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AKBAR JOHN . <akbarjohn@iium.edu.my> Thu, Jan 4, 2018 at 11:46 AM
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Dear Author,

Congratulation!!! According to my record, your manuscript, entitled **'Effect of Recent Flood on Icthyoplankton and Juvenile Fish Assemblage Changes in Kuantan River Revealed through DNA Barcoding'** has been accepted for publication in the Special issue of Jurnal Teknologi 2018 with minor revision.

Your manuscript has been subjected to a double-blind review process.

The reviewers recommend publication, following clarification of several points.

Which includes suggestions to improve the quality of the manuscript as listed below;

1. Kindly please label the photos in Figure 2 separately and briefly explain each photo in the caption.
2. Materials and Methods: Please state the country where does a modified Bubu light trap is manufactured.

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Once you have revised the manuscript, please e-mail it in MSWORD format to me at jtek2018@gmail.com on or before 9th October 2017, with a cover letter outlining the points on the revisions you have made in.

To speed up the publication process **please format the revised manuscrip** according to the Special Edition Jurnal Teknologi 2018 guidelines (**Refer attached files**).

Thank you very much for submitting your article to the Special issue of Jurnal Teknologi 2018. I look forward to receiving the revised version of your manuscript as soon as possible.

Best Wishes,

Zulkifli Yusof

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Full Paper

EFFECT OF RECENT FLOOD ON ICHTHYOPLANKTON AND JUVENILE FISH ASSEMBLAGE CHANGES IN KUANTAN RIVER REVEALED THROUGH DNA BARCODING

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Graphical abstract

Modified bubu light trap

Abstract

Identifying early life stages of fishes to the species level is one of the challenging task for the taxonomist as the information on key morphological characters are overlapping between genetically closer individuals. Universal DNA barcode (cytochrome oxidase C subunit 1) gene sequence helped tremendously in species level identification of early life stages of fishes. This study was aimed to address the effect of recent monsoon on assemblage changes in Ichthyoplankton and juvenile fishes of Kuantan river, Whereby, the samples were identified to the species level using COX1 gene sequencing. Samples were collected using modified bubu light trap net between April 2015 and December 2015 in Kuantan river and its all tributaries. Fish larvae/juvenile size ranged from 3mm to 100mm, with most abundant individual belong to size class <10mm. A total of 28 species belong to 15 families were successfully identified to the species level from the total of 58 barcodes generated from 372 larval/juvenile samples collected. Unlike the previous study, significant shift in species assemblage has been recorded in this study. Statistical analysis showed significant effect of salinity and suspended particulate on the species distribution and abundance. The dominant fishes belong to the cyprinidae family followed by toxotidae, ambasidae and eleotridae compared to the previous study (Ariidae, Lactaridae and Lutjanidae). It could be concluded that the recent flood (mid-December 2014 to January 2015) in east peninsular Malaysia will have greater positive impact on Kuantan river fish diversity in near future.

Keywords: DNA barcoding, Ichthyoplankton, Kuantan river, COX1 gene

1.0 INTRODUCTION

Malaysia has faced severe northeast monsoonal hit from mid-December 2014 to January 2015 with at least >60% above the normal precipitation rate. This has led to the uncontrolled discharge of organic and inorganic substances in to the river stream which are believed to be the limiting factor of sensitive organisms such as juvenile and Ichthyoplankton fishes. Numbers of studies have explained the significant effect of environmental variability on the larval distribution, abundance, assemblage structure and their biotic interactions [1]. Due to harsh Environments and extreme environmental conditions during flood, the Ichthyoplankton distribution and assemblage is strongly influenced by abiotic factors [2]. Though, many lotic organisms are adapted to harsh environmental conditions, many sensitive forms tend to get displaced during land runoff [3]. This is due to the alteration in water channel morphology due to heavy runoff which might kill or displace biota downstream and remove potential resources. Floods also can increase abundance and richness of fish where the water bodies are disconnected by sand bars or other disturbances by increasing the movement of fishes between water bodies which were previously impeded by barriers [4].

Ichthyoplankton are more sensitive life history stages exposed to number of environmental stresses in the aquatic water body [5]. In fact, the Ichthyoplankton distribution and in estuaries are more complex in terms of composition and abundance due to an interactive effects of various physical, chemical and biological factors [6] and hence they are sensitive to ambient water quality parameters. Identifying juvenile and Ichthyoplankton fishes is challenging due to overlapping morphological characters and non-differentiability between adult fishes. Accurate identification of Ichthyoplankton by DNA barcoding is an important tool for various conservation practices by implementing various management strategies. At present, no study had been carried out to determine the fish distribution in Kuantan river except a study conducted by Jalal *et al*. (2012) where the authors sampled adult fishes using different mesh size gill nets [7]. However, no studies on Ichthyoplankton and juvenile fishes in Kuantan river had been carried out in the past. Hence, the present study was aimed to identify juvenile and Ichthyoplankton of Kuantan river and its immediate tributaries using DNA barcoding techniques. The study also addresses the impact of recent flood on Ichthyoplankton assemblage changes and effect of environmental parameters over their distribution.

3.0 MATERIALS AND METHODS

Ichthyoplankton sampling was carried out in Kuantan river and its immediate tributaries during ebb tidal cycle (Fig 1).

Water quality parameters such as Temperature, Salinity, pH, Dissolved oxygen, conductivity and turbidity were recorded using hydrolab 4.0. Due to technical constrains such as high turbidity, uneven water depth in Kuantan river (varied from 4m to 13m) besides inefficiency of plankton sampling net (mesh size 500µ), a modified bubu light trap (Fig 2) was used during sampling from April to December 2015. Modified Bubu light trap used in this study is 5x4x3feet (LxWxH) size having conical shaped opening in one side towards the interior section of the trap. The skeleton is made up of bamboo or cylindrical wood which in turn covered completely by 2mm thickness stainless steel wire meshes with the mesh diameter of 2.5cm2. An underwater light was placed in transparent plastic container and the lid was sealed with commercially available silicone gel and parafilm to ensure maximum light emission. An anchor was tied at the bottom of the cage to make sure the cage does not wash away during water current. All materials and parts were purchased and assembled in Malaysia. The complete set up (modified bubu light trap) was cover by plastic window mesh sheet (mesh size <1mm) (Fig 2). The net was deployed under water at the depth of 4meter in sampling stations for 16hours overnight.

Ichthyoplankton and juvenile fish samples were measured under Dino Capture 2.0 portable microscope (or) standard scales (15cm) respectively and stored in 70% ethanol for further downstream application. Samples were identified morphologically to the lowest possible taxon using standard references and Taxonomic classification was according to [8]. Size classes of larvae were tabulated with observed physicochemical parameters of the ambient water for Pearson's correlation matrix analysis. Size class variation of Ichthyoplankton was represented in Mean ± SD.

DNA barcoding

Total genomic DNA was extracted using Geneaid DNA tissue isolation kitTM. The 5' end of cytochrome c oxidase subunit I gene region was amplified using the primer pair Fish F1: 5'-GGTCAACAAATCATAAAGATATTGG-3' and Fish R1: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR condition includes, hot start with 94˚C for 1 minute, 5 cycles of 94˚C for 30 seconds, annealing at 45˚C for 40 seconds, and extension at 72˚C for 1 minute, 35 cycles of 94˚C for 30 seconds, 51˚C for 40 seconds, and final extension at 72˚C for 10 minutes. The PCR products were gel eluded and sequenced based on the standard protocols previously described. DNA sequences were trimmed using chromas lite software v2.1.1. All the sequences were subjected to BOLD and NCBI BLAST analysis.

Data analysis

Scree plot and Pearson correlation Matrix (PCM) was used to determine the significant factors influencing Ichthyoplankton and juvenile fish distribution. Significant variations in physicochemical parameters collected from different sampling sites were analyzed

using multivariate analysis at 95% confidant interval. Size class variation of Ichthyoplankton was expressed in percentage. All data were expressed in Mean±SD. Probability value ($P < 0.05$) were considered statistically significant. All data analysis was conducted using Graph Pad prism v6.

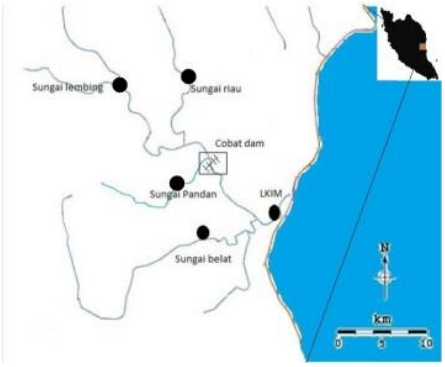

Figure 1: Location of the sampling sites at Kuantan river.

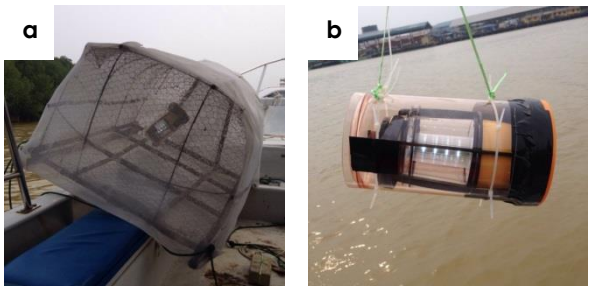

Figure 2: Modified bubu light trap (2a) and the prepared internal light source (2b) used to attract the Ichthyoplankton during sampling.

3.0 RESULTS AND DISCUSSION

A total of 28 species belong to 15 families were successfully identified to the species level from the total of 58 barcodes generated from 372 larval/juvenile samples (Table 1). BOLD and BLAST analysis clearly segregated individuals to their respective species with high percentage similarity score (> 90-100). The dominant families were cyprinidae (35%), toxotidae (24%), ambasidae (18%) and eleotridae (11%) compared to the previous report by Jalal *et al*. (2012), where they observed dominance of adult Ariidae (23%), Lactaridae (11 %) and Lutjanidae (8%) [7]. However, in the present study 28 different species of larvae were recorded unlike the previous report where only 19 species belong to 12 families were recorded [7]. Significant variation in physicochemical parameters between sampling stations were observed (Table 2) where salinity and suspended particles played a key role in altering Ichthyoplankton distribution as shown in scree plot and correlation matrix ($P<0.05$). The importance of hydrological disturbance in lotic environments has been broadly recognized because it produces a larger number of indicators and induces the reproduction of species with different reproductive strategies [9]. Many studies have shown the significant influence of salinity, DO and temperature on the larval abundance and temporal distribution in rivers [10,11]. Although the differences in environmental variables influence the abundance and composition of the Ichthyoplankton among the reproductive periods, they also observed some differences in larval assemblage composition among sections [12].

Mean standard length of larvae captured under the light trap was 9±5.3 mm which was significantly smaller than the samples caught from plankton net (0.5mm msh size). The advantage of this light trap method used in this study includes 1. The samples collected were not physically damaged and hence used for DNA isolation without other species contamination, 2. The efficiency of this light trap over bongo net or other plankton sampling net in order to get larval samples from areas were water depth and turbidity are the limiting factors for sampling. It should also be noted that the light trap method has also have demerits such as it does not attracts fish larvae which has negative phototaxtic behavior. For instance, Ariidae fishes which are benthic in nature having negative phototaxtic behavior and most of the adult Ariidae fishes are mouth-brooders. This key behavior has eventually reflected in result whereby no Ariidae fish larvae were recorded in this study [13,14,15]. Most of the Ichthyoplankton samples collected were between 0-10mm in total length with the percentage abundance of 34%, 60%, 17% and 10% in Kuantan river, belat river, Sg. Pandan and Riau river respectively (Figure 3).

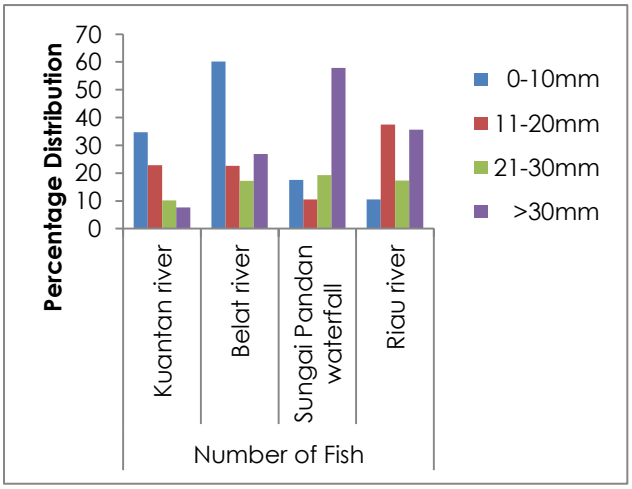

Figure 3: Percentage distribution of different size class Ichthyoplankton/juvenile fishes in sampling zones

Table 1: Ichthyoplankton and Juvenile fishes identified using DNA barcoding of Cytochrome oxidase C subunit 1 gene.

Family	Species Identified	IUC N Red List Status	BOLD/BLAST similarity percentage	Kuantan River	Belat River	Panching River	Riau River	Previous study (Jalal *et al*. 2012)
Ambassidae	*Ambassis marianus*	Least Concern	92	*				NF
Ambassidae	*Ambassis commersoni*	Least Concern	85		*			NF
Ambassidae	*Parambassis siamensis*	Least Concern	96			*		NF
Leiognathidae	*Secutor ruconius*	Not Evaluated	100	*	*			NF
Leiognathidae	*Photopectoralis bindus*	Not Evaluated	99		*			NF
Leiognathidae	*Leiognathus equulus*	Least Concern	99	*				NF
Lutjanidae	*Lutjanus johnii*	Not Evaluated	84	*				*
Lutjanidae	*Lutjanus russellii*	Not Evaluated	100	*				*
Eleotridae	*Butis gymnopomus*	Not Evaluated	100	*				NF
Eleotridae	*Prionobutis dasyrhynchus*	Not Evaluated	99	*			*	NF
Toxotidae	*Toxotes chatareus*	Not Evaluated	100		*			*
Toxotidae	*Toxotes jaculatrix*	Least Concern	99		*			*
Cyprinidae	*Poropuntius smedleyi*	Not Evaluated	99			*		NF
Cyprinidae	*Barbonymus schwanenfeldii*	Least Concern	100				*	NF
Cyprinidae	*Barbonymus gonionotus*	Least Concern	99				*	NF
Cyprinidae	*Neolissochilus stracheyi*	Least Concern	98			*		NF
Cyprinidae	*Barbodes binotatus*	Least Concern	99		*			NF
Cyprinidae	*Rasbora sumatrana*	Not Evaluated	99			*		NF
Cyprinidae	*Rasbora dusonensis*	Not Evaluated	99				*	NF
Cyprinidae	*Rasbora trilineata*	Least Concern	97				*	NF
Megalopidae	*Megalops cyprinoides*	Data deficient	100	*				*
Gobiidae	*Bathygobius laddi*	Not Evaluated	86	*				NF
Cichlidae	*Oreochromis niloticus*	Not Evaluated	84		*			*
Percichthyidae	*Gadopsis marmoratus*	Not Evaluated	85		*			NF
Monodactylidae	*Monodactylus argenteus*	Not Evaluated	90		*			NF
Scatophagidae	*Scatophagus argus*	Least Concern	99		*			NF
Zenarchopteridae	*Dermogenys pusilla*	Not Evaluated	99		*			NF
Hemiramphidae	*Hemirhamphodon pogonognathus*	Least Concern	100			*		NF
Balitoridae	*Balitora kwangsiensis*	Least Concern	83				*	NF

Note: * represents the inhabiting species in each sampling station. Species identified in this study was compared with previous study where adult fishes were sampled using gill net[7]. NF represent corresponding species in the row 'Not Found' during previous during previous study.

Table 2: Physicochemical parameters recorded in sampling stations (Data expressed in Mean±SD). Different alphabets between sampling zones showed significant variation between sampling sites

Sampling site	Number of fishes caught	GPS coordinate	Depth (m)	Temperature (°C)	Dissolved oxygen (mg/L)	Specific conductivity (mS/cm)	pH	Salinity	Turbidity (NTU)
Kuantan river	139	N 03° 47' 04.2" E103° 19' 05.1"	3.5	29.56±2.29[a]	5.01±1.7[a]	3.94±0.87[a]	7.10±1.67[a]	2.23±0.92[a]	40.7±3.81[a]
Belat river	74	N 03° 46' 15.7" E103° 17' 21.7"	4.0	29.60±3.15[a]	3.77±1.02[b]	12.8±2.11[b]	5.56±1.97[b]	6.58±1.63[b]	3.40±0.59[b]
Pandan river	46	N 03° 47' 4.65" E103° 08' 58.5"	0.8	24.90±1.39[b]	2.91±0.68[b]	0.12±0.01[c]	5.4±1.15[b]	0.22±0.001[c]	0.3±0.01[c]
Riau river	113	N 03° 51' 51.6" E103° 13' 32.4"	1.0	27.74±0.02[a]	4.53±0.57[a]	0.04±0.01[d]	6.23±0.07[a]	0.03±0.00[d]	35.9±0.87[d]

4.0 CONCLUSION

In conclusion, all the fishes sampled from Kuantan river and its tributaries were identified to the species level by DNA barcoding technique. A significant shift in species composition has occurred after the recent flood which is believed to have positive impact on Kuantan river fishing in near future. Unlike the previous study, diversified fish species were found during the present sampling. Constant monitoring would be a paramount important for sustainable fishery management practice in Kuantan river.

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