0.77 ^a	0.60 ^a	0.69
Sorensen's Similarity Index (Cs)	2.67	2.67	2.67

4. DISCUSSION

Species Composition and IUCN Red List Fishes

In this study, 14 fish species were classified belonging to nine (9) families and four (4) orders. In Bangalao, eight (8) species were commonly caught while Luga has 14 species where anguillids and clariids are more abundant here. Most eels, catfishes and mudfishes were caught by hook and line fishers with skiff boat. Result showed lower species composition than Lake Taal River Systems with 37 species belonging to 19 families ^[3] but higher than Tikub Lake ^[4] with 9 species from 7 families, both in Luzon Island, Philippines. The most dominant family is Osphronemidae followed by Cichlidae, Anguillidae, Channidae and Clariidae. In River Ore, Nigeria, clariids and channids were the most abundant and dominant groups.^[1] The result showed almost similar in species composition on the preliminary study at Aparri Cagayan River Estuary ^[5] with 15 finfishes caught by filter net due to the geographical location.

The Philippines is one of the world's major hotspots for the conservation of biological diversity. It hosts about 3,010 fish species with only 344 (10%) occurring in freshwaters, of which are 83 (24%) are endemic, 206 (62%) are native, 44 (15%) are introduced in the country, and 42 are of certain of status ^[2]. The L. plumbeus caught by gill net is endemic in the lake and other freshwaters of the country including Laguna de Bay, the largest lake in the Philippines. The presence of single specimen caught suggest that it is rare and endangered in the lake. And according to the fishermen, they can only encounter once or twice in a year. It was once abundant in the Tikub Lake but due to sulfur upwelling, the entire population of this rare species was totally wiped out ^[4]. It is is suspected to be threatened already but no data in CITES 2017^[6] and recorded as

Not Evaluated (NE) by IUCN Red List of Threatened Species.

Some 20 years ago, according to fishermen of LDC Lake, *Clarias macrocephalus* was once abundant but due to the introduction of Thailand hito, *C. batracus* an invasive alien species (IAS) is now more dominant species therefore, reduced the population of the native catfish. Further research across the species range is needed into the impacts of hybrids on the wild. Thus, the conservation efforts should ensure minimization of anthropogenic impacts, like the introduction of IAS.

Cagayan River, the longest river in the Philippines, is also habitat of the above two Near Threatened (NT) eel species including vulnerable (VU) species *Cyprinus carpio*. Although no population data exists, it is suspected that in the past 60 to 75 years within the native range, major threats are river channelization which impacts the species. Common carp needs flooded areas at very specific times to successfully spawn, and hybridization with introduced stock, has caused a population decline of over 30% ^[2]. Due to rising concerns over the increased exploitation of elvers, the Philippines issued a ban in 2012 on the trade of juvenile eels. Aside from this ban, which was largely driven by fisheries concerns, there are other conservation measures being taken for *Anguilla spp*.

LDC Lake has recently joined select areas in the country in the implementation of the Restoration and Enhancement of Fisheries in Lakes and Reservoirs, to capacitate the residents surrounding the lake on fisheries management, protection and conservation.

Diversity Indices

Results showed that Margalef's diversity index in Laguna de Cagayan varies from d=1.34 to 1.77. Diversity T-test results showed that Luga is significantly higher in species richness and abundance than Bangalao. Shannon-Weiner index H=1.59 means that the habitat structure is fairly stable and quite balanced. Comparing the diversity to Buguey Lagoon of a nearby town has higher species composition (n=43) with a stable ecosystem H=2.0 (author's unpublished paper presented WLC 2016, Bali, Indonesia). Lake Geriyo, Nigeria^[8] with H=2.48 indicated a fair fish diversity and Lake Taal River Systems^[3] with a range of H=2.32-3.05 as high diversity. The fair diversity estimate of this 200ha. lake could be explained by the very few fishermen with lesser fishing effort because they are busy with other livelihood like farming. They are not interested even in buying a motorized banca nor constructing one, again only two used a skiff boat. Most of the fishermen are about two km. away from the isolated deeper portion of the lake. However, because of the scenic beauty of this biodiverse lake including nearby verdant hills and numerous caves, it is where the said municipality celebrates the opening program and other fisheries activities of the national annual festival. Its theme emphasizes the management and conservation of terrestrial and aquatic ecosystems biodiversity.

High evenness values J'=0.69 was observed. The distribution of fish assemblage can be therefore

described as high evenness, reflecting fish assemblage co-existing within the lake. Result is quiet lower than Lake Taal River Systems ^[3] of which values ranged from J'=0.7 - 0.85.

5. CONCLUSION

Fourteen (14) species of freshwater fishes with a total of 1,714 individuals were classified at LDC Lake with good species richness and abundance Trichopodus *spp.* dominated the catch followed by cichlid, anguillids, channids and clariids. Cyprinus carpio (VU) is the only Red List species. More ever, native Anguilla bicolor pacifica celebesensis. Α. and Clarias macrocephalus are Near Threatened (NT) fishes already. Shannon-Wiener index estimate of fair habitat stability is for further study. Simpson's Reciprocal Index significantly showed 3-4 common fishes caught every fishing in Laguna de Cagayan Lake and suggest good result of the study. Consequently, Pielou's Evenness revealed more equal distribution of individuals or high evenness. Furthermore, Sorensen's Similarity Index showed that more than half of the fishes identified between sites are of the same species.

Further study should be conducted and coordinated to government line agencies like DA- BFAR and DENR for funding and better documentation. The generated data base will be presented to the Local Government Unit (LGU) of Sta. Teresita for information dissemination to increase awareness among fisher folks on the importance of conserving threatened, native and endemic fishes in maintaining ecosystem integrity and promote sustainable fishing. This study could serve as a baseline data in formulating management plan and conservation of fisheries resources of this water body.

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Bird abundance in the rice fields of Chor-Lae community,

Mae Rim District, Chiang Mai Province in Thailand

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ABSTRACT

The objective of this study was to investigate the abundance of birds in the rice fields and its surroundings during farming season. The survey was conducted from September to December 2016, by using the line transect technique along the rice fields. Data were collected from 0600 h to 0900 h and/or 1500 h to 1800 h, 16 times in total. Overall, seven orders consisting of 45 species within 24 families of birds were found. Thirty-two species were resident birds and 13 species were migratory birds. Passeriformes was the most common order of birds found in the area. Twelve bird species were categorized as abundant, for instance, Common Myna (*Acridotheres tristis*), White-vented Myna (*Acridotheres grandis*), Spotted Dove (*Streptopelia chinensis*), Rock Pigeon (*Columba livia*) and Eurasian Tree Sparrow (*Passer montanus*). Seven species were common; for example, White-breasted Waterhen (*Amaurornis phoenicurus*) and White Wagtail (*Motacilla alba*). Thirteen species were moderately common, such as Bronzed Drongo (*Dicrurus aeneus*). Other 13 species were uncommon, such as White-throated Kingfisher (*Halcyon smyrnensis*) and Intermediate Egret (*Mesophoyx intermedia*). The Shannon diversity index was 3.16 and the evenness index was 0.88. The data from this research will be useful for those interested in studying birds in the rice fields, wetlands or fresh water sources, and it would build good source of knowledge for community for planning conservation of natural resources in the future.

1. INTRODUCTION

Chor-Lae community is located in the basin of the Mae Ping and Mae Ngad River. It is an agricultural area with extensive vegetable gardens, fruit orchards and rice fields which are the good sources of food and habitat for birds. Many birds help to eliminate insects, worms and pests or help to spread pollination. During rainy and winter seasons, between August to December of every year, the large part of Chor-Lae community will become rice fields. When rice is planted, various living species, such as insects, mollusks, mice, etc., will come into the area due to the abundance of food and water. These organisms are abundant sources of food for many species of birds. Therefore, the objective of this study were to explore the diversity and abundance of birds in the rice fields and its surroundings, to gain knowledge for the use of community and those who are interested later on.

2. METHOD

Bird surveys were conducted along the rice fields in Chor-Lae community (19°08'30.5"N 99°00'48.2"E) by using the line transect technique. This technique is suitable for a complex environment or large areas with open spaces such as rice fields, wetlands, lowlands, etc., where birds are easily visible. The total distance observed was approximately 850 meters. The data were collected 16 times across the period of four months (four times every month) during September to December 2016, from 0600 h to 0900 h and/or 1500 h to 1800 h. The surveys were designed to explore both left and right sides vertically to the path not over 30 m. Binoculars and a digital camera were used to search for the location of birds from the ground level, in grass, bushes and up to the tall trees^[1]. Species, numbers and behaviour found were recorded. Bird species were identified by using the bird guide. The relative abundance^[2], Shannon diversity index (H') and Pielou's evenness index (J') were then calculated.

3. RESULTS

From the survey of birds found in the area from September to December 2016, there were seven orders consisting of 45 species from 24 families. Thirty-two of them were residents, such as Red Collared Dove (Streptopelia tranquebarica), White-throated Kingfisher (Halcyon smyrnensis), Green Bee-eater (Merops orientalis), White-breasted Waterhen (Amaurornis phoenicurus), Bronzed Drongo (Dicrurus aeneus), Scalybreasted Munia (Lonchura punctulata), Little Spiderhunter (Arachnothera longirostra), Olive-backed Sunbird (Nectarinia jugularis), Eurasian Tree Sparrow (Passer montanus), Baya Weaver (Ploceus philippinus), Streak-eared Bulbul (Pycnonotus blanfordi), Redwhiskered Bulbul (Pycnonotus jocosus), Sooty-headed Bulbul (Pycnonotus aurigaster), Black-collared Starling (Sturnus nigricollis) and Common Myna (Acridotheres tristis). The other 13 species were migratory birds or winter visitors, such as Chinese Pond Heron (Ardeola bacchus), Asian Openbill (Anastomus oscitans), Common Kingfisher (Alcedo atthis), Sand Martin (Riparia riparia), White Wagtail (Motacilla alba), Red-throated Flycatcher (Ficedula parva) and Japanese White-eye (Zosterops japonicas) (Fig.1). When considering the abundance of individual birds, across five categories of the abundance, 12 species were categorized as abundant, seven species were common, 13 species were moderately common and 13 species were uncommon (Fig.2). When analyzing the data drawn out from the recorded information, the diversity index was 3.16 and the evenness index was 0.88.



■ Residents ■ Winter visitors

Fig.1 Orders and status of birds found in the rice fields of Chor-Lae community during the farming season.



Fig.2 Relative abundance of birds found in the rice fields of Chor-Lae community during the farming season.

4. DISCUSSION

The most common bird species recorded are in order Passeriformes. They live in this area all year long and do not move or migrate to other areas. Thailand is in the tropical zone where warm weather is stable throughout the year. More than half of the birds found in Thailand are endemic. Each species adapted themselves to suit different habitats and adaptable birds are found in almost every season and habitat. However, the results shown that several species are abundant than others. They are Common Myna, White-vented Myna, Spotted Dove, Rock Pigeon and Eurasian Tree Sparrow. These birds are able to adapt to the changing environment, communities' livelihoods and the development of the country. By living in the suburbs where food and water sources are plentiful, the species can spread more widely^[3]. These birds also eat a variety of food and that makes more chance of survival than other species that eat specific kinds of food. Also, close to the rice fields, there are vegetable gardens, orchards and several ponds where food, fruits, seeds, grains, insects and small mollusks are abundant for them to eat. However, around the beginning of December, rice was harvested. There were more kinds of grains, insects and reptiles under the ground which brought more birds coming into the area, especially migratory birds such as White Wagtail, Common Kingfisher, Red-rumped Swallow, Sand Martin, Barn Swallow, Intermediate Egret and Little Egret.

Birds of interest in the area are Asian Pied Starling and Asian Openbill. Asian Pied Starling can be found in all parts of Thailand except the south and northeast. In the past, this species were rarely found in the north. However, this bird has successfully spread due to the breeding programme. Besides, this may be due to the abundance of resources in the northern part of Thailand, the species can find food more easily. The study area is also near the water sources and rice fields which are suitable for them to find food. We found the migration of Asian Openbill in the early period of the study. They will travel to Thailand around September or October and continue to live throughout the winter (November-February). This bird usually live by the rice fields, swamps, marshes, wetlands or fresh water sources where they feed on fish, mollusks, etc^[4].

Seasons in the year usually affect the number and density of birds. Overall, the Shannon diversity index of this study was 3.16. First couple of months of the study were the beginning of the rainy season and the area was generally watered. As a result, the abundance of bird habitats and food sources had increased. It caused more birds to spread out of the area and to other wetlands. By the end of the study, it was the beginning of the winter season and rice was harvested. The diversity of birds had increased because of the reproductive season of the resident birds and there were groups of migrant birds joined. Birds that eat grains and insects would be easily found such as Green Bee-eater, Sand Martin, Barn Swallow, Ashy Woodswallow, Red-throated Flycatcher and Common Tailorbird. For the species evenness, it was generally 0.88. Birds, that are living on top of tree canopy, by the roof of the house or walking along the ground and farmland, usually live together as a group. Most of them are found throughout the season, such as Eurasian Tree Sparrow, Scaly-breasted Munia, Black-collared Starling and Spotted Dove, resulting in high consistency. In open spaces and meadows, there were many small birds that were found in both grouped species and isolated species, such as Green Bee-eater, Common Tailorbird, Barn Swallow and Ashy Woodswallow. This makes them a little less consistency.

5. CONCLUSION

There are 45 species found in Chor-Lae community consisting of both resident and migratory birds. Most of them are in order Passeriformes. Each species has a different abundance caused by types of food, water sources, habitats, seasons and migration.

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青森県姉沼におけるイシガイ科二枚貝の脱落後の生態に関する研究

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キーワード:生物多様性評価,生態系機能,絶滅危惧種

抄録

イシガイ科二枚貝は、世界各地に広く分布し、国内では 15 種 1 亜種が確認されているが、近年、湖沼流域環境の劣 化の影響で 15 種中 11 種が絶滅を危惧される状態にある。そこでイシガイ科の保全を考慮し、本研究では、脱落後の 稚貝の生息適地に必要な餌料の解明を目指すことを目的とした。宿主魚類から稚貝を採取し、この稚貝を餌料が異な る底質で飼育することによって、その成長可否について調べた。解析の結果、底質にバイオフィルムが存在すると成長 量が大きくなる傾向がうかがえた。また、乾燥させたバイオフィルムも餌料として機能することが示唆された。足と消化管 内に繊毛を確認し、それらを用いて足弁摂餌およびそれらで水流を起こして周囲の餌料を摂食している様子が確認さ れた。これらのことから、イシガイ科稚貝は、バイオフィルムを餌料とし、足弁摂餌により摂食していると考えられる。

1. はじめに

軟体動物門に属するイシガイ科二枚貝(イシガイ科: Unionidae)は、世界各地に広く分布し、日本国内では 15種1 亜種が確認されている^[1]。近年,湖沼流域では, 開発や人工の増加等の社会・経済的な構造の変化によ って汚濁負荷が増加し、それと密接に関連した湖沼の 水域環境が損なわれてきており[2],これらの水域環境の 劣化によって 15 種中 11 種が絶滅を危惧される状態に ある[3]。本科二枚貝は、特定の宿主に対して寄生や産 卵場の提供を通じた共生関係[4]や大量の有機物を短期 的な物質循環のサイクルから取り除いて底土に集積する など生態的機能を有しているとともに, 浮遊物・金属イオ ンを取り除く[5]ことで水質浄化を行っているため、その保 全は必須である。本科二枚貝の保全を考慮した場合, 脱落後の稚貝の好適な環境を確保することで稚貝の生 残率を向上させることが重要な課題だが, 稚貝の生態に 関する知見は、成貝の生態に関する知見に比べて極め て少ない。中でも脱落後の稚貝の生息適地に必要な餌 料については、具体的にどの成分を吸収し、栄養分とし ているかは未だに不明な点が多い^[4]。そこで本研究で は,脱落後の稚貝を蓄養し,成長量を把握することで, 底質に含まれる自然環境下の餌料の解明および形態観 察により摂餌方法の解明を目指した。

2. 方法

対象地は,青森県東部に位置する姉沼とし,2017 年 6月27日,11月7日に宿主魚類の採捕を行った。宿主 魚類は定置網(周囲長 30 m, 目合い 5 mm)を用いて採 捕した。採捕する宿主魚種は,水質変化に比較的に強 く,長期的な飼育が容易なハゼ科(ウキゴリ,ジュズカケ ハゼ, ヌマチチブ)の魚種に限った。持ち帰った宿主魚 類は標準体長に合わせて 10~15 個体ずつ虫籠(190 mm×95 mm×90 mm 間隙寸法 2 mm) に入れ, それをエ アーポンプ(Suisaku 水心 SSPP-2S)で曝気を行っている 円筒形容器(直径 20 cm, 高さ 205 mm)に収容した。円 筒形容器は水温(20~23℃)を一定に保ったトロ箱(700 mm×410 mm×200 mm) に収容した。採捕日の翌日から 毎日,円筒形容器内の稚貝を含む沈殿物をプランクトン ネット(目合い112μm)で濾し取った。濾し取った沈殿物 は、プラスチック製のシャーレー(直径 60 mm)に移した 後,実体顕微鏡(SHIMIZU)で沈殿物から稚貝を探し出 し、パスツールピペット(先端直径 1 mm, 長さ 143 mm)を 用いて採取した。採取した稚貝は、採取時期により異な る方法で飼育を行った。稚貝の餌料には, 姉沼の沿岸 域の底質に堆積し、シルトが含まれるバイオフィルム (biofilm)を利用した。2017年6月28日,6月30日,7 月 6 日に宿主魚類から採取した稚貝(イケチョウガイ: Hyriopsis schlegeli)は、採取日ごとに地下水のみの飼育 ケース(以下,底質無し),姉沼で採取した砂礫・少量の 採取した状態の生のバイオフィルムから成る土を入れた 飼育ケース(以下,姉沼の土),採取した状態の生のバ イオフィルムを入れた飼育ケース(以下,生バイオフィル ム)の3種類に等分し、計9個の飼育ケースで飼育を行

った。2017年11月8日~11月17日に宿主魚類から採 取した稚貝 (カラスガイ: Cristaria plicata)は、個体ごとに 底質無し,加熱処理を行った砂礫を入れた飼育ケース (以下,砂礫),生バイオフィルム,乾燥させたバイオフィ ルムを入れた飼育ケース(以下,乾燥バイオフィルム)の 4種類に等分し、計27個の飼育ケースで飼育を行った。 6月,7月に採取した飼育個体(イケチョウガイ)は4日お きに、11月に採取した飼育個体(カラスガイ)は3日おき にデジタルマイクロスコープ(HIROX KH-8700)を用いて 殻長, 殻高を記録した。そこから, 稚貝を四角で囲った 殻面積を算出し, 稚貝の成長量とした。 脱落時に斃死し た個体や飼育中に斃死した個体は, 電子顕微鏡 (HITACHI TM3030Plus)を用いて形態観察を行った。 観察は, 稚貝の腐敗を避ける為に稚貝の斃死を確認し た当日に行った。また, 稚貝の成長量の観察を行う際に, デジタルマイクロスコープでも稚貝の形態観察を行った。 3. 結果

イケチョウガイ稚貝の成長観察について, 脱落後 12 日 目の成育条件ごとの成長量を図 1 に示した。イケチョウ ガイの脱落時の殻高×殻長は、271×227 µm である^[1]。 観察の結果, 姉沼の土で飼育した稚貝の成長量は, 底 質無しで飼育した稚貝の成長量に対して, 有意に大き いことが示された(Scheffe 検定, p < 0.05)。一方, 姉沼 の土で飼育した稚貝の成長量と生バイオフィルムで飼育 した稚貝の成長量には, 有意な差は示されなかった (Scheffe 検定, p > 0.05)。同じく, 底質無しで飼育した稚 貝の成長量と生バイオフィルムで飼育した稚 貝の成長量と生バイオフィルムで飼育した稚貝の成長量 にも, 有意な差は示されなかった(Scheffe 検定, p > 0.05)。

カラスガイ稚貝の成長観察について,成育条件ごとの 成長量を図2に示した。なお,図2では18日目の稚貝 の成長量が最大値の個体の成長過程を線で結んだ。カ ラスガイの脱落時の殻高×殻長は、317×292 µm である ^[1]。図2に示した折れ線グラフから,脱落18日目の稚貝 の成長量は、生バイオフィルムで飼育した個体が最も大 きく、殻面積0.24 mm²であった。次いで、乾燥バイオフィ ルムで飼育した個体は、殻面積0.23 mm²となり、生バイ オフィルムの殻面積に近い値となった。砂礫で飼育した 個体は、殻面積0.18 mm²であった。底質無しで飼育し た個体は、最も小さく殻面積0.11 mm²であった。 電子顕微鏡およびデジタルマイクロスコープでの観察 の結果,稚貝の足と消化管内に繊毛を確認した。さらに, デジタルマイクロスコープでの稚貝の形態観察では,足 に触れた泥状バイオフィルムは繊毛を利用し,足の表面 上を移動させて殻内部へ取り込む様子が確認された。ま た,消化管上にある繊毛で水流を起こし,周囲の浮遊 物・泥状バイオフィルムを殻内部へ取り込む様子が確認 された。



4. 考察

イケチョウガイ稚貝の成長観察およびカラスガイ稚貝の 成長観察から,底質に生バイオフィルムが存在すると稚 貝の成長量が大きくなる傾向がうかがえた。したがって, イシガイ科稚貝の餌料はバイオフィルムの可能性がある。 また,乾燥バイオフィルムで飼育した稚貝は,生バイオフ ィルムで飼育した稚貝に近い成長量を示したことから, 乾燥させたバイオフィルムも餌料として機能することが示 唆された。イケチョウガイの成長観察では,餌料に加え

て砂礫があると成長量が大きくなる傾向が示された。ま た、カラスガイにおいても砂礫で飼育した稚貝は、餌料 が存在しない環境であるものの, 脱落直後の2倍近い成 長量を示した。底質(細砂)がある条件下で Epiobasma copsaeformisと Villosa iris の稚貝を飼育した場合, 底質 が存在しない場合に比べて成長率と生残率が高いとさ れ、これは底質(細砂)がバクテリアなどの餌料の供給源 となっている可能性があることが報告されている^[6]。この ことから, 砂礫が稚貝の餌料となる生物・物質の発生・付 着する基質となり、それを稚貝が摂食したことで成長した と考えられる。イシガイ科稚貝の摂餌方法として, Yeager et al.^[7]によると Villosa iris は足で餌料を集めていること が示唆されており、Akiyama^[8]では、脱落直後のタガイ は粘性の強い粘液を吐き出し,周囲のデトリタスを軟体 部の周辺に吸着させる行動を行うとしている。また, 鰓の 発達が十分でないアサリは足弁摂餌「๑」を行うことが報告 されている。電子顕微鏡の形態観察およびデジタルマイ クロスコープでの観察の結果を踏まえると、イシガイ科成 貝に対し消化管が未発達なイシガイ科稚貝は、足と消 化管内にある繊毛を利用し, 足弁摂餌により餌料を摂食 していると推察される。

5. 結論

脱落後のイシガイ科稚貝の餌料は、バイオフィルムの 可能性が示唆され、底質に付着・堆積しているバイオフ ィルムを少なくとも足弁摂餌によって摂食していると考え られる。

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Biodiversity evaluation using Nature Index tool in

Chilika Lake, Odisha, India – A case study

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Keywords: Biodiversity evaluation and ecosystem services

ABSTRACT

Biodiversity is crucial for human well-being; it provides food, nutrition and livelihood security. In 2010, parties to the Convention on Biological Diversity (CBD) adopted the Strategic Plan for Biodiversity 2011-2020, a ten year framework for action by all the countries to safeguard biodiversity and halt biodiversity loss. A pilot study was carried out in Chilika Lake for evaluating the status of biodiversity using the **Nature Index (NI)** tool developed by the Norwegian Institute for Nature Research (NINA). Chilika is one of the largest lagoons in Asia and this is the first Ramsar site of India and boasts of rich floral and faunal diversity. For evaluating the biodiversity status in Chilika Lake, 25 indicator species were selected (15 species of fish & 10 species of birds) and the time series data of these indicator species were fed into the NI database. The NI values were calculated using the R-package and the status of biodiversity was visualized though a colour coded map (e.g. red-very poor; orange, yellow and green-gradual improvement; and blue-good). NI is a well-designed policy tool that synthesizes biodiversity data and helps in monitoring the status of biodiversity. This monitoring tool can be used for identifying the threat status of species/ecosystem and guides the policy makers to take important policy decisions towards conserving the biodiversity. NI tool can be used for assessing the biodiversity status of the an ecosystem, protected areas, lakes, rivers, hotspots etc.

1. INTRODUCTION

India is a mega diverse country harbouring 7-8% of all recorded species of the world, which includes 48,000 species of plants ^[1] and more than 97, 708 species of animals ^[2] India has 10 bio-geographic zones and is home for 8.6% of mammalian species, 13.7% avian species, 7.9% reptiles, 4.7% amphibians, 11.7% fishes and 11.8% plants in the world. India has more than 15,042 species of marine diversity ^[3]. Biodiversity plays a crucial role in the functioning of the ecosystems on which human beings depend for food, water, health, recreation and protection from the natural disasters. The loss of biodiversity affects cultural and spiritual values that are integral to human well-being. The global mission of the Strategic Plan for biodiversity 2011-2020^[4] is advocating countries to halt the loss of biodiversity and ensure that ecosystems are resilient

and continue to provide essential services, thereby securing the planet's variety of life.

The objective of the present study is to test the Norwegian Nature Index (NI) tool in Chilika Lake for evaluating the biodiversity status in collaboration with the Norwegian Institute for Nature Research and Chilika Development Authority. The NI is a policy tool provides an overview of the state and trend of biodiversity and it can be used for taking important policy decisions for conserving and managing the biodiversity in an ecosystem^[5]. The status of an indicator is calculated through a scaled value (ranging from 0 to 1) where the value zero means that the species is extinct and the value 1 means that the status of the indicator is very good. The NI is then calculated as the average over many such indicators. This tool can be applied in both data-rich and data-deficient areas and helps the policy makers and administrators to monitor the biodiversity status in an ecosystem.

2. METHOD

The NINA has developed a web-based information system for recording, storing and presenting NI data. The system consists of SQL relational database connected to a web server and the database has various modules such as (i) indicator type; (ii) type of organisms (mammals/reptiles/birds); (iii) red list status (critically endangered/vulnerable/ near threatened/least concern); (iv) reference value; (v) pressure factors (e.g. eutrophication, pollution, climate change, habitat destruction, human pressure and hydrological changes); (vi) ecosystem affinity ^[5]. The time series data collected from various secondary sources were fed into these modules. The Reference value for the indicators species were determined with the help of experts (expert judgement/opinion) and the catch data were collected during 2003 was used as a reference year. The NI values were analysed by NINA using R-scripts and the status of biodiversity is visualised using colour coded map. For example, red shows a very poor state and the blue a very good state. Orange, yellow and green signify gradual improvements towards blue^[6](Fig.1).





For monitoring the status of biodiversity in Chilika, 25 indicator species were selected which includes 15 fish species (Flat head mullet, Large scale mullet, Sea bass, Bengal corvine, Four finger threadfin, Pearl spot, Hilsa shad, Grey eel-catfish, Spot tail needle fish, Bloch's gizzard shad, Small Bengal silver-biddy, Long whiskers catfish, Gold lined sea bream, Soldier catfish and Striped snakehead and 10 bird species (Northern pintail, Eurasian wigeon, Gadwall, Black-tailed godwit, Brown-headed gull, Asian open-bill stork, Purple swamp hen, Common coot and Lesser whistling-duck). The polygon map of Chilika was divided into 5 regions *viz* (i) Southern Sector (SC); (ii) Central Sector (CS); (iii) Northern Sector (NS); (iv) Outer Channel Sector (OSC) and (v) Island based on salinity variation.

3. RESULT

In the Chilika lake the maximum NI value of (>0.8) of bird population was recorded in OSC, CS and Nalabana Island and the moderate values of >0.7 (SS) and >0.6 (NS) was recorded in other sectors. In the case of fish diversity, the OSC has recorded the maximum value of >0.8 and in other sectors the NI values have declined {SS (from >0.9 to <0.7); NS (from >0.7 to <0.6); CS (from >0.7 to <0.6)} between 2005 to 2015. The status of the biodiversity can be visualised through the colour coded maps depicted in Fig.2.





4. DISCUSSION

The biodiversity wealth of Chilika Lake has tremendously increased after the ecological restoration of the Lake (by opening a new mouth to the Bay of Bengal) during the year 2000. This has resulted in rejuvenation of lost species (6 species of fish, 4 species of prawn, 7 species of crab and 2 species of Indian spiny lobster have reappeared) and the Irrawaddy dolphin population was increased from 89 to 160 individual.

The present study has reported that in Chilika the bird population was showing an increasing trend in OSC, CS and Nalabana Island regions. Birds such as the Brown headed gull, Asian open bill stock, Purple swamp hen, Common coot, Lesser whistling duck and Little cormorant is showing a good population trend in all the sectors studied.

In the cases of fishes, the Pearl spot and Grey eel showed a maximum aggregation in all the sectors and it was noticed that, the Spot tail needle fish was maximum in the OSC and the Hilsa population was high the NS. The figure indicates that there is a declined in the biodiversity in the NS (fresh water zone) and this be due to various anthropogenic pressure and over harvesting of fish resources by the local fishers. In the other sectors the good biodiversity trend was maintained due to the some biodiversity friendly initiatives by the CDA which includes: (a) protection of migratory waterfowl from poaching; (b) eviction of the Illegal prawn gherries from Chilika;(c) maintenance and dredging of the feeder channel and creeks, which facilitates for migration of fish juveniles; (d) catchment area treatment; (e) weed control; (f) restoration of feeding and roasting habitat of water fowl; (g) pollution control and (h) encourage community participation.

5. CONCLUSION

The pilot study carried out in Chilika Lake helped us in monitoring the status of birds and fishes (Sector wise in Chilika Lake) from the year 2000 to 2015. The NI tool provides an overall picture of the changes in biodiversity over a period of time and provides details about the causative factors for taking corrective measures. This scientific tool can be used for evaluating the impact of climate change; monitoring the implementation of National Biodiversity Targets (NBTs) and Sustainable Development Goals (SDGs) etc.

This NI tool can be extensively used for assessing the biodiversity wealth of the Protected Areas (PAs) in the forestry sector. Similarly, NI can be used for evaluating the wealth of marine/inland and agriculture ecosystems. This scientific tool provide visual clarity (maps and graphs) to the policy makers for taking important policy decisions on species and ecosystem conservation related issues. Currently there is no other scientific tool is available to store, synthesis the time series biodiversity data showing the trends over time for assessing the biodiversity.

Hence, the Nature Index tool can be extensively used in evaluating biodiversity in national parks, sanctuaries, biodiversity hotspots, etc. The PA area managers can use this tool to monitoring the status of wildlife biodiversity inside the PAs. The NI methodology and database is very well suited to assess the state of biodiversity. The data generated under this study can also be used for the preparation of national reports, and Climate Change action plans and for undertaking studies for the Intergovernmental science-policy platform on biodiversity and ecosystem services, etc. As India pledged under several International agreements, the NI study can be used for monitoring the progress of the various global and national biodiversity targets (e.g., Aichi, National Biodiversity Action Plan and Sustainable Development Goals) towards addressing the biodiversity loss.

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Terrestrial and Freshwater Mollusks on the University of Tsukuba Campus

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Keywords: biodiversity evaluation, introduced species, nature conservation, education

ABSTRACT

Terrestrial and freshwater mollusks comprise one of the most diverse groups of animals. The mollusks are generally known as good ecological indicators and, because they are slow-moving and easy to observe, they have great potential for use in education. To describe the species composition and diversity, we surveyed terrestrial and freshwater mollusks on the University of Tsukuba campus in Ibaraki Prefecture, Japan. Between May 2017 and April 2018, we recorded 22 terrestrial and five freshwater molluscan species in 15 surveys at 28 sites. Commonly observed species were *Euhadra brandtii brandtii* (local name, Hitachimaimai), *Zonitoides arboreus* (Kohakugai), and *Acusta despecta sieboldiana* (Usukawamaimai) in terrestrial ecosystems and *Pseudosuccinea columella* (Habutaemonoaragai) and *Physa acuta* (Sakamakigai) in freshwater ones. Introduced species were also observed: six were terrestrial and two were freshwater species, accounting for about 30% of the total recorded species. The molluscan species are threatened globally by anthropogenic disturbances. Our results will be used for science and environmental education, management of introduced species and identification of important areas for biodiversity conservation.

1. INTRODUCTION

Although terrestrial and freshwater mollusks (i.e., nonmarine mollusks) are one of the most diverse groups of animals, they are threatened by anthropogenic disturbances worldwide ^[1]. The main causes of their decline are habitat loss, predation by introduced species, and overuse by humans ^[2]. Yet terrestrial and freshwater mollusks are generally known as good ecological indicators. In addition, because they are slow-moving and easily observed, they are good study organisms for education.

Here we describe our field surveys of terrestrial and freshwater mollusks at the University of Tsukuba in central Japan. Our aims were to describe their species composition and diversity on the campus and use the data for student education and conservation planning. This project is still ongoing, and we report the preliminary results here.

2. METHODS

Between May 2017 and April 2018, we conducted field surveys of terrestrial and freshwater mollusks on the University of Tsukuba campus in Tsukuba city, Ibaraki Prefecture, Japan. The campus is approximately 257 ha and contains various types of ecosystem, such as forests and grasslands, as well as street trees, gardens, ponds, artificial drainages, and land/water ecotones. We explored 28 sites during 15 field surveys and recorded the molluscan species observed by at least one of the authors.

3. RESULTS

Twenty-two species of terrestrial mollusk from nine families were recorded in total. Commonly observed species were Euhadra brandtii brandtii (local name, Hitachimaimai), Zonitoides arboreus (Kohakugai), and Acusta despecta sieboldiana (Usukawamaimai). We found relatively high species diversity in forest and grassland ecosystems, despite them being fragmented into small pieces by road and building construction. On the other hand, five species of freshwater mollusk were observed at three aquatic sites: Sinotaia quadrata historica (Himetanishi), Pseudosuccinea columella (Habutaemonoaragai), Physa acuta (Sakamakigai), Corbicula sp. (Mashijimi or Taiwanshijimi), and Laevapex nipponica (Kawakozaragai). Individuals of Corbicula sp. could not be distinguished between C. leana (native) and C. fluminea (introduced) because they are morphologically similar and we found only shells of dead individuals. With regard to introduced species, we recorded six terrestrial and two freshwater species, accounting for approximately 30% of the total recorded

species.

4. **DISCUSSION**

The University of Tsukuba campus was constructed approximately 45 years ago. Today many areas have been developed with roads and buildings, and more than 10,000 students attend the campus. The observed mollusks consist largely of native species, some of which (e.g., *E. brandtii brandtii*) are characteristic to the Kanto plain. We also recorded introduced species that are common in urbanized areas. These findings can be used for science and environmental education, management of introduced species, and identification of important areas for biodiversity conservation.

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Isolation, screening, and identification of potential antibiotic-producing fungi

from surface sediments of Lake Lanao in Lanao del Sur, Philippines

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ABSTRACT

To keep abreast with the need to discover new antibiotics and help address problem on antibiotic-resistance, this study was conducted to isolate, screen, and identify potential antibiotic-producing fungi from Lake Lanao along Ditsaan Ramain, Taraka, and Marawi City, Lanao del Sur, Philippines. Isolation of fungi from sediment samples followed Serial Dilution Pour Plate Method resulting to the isolation of 126 kinds of fungi based on colony, hyphae and reproductive spores in slide cultures. Antibiotic screeening was done using Cotton Swabbing Technique which showed 10 (7.94%) isolates with antibiosis activity based on the zone of inhibition (ZOI) around each fungal isolate against *Escherichia coli* and *Staphylococcus aureus*. *Acremonium* sp., *Pestalotia* sp. 1, *Sporotrichum* sp., and *Cladosporium* sp. inhibited both test bacteria with average ZOI of inhibition of 2-17.5 mm. *Pestalotia* sp. 2, *Scopulariopsis* sp., and *Aspergillus* sp. 2 inhibited *S. aureus* only whereas *Curvularia* sp., *Aspergillus* sp. 1, and *Penicillium* sp. inhibited *E. coli* only with 7-9.5 mm and 2.75-6.25 mm average zone of inhibition, respectively. The fungal isolates will be further tested against other pathogens and their antibiotics produced will be later purified and utilized for drug development, thus, could be of economic and medical importance.

1. INTRODUCTION

Antibiotic resistance has increasingly become a problem in recent years because the pace at which we are discovering novel antibiotics has slowed drastically, while antibiotic use is rising. Therefore, we will always need new antibiotics to keep up with resistant bacteria. And the most common source of antimicrobial compounds was isolated from soil but in the last two decades, however, the rate of discovery of novel compounds from this source has significantly declined, as demonstrated by the fact that extracts from soil-derived microorganisms have vielded high numbers of clinically unacceptable metabolites. The aquatic environment is now becoming increasingly recognized as a rich and untapped reservoir of useful novel natural products. Fungal habitats in lake ecosystems are numerous, diverse, and often hidden and due to the specific habitat of freshwater fungi, they might have biosynthetic capabilities different from those of terrestrial fungi. Hence, there is a high possibility of obtaining new antibiotic metabolites of medical importance from freshwater fungi. Thus, this study was conducted to isolate, characterize, screen for antibiosis activity, and identify up to the genus level the untapped pool of microbial sources from Lake Lanao, the second largest lake in the Philippines.

Fungi that displayed antibiosis can be potential sources of new antibiotics that can be

further tested against other microbial pathogens. These antimicrobial substances can then be further purified, elucidated and developed as new drugs for the treatment of various infectious diseases.

2. METHOD

This study made use of experimental research design and was conducted at the Microbiology Laboratory of the Biology Department, Mindanao State University, Marawi City, Lanao del Sur, Philippines. All glasswares and autoclavable materials were sterilized at 121°C, 15 psi for 15 minutes.

Sediment samples were collected from the littoral zone of Lake Lanao which lies between 8° N. Latitude and 124° E. Longitude. Three sampling sites were at the bank of barangays Ditsaan-Ramain, Taraka, and Marawi City (see Figure 1). Surface sediment samples were analyzed within 24 hours of collection.

Isolation of fungi from sediment samples was done using Serial Dilution Pour Plate Method with dilutions plated into Potato Dextrose Agar (PDA) with Cefalexin and then incubated for five to seven days. Fungal colonies were characterized, coded, and maintained in PDA slants ^[1].

Antibiosis assay was done through direct inoculation of each fungal isolate into nutrient agar plates. After four days, the test bacterium was swabbed around fungal growth and incubated for 48 hours at 30°C. Antibiosis activity was based on the presence of the zone of inhibition around each fungal isolate against *Escherichia coli* and *Staphylococcus aureus*.

Characterization and identification (up to the genus level) of antibiotic-producing fungi were done using both cultural and microscopic features of these isolates from slide culture. Manuals by Hauser (2006) ^[3], and Woodward (2001) ^[5], were used. Data on the zone of inhibition of the fungal

isolates against the two test bacteria were analyzed statistically.



Figure 1. Map showing Lake Lanao in the Philippines with the three selected sampling sites (Taraka, Ditsaan-Ramain, Marawi City) in red boxes (https://images.search.yahoo.com/search/images

3. RESULTS

A total of 126 kinds of fungal colonies were isolated, characterized, and tested for antibiosis activity against *Staphylococcus aureus* and *Escherichia coli*. Results showed that only ten (7.94%) exhibited growth inhibition to both or either of the test bacteria (see Table 1). These fungi belong to eight genera that include *Acremonium* sp., *Pestalotia* sp. 1, *Sporotrichum* sp., and *Cladosporium* sp. which showed average zone of inhibition of 3.25-17.5 mm and 2-13.75 mm against *S. aureus* and *E. coli*, respectively. Among the fungal isolates with antibiosis activity, *Acremonium* sp. showed relatively the widest inhibition to the growth of the two test bacteria. *Pestalotia* sp. 1, *Sporotrichum* sp., and *Cladosporium* sp showed comparable but lower antibiosis activity.

Pestalotia sp. 2, *Scopulariopsis* sp., and *Aspergillus* sp. 2 inhibited the Gram-positive *S. aureus* only with average ZOI of 7-9.5 mm. On the other hand, *Curvularia* sp., *Aspergillus* sp. 1, and *Penicillium* sp. inhibited the Gram-negative *E. coli* only with 2.75-6.25 mm average zone of inhibition.

FUNGAL	TEST BACTERIA		MEAN
ISOLATES	Staphylococcus	Escherichia	
	aureus	coli	
Acremonium sp.	17.5	13.75	15.62
Pestalotia sp. 1	7.25	3.5	5.38
Sporotrichum sp.	3.25	2	2.63
Cladosporium sp.	3.25	2	2.63
Pestalotia sp. 2	9.5	0	9.25
Scopulariopsis sp.	9	0	9
Aspergillus sp. 2	7	0	7
<i>Curvularia</i> sp.	0	6.25	6.25
Aspergillus sp. 1	0	4.25	4.25
Penicillium sp.	0	2.75	2.75
Grand Mean	8.107	4.929	6.50

Table 1. Zone of inhibition (in mm) of the ten fungal isolates

against the two test bacteria after 48 hours of incubation.

The 10 fungal isolates which showed antibiosis are maintained as pure cultures in PDA slants. Slide culture and microscopic examination showed their colony, hyphae, and spore features (Figure 2).



Figure 2. Colonies and spores (1,000x) of the 10 kinds of fungi which showed antibiosis (*Acremonium* sp.(a), *Pestalotia* sp. 1
(b), *Sporotrichum* sp. (c), *Cladosporium* sp. (d), *Pestalotia* sp. 2
(e), *Scopulariopsis* sp. (f), *Aspergillus* sp. 2
(g), Curvularia sp. (h), *Aspergillus* sp. 1
(i), and *Penicillium* sp. (j).

4. DISCUSSION

The 10 fungal isolates inhibited growth of *S. aureus* and/or *E. coli* through a mechanism called antibiosis.

Antibiotics act by either inhibiting synthesis of peptidoglycan in the cell wall and/or proteins and phospholipids in the cell membrane resulting to cell lysis; or may also inhibit enzyme activity, thus, causing cessation in metabolism resulting to cell death ^[4].

Four genera (*Acremonium*, *Pestalotia*, *Cladosporium*, *Sporotrichum*) that inhibited both *S*. *aureus* and *E*. *coli* are potential sources of broad-spectrum antibiotics effective against both Gram-positive and Gram-negative bacteria being comparable with streptomycin (ZOI:15 mm) and cephalosporin (ZOI:18 mm)^[2]. *Pestalotia* sp. 2, *Scopulariopsis* sp., and *Aspergillus* sp. 2 that inhibited *S*. *aureus* as well as *Curvularia* sp., *Aspergillus* sp. 1, *and Penicillium* sp. that inhibited *E*. *coli* could be possible sources of narrow-spectrum antibiotics.

5. CONCLUSION

With the main purpose to find sources of new antibiotics, this study was able to isolate *Acremonium* sp. with antimicrobial activity comparable to commercially sold antibiotics followed by *Pestalotia* sp. 1, *Cladosporium* sp., and *Sporotrichum* sp.; all these four fungal isolates are potential sources of broad-spectrum antibiotics. Six other isolates that include two species of *Aspergillus*, *Curvularia* sp., *Penicillium* sp., *Pestalotia* sp.2, and *Scopulariopsis* sp. could be sources of narrow-spectrum antibiotics. Further tests, however, should be done to establish safety in the use of these antimicrobials for future drug development. Also, more studies must be done on Lake Lanao particularly of its microbial community to assess its ecological status and safety for human uses.

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Evaluation of River Environment by Biological Indicators in Boso Peninsula

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Keywords : river environment, biological index, attached diatom, benthos, water quality, Boso peninsula

Abstract

In this study, rivers that flow down the Boso peninsula using biological indicators for river environmental assessment was conducted, and river environment map in the Boso peninsula drew up at the same time, aimed at the construction of biological environment information in Chiba Prefecture. The Boso peninsula is shown to be divided into three areas in the Northwestern (urban area), Northeastern (plains) and Southern parts (hilly area). The Northwest (urban area) and the Northeast (plains) were estimated as "dirty" and the South (hilly area) was estimated as "clean". In addition, there is the great gap on biotic assessment between benthos and attached diatom. In the future, traditionally from individuals, populations and water quality assessment and environmental assessment environmental DNA analysis be conducted from biological habitat quality and quantity and ecosystem composition, structure and functions point of view. Furthermore, development of the database is expected to the characteristics of environment information for ecosystem restoration and conservation by linking with evaluation based on sensibility such as Water Environmental Soundness Index.

1. INTRODUCTION

Utilization of biological indicators for water environmental evaluation is different from water quality indicator by chemical analysis. It has the advantage of reflection a relatively long-term environmental change and easy to convert as a influence to human. On one hand, there is a big problem that they require skill in the classification, identification and counting, and susceptible to the influence of analysis of individual differences and method of investigation. In Japan, when evaluating rivers, chemical water quality survey is main investigation but biological water quality survey is not, as well as in Chiba prefecture. Then, it is thought that comprehensive biological water quality survey in Chiba prefecture will be possible by using attached diatom as a producer and benthos as higher-level consumers. In this study, rivers that flow down the Boso peninsula using biological indicators for river environmental assessment was conducted, and river environment map in the Boso peninsula drew up at the same time, aimed at the construction of biological environment information in Chiba prefecture.

2. RESEARCH METHODS

2.1 Rivers to survey

Kikuta River (2005), Koito River (2009), Obitsu River (2010), Yoro River (2011), Inba marsh inflow and outflow Rivers(Shinkawa River, Hanami River, Kashima River) (2012, 2016), Tega marsh inflow and outflow rivers(Ohori River, Ohtsu River, Someiiriotoshi, Tega River) (2013), Yatsu tidal flats inflow in and out Rivers(Yatsu River, Takase River) (2013), Kuriyama River (2014), Ebi River (2014), Isumi River (2015), Sakuta River (2016), Ichinomiya River (2017), Minato River (2018), and their watershed were surveyed. It was carried out four times a year at each of three to seven points of each river by season. The parentheses are the years studied.

2.2 Attached diatom investigation as a producer

Attached diatom was collected using a quadrate frame of 5 cm \times 5 cm from the bottom of the river, and It was classified, identified and counted using a biological microscope (Nikon Ecripse E 800).

DAIpo (Diatom Assemblage Index to organic water pollution) and RPId (River Pollution Index based on DCI) were calculated, and the river environment was evaluated by these indicators.

1) DAIpo

DAIpo is an organic pollution index based on attached diatom community, and is used as a biological index for organic contamination, common to still water areas (lakes) and running water areas (rivers).

Attached diatom is decided to investigate belong to what a biotic community (good clear water species, wide adaptability species, good pollution species), and calculated from equation (1).

$$DAIpo=50 + ((A - B)/2) \dots (1)$$

Here,

- A: The sum of the relative frequency (%) of all saproxenous taxa at the survey site
- B: The sum of the relative frequency (%) of all saprophilous taxa at the survey site

Table 1 shows the relationship between DAIpo, BOD, and pollution class.

2) RPId

RPId is a total evaluation value of river based on DAIpo, and by calculating the RPId, it is possible to compare the pollution of rivers different flow path length. It is calculated the area of the trapezoid created by plotting the value of DAIpo on the graph and drawing a perpendicular from the plot and flow path length, by equation (2)

$$RPId=S/L$$
 (2)

Here,

- S: The area of figure consisting of DAIpo (horizontal axis) at each survey site and channel length (vertical axis) of the survey river
- L: The flow path length of the river surveyed (Distance between the most upstream point and the most downstream point among the surveyed points)

2.3 Benthos survey as higher-end consumers

The benthos was collected by D-frame net or Ekman-Birge mud sampler, and classified, identified, and counted using a substantive microscope (Nikon smz445). ASPT (average score per taxon) value was calculated the by BMWP score (Biological Monitoring Working Party score) method, and evaluated for the river environment.

1) BMWP score method

The BMWP score method is improved BMWP method for Japan. The Working Group organized in the United Kingdom (Biological Monitoring Work Party) advocated standardize biological water quality evaluation methods. This method is a new indicator for biological water quality survey "River Water Environmental Evaluation Manual (draft)" created by the Ministry of Environment, Japan.

2) ASPT

ASPT (Average Score Per Taxon) identify and classify enthos into the family, apply the benthos of 62 families to the score table classified as a score of 1 to 10. ASPT is obtained by calculating from equation (3).

$$ASPT = TS / TF \dots$$
 (3)

Here.

TS: Total score

TF: Total number of families

The survey point was evaluated from 1 to 10 points by the ASPT value (Table 2).

2.4 Water quality survey as a nutritional substrate

The surface water was collected with a heyroht water sampler and measured BOD, COD, nutrient salt, Chl.a, SS, pH, DO, water temperature, transparency degree according to JIS-K0102 of the factory wastewater test method and the ocean observation guidelines.

3. RESULTS

Figs. 1, 2, 3, and 4 show the results of RPId at each research site, BOD as a nutrient substrate,

Table 1 Relationship between DAIpo, BOD, and pollution class

	-	
DAIpo	BOD	The pollution class
100~85	$0\sim 0.0625$	Destitute water
85~70	$0.0625 \sim 1.25$	β-Oligosaprobic water
70~50	$1.25 \sim 2.5$	α-Oligosaprobic water
50~30	2.5~5.0	β-Mesosaprobity water
30~15	5.0~10.0	α-Mesosaprobity water
15~0	>10	Polysaprobity water

Table 2 Evaluation of water environment from ASPT value (provisional)

	=
ASPT value	Expression(Provisional)
> 7	Clear water
$6{\sim}7$	Somewhat clean water
$5 \sim 6$	Somewhat polluted water
< 5	Polluted water



Fig.1 RPId value in each research point

attached diatom as a producer, and benthos as a high-level consumer.

The Boso peninsula was shown to be divided into three areas, that is the Northwestern (urban area), Northeastern (plains) and Southern parts (hilly area). The Northwest (urban area) and the Northeast (plains) were estimated as "dirty" and the South (hilly area) was estimated as "clean", and it was shown that rich nature was left in the Boso hills.

Although, the BOD was evaluated as "slightly clean" in the Northwestern part (urban area), Northeast (plain), it was not consistent with the evaluation by indicator organisms.

In addition, there was the great gap between the evaluation benthos and attached diatom.

4. DISCUSSION

The Northwest (urban area), where many large cities are gathered, were evaluated as "polluted". It was considered due to the pollution caused a vibrant urban economy. Despite the big city does not exist, Northeast (Plains) was assessed as "polluted". It was considered nonpoint sources from paddy fields because fluctuation of pollution connected farm work. BOD becomes somewhat clean reputation in the Northwest (urban areas), and Northeastern (Plains), and BOD and biological indicators were not consistent. The water quality is evaluated from instantaneous sampling, however, biological indicators for are the evaluation of long-lasting water quality of river. The difference between benthos and attached diatom assessment are time lag before change of water quality was reflected by bottom material, because water quality of rivers had a big-time change but bottom material had an influence for a long term. Therefore, it may show improvement from pollution state.

5. CONCLUSIONS

- 1) The Boso peninsula was shown to be divided into three areas of the Northwestern (urban area), Northeastern (plains) and Southern parts (hilly area).
- 2) The Northwestern part (urban area) and the Northeastren part (plains) were estimated as "dirty" and the Southern part (hilly area) was estimated as "clean", respectively.
- 3) There was the great gap between the evaluation by benthos and attached diatom as biological indicators, and from this reason, it is necessary to evaluate both indicators comprehensively.

6. FUTURE PROSPECT

In the future, using assessment by existing individuals, population and water quality and analysis by DNA (eDNA), more detailed environmental assessment will be conducted considering quality/quantity of existing creature and construction/structure/function of ecosystem. Furthermore, conducting assessment by sensitivity like Water Environmental Soundness Index at the same time, it is anticipated that construction of database relating to acquisition environmental information to ecosystem restoration and characteristic of location become possible.

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Fig.2 Environmental condition map of Boso peninsula by BOD



Fig.3 Environmental condition map of Boso peninsula by diatom



Fig.4 Environmental condition map of Boso peninsula by benthos

The Diversity of phytoplankton in some check dams of Chiang Mai Province

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Keywords: Diversity, Phytoplankton, Check Dams

ABSTRACT

The study aims to explore the diversity of phytoplankton and their relationships with some environment parameters such as physical and chemistry properties. Ten different type of check dams in Chiang Mai province were selected. The results showed that 5 divisions of phytoplankton were found including Bacillariophyta (66%), Chlorophyta (28%), Cyanophyta (3%), Euglenophyta (2%) and Chrysophyta (1%) respectively. The phytoplankton diversity and their abundance were able to reflex the environments varieties. Not only the physical and Chemical parameter that impacted to the check dams but the other factors such as seasonal variation, type volume and size of check dams were also influence to the distribution of phytoplankton

1. INTRODUCTION

The current natural resources problems is getting worse as deforestation, expansion and pollution. The natural resources were extremely negatively impact not only represent in the term of environmental loss but also impact to the human as flooding, drought and water shortage. Therefore, one of those problem solutions was the check dam project. The check dam was support not only storage water in dry season but help in the replenishment of water resources and restore moisture to the local ecosystem. The check dam was acting as the natural water tank that can be used to modify the velocity of stream flow which can be help to reduce erosion. Moreover, the check dam were prevent to solved the flooding problem in rainy season as well as the drought in dry season.

Check dams refer to the construction that lay cross section or block on the stream. bed check dams is usually blocked the gully brook in the area or that is highly slope. The water flow was slowed down and the sediment was settled down to the bottom. However, the check dams might have an impact to the environment. The most impacted of check dam to environment were slowing flow and increasing the matter sediment accumulation. Another impacted were the water level. The water level usually raised up, which will affect to the living things no only producer as an algae, phytoplankton but the consumer as zooplankton and small invertebrate. The migration of organisms might obstruct check dams wall. The impact might more or less depend on the type of check dams such as the height and shapes, etc. Therefore, prior to building check dams to get the maximum positive and minimal negative effects were needed to analyzed those impact caused from check dams throughout by research. One to the potential assess of impact is aquatic bioindicator such as phytoplankton as this organism that sensitivity to water quality.

Therefore, this research study were focused to the diversity of phytoplankton and their relationship with the environment and physical- chemical parameters and were evaluated the impacted of the check dams in the area of upstream in Chiang Mai province. The result might use as a basic information for other check dam construction.

2. METHOD

The water and phytoplankton samples were collected from middle points of the all 10 check dams.(Fig 1, Table 1) The water quality as physical and chemical properties were investigated at sites as water temperature, air temperature, color, velocity, pH, conductivity, DO, TDS, turbidity, BOD, volume of water, surface area, nitrate-nitrogen, ammonia – nitrogen and orthophosphate. 10 liters of water sampled was filtered through 10 μ m mesh plankton net and keep it in a bottles and preserved with Lugal's solution. The phytoplankton was identified and counted in the laboratory. The diversity index, species evenness, species richness and the relationship between the physical and chemical diversity of phytoplankton were investigated by Past3 version 3.14 program.

Table 1 Type and description totals 10 check dams

Type of	Description	Check-
check dam		dams
		code
Indigenous	- Use the natural materials	PY, PK,
(A)	such as bamboo rock, etc.	DK
	- Appropriate to the gully,	
	twigs, creek, small stream	
	size.	
Semi-	- The combination of the	OR, SNG,
permanent	natural materials and the	YK, YK2
(B)	material that is not a natural	
	such as concrete with a stone	
	- Appropriate to the stream	
	or small rive	
Permanent	- Reinforced concrete	MH, MHL,
(C)	- Appropriate to the streams	MLM
	and small river (large size	
	with fast water flow)	





Figure 1 Example of different type of the check dams A: The local indigenous check dams (PK, PK, DK) B: The integrated check dams (OR, SNG, YK, YK2) C: The modern check dams (MH, MHL, MLM)

3. RESULT

Five division of phytoplankton were found in Division Bacillariophyta (66%), Division Chlorophyta (28%), Division Cyanophyta (3%), Division Euglenophyta (2%) and Division Chrysophyta (1%) respectively. The highest amount of individual phytoplankton belong to Division Baciilriophyta except upstream check dams as sites YK2, MH, MHL that the Division Chlorophya were found as the dominant group.



Figure 2 The number of phytoplankton totals 10 check dams

The diversity index, evenness and number of species of phytoplankton in 10 check dams were investigated. Site DK was a highest diversity index, species evenness and number of species richness as 3.21503, 0.93624 and 31 respectively. (Table 2)

Table 2 Diversity index, Species evenness and Species richness totals 10 check dams

Type of	Name	Diversity	Species	Number
check dams		index	evenness	of
				species
Indigenous	PY	2.57978	0.82277	23
	РК	2.56334	0.88685	18
	DK	3.21503**	0.93624**	31**
semi-	OR	2.41727	0.85319	17
permanent	SNG	0.42685*	0.23823*	6
	YK	0.76684	0.47646	5*
	YK2	2.00226	0.80577	12
permanent	MH	2.49162	0.80608	22
	MHL	2.61448	0.84583	22
	MLM	1.13827	0.38658	19
** Maximum		* Minin	num	

** Maximum

The relationship of the physical and chemical and diversity of phytoplankton in 10 sampling sites were investigated. The relationship between the water quality physical and chemical properties could be grouped by the cluster distance 1.0 as divided to two. Firstly turbidity had a positive relationships with site MH and MHL. Thus the conductivity and TDS has a positive relationships with sites SNG and OR in second group. (Fig. 3) On the other hand, the diversity of phytoplankton to check dams could be grouped by the cluster distance 7.5 as 8 groups. Each group has a difference dominant phytoplankton such as site MH, MHL were *staurastrum tetracerum*, MLM was Navicula sp., PY, YK2 weas *Coelastrum reticulatrum* (dangeard) Senn, DK was *Pandorina* sp. and PK was *Cyclotella* sp. (Fig. 4)







Figure 4 The cluster plot show the relationship diversity index of phytoplankton in 10 check dams.

4. **DISCUSSION**

The diversity and distribution of phytoplankton in sampling sites were different as well as the water condition.^[1] The water composition from each sampling point were unique characteristics and influence to the phytoplankton communities.^[2] The other factor as a seasonal condition might impact to check dams especially, small size and low volume capacity. Moreover, the seasonal influenced to the distribution of phytoplankton as higher abundant in rainy season as well as the richness more than the dry season. ^[3]

In this research the relationship between the physical and chemical and some algae were investigated. The sites MH MLM that had a high turbidity would found *staurastrum tetracerum* as the dominant species. (Fig. 3, 4)

5. CONCLUSION

The physical and chemical properties of all 10 sampling check dams sites that had and unique and different characteristics. The other factors such as the season, the volume and size of check dams were also impact to the diversity of phytoplankton were also different depended on the characteristic of the check dams. Thus, the construction of the check dams should be aware about the impacted to ecosystem and environment.

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P1-21

A brief introduction of biodiversity in Dongting Lake

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Keywords: Dongting Lake; Biodiversity; Invasive plants; Poplar plantation

ABSTRACT

Dongting Lake, the second largest freshwater lake in China, locates in the northern Hunan Province and connectes to the Yangtze River. The lake has three Ramsar wetlands of international importance and shows rich biodiversity.

The vegetation in Dongting Lake composes by 229 seed plants belong to 58 families and 150 genera. Among these plants, 97% of species belongs to herbs. However, the number of the invasive plants reaches 43 species, belong to 34 genera and 19 families. The most typical invasive plant in the Dongting Lake is poplar plantations and their effects on the native plant diversity were investigated. On the whole, large-area of poplar plantations increased species richness and diversity index. However, they changed the species composition and decrease the number of aquatic species and native species. Therefore, biodiversity protection must be enhanced in the Dongting Lake wetlands.

1. INTRODUCTION

Dongting Lake (28°30′-30°20′N, 111°40′-113°10′E), the second largest freshwater lake in China (2,691 km²), is located in northern Hunan Province in a basin south of the Yangtze River and is connected to the Yangtze by distributary channels. Three major sections of the lake (eastern, southern, and western) have been designated as Wetlands of International Importance by the Ramsar Convention. The mean water depth is 6.7m and with deepest 30.8m, the mean annual runoff is 312.6 billion m³. The lake has seven water inlets, including three channels of the Yangtze River (Songzi, Taiping and Ouchi) and four rivers (Rivers Xiang, Zi, Yuan and Li). The lake has only one outlet, which discharges into the Yangtze River at Chenglingji. Annual fluctuation in water level is approximately 12-14 m, with the maxima in August and minima typically in January or February, which provides the basic hydrological regime for maintaining large areas of beach wetlands, the most dynamic landscape within the Dongting Lake area. The mean annual temperature in the area is 16.4-17°C, with the coldest temperatures in January (3.9–4.5°C) and the hottest in July (28.6–29.1°C). Annual precipitation is 1,382 mm, with more than 60 % falling during the period from April to August. Alluvial soil is the primary soil type in the Dongting Lake wetlands.

The region has also been recognized as one of the 200 global conservation priority eco-regions proposed by the World Wide Fund for Nature, due to its valuable and exclusive biodiversity. The catchment has 1428 herbaceous species, 114 fish species, and 217 bird species.

2. METHOD

The nested quadrats sampling design suggested by Avery and Burkhart33 was used to form sampling units to determine plant diversity. In each vegetated area, three plots (20 m 330 m) with equal spacing were established as replicates. In each plot, five quadrats (2 mX2 m) with equal spacing (5-6 m) were chosen for investigation.

3. RESULTS

With the field investigation, totally 235 species were found in the Dongting Lake wetland. Among these species, 97.9% were herbaceous plants. The number of seed plants was 229 species, belonging to 58 families and 150 genera. Additionally, we analyzed the floristic type according to the floristic areal-type method. In family level, the plants contents 38 families belonging to cosmopolitan type. Poaceae is the biggest family, followed by *Asteraceae*. In genus level, the plants contents 52 genera belonging to cosmopolitan type and Polygonum is the biggest type. From the observation results, most plants in Dongting Lake belong to the cosmopolitan type and temperate flora.

Then, we would introduce the invasive plants in Dongting Lake and take poplar as an example. Totally, we found 43 invasive plant species from 19 families and 34 genera. The biggest invasive family is Compositae and contents 7 species, which accounts to 16.2% of the total species. Among these invasive species, 24 species are originated from America. The 17 species, including Erigeron annuus, Alternanthera philoxeroides, and Populus euramevicana are most common invasive plants in Dongting Lake. 27 species distribute along the flood control levee and road edge. 37 species belong to herbaceous type, in which, 29 species are annuals or biennials. The invasive species are adaptable and short in growth cycle and easy to be spread by water flow in Dongting Lake. On the whole, 48.8% plant species were introduced by intentional human activities and or 37.2% by unconscious human activities.

Now, we take Poplar (*Populus deltoides*) as an example. In 1970s, *P. deltoides* was introduced into the Dongting Lake area for making paper. In 2007, the area increased from 87 km² in 1983 to 640 km² (accounting for 26% of the total lake area). The study shows that although species number and Shannon's diversity were higher in young poplar versus reedy areas, poplar plantations had different effects on relative proportions of species composition: a higher ratio of mesophytes but a lower ratio of hygrophytes, and a higher ratio of heliophytes but a lower ratio of neutrophilous or shade plants. Changes in relative proportions of species also reflect the changes in understory environmental conditions. Therefore, species richness alone is incomplete and species composition of different ecotypes or life forms cannot be neglected when evaluating the extent to which understory plant diversity is affected by fast-growing tree plantations. Additionally, poplar plantations might also have far-reaching effects on the diversity of animal species. In the South Dongting Lake wetlands, poplar plantations have led to a significantly lower diversity of migratory birds.

4. CONCLUSION

Considering the wetland of international importance, the biodiversity in Dongting Lake region should be protected or strengthen by taking artificial and natural measures to restore the degraded habitat

P1-22

Paper title : Do winter-flooded paddies serve as overwintering sites for aquatic animal communities?

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Keywords: eco-friendly rice paddy, biodiversity, winter-flooded rice cultivation, aquatic animal community

ABSTRACT

For successful reintroduction of storks, winter-flooded rice cultivation has been introduced widely in paddy fields in the Toyooka Basin of the Hyogo prefecture, Japan. We studied the overwintering status of aquatic animal communities in winter-flooded paddies (WFP). The number of taxa of aquatic animals in winter-flooded paddies did not differ from that of conventional water management paddies (CWMP), fallow-field biotopes (FFB), and canal-type biotopes (CTB). Aquatic animals were more abundant in CWMP than in WEP. In addition, community structures of Odonata, Hemiptera, and Coleoptera were roughly divided into CWMP and WFP, and FFB and CTB, respectively. Our results show that winter-flooded rice cultivation is not very effective for the overwintering of aquatic animals, compared with conventional paddies and fallow-field biotopes. Therefore, it may be necessary to develop various types of overwintering environments at the regional scale in paddy fields for conservation of the aquatic animal communities.

1. INTRODUCTION

The Oriental white stork (Ciconia boyciana) inhabits open freshwater wetlands, such as paddy fields and floodplains, and preys on various animal species (e.g., fish, insects, frogs, tadpoles, snakes, crayfishes, and small mammals) there ^[1]. The Japanese wild population of the Oriental white stork became extinct in 1971. One major reason for this extinction was the sharp decrease in the number of aquatic animal species in paddy fields due to the spread of agrochemicals and agricultural field improvements in the 1960s ^[2]. Since 2005, Oriental white storks have been continuously reintroduced in the Toyooka Basin, the final habitat of wild storks in Japan. Reintroduced storks have bred every year since 2007. For successful reintroduction of storks, various eco-friendly rice farming, i.e., "White Stork-friendly Farming Method", have been introduced in paddy fields of the Toyooka Basin. Among them, winterflooded rice cultivation is especially believed to contribute to the conservation of paddy biodiversity and restoration of feeding habitats of storks in winter; however, to our knowledge, no study has quantitatively analyzed its effects. Therefore, we examined the impact of winter-flooded rice cultivation on overwintering in aquatic animal communities.

2. METHOD

We studied one paddy field area (35°55 N, 134°86 E) in the Toyooka Basin, of the Hyogo prefecture, Japan. This has approximately 50 modernized structural paddy fields. Small streams are utilized for irrigation, and water is supplied to each paddy through concrete creeks spread throughout the district. Winter-flooded rice cultivation is carried out in half of all paddies in this area. Conventional water management paddies (CWMP) are flooded only during the cropping period (from late April to late August), but some puddles remain in each paddy because of poor drainage and heavy precipitation during the non-cropping period (Fig. 1a). On the other hand, winter-flooded paddies (WFP) are flooded not only during the cropping period but also during the non-cropping period (from early December to late March, Fig. 1b). Besides WFP, 20 fallow-field biotopes (FFB) and eight canal-type biotopes (CTB) adjacent to paddies have been developed for conservation of aquatic animal habitats and as foraging sites of storks. FFB are flooded during all seasons and CTB are flooded during the same season as WFP. Four types of study sites were selected: (1) WFP, (2) CWMP, (3) CTB, and (4) FFB. We set three control sections for each site type to have 12 study sites in total. To capturing aquatic animals, we conducted sweeping survey with a Dframe hand net (35-cm frame diameter, 1-mm mesh size). At each study site, we set eight sampling points and swept approximately 30 cm toward the shoreline. Aquatic animals were counted for each taxon and were discharged into the sampling point. All surveys were conducted from January 19 to January 30, 2015.

area is one of the main habitats of reintroduced storks and



Fig. 1 Pictures of study paddies during the non-cropping season (January 30, 2015) in the Toyooka Basin, (a) the CWMP and (b) the WFP.

3. RESULTS

A total of 2,915 aquatic animals belonging to 38 taxa were collected at the 12 study sites. The number of Oligochaeta and Chironomidae larvae were 1349 and 754, respectively, which accounted for 72% of captured aquatic animals. In terms of taxa, aquatic insects were dominant (24 taxa, 63%). The number of taxa of aquatic animals in WFP did not differ from that in other study sites. Community structures of Odonata, Hemiptera, and Coleoptera, which are the indicator fauna in paddy fields (Nakanishi et al., 2009^[3]), were roughly divided into CWMP and WFP, and FFB and CTB, respectively (Fig. 2). Oligochaeta, Chironomidae larvae, and Hirudinea were more abundant in CWMP than in other study sites. Aquatic Hemiptera Sigara spp. showed a similar abundance in CWMP and WFP, they did not appear in FFB and CTB. Viviparidae were more abundant in CWMP, WFP, and CTB than in FFB, while Veneroida showed the opposite trend.

4. DISCUSSION

Aquatic animals in WFP were not more diverse than those in other study sites. Tawa et al. (2013)^[4] reported that illdrained paddy fields where water puddles of various depths remain during the non-agricultural period can serve as wintering habitats for aquatic animal communities. In the WFP, the whole surface was filled with water after flatting the field, so a water environment of constant depth was formed there. Hence, the WFP plays a role as an overwintering habitat for the aquatic animals, but it is considered to be less likely to function as an effective overwintering site than the CWMP.

17th World Lake Conference, Lake Kasumigaura, Ibaraki, Japan, 2018



Fig. 2 Nonmetric multidimensional scaling (NMDS) plot of aquatic insect communities in study sites. Different types of study sites are denoted by different symbol shapes.

5. CONCLUSION

For conservation of aquatic animal communities in paddy fields at the regional scale, it is necessary to create various types of overwintering environments rather than introducing specific environmentally friendly farming methods (e.g., WFP). Even in modernized structural paddy fields, water puddles that serve as overwintering habitats for aquatic animals may be formed depending on topography. Therefore, it is also important to not only implement new eco-friendly rice farming but also make use of existing paddy fields as overwintering sites for aquatic animals.

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P1-23

Paper Title: Estimation of ecological carrying capacity of tilapia (Oreochromis

niloticus) cage culture in Lake Volta using phosphorus mass balance.

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ABSTRACT

Development of intensive fish cage farming in reservoirs and lakes could pose threats to the environment. Ecological carrying capacity assessment is useful for sustainable aquaculture production and rational use of water bodies. The current study estimated the ecological carrying capacity for a tilapia cage culture on Lake Volta. The ecological carrying capacity was predicted using Dillon and Rigler mass balance model, taking into account water quality data and average production data of feed and fish compositions and feed conversion ratio (FCR). The phosphorus load to the environment estimated per tonne of fish produced was 22.77 kg P t⁻¹ for the farm. The calculated capacity was 6,363 ty⁻¹ for the farm area. The estimated capacity was about 14 times higher than the present production (450 ty⁻¹) in the zone. This suggests that further cage culture of tilapia could be established in the lake without compromising the water quality and the ecosystem. However, monitoring of water quality should be undertaken periodically to accurately determine the state of the lake in order to both confirm and refine predictions.

1. INTRODUCTION

Lake Volta, a multipurpose water body supports a range of interests including wild capture fisheries, hydropower, irrigation and water supply. Currently, cage fish farming of tilapia is fast expanding on the lake. Large quantities of waste (uneaten food and fish faeces) are produced as a result of intensive cage fish culture^[1]. Excess phosphorus loading from fish feed can cause eutrophication in lakes. Thus, mismanagement of intensive cage farming can negatively impact the lake water.

The estimation of ecological carrying capacity of a specific site is essential to sustain culture, protect the environment and reduce risks of eutrophication that may occur^[2]. A phosphorus mass balance model for freshwater lakes by Dillon and Rigler is widely used for estimating phosphorus levels in lakes and reservoirs in different areas, as well as other waters with cage fish culture. The carrying capacity of Lake Volta is unknown. There is a need to have estimates for the lake's carrying capacity for aquaculture development.

The study is aimed at estimating the ecological carrying capacity of important areas for aquaculture development in Lake Volta. This is done as a basis for planning, decision making and integrated management of sustainable aquaculture in the lake.

2. METHOD

The Lake Volta is a tropical man-made lake that was created by damming River Volta in 1964 to provide hydroelectric power. Geographically, it lies between longitude $1^{\circ} 30^{\circ}$ W and $0^{\circ} 20^{\circ}$ E and Latitude $6^{\circ} 15'$ N and $9^{\circ} 10'$ N (Figure 1). At a maximum level, the lake has a volume of 149 km³, a surface area of about 8,500 km² and its length is 400 km.

For the purposes of estimating the ecological carrying capacity, a medium-scale farm with 80 cages of 5m x 5m x5 m was chosen. The farm is located close to the dead end of a side channel of Lake Volta, near to Akosombo, an

inland port with a mean depth of 20 m. (Figure 2). The farm practiced intensive system of farming and used predominantly locally manufactured extruded feed. The total production of the farm was estimated at 106 tonnes in 2014 with feed input of 211 tonnes per annum and FCR of 1.99.

The Dillon and Rigler model states that at steady state "the concentration of total-P in a water body, [P], is determined by the P loading, the size of the lake (area, mean depth), the flushing rate (i.e. the fraction of the water body lost annually through the outflow) and the fraction of P lost permanently to the sediments"^[1]

$$[\mathbf{P}] = \frac{L(I-R)}{Z\rho}$$

Where [P] is in μ g/L total-P or TP; L = the total-P loading in gm⁻² per year; Z= is the mean depth in m; R = the fraction of total-P retained by the sediments; and ρ = the flushing rate in volumes per year.



Fig. 1: Map of L. Volta showing the study area

3. RESULTS

The results of ecological carrying capacity estimation using Dillon Rigler phosphorus mass balance are shown in the steps below:

i. Determination of the steady-state total-P concentration. In tropical lakes and reservoirs, [P] is taken as the annual average total P concentration of surface waters



Fig. 2: Google map of section of L. Volta (marked yellow) where carrying capacity determined

and should be based on a number of samples taken during the year. The average phosphorus concentration of the lake water samples was $[P]_i =$ $46\mu g/L$ at the reference site in the study period in 2013.

- ii. The capacity of lake or reservoir for intensive cage culture is the difference $[\Delta P]$ between $[P]_i$ prior to exploitation and the final desired/acceptable level of productivity, $[P]_f$. A 100 µg/L is chosen as the value for maximum acceptable $[P]_f$ in tropical inland water bodies used for the culture of tilapia ^[3]
- iii. Determine Δ [P];

$$\begin{split} &\Delta \ [P] = [P]_f \text{ - } [P]_i \ = 100 - 46 = 54 \ \mu g/L \\ &L_{fish} = \Delta \ [P] \ z\rho/(1 \text{ - } R_{fish}) \end{split}$$

Table 1: Morphometric, hydrodynamic and
phosphorus load of cage farm on Lake Volta

Characteristics	Units	Symbols	Values
Surface area	km ²	Ao	5.365
Lake volume	$10^{6}m^{3}$	Vo	107.3
Mean depth	m	Ζ	20
Total outflow	$10^6 \mathrm{m}^3$	Q	939
Flushing rate	y ⁻¹	$\rho = Q/V_o$	8.75
Residence time	У	$T_{\rm w}$	0.1143
Phosphorus retention			
coefficient		R	0.308
Phosphorus in feed		%	1.35

Feed conversion ratio		1.99
(FCR)		
Phosphorus content	%	0.41
in fish		
Phosphorus load to	kgt ⁻¹	22.77
environment		
Tot. Phosphorus load	kgy ⁻¹	2,416
to environment		

 R_{fish} is the most difficult parameter to estimate. At least 45-55% of the total-P wastes from cage rainbow trout are likely to be permanently lost to sediments as a result of solids deposition. In the absence of any other data, these values are also used for cage tilapia and carp, and calculated as $R_{fish} = x + [(1 - x)] R$

Where x is the net proportion of total-P lost permanently to the sediments as a result of solids deposition (0.45-0.55) and R is proportion of dissolved total-P lost to the sediments i.e. phosphorus retention coefficient.

X was calculated to be 0.50

Thus, R is calculated from equation $R = 1/1 + 0.747\rho$ 0.507

R = 0.308

Thus, using above equation, R_{fish} is calculated as 0.65.

$$z = 20 \text{ m} \text{ (Table 1)}$$

$$\rho = 8.75 \text{ y}^{-1} \text{ (Table 1)}$$

 $L_{fish} = (54 \text{ x } 20 \text{ x } 8.75) / (1 - 0.65)$

 $= 27,000 \text{ mg m}^{-2} \text{ y}^{-1}$

Equivalent to 27.0 g m⁻² y⁻¹

iv. Since the area under operation (Farm area) of the lake has a surface area of 5.365 x 10^6 m², the total loading, L_a, is

 $L_a = 27.0 \text{ g m}^{-2} \text{ y}^{-1} \text{x} 5.365 \text{ x} 10^6 \text{ m}^2$

$$L_a = 27.0 \text{ g y}^{-1} \text{x} 5.365 \text{ x} 10^6$$

$$L_a = 144.86 \text{ x } 10^6 \text{ g y}^{-1}$$

v. Having calculated the total acceptable P loading $L_{\rm fish}$, this value is divided by the average total P waste to the environment per tonne fish production as a result of the cage culture to obtain the carrying capacity of the area.

The tonnage of fish of P loading from Farm was calculated as 22.765 kg t⁻¹ (Table 1). Therefore,

Carrying capacity of Farm area of lake = 144.86×10^{6} g y⁻¹/ 22765 g t⁻¹

 $= 6,363 \text{ t y}^{-1}$

4. DISCUSSION

The present production levels of tilapia of 450 t for the farm is over 14 times higher than the predicted ecological carrying capacity. Despite the rapid expansion of cage culture, the present biomass and impact to the lake's ecosystem is still acceptable. Cage aquaculture has the potential to continue on its current trend and increase without substantially altering the lakes ecosystem. With the government's proposal to increase aquaculture production from 10, 200 tonnes to 100,000 tonnes, and from the results of this study, this may be still within acceptable ecological limits, since there are other zones in the lake to accommodate the required expansion.

5. CONCLUSION

The results of this work indicated that the carrying capacity of the zone studied on the lake has not yet been reached. Further cage culture of tilapia could be established in the lake without compromising the water quality and the ecosystem. This calculated value can be taken as an indicator of a possible ecologically sustainable aquaculture production level for Lake Volta area. However, monitoring of water quality should be undertaken periodically to accurately determine the current state of the lake in order to both confirm and refine predictions.

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青森県姉沼におけるイシガイ科の生息環境評価

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抄録

イシガイ科の持つ生態的機能は、水域生態系において重要な役割を担っている。イシガイ科は、国内で15種確認さ れており、内、11種が環境省レッドデータブックに指定されている。国内において、イシガイ科の保全は急務である。本 研究では、日本の本州北部に位置する姉沼を対象水域とした。Habitat Evaluation Procedure (以下、HEP)を用いて、 イケチョウガイ Hyroipsis schlegeli、ヨコハマシジラガイ Inversiunio jokohamensis、ドブガイ属 Sinanodonta spp.を対象に、 生息分布が成立する環境を評価した。HEP を用いて評価を行う際、6 つの因子(水深、DO、クロロフィル a、ヨシ帯から の距離、底質の粒度)を選定し評価を行った。その結果、HEPを用いて作成したイシガイ科3種の生息分布モデルは、 現地での生息分布とおおむね一致した。本研究で、イシガイ科の生息環境評価手法の開発事例が得られたのでここ に報告する。

1. はじめに

イシガイ科は, 雌雄異体の卵胎生であり, 繁殖時期に なると, 雄個体の出水管から放出される精子球が, 雌個 体の入水管に取り込まれることで, 雌個体の育児嚢中の 卵が受精し、グロキディウム幼生が発生する。その成熟 したグロキディウム幼生が水中に放出され,宿主となる魚 類の鰓や鰭などに寄生する。寄生期間を終えると、グロ キディウム幼生が変態して稚貝となり、宿主魚類から脱 落,底生生活を送り,幼貝,成貝へと成長する。また,イ シガイ科は生態的機能としてコイ科タナゴ亜科 (Acheilognathinae)の産卵母貝としての利用や, 貝殻に よる隠れ場など,他生物の生息場所の創出,貝殻の内 側に魚類の産卵場所の提供, 排泄物や偽糞の供給によ る低質への堆積,移動による低質変化・攪乱などを介し て,水域生態系において重要な役割を担っている。イシ ガイ科は,国内で15種確認されており,内,11種が環境 省レッドデータブックに指定されている[1][2][3][4]。イシガイ 科の保全は急務であり、本科が豊富に残存している水 域で良好な生息環境について調べることは、本科の保 全にとって重要である。

本研究の対象水域である青森県東部に位置する姉 沼では、イケチョウガイ Hyroipsis schlegeli、ヨコハマシ ジラガイ Inversiunio jokohamensis、カラスガイ Cristaria plicata、フネドブガイ Anemina arcaeformis、タガイ Sinanodonta japonica、とヌマガイ Sinanodonta lauta を 含むドブガイ属 Sinanodonta spp. といった多くの種が生 息し,種によっては多数の生息分布が確認されている ^{[6][7][8]}。本水域では,前述の複数種にとって良好な環境 が担保されていることがうかがわれるが,種ごとの生息環 境の違いや,分布の要因は明らかになっていない。種数 および種ごとの生息個体数が多い水域での生息環境を 把握することは,生息環境が劣化している他水域の本科 の保全に役立つ。

本研究は、湖沼のイシガイ科の生息環境評価法の開 発を目指した基礎研究と位置づけ、青森県東部に位置 する姉沼を事例として、夏季における宿主魚種の把握 および分布の推定、水質、物理条件、餌条件を把握し、 Habitat Evaluation Procedure (以下、HEP)を用いて、イ シガイ科の生息分布が成立する環境を評価することを目 的とした。

2. 対象水域および方法

対象水域を,青森県東部に位置する姉沼(40°42'N, 141°20'E)とした。総面積1.56km²,湖岸長7.6km,最大 水深4.3m,平均水深1.3m,の淡水湖である^[9]。本水域 は,南側から姉沼側と中津川が流入し,下流側で汽水 湖である小川原湖につながっている。沿岸には人工護 岸が施されておらず,ヨシ Phragmites austrailis やガマ Typha latifolia が多数繁茂しており,沿岸帯からやや離 れた地点には,ヒシ Trapa japonica の小群落が点在す る。

本研究では,評価手法として HEP 用いた。HEP は環 境要因ごとの適正指数(Suitability Index 以下, SI)と, それらを結合したハビタット適正指数(Habitat Suitability Index 以下, HSI)で表される。また, 適正指数はそれぞれ 0(不適)~1(最適)で表される。

HEPの手順に従い、姉沼で採取した、イシガイ科3種 (イケチョウガイ、ヨコハマシジラガイ、ドブガイ属)を対象 に、生息に必要な生存条件を既存の資料や文献によっ て整理し^{[1][10][11][12][13][14][15][16][17]、SIの適正値を決定し た。また、SIを目的変数とし、生存必須条件(水深、DO、 クロロフィルa、ヨシ帯からの距離、底質の粒度)を説明変 数とした。}

姉沼全域(36 地点)にて,説明変数である生息必須 条件について測定を行った。生存必須条件の一つであ る「ヨシ帯からの距離」には、宿主魚種の生息分布が反 映されている。イシガイ科は植物プランクトンを餌資源と している可能性が高いため、クロロフィルaを生息必須条 件の1つとした。イケチョウガイ、ドブガイ属とヨコハマシジ ラガイでは,底質粒度において生存必須条件が異なっ ている知見を得ため,異なる SI の適正値を設定した。な お,水深は,2016年7月12日,9月27日に超音波ドッ プラー流向流速計(SonTek/YSI 社 Hydro Surveyor M9) を用いて測定し、DO およびクロロフィル a は、2016年9 月 16 日に多項目水質系(Hydrolab/DS5X)を用いて測 定を行った。底質粒度は、2016年9月16日にエクマン バージ採泥器(15×15×15 cm)を用いて底質を採取し, 採取した底質を乾燥機で約110℃,24時間乾燥させた。 乾燥させた底質を,篩にかけ,粒形ごとの重量構成割合 を構成した。篩はそれぞれ、19mm(粗礫), 4.7mm(中 礫), 2mm(細礫), 0.85mm(粗砂), 0.25mm(中砂), 0.075 mm(細砂)を用いた。

前述の方法で得られた測定値を用いて、SIを算出し SI モデルを作成した。これらの SI を結合させる際に、生 存必須条件のどれか一つを欠けるとなると、本科の生息 地に不適であると評価できるように、本研究では幾何平 均法を用いて HSI を算出し、HSI モデルを作成した。ま た、6 次メッシュ(1 区画 125×125m)で作成した姉沼マ ップを図示し、グリッドごとで評価値に応じて色分けを行 い、イシガイ科3種の生息分布ポテンシャルを評価した。

本研究で行った評価が適切かどうか判断するため, 検証可能なグリッド,すなわち,HSI評価されたグリッドに おいて,イシガイ科が実際に採捕されたか否かを,既存 のデータを踏まえて検証した。

3. 結果および考察

SI の適正値は、水深 0m の時に SI を 0 とし、40 cm以上の時 SI を 1 とした。DO は 0mg/lの時に SI を 0 とし、

5mg/l以上では DO が高く、本科の生息密度が高いこと いう知見を得たことから、SI を 1 とした。クロロフィル a が 5µg/l未満の地点では本科は生息しないとの知見から、 クロロフィル a が 5µg 以上の時は SI が上昇し、10µg/l以 上を 1 とした。宿主魚類のヨシ帯との距離は、宿主魚類 がヨシ帯から離れるほど本科の生息密度が低くなること から、ヨシ帯からの距離が 770m 以上では、SI を 0 とし、 0m では SI を 1 とした。底質の粒度は、イケチョウガイとド ブガイ属は泥質環境を好むことから、シルト、砂では SI を 1とし、礫では 0.2 とした。ヨコハマシジラガイは、砂礫底 の環境を好むので、砂、礫では SI を 1 とし、シルトでは SI を 0.3 とした。

SIを結合させ、姉沼の36地点各々のHSIを算出した 結果、イケチョウガイ、ドブガイ属はHSIが0~0.77(標準 偏差0.29)であり、ヨコハマシジラガイでは0~0.91(標準 偏差0.30)であった。これらの値を用いて、HSIモデルを 作成し、イシガイ科を評価した結果(図1,2)、沿岸帯で はHSIが高く、特に南西部はイシガイ科3種類において HSIが高かった(0.77~0.91)。また、ヨコハマシジラガイ は北部において生息しにくい環境であるという評価となり、 イケチョウガイ、ドブガイ属はやや沖帯では、生息しにく いという評価となった。イシガイ科3種において沿岸帯か らグリッドまでの距離が離れるほど HSI が低くなることが 分かった。また、グリッドごとで沿岸帯から沖帯にはなれ るほど SI 値が低くなったため、沖帯の環境ではイシガイ 科の生息分布ポテンシャルは低いと考えられた。

本研究の評価が適切かどうか判断するため、イシガイ 科が、実際に採捕されたか否かを検証した結果、沿岸 帯で確認されたイシガイ科 3 種は HSI モデルに概ね適 合すると考えられた。ただし、姉沼側河口付近は適合し なかった。これは、姉沼川が増水する際にこの範囲の掃 流力が高まり、貝が沖帯へと流されている可能性、もしく は河口付近は貧栄養によって貝にとって生息に適さな いためであると考えられる。

SI モデル作成時に他水域の知見データが組み込まれているため、本研究で得られたHSIモデルは、他水域においても、本科の生息分布が成立する環境を評価できると考えられた。

4. 課題

本研究では、南東の姉沼側のような外部の水域による 影響を考慮しておらず、今後その影響に対する補正が 必要である。また、より正確なモデルを作成するために、 生息必須条件に挙げられている宿主魚種の分布につい てより詳細なデータを得ることが今後の課題である。

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