Environmental DNA (eDNA); Monitoring and management of fisheries and water resources in Paunglaung River and Mekong River

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Abstract

Nowadays, the role of Environmental DNA (eDNA) is vital importance to conserve as sustainable state for fisheries and water resources regarding aquatic ecosystem as well as biomass in it. In doing so, research works regarding abundance of fisheries and water resources are carrying out in two the study areas; Paunglaung River and Mekong River. According to Anaeis *et al*., (2016), Environmental DNA (eDNA) promises to ease non-invasive quantification of fish biomass or abundance, but its integration within conservation and fisheries management is currently limited by a lack of understanding of the influence of eDNA collection method and environmental conditions on eDNA concentrations in water samples. Water temperature is known to influence the metabolism of fish and consequently could strongly affect eDNA release rate. Therefore, in this research work, it is necessary to observe abundance of fish species and water resource management in the study areas; Paunglaung River and Mekong River (here after, study areas), where hydropower projects are similarly found along the rivers. Environmental conditions are seriously effect on the natural resources against sustainable state concerning for the sake of all community's socioeconomic development of local indigenous groups. Griggs et al., mentioned in (2013), the SDGs are important for freshwater biodiversity because they include explicit commitments to the conservation, restoration and sustainable use of fresh water linking for the first time ecosystem health to human well-being into an agenda agreed by all United Nations members. Therefore, this research work is implementing with effective ecosystem management framework in the study areas to be sustainable riverine ecosystem with all key stakeholders mapping as interdisciplinary approach by focusing on environmental contaminants and its effect, biological indicators, and physico-chemical indicators regarding Sustainable Development Goals (SDGs).

Keywords: Environmental DNA (eDNA), local indigenous groups, effective ecosystem management framework, stakeholders mapping, Sustainable Development Goals (SDGs)

Introduction

Riverine effective ecosystem management study areas are Paunglaung River (17° 10' 00" N, 96° 58' 00" E) as the length of 320 Km, and Mekong River (33°42.5′N, 94°41.7′E) as the length of 4,350 km (2,703 miles) but Mekong River (within Myanmar territory) is 350 Km that plays a crucial role with the deep observation of advanced Environmental DNA (eDNA) in order to sustain fisheries richness as well as water sanitation. In this regards, it is necessary to implement systematic analysis on aquatic ecosystem and biomass survival especially fisheries resources and its aquatic environment is as efficient research tasks. According to Jane et al., 2014, Stream characteristics such as flow rate, dissolved oxygen, pH, temperature, and turbidity were also measured at each site to determine if site-specific features may either inhibit or reduce the efficiency of DNA amplification. The concentration of eDNA varies as a function of the rate of eDNA release from the organism and the rate of degradation in the environment, both of which are expected to be the result of complex interactions between environmental conditions, metabolism and the ecology of the targeted species (Barnes et al. 2014; Strickler et al. 2015).

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Therefore, in this research tasks, it is time to focus on the degree of environmental degradations and its main sources as well as the survival rate of aquatic life because of environmental challenges on it. Turak, E., et al., 2016, mentioned Global biodiversity declined between 2000 and 2010, and there are indications that the decline was greater in freshwater than in terrestrial or marine systems. Global biodiversity loss represents one of the most serious environmental crises of the 20th and 21st centuries, with considerable impact on both ecosystem services and the health of our planet (Pimm et al. 2014). An overall biodiversity decline of 52% was recorded between 1970 and 2010, and this loss was even higher for freshwater populations than for marine or terrestrial ecosystems (WWF 2014). Global freshwater biodiversity continues to decline at an alarming rate (Collen et al., 2014) despite efforts to prevent such loss. Fukumoto et al., 2015; Boothroyd et al., 2016 explained improvements in lab methods based on microbiology and ancient DNA research have enabled the detection of small quantities of DNA through analysis of water samples, as all animals deposit their DNA in the environment around them as a result of regular biological processes such as sloughing of skin cells or urination. This data source is termed "environmental DNA" (eDNA) and has recently emerged as an effective method for assessing species presence/absence in a wide range of aquatic habitats, including threatened species of conservation significance. Hänfling et al., (2016) stated organisms continuously release DNA into their environments via shed cells, excreta, gametes and decaying material. Analysis of this "environmental DNA" (eDNA) is revolutionising biodiversity monitoring. Regarding all this points, fisheries and water resources data were collected in both study areas to analyze species index and its surrounding water, in doing so, new findings are observed as new fish species as well as fish's habitat preference as recorded carefully in order to report the International Union for Conservation of Nature (IUCN), as goals of researchers and scientists. The most important source of regularly updated information on species' distributions and conservation status is the IUCN Red List of Threatened Species (IUCN, 2016). Therefore, in the present work that needs to implement effective ecosystem research framework with advanced techniques with all stakeholders mapping in order to be effective natural resource management not only Paunglaung River but also Mekong River as case studies yet the sustainability of these resources are threatened by over-exploitation, development and hydropower projects. Environmental DNA (eDNA) sampling has proven to be a valuable tool for detecting species in aquatic ecosystems. Within this rapidly evolving field, a promising application is the ability to obtain quantitative estimates of relative species abundance based on eDNA concentration rather than traditionally labor-intensive methods (Can. J. Fish (2017). Carina Tetzlaff, Dietrich Mäde (2016) mentioned from the nutritional point of view, fish is a valuable food supplying the human with essential amino acids, lipid-soluble vitamins and ω-3 fatty acids. Besides its nutritional benefits, fish is as well one of the most important triggers of food-allergic reactions. The consumption of fish by affected persons may cause severe anaphylactic shocks with probably fatal consequences. Therefore, in this regards, comprehensive and systematic population genetic analyses of fisheries and water resources with representative natural history characteristics by ensuring these resource management as well as by extending this knowledge to government, academic institutions and the general populace through aggressive outreach activities for the sake of community.

Aims and Objectives

1. To explore substantially strengthen knowledge of the life history characteristics of representative important fisheries and water resources
2. To observe population genetic data available for important fish species with clearest understanding on the degree of population structure and gene flow
3. To compact network of regional collaboration of all stakeholder mapping on conservation natural resource management with capacity building enhancement
4. To encourage monitoring and strengthening fisheries as well as water resources management concerning potential impacts of dams on the populations of fishes
5. To inform all stakeholders; academic, government, non-governmental and intergovernmental organizations about baseline of natural resource conservation management

Materials and Methods

Project Design

Collections fish species and water resource data, Laboratory work, DNA sequencing, Analyses and reporting of sequencing results

Target sites and species

Species and site selection, life history strategies and habitat preference, meetings with stakeholders for dissemination of the most up-to-date results and current project goals

Data and infrastructure capacity

Widen field survey, preserved biological tissues, DNA extractions, sequencing primers and protocols, nucleotide sequences, output from sequence and statistical analyses, policy making process, private sector implementation and technology adoption, community engagement, and capacity building enhancement

Laboratory Procedures

1. Samples collections, metabolic waste of species, muscle tissue (95% ethanol) or (RNA/DNA Shield (ZymoResearch)
2. DNA were extracted from tissues using Qiagen DNeasy ® kits, quantified, and stored at 0 C, double-digest restriction site-associated DNA (ddRAD) library preparation as non-model organisms with unknown genome sizes
3. Final quality assurance and sequenced on an Illumina HiSeq 4000

Analyses

1. Initial processing of raw sequences and the discovery of single nucleotide polymorphisms (SNPs) **or** VCFtools for SNP filtering,
2. Genetic diversity and fixation indices
3. Analysis of Molecular Variance (AMOVA) **or** Structure v2.3.4 to explore population structuring,
4. Ne Estimator to estimate effective population sizes
5. Physico-chemical indicator, D.O, B.O.D, Heavy metal examination regarding water resource management

Dissemination plan

1. Preserved biological tissues and DNA extractions
2. Tissues were stored in 95% molecular grade ethanol or in DNA/RNA Shield (Zymo Research)
3. Extractions were eluted in salt buffer to prevent enzyme degradation
4. Both tissues and extractions in -20°C freezers for long-term preservation
5. Field notes, laboratory work and protocols were recorded and retained

6. Electronic data such as specimen catalog information, gel photos, and analyses
7. Molecular sequences with other standardized metadata
8. Data and dissemination to the scientific community
9. Molecular protocols and any specific primers designed
10. Outreaching seminars for the relevant academic, government, non-governmental organizations
11. Compact network of regional collaborators; build the capacity for advanced genomic research, and elucidate the impacts of dams on the fish populations of the Rivers
12. Government agencies, academic institutions, and stakeholder groups to engage in each community & anticipate identifying additional local institutions and agencies and local news outlets

Analysis of Key factors

It is necessary to analyze all key points according to research schedules in the study areas, in doing so, analysis of biological resources especially for fisheries resources and their survival rate, water resources especially for main causes of pollution and its effects on natural resources, analysis of genetic composition and its consequence effects on aquatic ecosystem with effective monitoring as well as all key stakeholders involvement. Turak, E., et al., (2016) mentioned analysis of genetic composition is rarely included as an objective in biodiversity monitoring programs, especially in freshwater environments. These include the completion of a global database of stream and river networks (Lehner and Grill, 2013), a more comprehensive database on the location of large dams and reservoirs as well as new regional assessments of freshwater biodiversity (e.g. Darwall et al., 2011). In the study areas, it is necessary to compare before and after the situations of implementation of developmental projects in the riverine ecosystem management not only in laboratory experiments but also in field works. In Myanmar, fisheries is main source of nutrients, therefore, it is important to analyze genomic mapping of fisheries and their life history, ecology, classification of fish especially for economic important fish to fully understand in order to inform all stakeholder levels. Houhoula D., et al (2015) stated the RT PCR assay proved to be a potential tool for the detection and label management of fish allergens in food.

In the study areas, effective monitoring and efficient management is weak, therefore, it need more attention with analytical environmental awareness to conserve biodiversity richness especially for aquatic life and their habitat preference, reasons of population decline and disappear, environmental impacts on water bodies regarding upstream, downstream, related streams and creeks, as well as watershed and reservoir. Allendorf et al., (2010) observed genetic sampling of wild populations can help us to address questions of demography, individual relatedness, population structure, and other important aspects of biodiversity that cannot be answered by behavioral monitoring alone. Megan L. Aylward, et al., (2018) explained non-invasive sampling is an important development in population genetic monitoring of 28 wild animals. Therefore, not only in the study areas but also all over Myanmar, the most effective ecosystem management research framework is vital for sustainable aquatic life with healthy fisheries populations and its community as well as clean water sanitation regarding biodiversity richness in riverine as a sustainable state. Because of climate change all over the world including Myanmar, the populations of all species are decreasing day by day with the lack of environmental awareness. In doing do, need to analyze on possible impacts and speciation of target species. The Essential

Biodiversity Variables (EBVs) framework (Pereira et al., 2013), six broad classes of EBVs have been proposed, each representing a major component of biodiversity: genetic composition: species populations; species traits; community composition; ecosystem structure; and ecosystem function.

In the riverine ecosystem of the study areas, some fish species, for example tilapia, was introduced in the natural ecosystem, in doing so, their fecundity rate is enormous that enough to be threatened of local indigenous fish species by doing competitions for foods of their survival, shelters for their hiding from enemies as well as breeding grounds including nursery of fingerlings, occupying wandering places, therefore, local endemic fishes are facing challenges with full of stress that is leading towards the increasing of mortality rate and finally eradication of endemic fish which gradually leads endangered , threatened, and finally extinctions as loss of biodiversity richness. Fernandez et al., (2018) mentioned many fish species have been introduced in wild ecosystems around the world to provide food or leisure, deliberately or from farm escapes. Some of those introductions have had large ecological effects. Detecting native and invasive fish populations in ecosystem monitoring is crucial, but it may be difficult from conventional sampling methods such as electrofishing. Rheyda Hinlo et al., (2017) concluded that eDNA survey results are more powerful when used in conjunction with other survey methods as a way to enhance detection rates and increase confidence in the monitoring results. Hinlo R et al., (2017) examined the environmental DNA (eDNA) method is a detection technique that is rapidly gaining credibility as a sensitive tool useful in the surveillance and monitoring of invasive and threatened species.

Noble, T.H et al., 2015 explored invasive fishes pose a major threat to aquatic ecosystems worldwide. Environmental DNA technology was successfully adapted for the specific purpose of tilapia surveillance and this has resulted in a high quality service that will be beneficial to many organizations and associations to help early detection of tilapia incursions. Therefore, nowadays, we all researchers need to focus the most effective ways of using methods; most accurate, faster, cheaper, effective ways to conserve species richness and water resource management. In this regards, in the study areas (Paunglaung River and Mekong River), collections of fisheries resource and sampling water resources for examination regarding field works and lab tasks to explore new findings concerning fish species and their habitat preference as well as main causes of pollutions for water bodies. Rocky Mountain Research Station (2017) stated aquatic species shed DNA material which disperses throughout the water column. This external DNA is called environmental DNA (eDNA). Compared to traditional methods, eDNA sampling is a faster, less expensive, and more sensitive way to assess species presence. The detection of eDNA represents a major advance in monitoring species in freshwater environments. By filtering water samples and analyzing them for eDNA, one can determine whether a species is pre-sent without actually capturing or seeing an individual. Different species can be identified by using genetic markers that are unique to them.

Significance of research

Environmental deterioration is gradually facing as main challenges against sustainable state because of lack of environmental awareness, extreme harvesting of natural resources as illegal logging, illegal, unreported, and unregulated (IUU) fishing, advanced civilizations, and awful events are resulting in diminishing the degree of biological richness, unsustainable aquatic ecosystems, adverse severe alteration of freshwater fish population and its fecundity, and reducing abundance of vital key

species in aquatic ecosystem especially riparian corridors of study areas. Regarding all this points to recover again, it is necessary to use advanced methods to conserve aquatic life and its ecosystem in time, so, it is essential to understand the importance and usefulness of Environmental DNA (eDNA) for sustainable management. Carim et al., (2016) declared Environmental DNA (eDNA) is DNA that has been released by an organism into its environment, such that the DNA can be found in air, water, or soil. In aquatic systems, eDNA has been shown to provide a sampling approach that is more sensitive for detecting target organisms faster, and less expensively than previous approaches.

The result of research will be completely enhanced to recover the richness of all aquatic life by tracing as well as focusing the main causes of environmental contaminants and its effect on aquatic life in order to cure the current situations of reversing the decline of species population. It is sure that to inform all update results to related academic, government, non-governmental and intergovernmental organizations in Myanmar and all ASEAN community by enhancing to have compact network of regional collaborators in order to engage in each community as well as anticipate identifying additional local institutions and agencies concerning local news outlets by making meeting, presentations, workshops, symposiums with interdisciplinary approach for managing environmental contaminants, sustaining all natural resources, recovering biological richness, and observing riparian corridors with new ideas of scaling innovative sustainable development solutions that to be proven new findings to encourage more powerful interdisciplinary approach, that may be useful in policy making progress for the sake of all community.

Conclusion

Regarding the criteria of Sustainable Development Goals (SDGs), this research work is securing the fisheries resources as well as water resource (case study; Paunglaung River and Mekong River) from the conservational eDNA point of view, it is explored proposed new species of fish and new findings on habitat preference in their ecosystem of some fish species. Similarly, new methods and new research framework are used to conserve aquatic life in the riverine as well as new findings on main sources of water pollutions and it impacts on aquatic life by making effective assessment of water quality analysis with better understanding of comprehensive knowledge and compact scientific foundation with new application of alternative ideas and the highest consideration of the criteria of SDGs. The overall destination of this research work is accomplished by having better understanding of value of natural resources with environmental ethics by informing environmental awareness to local community as well as all levels effectively.

Appendix

Figure 1.**F**isheries resource in Paunglaung River; *Puntius stoliczkanus* (Ng—Khone-Ma), *Devario* sp. (Nga-La-War), *Nemacheilus pallidus*, *Hypsibarbus* sp. (Nga-Ga-Lain), *Cirrhinus molitorella*, *Macrognathus semiocellatus*

Figure 2. Paunglaung River; collections of water sample by Mie Mie Kyaw

Figure 3. Mekong River; research works for fisheries resources

Figure 4. Collections of target fish species at Mekong River area, Myanmar

Figure 5.Public meeting with village leaders & local community, Mekong area

Table 1. Physical and Chemical Examination of Water in the Vicinity of the Middle Paunglaung Hydropower, Myanmar

Parameter	Unit	Lin War Stream	Ye Pu Stream	Paunglaung River	W.H.O Std.
pH value	Scale	6.9	6.9	6.9	7-8.5
Colour	Units	>50	>50	>50	5
Turbidity	N.T.U	2.03	8.81	5.23	5
Conductivity	MicroS/cm	203	197.2	220	80
Total dissolved solids	mg/l	108.5	105.6	118.3	80
Total suspended solids	mg/l	16	7	5	2
Calcium, Ca	mg/l	24	19	40	75
Hardness, CaCo3	mg/l	100	80	140	100
Magnesium, Mg	mg/l	10	8	10	30
Chloride, Cl	mg/l	8	8	8	200
Total Alkalinity	mg/l	120	100	120	200

Iron, Fe	**mg/l**	**>0.2**	**>0.2**	**>0.2**	**0.1**
Manganese, Mn	**mg/l**	**0.03**	**0.03**	**0.03**	**0.05**
Sulphate, So4	**mg/l**	**<200**	**<200**	**<200**	**200**
Nitrogen Nitrate, N-NO3	**mg/l**	**22**	**22**	**22**	**-**

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