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REVIEW

Lobster Fishery Connectivity and Management In Indonesia Waters

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ABSTRACT

The distribution of lobsters in Indonesian waters is very wide, even lobster species in Indonesia are also scattered in the tropical waters of the western Pacific Ocean, the Indian Ocean, Africa to Japanese waters. Indonesian waters are divided into 11 (eleven) Fishery Management Zone (FMZ). Lobsters in Indonesia may come from various water areas, both national and regional water zones, and they're called the sink population. Its spread is influenced by the movement of the current. Lobster seed is nurtured by nature through ocean currents from Australia, East Indonesia, Japan, then back to Australia. Lobsters have a complex life cycle, where adult lobsters inhabit coral reefs as a place to lay eggs, then hatch into planktonic larvae, and grow up in open seas and carry out diurnal and ontogenetic vertical migrations before returning to nurseries in shallow coastal areas and reefs, coral, as well as habitat by the type of species. Literature research had used at least two methodologies to estimate the distribution and connection sensitivity matrices of marine organism larvae. The two most common approaches are using genetic markers and numerical biophysical modeling. Thus, this research uses molecular genetic techniques to explain the genetic structure of lobster populations using a biophysical model approach that can explain the genetic structure of lobsters, as well as the distribution based on regional oceanographic synthesis data and lobster biology known in Indonesian waters. This model has four components, namely: 1) a benthic module based on a Geographical Information System (GIS) which is a lobster habitat in the spawning and recruitment process, 2) a physical oceanography module containing daily velocity in the form of a three-dimensional hydrodynamic model, 3) a larva biology module that describes larval life history characteristics, and 4) a Lagrangian Stochastic module that tracks the individual trajectories of larvae.

1. Background

Lobster in English is known as *Crayfish* or *Spiny Lobster*, while in Indonesia it is known as the *Crayfish* or *Barong Shrimp*. A name scientifically well-known is Panulirus belonging to the *Palinuridae family* [1]. In Indonesia, six types of *Crayfish* are found, that are scattered from western waters to eastern Indonesian waters with conditions different habitats. They are Barong Shrimp (*Panulirus Versicolor*), Stone Shrimp (*Panulirus paniculate*), King Shrimp (*Panulirus longipes*), Ketangan Shrimp, Pine Shrimp or Pearl Lobster (*Panulirus ornatus*), Jatropha shrimp (*Panulirus polyphagus*), Green Sand Lobster

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(*Panulirus hommarus*) [2,3,4,5].

The distribution of lobsters in Indonesian waters is very wide, even lobster species in Indonesia are also scattered in the tropical waters of the western Pacific Ocean, the Indian Ocean, Africa to Japanese waters [6]. Indonesian waters are divided into 11 (eleven) Fishery Management Zone (FMZ), namely FMZ 571 (Malacca Strait and Andaman Sea waters), FMZ 572 (Indian Ocean waters next door West Sumatera and the Sunda Strait), FMZ 573 (Southern Indian Ocean waters Java to the south of Nusa Tenggara, the Savu Sea, and parts of the West Timor Sea), FMZ 711 (Karimata Strait, Natuna Sea, and the South China Sea), FMZ 712 (Java Sea waters), FMZ 713 (Makassar Strait waters, Bone Bay, Flores Sea, and the Bali Sea), FMZ 714 covering the waters of Tolo Bay and the Banda Sea), FMZ 715 (waters of Tomini Bay, Maluku Sea, Halmahera Sea, Seram Sea, and Berau Bay), FMZ 716 covering waters of the Sulawesi Sea and north of Halmahera Island), FMZ 717 covering the waters of the Cendrawasih Bay and the Pacific Ocean), and FMZ 718 (Sea waters Aru, Arafuru Sea, and the East Timor Sea) [7]. Any FMZ existing in Indonesia has the different potential abundance and types of lobster resources. The potential for lobsters in each FMZ has estimated, of which 11 FMZ are classified into 3 (three) clusters of water areas, namely was Indian Ocean Cluster (FMZ 571, 572, 573), Sunda Shelf cluster (FMZ 711, 712, 713) and the Sahul Exposure cluster (FMZ 714, 715, 716, 717, 718). Estimation results show that the potential of lobster seeds in the Indian Ocean cluster reaches 3,128 tons. From this potential value, about 1,563 tons are female lobsters. If 10% of female lobster seeds to grow into broodstock lobsters, the potential for lobster broodstock females in the Indian Ocean cluster reached 15,630 tons or it can be estimated that they reached 11,265 female lobster broods. Thus, the total female lobster broodstock can produce as many as 7,815,000,000 eggs. However, like all the eggs that exist, about 1% can survive for each cycle, leaving only about 78,150,000 lobster eggs in the cluster Indian Ocean. Likewise with the potential for lobster seeds in the Sunda Shelf cluster reach around 3,337 tons with an estimated number of female seeds as much as 1,669 tons and an estimate of which become breeders of 16,685 tails, with a potential number of capable eggs gradually life reaches 83,425,000 eggs. While the potential number of broodstock lobsters in the Sahul Exposure cluster totaled 117,375,000, thus estimates the potential for lobster in all FMZ in Indonesia is around 278 billion eggs, 250 billion larvae and the estimated number of lobster seeds (Peurulus) reached 12.5 billion. The assumed data on the potential lobster seeds in each FMZ Indonesia are shown in Table 1 [8].

Lobsters in Indonesia may come from various water areas, both national and regional water zones, and they're called the sink population. Its spread is influenced by the movement of the current. Lobster seeds nurtured by nature through ocean currents from Australia, East Indonesia, Japan, then returned to Australia [9,10]. Lobsters have a complex life cycle, where adult lobsters inhabit coral reefs as a place to lay eggs, then hatch into planktonic larvae, and grow adults in the open ocean and perform diurnal and ontogenetic vertical migrations before return to the nursery area in shallow coastal areas and coral reefs, and habitats suitable for the species [11]. On when it becomes a planktonic larva, it has a sufficient duration of pelagic larvae length which could take around 5-9 months (depending on species), as planktonic larvae [12], where these larvae have the potential to spread among lobster populations in all waters [13]. For example, that stock of lobster seeds in each FMZ in Indonesia in terms of type, quantity, and source of seed origin different. This corresponds to the *sink population* in which the planktonic larvae will be spread to other areas along with the movement and circulation of currents in the region of Indonesian waters. When viewed geographically, Indonesian waters are located in tropical regions, it can be assumed that two water areas function as spawning (*spawning ground*) lobster, which is in the waters between Papua New Gini and Australia, as well as Philippine waters. Lobsters will spawn in the waters between them Papua New Gini and Australia, then the larvae will be scattered in the direction of and movement of currents to the north through the Solomon Sea, the Bismarck Sea north of Papua New Gini, and northern Papua, which crosses the western Pacific Ocean to the water Philippines. In the Philippine Sea, there is a *sink population* and it spreads back into two water areas, the first is to spread towards the Makassar Sea and the second is towards South China Sea (including Hong Kong, Vietnam, and Malaysia) and towards the Sea Java. The movement of lobsters across the Makassar Sea and the South China Sea both meets and gathers (*sink population*s) in the waters of West Nusa Tenggara, especially in Lombok Strait. The lobster population that met in the Lombok Strait then spread in two directions across the Indian Ocean, the first is to spread out and back again towards the waters between Papua New Gini and Australia, and the second is to the west across the Indian Ocean to the west of Sumatera. The dispersal and genetics of lobster in Indonesian waters is shown in Figure 1 [9,10].

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Table 1. The assumed data on the potential lobster seeds in each FMZ Indonesia

FMZ	Potency (ton)	Concrete estimation (ton)	Female estimate will be parent 10% (ton)	The estimate number of broodstock (lobsters)	Estimated number of eggs	SR 1% (per per sycle all type of lobsters)	Species of pearl and sand lobsters (10% of all type of lobsters)	10%	20%	50%
HINDIA OCEAN CLUSTER										
571	673	336.5	3.365	3,365	1,682,500,000	16,825,000	1,682,500	168,250	336,500	84,250
572	1483	741.5	7.415	7,415	3,707,500,000	37,075,000	3,707,500	370,750	741,500	1,853,750
573	970	485	4.850	4.850	2,425,000,000	24,250,000	2,425,000	242,500	485,000	1,212,500
SAHUL SHELF CLUSTER										
711	1421	710.5	7.105	7,105	3,552,500,000	35,525,000	3,552,500	355,250	710,500	1,776,250
712	989	494.5	4.945	4,945	2,472,500,000	24,725,000	2,472,500	247,250	494,500	1,236,250
713	927	463.5	4.635	4,635	2,317,500,000	23,175,000	2,317,500	231,750	463,500	1,158,750
SAHUL SHELF CLUSTER										
714	724	362	3.62	3,620	1,810,000,000	18,100,000	1,810,000	181,000	362,000	905,000
715	846	423	4.23	4,230	2,115,000,000	21,150,000	2,115,000	211,500	423,000	1,057,500
716	894	447	4.47	4,470	2,235,000,000	22,350,000	2,235,000	223,500	447,000	1,117,500
717	1044	522	5.22	5,220	2,610,000,000	26,100,000	2,610,000	261,000	522,000	1,305,000
718	1187	593.5	5.935	5,935	2,967,500.000	29,675,000	2,967,500	296,750	593,500	1,483,750

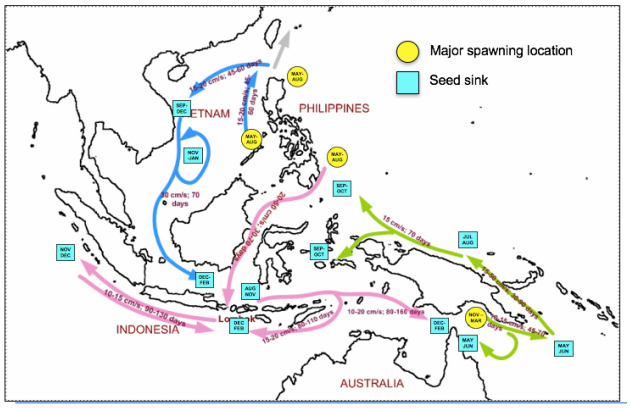

Figure 1. The dispersal and genetics of lobster in Indonesia waters [9,10]

Based on the distribution pattern of larvae originating from the northern region of Australia to the Philippine Sea crosses the western Pacific Ocean, South China Sea, Java Sea, and the Indian Ocean, there are two very important groups of events of distribution lobster larvae, namely spawning (*major spawning ground*) and foraging places (*seed sinking*) for the lobster. The time it takes to do trips starting from spawning ground in the territorial waters of Australia, Pacific Ocean western part, Philippines, South China Sea, Makassar Sea, Java Sea, and Ocean The Indies to return to Australia required a certain speed of spread as well as a very long duration. Broadly speaking, lobsters will spawn in Australian waters in November-March, while spawning in waters Philippines occurs in May-August. Based on the spawning time, you can assume that general lobsters will spawn twice deep a year [10]. Based on the distribution of lobsters that cross and enter FMZ in Indonesia, four main areas become the *sink population*, namely Sumbawa Island (9 locations, with a total area of 249.93 km^2), Lombok (14 locations, with an area a total of 80.94 km^2), Bali (2 locations, with a total area of 68 km^2) and Java Island (58 locations, with a total area of 1,198.36 km^2). The total area of *sink population* on the Java and Bali Islands is shown in Table 2 and Figure 2 [14].

The life cycle of most marine animals including lobsters starts from the larval stage planktonic last for hours to months connecting populations one another between regions. Hence, knowledge about connectivity larvae is very important for understanding population dynamics and managing the ocean sustainable based on biogeographically dispersed taxa. Recent studies on larval connectivity using natural or artificial tagging [15,16,17], biophysical modeling [18,19,20], larvae tracking methods [21], and analysis genetic [22,23,24], suggests that the population recruitment rate depends on exogenous supply and larvae in the sea [25]. The ability to predict the spread of larvae that is in the waters starting from the spawning grounds to the nursery is level science which is quite rare and interesting. Therefore, it needs to be studied more deeply to explain how a biophysical model is based on oceanographic parameters and biology empirically to provide estimates of larvae

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supply and can be used to determine the origin of larvae, destination, and distribution path of lobster larvae in Indonesia FMZ.

Table 2. The total area of *sink population* on the Java and Bali Islands

No	Location	Number of *sink population*	Percentage of number location (%)	Area (km^2)	Percentage of area (%)
1	Sumbawa	9	11	249.93	16
2	Lombok	14	17	80.94	5
3	Bali	2	2	68.00	4
4	Java	58	70	1,198.36	75
Total		83	100	1,597.23	100

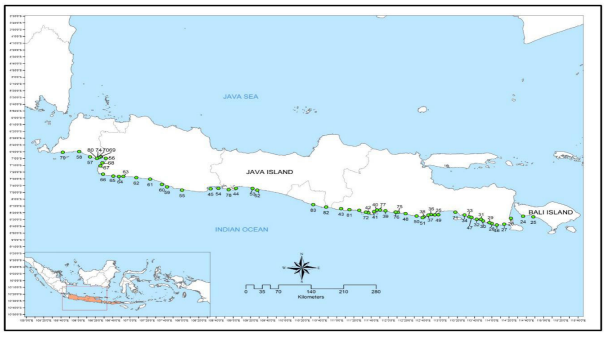

Figure 2. The total area of *sink population* on the Java and Bali Islands

Demographic connectivity studies have mostly focused on taxa with planktonic larvae (e.g. bivalves and reef fish) have contributed substantially scientifically, although there is a considerable bias over the broader spatial scale [26]. Demographic population connectivity between populations at scale spatial area (> 1000 km) is based on tagging and genetic methods undetectable [27,28]. Several approaches have also been taken to evaluate connectivity between populations of marine organisms, including genetic markers (e.g. mitochondrial DNA or microsatellites), geochemical markers (e.g. deep microchemical signatures shells), and/or the use of high-resolution biophysical models, but not one Neither of these approaches can be convincing in isolation and in knowing connectivity between populations [29,30,31]. Currently, the studies that have been conducted are limited to the dispersion model hydrodynamic species *Panulirus ornatus* in a limited area of species distribution. Previous studies in eastern Australian waters and in the Philippines focus on short-term larval dispersal within one generation *Panulirus ornatus* (e.g. from spawning grounds to nursery sites) [32,33]. According to in this model most of the larvae are released from the spawning grounds in the Coral Sea, such as The Gulf of Papua, will be brought back to the northeast Queensland coastline, in the interim some of them can move north to the Vitiaz Strait of eastern Papua New Gini within three months [32]. From different spawning places on the west and east coast of the Philippines, the larvae will be scattered towards the north of Taiwan, then spread towards the South China Sea, or spread into the interior of the Sulawesi Sea. Will but based on this study has not been able to answer the problem of connectivity *Panulirus ornatus* in the wider Southeast Asian archipelago [33].

The bathymetry and oceanography of the Southeast Asian archipelago are complex, with numerous shoals, straits, islands, coral reefs, and semi-enclosed seas, and mass flows water is carried by currents between the Pacific and the Indian Ocean. There is currently no scale model Oceanography covers the entire archipelago to aid in understanding that in-depth related factors that affect the genetic structure of *Panulirus ornatus*, or even the connectivity of other types of marine organisms. Existing oceanographic models includes several small-scale models that are the focus of conservation areas in the region the archipelago [34], and a medium-scale model of the entire domain [35] which has a grid size that is too coarse to complete the mass flux of water that goes through the Philippine Strait, so that produces an analysis that ignores connectivity between the Philippine Sea and the China Sea South and western Pacific Ocean [36]. For example, adult *Panulirus ornatus* was found in waters with depths of 1-50 m and occupy a variety of habitats such as sandy and muddy substrates, coral reefs, rocky bottoms, and even murky coastal waters [37]. *Panulirus ornatus* is known to migrate by walking along the seafloor as far hundreds of kilometers from fairly extensive spawning aggregations, for example, *Panulirus* adult *ornatus* from Torres Strait, Australia, migrate up to 500 km to the place spawning near Yule Island in the Gulf of Papua [38,39,40]. Furthermore, P. ornatus larvae have phases planktonic length that lasts 135-210 days [38,39,40,41]. Before completion, the larvae metamorphose to the puerulus stage, which is the end of the larval stage with strong swimming abilities, this phase lasts between 9-25 days [38,42]. The larval development period is this length, puerulus swimming ability, and the potential for phyllosoma admixture (planktonic larvae) in the Southeast Asian Archipelago region will produce levels low population genetic structure [43]. However, the hypothesis until recently has not been tested. Therefore, some methods are both quick and affordable and not limited by phyllosoma (planktonic larvae), namely the biophysical modeling method [44,45]. Thus, this study uses techniques of molecular genetics to explain the genetic structure of lobster populations by approaching biophysical models that can explain the genetic structure of lobsters, as well

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as pathways distribution based on regional oceanographic synthesis data and lobster biology known in the Indonesian FMZ. This model has four components, namely: 1) benthic module based on Geographical Information System (GIS) which is the habitat for deep lobsters spawning and recruitment process, 2) physical oceanography module contains velocity daily in the form of three-dimensional hydrodynamic models, 3) larvae biology module describe the life history characteristics of larvae, and 4) the Lagrangian Stochastic module which tracks the individual trajectories of the larvae.

In making a model of connectivity between populations, a parameter model is made based on spatial-temporal spawning and planktonic larvae behavior patterns, then verify the model by comparing the simulation results with the data empirical on the spatial-temporal pattern of larval supply in several FMZs in Indonesia. Oceanographic circulation models in three-dimensional form as well as larvae behavior models, where both affect the trajectory of the dispersal process [46].

2. Formulation of the Problem

Most of the marine macroinvertebrates, one of which is lobsters, in general, has a two-phase life cycle, consisting of the adult benthic phase and the pelagic larvae has the potential to spread from one region to another due to its existence movement of currents so that the benthic population has the potential to have a level of relationship genetically between populations and regions. To find out the level of population relations between regions genetically, a fundamental understanding of the flow is needed genes and adaptations of marine organisms. The basic thing that must be considered is the biological parameters of organisms that interact with physical and oceanographic parameters chemistry as a driver in the transfer of larvae between regions.

Indonesia's territorial waters are classified into eleven territories Fisheries Management Area (FMZ) with quite complex physical oceanographic characteristics, it is very likely that these oceanographic conditions will assist the process spread of larvae of marine organisms such as lobsters from one FMZ area to the FMZ others, so that the stock of lobster seeds in each FMZ in Indonesia, in terms of type, quantity and the source of origin of the seeds is different. This is related to the *sink population* where the larvae are planktonic will spread to other areas along with the movement and circulation of currents in Indonesian territorial waters. When viewed geographically, Indonesian waters located in the tropics, it can be assumed that two water areas serve as a *spawning ground* (*spawning ground*) lobsters, namely in the waters between Papua New Gini and Australia, as well as Philippine waters. The lobsters will do spawning in the waters between Papua New Gini and Australia, then the larvae will be scattered following the direction and movement of currents towards the north through the Solomon Sea, Bismarck Sea north of Papua New Gini and north of Papua which crosses the western Pacific Ocean up to Philippine waters. In the Philippine Sea, there is a *sink population* and it is spreading back into two water areas, the first is to spread out to the sea Makassar and the second to the South China Sea (including Hong Kong, Vietnam, and Malaysia) and towards the Java Sea. Movement of lobsters across the Makassar Sea and The two South China Sea meet and gather (*sink population*s) in the waters of Nusa West Southeast especially in the Lombok Strait. Lobster populations that meet in the Strait Lombok then spread in two directions across the Indian Ocean, the first is spreading and returning to the waters between Papua New Gini and Australia, and the second is westward across the Indian Ocean to the West Sumatera [9,10] (Dao *et al* ., 2015; Priyambodo, 2020). Based on the distribution of the lobster crossing and entering FMZ in Indonesia, four main areas become *sink population* namely Sumbawa Island (9 locations), Lombok (14 locations), Bali (2 locations), and south coast of Java Island (58 locations) [14].

Several research results have been carried out to determine inter-connectivity population based on genetic and oceanographic factors, although still on a spatial scale narrower, and focus on a single species, so several approaches have not been able to convince in knowing the connectivity between population [29,30,31]. Several methods have been developed including visual tracking marine larvae, artificial tagging, and natural tagging, and biophysical modeling numeric. Visual tracking of individual larvae is the only method applied directly, but can only be applied to large larvae with larval duration pelagic short, so its application is limited. There are also many other methods used, but the level of confidence is still small because there are still many factors in biology that have not been considered such as certain life-history traits, physiology, or anatomy of the study target. All methods have an intrinsic uncertainty depending on the type of marker, the analysis procedure, and the statistical methodology used.

Literature research has been conducted which collected a total of 507 research articles published since 1990 demonstrated that 41 studies have used at least two methodologies for estimating dispersion and connection sensitivity matrices of marine organism larvae. Two the most common approach is to use genetic markers and modeling numerical biophysics. Thus, this research uses molecular genetic techniques to explain the genet-

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ic structure of lobster populations using a biophysical model approach which can explain the genetic structure of lobsters, as well as the distribution pathways based on regional oceanographic synthesis data and known lobster biology in FMZ Indonesia. This model has four components, namely: 1) benthic based module Geographical Information System (GIS) which is the lobster habitat in the spawning process and recruitment, 2) physical oceanography module contains daily speed in the form of three hydrodynamic model dimensions, 3) larvae biology module that describes the characteristics life history of larvae, and 4) Lagrangian Stochastic modules that track individual trajectories larva [47].

3. The Urgency of Future Research

The urgency of future research is determined: 1) lobsters distribution in Indonesian FMZ based on genetic analysis, 2) lobster connectivity in Indonesia FMZ based on oceanographic data and lobster biology.

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ARTICLE

Occurrence of *Anguilla luzonensis* in the Tributaries along the Lagonoy Gulf, Philippines

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ABSTRACT

Anguillids are a valuable fish commodity worldwide. Although *Anguilla luzonensis* have been abundantly found in the northern Philippines and collected for trade, no available records show that it recruited in the mid-part where Lagonoy Gulf, Bicol is situated. In this study, we investigated the occurrence of *A. luzonensis* in the tributaries along the Lagonoy Gulf, Philippines using molecular tools. Glass eel specimens were collected in 2018–2019 from the Comun river, Albay; the Lagonoy river, Camarines Sur; and the Bato river, Catanduanes. *Anguilla luzonensis* was first reported in Lagonoy Gulf using molecular analysis. *A. luzonensis* was the second most abundant species in the Comun and Lagonoy rivers (9.5 and 22.4 %, respectively). *Anguilla luzonensis* collected from the Comun and Lagonoy rivers did not show a significant difference (FST= 0.00825, p>0.05). *Anguilla marmorata* was the most dominant species in all tributaries (71.1–98.0 %). In the Comun and Lagonoy rivers, *A. bicolor pacifica* was the third most abundant species (7.7 and 6.5 %, respectively). In addition, *Anguilla celebesensis* was only found rarely in the Comun river (0.9 %). This study provides important information for sustainable resource management and effective utilization of the eel species in these regions.

1. Introduction

The Philippines can be considered a species-rich habitat for Anguillid eels, with the total of six species (*A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, *A. bicolor bicolor*, *A. japonica*, and *A. celebesensis*) among the 19 species/subspecies of the genus *Anguilla* [1-7]. Anguillids from the Philippines are a commercially important commodity for export [8]. *Anguilla* luzonensis was discovered in Cagayan and described as a new species in 2009 using adult specimens (244-682 mm) [9]. *Anguilla* luzonensis has potential as a fishery product since trade to eel-consuming countries in the East Asia has been reported [7, 10-11]. Information on its population dynamics, stock status, and utilization is limited [12]. Due to the limited known range of *A. luzonensis*, it was recently assessed by CITES as a

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'Vulnerable' (VU) species under category D2 [13]. Therefore, resource management strategies in support of effective utilization, economic potentials, and sustainability of *A. luzonensis* bioresources are needed.

Anguilla luzonensis may be widely distributed in the Western Pacific with reports of its occurrence in Cagayan [1-5], Mindanao [2-3, 5-6], Taiwan [2-3], and Okinawa, Ryuku archipelago [14]. Abundance of *A. luzonensis* was reported in Cagayan [1, 3]. *Anguilla* luzonensis is presumed to have spawned in the North Equatorial Current (NEC) [3, 15]. One of the NEC bifurcates, the Kuroshio Current [16], transported *A. luzonensis* to Eastern and Northern Luzon and Taiwan [3]. As the Bicol region faces the Kuroshio Current, it is highly possible that these species may also occur in the Lagonoy Gulf in the same region. The Bicol region ranked 2nd for highest municipal fish production [17]. Therefore, the *A. luzonensis* glass eel might be one of the important target species for fishermen, but no fishery data are available. For resource management, the precise occurrence of *A. luzonensis* and its species composition must be clarified.

In our recent study, the species of freshwater eels recruited in the tributaries along the Lagonoy Gulf, Bicol were investigated [18]. Freshwater eel species were identified based on pigmentation patterns using an illustration [19]. Although the illustrations for *A. marmorata*, *A. bicolor pacifica*, and *A. luzonensis* showed that these species have pigmentation, they were difficult to identify. Consequently, among more than 4,000 identified pigmented glass eels, 89.8 % were grouped as *A. marmorata*, 10.1 % as *A. bicolor pacifica*, and none as *A. luzonensis*. No *A. luzonensis* individuals were found to recruit in the Lagonoy Gulf. The use of morphological identification alone was not enough to distinguish the pigmented Anguillid species; hence, molecular identification may be used for precise species identification and confirmation of species composition.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a fast and reliable technique, could identify 14 known *Anguilla* species [19-23] among the 18 known species and subspecies. Although *A. bicolor* subspecies cannot be distinguished, PCR-RFLP technique was suggested to be refined using a large number of samples to identify any eel species [22-23]. There may be cases where PCR-RFLP could not identify a specimen; hence, DNA sequencing may be used for further species identification [24]. The DNA sequences were also used in investigating molecular evolution [24-25]. Thus, *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, *A. bicolor bicolor*, *A. japonica*, and *A. celebesensis*, commonly found in Luzon, Philippines, may be identified by PCR-RFLP and DNA sequencing analysis.

In this study, we clarified the occurrence of *A. luzonensis* in Lagonoy Gulf using molecular tools. The species composition of six target species in the tributaries along Lagonoy Gulf was clarified. *Anguilla* luzonensis population structure was also confirmed.

2. Materials and Methods

2.1 Study Site and Sample Collection

The specimens analyzed in this study were glass eels that were identified on the basis of morphology [18] and collected from three main rivers, namely the Comun river (13°25'09.1" N 123°42'44.6" E) in Albay, the Lagonoy river (13°43'37.9" N 123°35'11.0" E) in Camarines Sur, and the Bato river (13˚35'40.0" N, 124˚17'10.0" E) in Catanduanes (Figure 1).

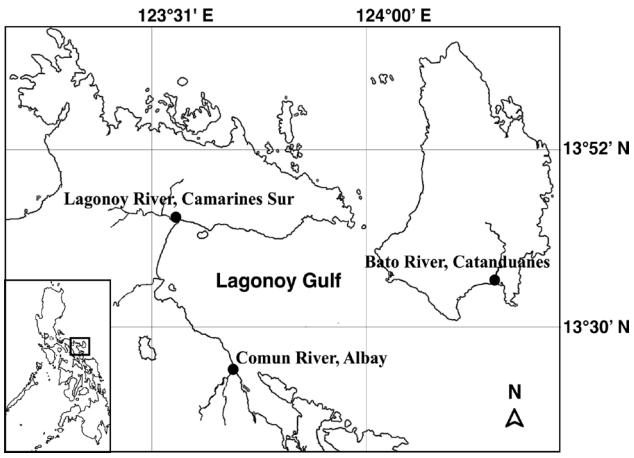

Figure 1. Map showing the study area in Lagonoy Gulf, Philippines, (●) indicates the sampling sites in Albay, Camarines Sur and Catanduanes

The glass eels were collected using a modified fyke net that was 1.15 m in height (H) and 10 m in length (L), with 2-8 m long wings and a 2.5 m-long catching bag [oval-shaped opening dimensions - 1.15 m H and 3 m width (W)] installed near or at a 3-4 m distance from the river mouth at a water depth of 1-1.2 m in the Comun river and Lagonoy river. A push net with dimensions 1.0 m H by 1.15 m W was positioned at a 3-4 m distance from the river mouth and a water depth of 1-1.2 m in the Bato river.

A one-day sample collection was conducted beginning at 18:00 h for 2-4 h during the new Moon phase between August 2018 and August 2019 in the Comun river, July 2018 and July 2019 in the Lagonoy river, and December 2018 and May/July 2019 in the Bato river. The collected glass eels were preserved in 95 % ethanol for subsequent analysis. Among the morphologically identified pigmented glass eels, approximately 10 % (n = 554; Comun= 220,

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Lagonoy= 232, Catanduanes= 102) were randomly selected for molecular analysis.

2.2 DNA Extraction and PCR Amplification

Approximately 25 mg of the middle-part of a glass eel was cut into small pieces. These were lysed overnight, and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) or FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., MI, USA). The cytochrome c oxidase 1 (COI) target fragment gene was amplified using the designed primers 5503F1 (5'-CCGCTTAAACATTCAGCC-3') and 7138R1 (5'-GGGGGTTCAATTCCTTCC-3'). PCR was performed in a 25 µl mixture containing 1.0 µl DNA template, 2.5 µl 10× buffer [magnesium (Mg^{2+}) plus], 2.0 µl 2.5 mM deoxynucleoside triphosphate, 1.25 µl 10 µM of each primer, 0.125 µl Taq polymerase (Takara Bio Inc., Shiga, Japan), and double-distilled water. The thermal cycler profile included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, elongation at 72 °C for 60 s, and final extension of 72 °C for 10 min. The PCR amplicons were visualized on 1 % agarose Tris-acetate-EDTA (TAE) gel electrophoresis with GelRed Nucleic Acid Gel Stain (Biotium, CA, USA).

2.3 Restriction Fragment Length Polymorphism Analysis

The simulation of restriction digestion was carried out for the target species *A. marmorata*, *A. bicolor pacifica*, *A. luzonensis*, *A. japonica*, *A. bicolor bicolor*, and *A. celebesensis* found in the Philippines. The complete mitochondrial genome of these six Anguillid species (Accession nos. AB469437, AP007242, AP007237, AP007236, AP007239, AB038556) was downloaded from the National Center for Biotechnology Information (NCBI) and aligned using MEGA X software [26]. Thirty-two restriction enzymes with clear and identifiable cleavage sites within COI fragment genes were used for virtual digestion. Based on its ability to cut DNA into fragments and the patterns produced, *Msp* I was used for species identification. *Dde* I was used to distinguish *A. bicolor pacifica* from *A. bicolor bicolor* since *Msp* I cannot distinguish these two species. Restriction digestion was carried out in a 10 µl reaction mixture with 80-100 ng amplified product according to the *Msp* I and *Dde* I protocol (Takara Bio, Japan). The RFLP patterns were visualized in a 2 % agarose Tris-Borate-EDTA (TBE) gel electrophoresis then photographed. Expected RFLP patterns for the specimens were used for species identification. The PCR-RFLP profiles of several samples were visualized using DNA1000 LabChips (Agilent Technologies, Inc.) with the 2100 Bioanalyzer microchip capillary electrophoresis system according to the manufacturer's instructions. The obtained RFLP patterns were analyzed using 2100 expert software.

2.4 DNA Sequencing and Phylogenetic Analysis

The designed four oligonucleotide primers, 5511F2 (5'-ACATTCAGCCATCTTACC-3'), 6468R3 (5'-TGCRATGATTATTGTGGC-3), 6126F3 (5'-VCCAGTCCTAGCTGCAGG-3'), and 7131R2 (5'-CAATTCCTTCCTTTCTTG-3') were used to sequence the COI target fragment gene. The PCR product purified by Agencourt AMPure XP (Beckman Coulter, CA, USA) was used for direct cycle sequencing with the ABI BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequences generated from the 3130 Genetic Analyzer (Applied Biosystems, CA, USA) (Accession Numbers: LC588356- LC588371, LC588373-LC588374) were edited and manually aligned using Chromas version 2.6.6 and the MEGA X [26] software, obtaining 1431 bp after truncation. The two specimens that failed to show the high-intensity PCR bands required for RFLP were directly sequenced. A Maximum Likelihood tree was constructed using the HKY+G+I model with *Stemonidium hypomelas* (NC013628) as an outgroup using MEGA X [26] with 1000 bootstrap probabilities for species identification of unknown RFLP patterns and confirmation of representative specimens with expected ones.

2.5 Mitochondrial DNA Analysis

All (72 individuals) *A. luzonensis* partial COI target fragment genes were directly sequenced using the designed primer 6126F3 (5'-VCCAGTCCTAGCTGCAGG-3') by ABI BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and were manually aligned using Chromas version 2.6.6 and the MEGA X [26] software, obtaining 663 bp after truncation. A model test was conducted using MEGA X [26] and the model with lowest Bayesian Information Criterion (BIC) score was selected. A Maximum Likelihood tree was constructed using T92 (lowest BIC score) with *A. japonica* (AB038556.2) as an outgroup using MEGA X [26] with 1000 bootstrap probabilities to examine the geographical similarities between individuals and the phylogenetic relationship.

2.6 Statistical Analysis

Genetic variability was characterized by the amount of nucleotide substitutions calculated by the ARLEQUIN

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3.5.2.2 software [27]. The genetic diversity between rivers was investigated using the fixation index (F_{ST}) for all pairwise comparison (10230 permutations) using the ARLEQUIN 3.5.2.2 software [27]. The Bato river was excluded in computations since only 1 *A. luzonensis* individual was identified there.

3. Results

3.1 Species Identification

Three patterns were observed among the expected PCR-RFLP patterns for six Anguillid species (lanes 1-3, Figure 2a). Lane 1 was similar to the *A. luzonensis* pattern, and the band sizes were 669, 367, 344, 171 and 110 bp. Lane 2 was similar to the *A. marmorata* pattern, and the band sizes were 837, 366, and 341 bp. Lane 3 had a similar pattern to A bicolor pacifica or *A. bicolor bicolor*, with band sizes of 824 and 681 bp. Lane 1 (Figure 2b) also showed a similar pattern to *A. bicolor pacifica*, and the band sizes of 854, 278, and 223 bp confirmed that we were able to identify almost all *A. bicolor pacifica* (lane 1, Figure 2b). There were 536 individuals identified by PCR-RFLP using *Msp* I. The 33 specimens distinguished as *A. bicolor pacifica* or *A. bicolor bicolor* by *Msp* I (lane 1; Figure 2a) were confirmed by *Dde* I as *A. bicolor pacifica* (lane 1, Figure 2b). There were 16 specimens showing one of the unknown RFLP patterns similar to those in lanes 4-8 (Figure 2a). No expected patterns were observed for *A. celebesensis* and *A. japonica*.

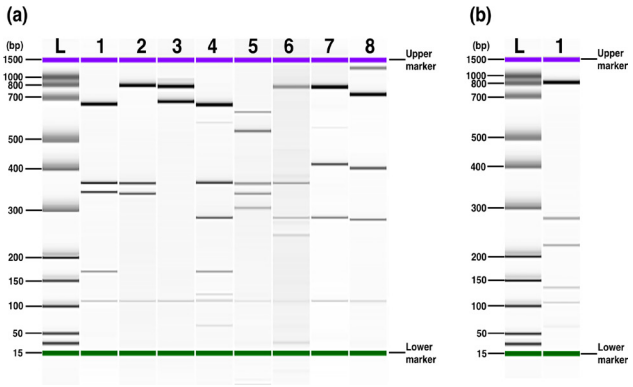

Figure 2. Restriction digestion by (a) *Msp* I for *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica* or *A. bicolor bicolor* and unknown PCR-RFLP patterns of *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, and *A. celebesensis*. L, Ladder; lane 1, *A. luzonensis*; 2, *A. marmorata*; 3, *A. bicolor pacifica* (expected). Lane 4, *A. luzonensis*; 5-6, *A. marmorata*; 7, *A. bicolor pacifica*; 8, *A. celebesensis*; confirmed by DNA sequencing analysis. (b) *Dde* I patterns for *A. bicolor pacifica*, lane 1; bp, base pairs

The phylogenetic tree (Figure 3) with *Stemonidium hypomelas* as an outgroup showed that DNA sequences with expected and unknown RFLP patterns were grouped under its specific reference sequences. Individuals with an expected RFLP pattern similar to *A. luzonensis* (lane 1, Figure 2a; LC588366-LC588367), the unknown pattern in lane 4 (Figure 2a; LC588368), and a directly sequenced specimen (LC588369) were grouped under the *A. luzonensis* clade (AB469437; Figure 3). The individuals with expected RFLP similar to *A. marmorata* (lane 2, Figure 2a; LC588356-LC588358, LC588363- LC588364), unknown (lanes 5-6, Figure 2a; LC588359-LC588362), and another individual directly sequenced pattern (LC588365) were grouped with the *A. marmorata* reference sequence (AP007242). Furthermore, individuals with a pattern similar to *A. bicolor pacifica* (lane 1, Figure 2b; LC588370-LC588371) and unknown (lane 7, Figure 2a; LC588373) were grouped under the clade of *A. bicolor pacifica* (AP007237). One of the sequenced unknowns (lane 8, Figure 2a; LC588374) belonged to the clade of *A. celebesensis* (AP007239).

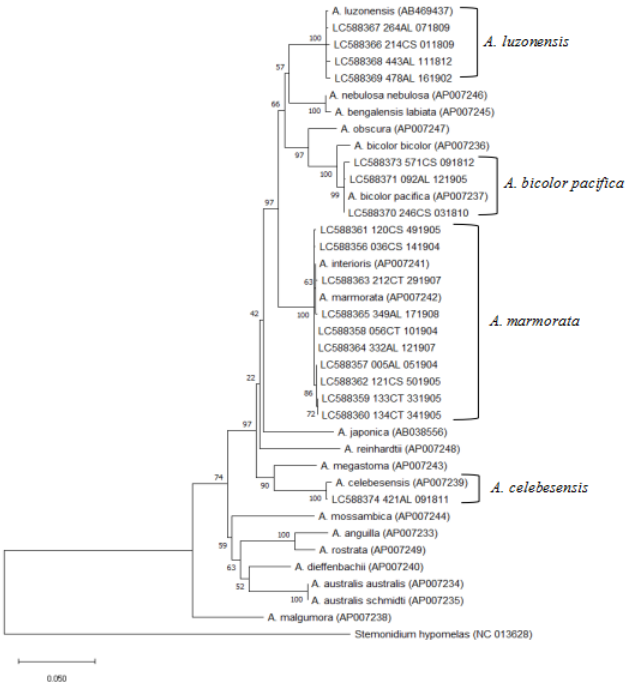

Figure 3. Maximum likelihood tree of the DNA sequences of specimens showing typical and unknown RFLP patterns.

3.2 Species Composition

Anguilla luzonensis was found to recruit in the Lagonoy Gulf, mainly in the Comun and Lagonoy rivers, in addition to *A. marmorata* and *A. bicolor pacifica* (Figure 4). Further, *A. luzonensis* was the second most abundant species in the Comun (9.5 %) and Lagonoy (22.4 %) riv-

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ers, next to *A. marmorata*. *Anguilla marmorata* dominantly occur in the Comun (81.8 %), Lagonoy (71.1 %), and Bato (98.0 %) rivers, while *Anguilla* bicolor pacifica was the third most abundant species in the Comun (7.7 %) and Lagonoy (6.5 %) rivers. In the Bato river, *A. luzonensis* and *A. bicolor pacifica* (1.0 %) recruited at a considerably low percentage. In addition, a rare occurrence of *A. celebesensis* (0.9 %) was observed only in the Comun river.

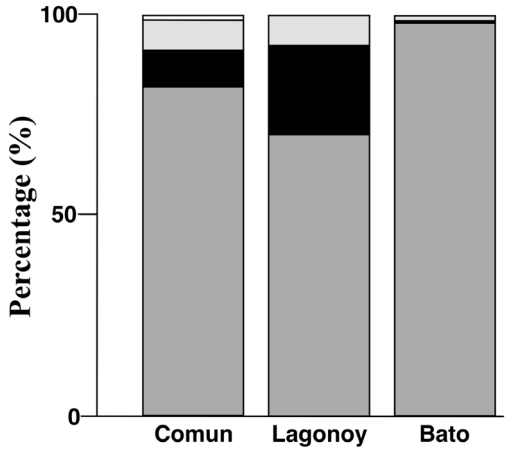

Figure 4. Percent (%) composition of freshwater eels recruiting in each of the rivers. ■, *A. luzonensis*; ▒, *A. marmorata*; ▒, *A. bicolor pacifica*; □, *A. celebesensis*

3.3 Population Structure of *A. luzonensis*

When examining the genetic variability among the 663 nucleotide sequence sites obtained from 72 individuals, a total of 24 variable positions and 20 haplotypes were observed. The pairwise comparison based on the partial COI sequences of *A. luzonensis* from Comun and Lagonoy rivers showed low genetic diversity with an F_{ST} value of 0.00825, which was not significantly different (P > 0.05) (Table 1).

Table 1. Genetic diversity based on the partial COI (663 bp) gene of *A. luzonensis* individuals collected from Comun and Lagonoy rivers.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation	Fixation index (F_{ST})
Among populations	1	1.234	0.00823	0.82	
Within populations	70	69.252	0.98932	99.18	0.00825

p-value>0.05; 10230 permutations

The phylogenetic tree with *A. japonica* as an outgroup (Figure 5) showed that almost all *A. luzonensis* individuals from Comun and Lagonoy and one individual from the Bato river formed a monophyly in one clade (B) with one individual diverged first (A) but node is only supported by 54 % bootstrap value which is weak. In addition, the two reference sequences downloaded from NCBI, which were found in the Cagayan river (*) also belonged to this large clade (B).

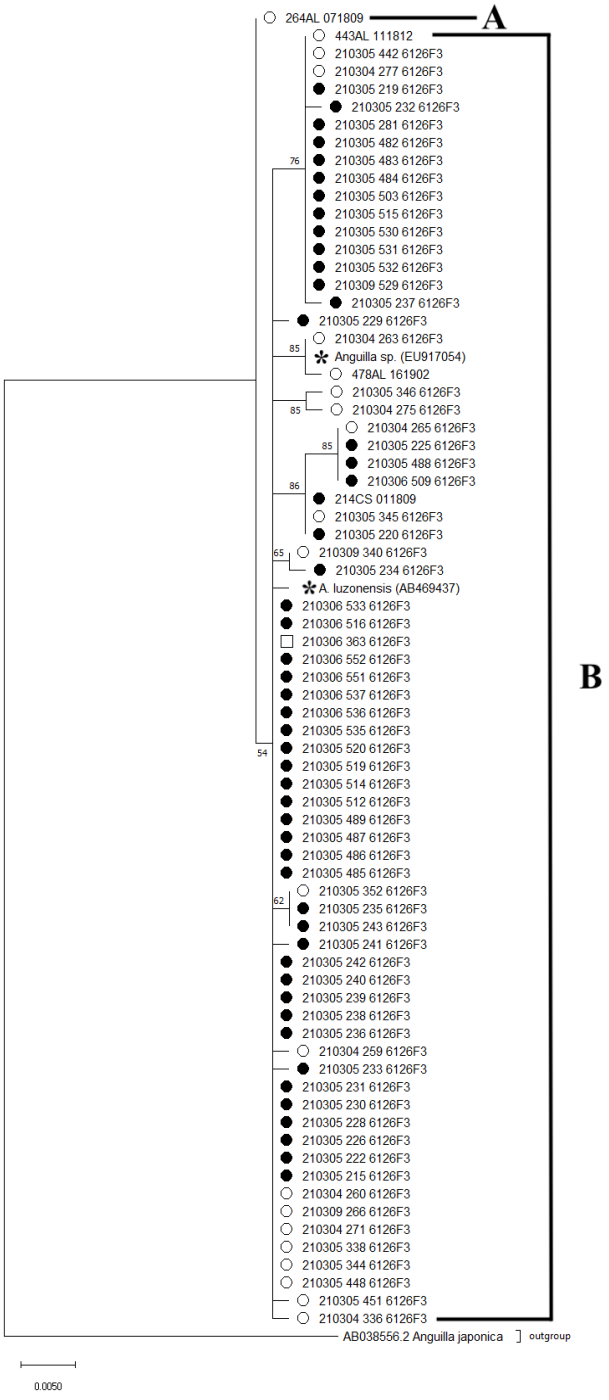

Figure 5. Maximum likelihood tree showing phylogenetic relationships between *A. luzonensis* collected mainly from Comun (○) and Lagonoy (●) and one individual from Bato (□) river. Reference sequences (AB469437, EU917054) of *A. luzonensis* from Cagayan (*) downloaded from NCBI was also included.

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4. Discussion

4.1 Species Identification

Anguilla luzonensis was found in the Lagonoy Gulf by molecular analysis. The unknown RFLP patterns showed that lane 4 (Figure 2a) had a largest band of around 671 bp, which was similar to a band detected in *A. luzonensis* (lane 1, Figure 2a). *A. luzonensis* individuals showing expected and unknown patterns were grouped in one distinct clade, implying that these are the same species supported by 100 % bootstrap value.

Anguilla marmorata was also identified. Lanes 6 had a largest band of more than 800 bp, similar to the largest band for both *A. marmorata* (lane 2) and *A. bicolor pacifica* or *A. bicolor bicolor* (lane 3) (Figure 2a), hence, could not be identified. Doublets between 300-400 bp observed in lane 5 were similar to the doublets of *A. luzonensis* (lane 1) and *A. marmorata* (lane 2) (Figure 2a). However, the sequences of *A. marmorata* individuals with expected and unknown patterns that were grouped into one clade were the same species (100 % bootstrap value).

In the case of *A. bicolor pacifica*, the use of *Dde* I in addition to *Msp* I was able to confirm that the 33 individuals identified as *A. bicolor pacifica* or *A. bicolor bicolor* were *A. bicolor pacifica* individuals, and not *A. bicolor bicolor* (Figure 2b). The largest band of lane 7 (more than 800 bp) is similar to the largest band for both *A. marmorata* (lane 2) and *A. bicolor pacifica* or *A. bicolor bicolor* (lane 3) (Figure 2a), however, the DNA sequence was grouped with the specimens having expected RFLP patterns and reference sequence indicated that these are identical species. It may also be noted that, all detected bands of the *Msp* I digests of the three species the and the *Dde* I digest of *A. bicolor pacifica* were ranged within ±10 % band sizing accuracy of the expected band sizes. Almost all individuals (536) were identified by PCR-RFLP using *Msp* I. Although we were not able to observe the expected pattern for *A. celebesensis*, we found an unknown individual (lane 8, Figure 2a; LC588374) was *A. celebesensis*, since it was under the clade of its reference sequence (AP007239; Figure 3).

PCR-RFLP using 16S rRNA have been reported appropriate for identification of the genus *Anguilla* [20, 22-23] before *A. luzonensis* was discovered as a new species in 2009 [9]. No available study used PCR-RFLP to identify *A. luzonensis*, hence, we simulated the restriction pattern for COI gene, in addition, to 16S rRNA for six target species and found that COI gene digested by *Msp* I and *Dde* I produced distinctive RFLP pattern for *A. luzonensis* and all the other five species. The combination of PCR-RFLP for COI gene by *Msp* I and *Dde* I and for unknown patterns using DNA sequencing analysis, finally, we were able to completely identify four Anguillid species in Lagonoy Gulf.

4.2 Species Composition

It is interesting to note that *A. luzonensis* was found to be the 2nd most abundant species in the Comun and Lagonoy rivers. *Anguilla luzonensis* abundance and its stable recruitment for two periods of study were reported in Cagayan [1] and its rare occurrence in Mindanao [6]. Our study and the references indicated the abundance of *A. luzonensis* in the northern and eastern part of the Philippines and its rarity in the south.

In the case of *A. marmorata*, decrease on the percentage of recruitment in Comun, Lagonoy and Bato rivers (87.5 %, 82.3 %, and 99.7 % respectively) reported by morphology [14] was due to the occurrence of *A. luzonensis* (9.5 %, 22.4 %, and 1 %, respectively). Similarly, *A. marmorata* has dominantly recruited in Cagayan [1]and Mindanao [6]. This means that *A. marmorata* is widely distributed in the Philippines.

For *A. bicolor pacifica*, molecular analysis found lower than 10 % recruitment in Comun (7.7 %) and Lagonoy (6.5 %), which is less than the occurrence reported by morphological identification (12.4 % and 17.5 %, respectively) [14] since we found that some *A. luzonensis* individuals were misidentified as *A. bicolor pacifica* by morphology. In Cagayan, an increase in the annual percentage of recruitment during two periods of their study was observed for *A. bicolor pacifica* [1]. *Anguilla bicolor pacifica* was the 2nd most abundant species occurred in Mindanao [6]. Based on our results and other studies, among *A. bicolor* subspecies, *A. bicolor pacifica* is abundant in the whole Philippines. The decrease in the percent composition reported by morphology for *A. marmorata* and *A. bicolor pacifica* was due to the occurrence of *A. luzonensis* in Lagonoy Gulf.

In the Bato river, the *A. luzonensis* and *A. bicolor pacifica* species with low percentage occurrence may recruit during months when no samples were collected, since these were found to recruit in the Comun and Lagonoy rivers. *A. celebesensis* was rarely observed in the Comun river only, which is similar in Cagayan wherein this species was extremely rare [1]. The use of morphology specific to pigmentation patterns was not able to distinguish *A. luzonensis* from *A. marmorata* [18]. Pigmentation patterns specific to *A. luzonensis*, *A marmorata*, and *A. celebesensis* are not yet established [7]. The molecular analysis we carried out affirmed that pigmentation pattern alone is not enough to distinguish *A. luzonensis* among the pigmented eels [28]. The species composition based on morphology can be confirmed and revised by molecular techniques. In

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addition, we were also able to confirm that no *A. bicolor bicolor* and only *A. bicolor pacifica* recruit in the Lagonoy Gulf. Hence, molecular analysis has provided a more precise estimate of Anguillid species composition in the tributaries along Lagonoy Gulf.

4.3 Population Structure of *A. luzonensis*

Anguilla luzonensis was mostly found in two rivers, Comun and Lagonoy, along the Lagonoy Gulf. Individuals of *A. luzonensis* recruited in Comun and Lagonoy seem to have genetically variable sites and haplotypes. In addition, *A. luzonensis* between these two rivers were found to have low genetic diversity, which was not statistically significant (Table 1), possibly indicating a panmictic population. The dispersed *A. luzonensis* individuals from the Comun and Lagonoy rivers in clade B (Figure 5), including the two DNA sequences downloaded from NCBI, which were collected from Cagayan, could imply that these individuals share similar genetic materials. *Anguilla luzonensis* and *A. japonica* were presumed to have similar spawning area [3,15], experience similar oceanographic features [3], though distribution are different, wherein the latter was reported to have a single panmictic population in the East Asia [29]. Although our study was only in Lagonoy Gulf, *A. luzonensis* might have a similar case with *A. japonica* having panmictic population. Since this is the first investigation of the population structure of *A. luzonensis* recruited in tributaries along the Lagonoy Gulf and being the 2nd abundant species that may have high economic importance, this study could indicate important implications for resource management of this species. Furthermore, genetic differences and population structure of *A. luzonensis* from Luzon, Mindanao, Philippines, Taiwan, and Okinawa, Ryuku archipelago may be compared and studied in the future.

5. Conclusions

Our study using molecular analysis found *A. luzonensis* in the Lagonoy Gulf along the reported *A. marmorata* and *A. bicolor pacifica*. Interestingly, *A. luzonensis* was identified as the 2nd most abundant species in the Comun and Lagonoy rivers. Although genetic variability was found in *A. luzonensis* individuals, genetic diversity was very low and not significantly different, which was inferred from the partial COI gene fragment. It maybe noted that our current study was not able to find enough *A. luzonensis* individuals from Bato river but highly possible to occur during months with no samples were collected; therefore, this study will be further continued. With *A. luzonensis* classified as a vulnerable species, this study provides information that will be of help to effective management and utilization of freshwater eels.

Acknowledgments

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ARTICLE

Culture Performance and Economic Return of Brown Shrimp (*Metapenaeus Monoceros*) at Different Stocking Densities Reared in Brackishwater Pond

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ABSTRACT

Shrimps are recognized as the white gold of Bangladesh because it is the second largest export earning product after garments sector. The brown shrimp (*M. monoceros*) have high growth rates together with that they tolerate wide ranges of salinity and environmental parameters which makes them highly attractive for culture purposes. The purposes of this research were to assess the cultural performance and economic profitability of brown shrimp (*Metapenaeus monoceros*) in brackish water ponds. This research lasted from February to June 2020 under three different stocking densities such as 35, 45 and 55 individuals/m2 in treatments T1, T2 and T3 at Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna. After 90 days culture periods the total production was 1703.32±144.48, 2768.25±167.63 and 2535.03±253.52 kg/ha in T1, T2 and T3 respectively which was significantly higher ($p<0.05$) in T2 compared to T1 and T2. Benefit cost ratio (BCR) was 0.32, 0.87 and 0.52 in T1, T2 and T3 respectively and found significantly higher ($p<0.05$) in T2 than T1 and T3. Both cultural performance and economic analysis imply that brown shrimp (*M. monoceros*) with a stocking density of 450000 individuals/ha might be environment conciliatory and economically enduring in coastal areas of Bangladesh.

1. Introduction

Shrimp is contributing significantly to the national economy of Bangladesh through export earnings and creating employment opportunity. It is the second largest foreign exchange earning source in Bangladesh and 97% of the produced shrimp being exported [1]. In 2016-17, production of shrimp in Bangladesh was about 246188 tons through culture in about 275509 ha impoundments (locally called ghers) resulting production rate of 456 kg/ha [2]. Black tiger shrimp (*Penaeus monodon*, locally named as Bagda) is particularly stocked in these ghers. But this valuable shrimp industry faces massive production losses due to invasion of virus (WSSV) and AHPND (Acute Hepatopancreatic Necrosis Disease) [3]. Therefore, farmers have become highly farsighted about stocking of this shrimp species in their ghers and a large number of tiger shrimp farmers has already meant to shift their cultural pattern as well as searching for suitable species for stocking to their ghers. In this context, the brown shrimp, *Metapenaeus monoceros* (Fab.) (Locally called Harina)

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can be a suitable candidate for culture in the brackishwater ghers. This shrimp offers a good potential for large scale commercial aquaculture primarily because of available natural seed and demand in the international market. The severity of disease incidence in this shrimp is not so alarming like that of *P. monodon*. Requirement of oxygen of this species is also very low and increases with the increase in salinity. In spite of having many advantages of production, the culture technology of the species has not yet developed in Bangladesh. *M. monoceros* grows up to 10.7 cm [4]. The food and feeding habits of *M. monoceros* in its juvenile phase was found to be a selective carnivore and a benthic feeder, active mainly during night [5]. Total consumption of oxygen in the penaeid prawn, *M. monoceros* was very low when exposed to 2 ppt salinity and very high in 25 ppt salinity [6]. They survived for a longer period when kept in higher salinities (12 ppt to 36 ppt) on acclimatization from lower to higher succeeding grades [7].

2. Materials and Methods

2.1 Study Location and Experimental Design

The research was performed in the pond complex of Bangladesh Fisheries Research Institute, Brackishwater Station (27.2046°N, 77.4977°E); Paikgacha under Khulna district, Bangladesh. The study was arranged in nine on-station earthen ponds of 0.1 ha each. The experiment was designed in three treatments with three different stocking densities (T1=35; T2=45 and T3=55 individuals/m^2 respectively) with three replications for each. However, a control experiment was also performed where the stocking density was maintained at the rate of 45 individuals/m^2, no water has been exchanged and no lime, fertilizer and feed were provided. The initial stocking size (length and weight) for the experiment was 1.5 cm and 0.05 g respectively.

2.2 Pond Management and Husbandry

Before stocking PL, the ponds were prepared according to the standard process described by [8]. An in-pond nursery was built in one corner of each pond made of a nylon net attached to a bamboo frame. After 4th days of fertilization and sufficient plankton production, the required amount of shrimp PL was acclimated with pond water and stocked in April 2020. A commercial feed (Quality Shrimp Feed) was used to feed the shrimp at satiation. After the third week of nursery rearing, the juveniles were released into the entire pond by folding up the enclosure. At least 50 shrimps were sampled to check the feeding behavior and health condition of shrimp in fortnight intervals. After 90 days of culture, the shrimps were harvested by dewatering the ponds.

2.3 Hydrological Parameters

Physical and chemical parameters of water *viz*. dissolved oxygen, salinity, depth, alkalinity, temperature, P^H were analyzed in weekly basis according to the standard protocol (APHA 1992) [9]. DO was monitored by DO meter, model DO 175, Hach; salinity by an optical Refractometer (Atago, Japan); depth of water by a depth gauge; total alkalinity by titrimetric method in the morning and as mentioned in APHA (2005) just before sunrise; temperature was monitored by a mercury thermometer; transparency by a Secchi disk; and pH by a digital pH meter [10].

2.4 Observed Research Parameters

The observed parameters were the growth performance of the experimental shrimp and economic profitability parameters. The growth performance parameters include the survival rate (SR, %), specific growth rate (SGR, % bw/day), and feed conversion ratio (FCR) calculated according to the following equations:

$$\text{Survival rate, SR (\%)} = \frac{\text{Final population}}{\text{Initial population}} \times 100$$

$$\text{Specific growth rate, SGR}\left(\%\ \text{bw day}^{-1}\right) = \frac{\ln\left(\text{final weight}\right) - \ln\left(\text{initial weight}\right)}{\text{days of culture}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total weight of consumed feed}}{\text{Weight gain of shrimp}}$$

The economic profitability was estimated using the shrimp price in Paikgacha fish retail market, Khulna, Bangladesh in 2020. The economic profitability parameters were gross return, net profit and benefit cost ratio (BCR) calculated based on the following formulas:

$$\text{Total production cost} = \text{Total variable cost} + \text{Total fixed cost}$$

$$\text{Gross return} = \text{Total shrimp yield} \times \text{price of per kg of shrimp}$$

$$\text{Net profit} = \text{Gross return} - \text{total production cost}$$

$$\text{Benefit cost ration (BCR)} = \frac{\text{Net profit}}{\text{Total production cost}}$$

2.5 Proximate Analysis

For proximate composition, hot air oven method (dry-

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ing the sample at 105°C±2°C) was used to determine the moisture content until a constant weight was obtained (AOAC 1990) [11]. The crude lipid was estimated by extracting a weighed amount of sample with acetone in the Soxhlet extraction unit (model 1045) [12]. The crude protein content was analyzed by altering the nitrogen content acquired by the Kjeldahl method (Nx6.25) [13]. The ash content was determined after burning for 20 hours at 550°C [11]. Total carbohydrates were analyzed by subtracting the sum of the moisture, fat content, protein content and ash content from 100 [14]. The crude fibre content was determined according to the protocol mentioned by (Ayuba and Iorkohol 2010) [12]. Calcium was estimated through atomic absorption spectrophotometry and phosphorus was analyzed photometrically [15]. For the estimation of proximate composition three different samples were taken to determine each content.

2.6 Data Analysis

After 90 days of culture periods all the shrimp were harvested and the collected data were statistically analyzed with MS Excel and SPSS (Statistical Product and Service Solutions) version-20 to express the research findings in a meaningful way. One-way analysis of variance (ANOVA) and Duncan multiple range test was carried out to compare the treatments and significance level were assigned at 5% (P>0.05).

3. Results

3.1 Water Physico-chemical Characteristics

The changing pattern of physico-chemical parameters of water studied in this research viz. dissolved oxygen, salinity, depth, temperature, alkalinity and P^H recorded during the culture period in weekly basis were presented in Figure 1. The range and Mean±SD values of water quality parameters in three different treatments were presented in Table 1. Interestingly, all the hydrological parameters of the experimental ponds were within the favorable condition of Harina shrimp culture. The DO level of the experimental ponds varied between 5.13-10.97 mg/l (Figure 1A) with a mean±SD values of 8.11±1.10, 7.93±0.91 and 8.65±1.21 in T1, T2 and T3; respectively (Table 1).

Salinity is considered as one of the most fundamental factor for shrimp culture. The salinity level in different experimental ponds varied between (7-16) ppt (Figure 1B) with a mean±SD values of 11.04±2.27, 11.02±2.20 and 11.10±2.26 in T1, T2 and T3; respectively (Table 1). Salinity level was increased unwaveringly from April until reached its peak in June (16 ppt) and then it showed gradual decrement with least variation between the treatments. The depth level of the experimental ponds varied from (85-140) cm (Figure 1C) with a mean±SD values of 107.32±3.94, 98.00±4.33 and 132.71±4.36 in T1, T2 and T3; respectively (Table 1).

Temperature is also one of the critical physical modifiers that affects the growth, energy flow and biological effects in marine organisms. There was found least variation of temperature among the experimental ponds. The temperature of the experimental ponds varied between 30-35℃ (Figure 1D) with a mean±SD values of 33.31±1.94, 32.92±1.66 and 32.97±1.66 in T1, T2 and T3; respectively (Table 1).

The recorded alkalinity of the study ponds were within 90-162 mg/l (Figure 1E) with a mean±SD values of 111.81±12.13, 111.78±12.28 and 114.64±16.98 in T1, T2 and T3; respectively (Table 1). The P^H range of the experimental ponds were within 7.63-8.80 (Figure 1F) with a mean±SD values of 8.43±0.2, 8.33±0.13 and 8.46±0.17a in T1, T2 and T3; respectively (Table 1).

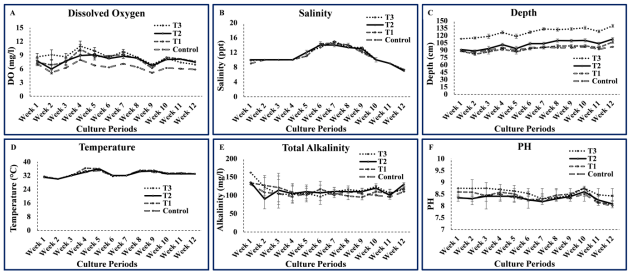

Figure 1. Water quality parameters of different treatments in weekly basis (A. Dissolved Oxygen; B. Salinity; C. Depth; D. Temperature; E. Total Alkalinity; F. PH)

3.2 Growth Performance of Brown Shrimp

The study was conducted in the pond complex (0.1 ha each) of the Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna. The growth performance of Harina shrimp was monitored fortnightly (Figure 2). The average body weight was always higher in T2 in comparison with T1 and T3, respectively (Figure 2A). The average body weight of shrimp PL during stocking was same of 0.05g in all treatments and after 90 days of culture period brown shrimp attained an ABW of 9.15±0.6, 10.42±0.5 and 8.38±0.7 in T1, T2 and T3; respectively (Table 2). In three different stocking densities, ABW of brown shrimp in T2 was significantly higher ($p<0.05$) than the other two treatments (Table 2). Survival of brown shrimp in T2 was 59.04% which was found highest and significantly higher ($p<0.05$) than the other two treatments as the survival rate of brown shrimp were 53.19% and 55.00% in T1 and T3; respectively (Table 2).

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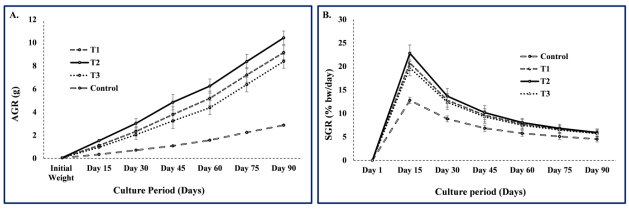

Figure 2. Growth performance of brown shrimp in different treatments during the complete experimental period (A) average body weight and (B) specific growth rate.

As shown in Figure 2B, SGR was highest of 19.70-22.81% in the 1st fortnight in the experimental ponds. The SGR declined with the progress of culture period and declined sharply to 7.45-8.04% up to 60 days of culture. After then, the SGR was almost straight for the rest of the culture period and finally declined to 5.69-5.93% in the last fortnight of culture. The SGR of brown shrimp after 90 days of culture period was 5.78±0.61, 5.93±0.56 and 5.69±0.71% in T1, T2 and T3; respectively. Total production was recorded as 1703.32±144.48, 2768.25±167.63 and 2535.03±253.52 kg/ha at T1, T2 and T3; respectively. The highest production obtained in T2 (45 individuals/m2) which was significantly higher ($p<0.05$) than T1 and T3; respectively. FCR value in T2 was 1.11 which was found lowest and significantly lower ($p<0.05$) than the other two treatments as the FCR of T1 and T3 were 1.51 and 1.41; respectively (Table 2).

3.3 Proximate Composition of the Commercial Diets

Moisture content ranged from 10 to 11%, crude protein ranged from 32 to 36%, fat content ranged from 7 to 8%, crude fibre ranged from 2.0 to 3.5%, ash ranged from 10 to 11%, calcium ranged from 3.0 to 3.2% and phosphorus ranged from 1.5 to 2.5% of the diets used in different stage of culture period (Table 3). However, crude protein content in pre-nursery diet (36±0.07%) was significantly ($P<0.05$) higher than that of nursery and growout diets. There was no significant ($P<0.05$) difference between the carbohydrate, ash and calcium content among three different diets respectively (Table 3). Besides, phosphorus content in each diet significantly ($P<0.05$) differs from each other.

Table 1. Water quality characteristics in different treatments during culture periods

Parameters	Treatment 1		Treatment 2		Treatment 3	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Dissolved Oxygen (mg/l)	6.17-10.29	$8.11±1.10^{b}$	5.13-9.08	$7.93±0.91^{c}$	6.95-10.97	$8.65±1.21^{a}$
Salinity (ppt)	7-16	$11.04±2.27^{b}$	7-16	$11.02±2.20^{b}$	7-15	$11.10±2.26^{a}$
Depth (cm)	85-107	$98.00±4.33^{c}$	88-113	$107.32±3.94^{b}$	113-140	$132.71±4.36^{a}$
Alkalinity (mg/l)	95-138	$111.81±12.13^{b}$	90-140	$111.78±12.28^{c}$	96-162	$114.64±16.98^{a}$
P^{H}	7.91-8.76	$8.43±0.2^{b}$	7.72-8.80	$8.33±0.13^{c}$	7.63-8.72	$8.46±0.17^{a}$
Temperature (℃)	30-35	$33.31±1.94^{a}$	30-35	$32.92±1.66^{c}$	30-35	$32.97±1.66^{b}$

*Different letter superscripts in the same column indicate significant difference ($p<0.05$).

Table 2. Growth and production of brown shrimp (*M. monoceros*) in different treatments

Treatments	Stocking Density (m^2)	Initial Length (cm)	Initial Weight (g)	Final Length (cm)	Final ABW (g)	Survival (%)	SGR (%)	Production (Kg/ha)	FCR
T1	35	1.5	0.05	12.17±0.7	9.15±0.6	53.19^{b}	10.55^{b}	$1703.32±144.48^{c}$	1.51^{b}
T2	45	1.5	0.05	15.63±1.9	10.42±0.5	59.04^{a}	11.24^{a}	$2768.25±167.63^{a}$	1.11^{a}
T3	55	1.5	0.05	11.34±2.6	8.38±0.7	55.00^{b}	10.16^{c}	$2535.03±253.52^{b}$	1.41^{b}

*Different letter superscripts in the same column indicate significant difference ($p<0.05$).

Table 3. Proximate composition of different feed used for the culture of brown shrimp

Parameters	Pre-nursery	Nursery	Growout
Moisture (%)	$10±0.02^{b}$	$11±0.01^{a}$	$11±0.01^{a}$
Protein (%)	$36±0.07^{a}$	$32±0.05^{b}$	$32±0.08^{b}$
Fat (%)	$7±0.02^{b}$	$8±0.03^{a}$	$7±0.01^{b}$
Carbohydrate (%)	$22±0.03^{a}$	$22±0.02^{a}$	$22±0.03^{a}$
Fibre (%)	$2±0.01^{b}$	$3±0.02^{a}$	$3.5±0.02^{a}$
Ash (%)	$11±0.05^{a}$	$10±0.08^{a}$	$10±0.04^{a}$
Calcium (%)	$3.2±0.02^{a}$	$3±0.01^{a}$	$3±0.03^{a}$
Phosphorus (%)	$2.1±0.01^{b}$	$2.5±0.02^{a}$	$1.5±0.01^{c}$

*Different letter superscripts in the same column indicate significant difference ($p<0.05$).

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3.4 Cost-Benefit Analysis

A benefit-cost ratio (BCR) is a ratio used in a cost-benefit analysis to summarize the overall relationship between the relative costs and benefits of a project. The cost and economic benefit analysis of this research showed that the higher net profit was achieved in treatment T_2 (where stocking density was 450000 individuals/ha) than T_1 and T_3 (Table 4). Total cost (TC) is the total economic cost of production and is made up of variable cost, which varies according to the quantity of a good produced and includes inputs such as labour, land lease cost and raw materials, plus fixed cost. Total cost of production was recorded 514282 BDT/ha, 593388 BDT/ha and 667503 BDT/ha in T_1, T_2 and T_3 respectively (Table 4). However, highest return (1107300 BDT) and net

Table 4. Production performance and cost-benefit ratio of brown shrimp (*M. monoceros*) under three different stocking densities

Particulars	Quantity	Rate (BDT)	Treatments		
			T_1 (35ind./m^2)	T_2 (45ind./m^2)	T_3 (55ind./m^2)
Variable cost					
Pond preparation (man days)	130	350	45500	45500	45500
Eradication (kg bleaching)	55	40	2200	2200	2200
Labour charge for eradication	10	350	3500	3500	3500
Lime (kg)	400	25	10000	10000	10000
Dolomite (kg)	150	18	2700	2700	2700
Urea (kg)	50	16	800	800	800
Triple super phosphate (kg)	100	22	2200	2200	2200
Shrimp PL		0.3	105000	135000	165000
Shrimp feed (35% Protein)		75	87000	116200	124500
Shrimp feed (30% Protein)		70	98700	105000	133700
Molasses (kg)	150	35	5250	5250	5250
Salary of farm assistant (BDT)	3	10000	30000	30000	30000
Power cost (units)	250	7.5	1875	1875	1875
Land lease			50000	50000	50000
Harvest cost (BDT/kg)		5	8516	13841	12675
Fuel cost (kg)	50 L	65	3250	3250	3250
Total variable costs			456491	527316	593150
Fixed costs					
Interest		10%	46093	52718	59343
Depreciation			11698	13354	15010
Total fixed costs			57791	66072	74353
Production					
Total shrimp yield			1703.32	2768.25	2535.03
Price of shrimp			400	400	400
Economic analysis					
Total production costs			514282	593388	667503
Gross return			681328^c	1107300^a	1014012^b
Net profit			167046^c	513912^a	346509^b
Benefit/cost ratio			0.32^c	0.87^a	0.52^b

*Different letter superscripts in the same column indicate significant difference ($p<0.05$).

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profit (513912 BDT) was obtained in T2 at SD 45 individuals/m^2 followed by T3 (1014012 BDT and 346509 BDT) at SD 45 individuals/m^2 and T1 (681328 BDT and 167046 BDT) at SD 35 individuals/m^2 and they were significantly ($P<0.05$) different from each other (Table 4). Cost-benefit ratio (BCR) was 0.32, 0.87 and 0.52 in T1, T2 and T3; respectively. BCR value of T2 was significantly higher ($p<0.5$) than T1 and T3 implies that net economic return is higher in treatment with 450000 individuals/ha stocking densities.

4. Discussion

Development of technique for the culture of brown shrimp (*M. monoceros*) in nine earthen brackishwater ponds under three different stocking density showed the feasibility of producing of shrimp product with good growth, survival, FCR and yield. Water quality parameters during the culture periods were within the acceptable range for growth and survival of normal shrimp production. Shofiquzzoha *et al.* (2001) reported that dissolved oxygen content of a shrimp farm should be greater than 4.0 mg/l [16]. Dissolved oxygen content during culture periods was well enough due to the use of paddle wheel aerator throughout the culture period (Figure 1A). The favorable salinity range for the growth of shrimp was 7 to 25 ppt [17]. This research was carried out before starting of rainy season and ends during the rainy season and there was much rain at the end time so it made salinity level below 10 ppt. Although the range of salinity varied between 7-16 ppt (Figure 1B) with an average value of 11 ppt (Table 1) during the culture period which is favorable for brown shrimp. Islam *et al.* (2004) reported that depth of a shrimp farm should be 80-120 cm [18]. The depth of the research ponds gradually increases with the arrival of rainy season. The depth become slightly higher (Figure 1C) than the range mentioned by (Islam *et al.* 2004) for a few days of culture period [18]. Although it was not hampered in the survival and growth of cultured shrimp. The optimum temperature for small shrimp is greater than 30℃ (less than 5 g) while for large shrimp the optimum temperature is about 27℃ [19]. The optimum temperature range of both shrimp and prawn found at 28-35℃ [20]. The optimum range of alkalinity for brown shrimp farming was 80-200 mg/l [21]. The optimum range of water P^H for shrimp culture is 7-9 [22]. Besides several authors have reported a wide variation in P^H 7.5-9.2 [23] and 7.68-8.35 [16] in shrimp farms and found favorable for shrimp culture. Water physico-chemical parameters such as temperature, alkalinity and P^H were in the suitable range for shrimp growth throughout the culture period (Table 1).

Growth pattern of brown shrimp, *M. monoceros* showed that average growth rate (AGR), specific growth rate (SGR), total production, survival rate (SR) and feed conversion ratio (FCR) were a worthy performance of shrimp growth in brackishwater ponds (Table 2, Figure A-B). Begum *et al.* (2020) reported that after 90 days of culture period of brown shrimp in brackishwater ponds the average body weight was 8.2±1.6, 7.3±2.4, and 5.1±2.8 g; mean FCR value was 1.2±0.15, 1.2±0.3 and 1.1±0.1; specific growth rate was 6.9±0.2, 6.8±0.4 and 6.5±0.1 with total production of 577.0±48.0, 608.0±62.0 and 764.0±72.0 kg/ha at 10, 20 and 30 individuals/m^2 stocking density respectively [8]. In this research the ABW, SGR, FCR and total mass weight was significantly higher (Table 2) than Begum *et al.* (2020) [8] due to high stocking density.

In the present study, the brown shrimp were fed with commercial feed (Quality) and proximate analysis revealed that maximum protein, ash and calcium percentage was recorded in pre-nursery feed, highest fat and phosphorus percentage recorded in nursery feed and maximum fibre content was found in growout feed (Table 3). Washim *et al.* (2016) reported that net benefit was 106484 BDT after 63 days of culture of black tiger shrimp where stocking density was 7 individuals/m^2 and BCR value was 1.41 [24]. Saha *et al.* (2016) also found net benefit BDT 79368 at 50000/ha density in 120 days culture period and BCR value was 1.29 [25]. The net benefit in this research found 167046, 513912, 346509 BDT and BCR value was recorded 0.32, 0.87 and 0.52 in T1, T2 and T3 respectively (Table 4) which implies treatment with 45 individuals/m^2 stocking density provides maximum return.

5. Conclusions

Highest body weight gain, survival, production, net economic return and BCR were achieved under the stocking densities of 45 individuals/m^2 indicated its superiority over other two stocking densities. From this research, it is concluded that culture of brown shrimp (*M. monoceros*) with 45 individuals/m^2 stocking density would be environment friendly and economically viable in brackishwater areas of Bangladesh. Validation through repetition of this density, enhancement of natural food production in culture ponds and use of low cost feeds needed to be tested to maximize the profit level before extension to farmer's field.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All the authors were an active part of the concept, design, analysis of data, drafting and revising the manuscript, while the SA and MLI performed the entire work together.

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ARTICLE

Study of Condition Indices in Goby, *Parachaeturichthys Ocellatus* (Day, 1873) from the Creeks of Mumbai

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ABSTRACT

Condition indices study like RNA content, DNA content, RNA: DNA, RNA: protein, RNA: lipid was carried out in goby, *Parachaeturichthys ocellatus* from the creeks of Mumbai to assess its nutritional status in different months. The study was carried out from June 2010 to September 2011.The range of RNA content in male was 72-185.6 µg/100 mg while in female was 82-145.46 µg/100 mg. RNA content was high during spawning months. The DNA content showed slight variations with range of 22.56-39.31 µg/100 mg in males and 25.20-32.52 µg/100 mg in females. The range of ratio of RNA: DNA in males was 2.08-5.13 with an average of 3.74 while in female was 2.92-5.07 with an average of 3.99. The ratio above 2 indicates good condition. The RNA: protein showed an average of 0.0015 in males and 0.0017 in females while the average of RNA: lipid was 0.0176 in males and 0.0127 in females. RNA: protein and RNA: lipid showed the lowest values in post reproductive stages while it increased with the onset of reproductive cycles. The condition indices study showed that *P. ocellatus* was in good condition throughout the year and the creeks of Mumbai were suitable habitat for feeding and reproduction.

1. Introduction

Macromolecular indices like RNA concentration, RNA: DNA ratios, RNA: protein ratios and protein: DNA ratios are frequently measured as indicators of protein synthesis potential and growth in marine fishes and invertebrates [1,2,3]. These indices are particularly useful for evaluating recent environmental conditions as they reflect differences in growth rates over a period of several days [4,3,5] Individuals in good condition tend to have higher RNA : DNA ratios than those in poorer condition [6]. It is valuable for managers of aquatic ecosystems for assessment of the health status of populations [7]. The ratio is thus used to give measure of instantaneous growth in the field, thus avoiding the need for repeated measurements [3]. It is a useful technique to evaluate physiological condition in a short period and could be utilized as nutritional condition and/or instantaneous growth for routine check to verify health status in early life of cultivated species [8].

Paracheaturichthys ocellatus is a native fish from the creeks of Mumbai. This fish forms part of creek fishery and is consumed widely by the people living along the coast. The present study of condition indices in *P. ocellatus* has been carried out to establish their relation to growth and nutritional conditions by determining indices like RNA and DNA content, RNA : DNA, RNA : protein and RNA : lipid ratios from the muscles of male and female.

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2. Materials and Methods

The samples for the present study were collected every fortnight at regular intervals from Malad, Vasai, Thane and Mahul creek during the period from June 2010 to September 2011. The fish samples were brought to the laboratory and thoroughly washed, cleaned and wiped. Total length was measured from the tip of the snout to the tip of the caudal fin in centimetres and weight was noted to the nearest gram. The fish was cut open and the sex was noted by the macroscopic examination of gonads.

The skeletal muscle was excised from the epaxial region of the trunk below the place of the origin of dorsal fin. Known weights of the tissue were processed for the extraction of RNA, DNA and Protein [9]. Techniques of Schneider [10] were followed for extraction of RNA from the tissues and its concentration was determined by the orcinol reaction. The values were read against standard curve prepared by relating the colour intensity to different concentrations of purified yeast RNA.

DNA was extracted according to the method described by Webb and Levy [11] and its quantity was estimated by the methodology of Ashwell [12]. Highly polymerised calf-thymus DNA was used for preparation of calibration curve.

Protein was assayed according to the method of Lowry [13] .Bovine serum albumin was used as the standard. Lipid was estimated by the method of Folch [14]. DNA, RNA, protein and lipid were expressed as µg/100 mg dry tissue.

3. Results and Discussions

Table 1. RNA, DNA, RNA: DNA, RNA: protein, RNA: lipid ratios from muscles of male *P. ocellatus* during different months.

Months	RNA µg/100 mg dry wt	DNA µg/100 mg dry wt	RNA: DNA	RNA: protein	RNA: lipid
Jun-10	122.45	28.5	4.2965	0.0016	0.0277
Jul-10	118.23	32.4	3.6491	0.0015	0.0182
Aug-10	98.22	24.5	4.0090	0.0013	0.0137
Sept-10	96.00	33.7	2.8487	0.0014	0.0098
Oct-10	82.00	39.31	2.0860	0.0011	0.0090
Nov-10	72.00	30.5	2.3607	0.0010	0.0078
Dec-10	89.93	31.56	2.8495	0.0012	0.0144
Jan-11	142.86	31.56	4.5266	0.0020	0.0169
Feb-11	185.6	36.19	5.1285	0.0026	0.0305
Mar-11	176.66	34.43	5.1310	0.0024	0.0236
Apr-11	146	28.82	5.0659	0.0020	0.0138
May-11	106.66	27.21	3.9199	0.0013	0.0270
Jun-11	104.26	26.4	3.9492	0.0013	0.0261
Jul-11	123.22	35.23	3.4976	0.0017	0.0220
Aug-11	99.34	32.45	3.0613	0.0013	0.0142
Sept-11	79	22.56	3.5018	0.0010	0.0081
Avg	115.15	30.95	3.7425	0.0015	0.0176

Table 2. RNA, DNA, RNA: DNA, RNA: protein, RNA: lipid ratios from muscles of female *P. ocellatus* during different months.

Months	RNA µg/100mg dry wt	DNA µg/100mg dry wt	RNA: DNA	RNA: protein	RNA: lipid
Jun-10	98.00	25.20	3.8889	0.0014	0.0102
Jul-10	125.00	32.45	3.8521	0.0017	0.0125
Aug-10	122.00	32.52	3.7515	0.0017	0.0124
Sept-10	94.00	28.05	3.3512	0.0014	0.0104
Oct-10	82.00	28.05	2.9234	0.0012	0.0077
Nov-10	75.00	25.27	2.9679	0.0011	0.0056
Dec-10	98.66	26.69	3.6965	0.0015	0.0061
Jan-11	112.53	25.45	4.4216	0.0015	0.0096
Feb-11	160.8	31.93	5.0360	0.0022	0.0151
Mar-11	145.46	28.69	5.0701	0.0019	0.0241
Apr-11	134.93	28.95	4.6608	0.0019	0.0150
May-11	128.40	29.33	4.3778	0.0017	0.0290
Jun-11	138.20	32.42	4.2628	0.0019	0.0142
Jul-11	142.40	30.34	4.6935	0.0022	0.0101
Aug-11	122.50	32.45	3.7750	0.0018	0.0115
Sept-11	95.50	30.25	3.1570	0.0014	0.0103
Avg	117.21	29.25	3.9929	0.0017	0.0127

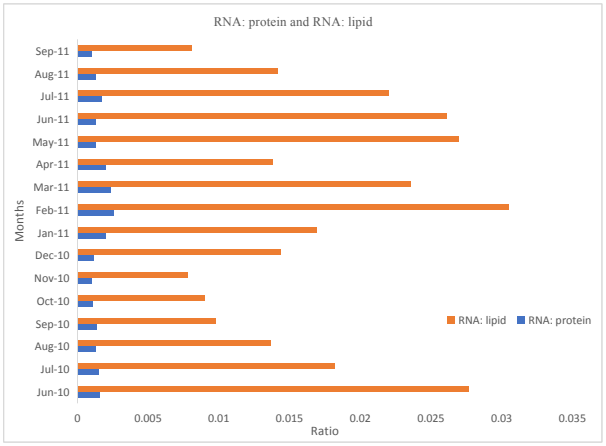

Figure 1. RNA: protein and RNA: lipid ratio in male *P. ocellatus*

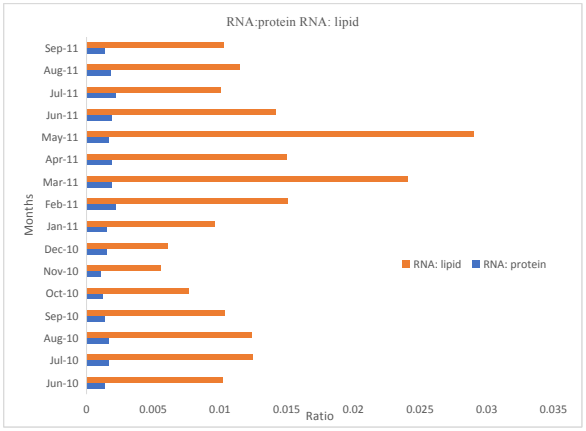

Figure 2. RNA: protein and RNA: lipid ratio in female *P. ocellatus*

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Variations in RNA and DNA content, RNA : DNA, RNA : protein, RNA : lipid in the muscles of male and female *P. ocellatus* during different months is presented in Table 1 and Table 2. This was estimated to determine condition indices in *P. ocellatus* male and female monthly. The total amount of DNA in a defined amount of dry tissue will depend on cell size which changes with age, stage of the reproductive cycle and nutritional status [15]. RNA concentrations depend on factors that affect cell size and are usually more variable than DNA concentration because RNA is required for protein synthesis, which responds quickly to changes in environmental condition [1]. The RNA content was maximum in February 2011 and minimum in November 2010, in male and female. The range of RNA content in male was 72-185.6 µg/100 mg and in female was 82-145.46 µg/100mg .The RNA content in male was observed to increase from November 2010 to February 2011and then decrease gradually till June 2011. In female RNA content was found to increase from November 2010 to February 2011 and decreased progressively till May 2011. This increase in RNA content may probably be due to increase in protein synthesis in male and female for the spawning period. The DNA content was maximum in male in October 2010 and minimum in September 2011. In female maximum value was observed in August 2010 and minimum in June 2010. The range of DNA content in male was 22.56- 39.31 µg/100 mg and in female was 25.20-32.52 µg/100 mg. In male DNA content was found to increase from November 2010 to February 2011 and then decreased gradually till June 2011. In females the values increased in February 2011 and decreased till May 2011 and further increased in June 2011. DNA content in females showed slight variations every month.

The RNA : DNA ratios ranged between 2.08-5.13 in males and 2.92-5.07 in females. In fish RNA : DNA ratio having values lower than 2 have usually been associated with prolonged fasting and an enhanced risk of mortality [16]. Thus biochemical indices reflect on a very good condition indices of the fish throughout the period of present study from June 2010 to September 2011 Therefore the creeks of Mumbai appears to be suitable nursery ground for this species.

The average RNA : DNA in *P. ocellatus* female was slightly higher compared to that in males. Similar findings were recorded in goby *Pomatochistus microps* [17]. The range for the RNA : DNA in the females was also narrow with the range in the males at 2.08-5.13 while in female it was 2.92-5.07. The lowest value in females is nearing the index 3 while that in the male is nearing 2. Value of RNA : DNA lower than 2 indicates prolonged fasting. Thus though the ratio is nearing 2 it was not below 2 and hence though in some months the ratio indicates poor condition, the average index in male is 3.74. The males enjoy good condition indices. The average ratio in female was 3.99 indicating a good condition of females than males. Maturation and reproduction differ between males and females and often require different amounts of energy and the reproductive costs are much greater for females than for males, which imply protein synthesis and therefore RNA : DNA ratio is greater in females than in males [18,19].

An analysis of monthly variation showed that condition values in terms of RNA : DNA ratio increased from October 2010 to March 2011 in both male and female. The value further decreased till June 2011 in male and May 2011 in female. The increase in protein synthesis associated with the development in gonads from November to February might have resulted in the increasing RNA : DNA ratio. The lower values in November and December are in accordance with that observed in *Mysis diliviana* [20] during the pre-reproductive season. The RNA : DNA values were found to be higher in reproductive season extending from January 2011 to April 2011 in *P. ocellatus*. RNA : DNA ratio increased during gonad development gradually from the developing to the spent stage [21]. As the fish mature, seasonal cycles of temperature and gonad development have large effects on nucleic acid levels in different tissues [3]. Increase in RNA : DNA ratio in recovering fishes can be considered as an indicator of protein synthesis and growth [22].

Figure 1 and Figure 2 shows the variation in RNA: Protein and RNA: Lipid ratio during various months of study. The RNA : protein ratio showed an average of 0.0015 in males and 0.0017 in females. Monthly variation in terms of RNA : protein showed minimum value in November 2010 in male and female and again in September 2011 in male. The maximum value for the ratio was observed in February 2011 in both male and female *P. ocellatus*. In male and female *P. ocellatus* RNA : protein ratio increased from November 2010 to February 2011 which is the progression from resting stage to spawning stage. The RNA : protein ratio further decreases in the post spawning stage of male. In female it was observed to show an increase in July 2011 probably due to recovery of spent stage. The maximum number of matured male and female *P. ocellatus* were observed in February 2011 and spent stages were observed in November 2010 [23]. The RNA : protein ratios along with RNA : DNA ratios reflect protein synthetic rates in organisms [24,25]. Condition indices shows a significant relationship with protein synthesis and growth rates [25,26].

The RNA : lipid ratio showed minimum values in No-

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vember 2010 in male and female *P. ocellatus*. Maximum values were recorded in June 2010 in males, May 2011 in females. The increase in RNA: lipid in female fishes in the month of May 2011 may be due to recovery of spent stage with increase in fat . The seasonal variations in the lipid content were related to reproduction and food availability in cod fish Gadus morhua [27].

4. Conclusions

Thus RNA : DNA, RNA : protein and RNA : lipid ratios can be used to evaluate conditional status of fishes in general. It is an indicator of the potential for protein and lipid synthesis. It also reflects the feeding, nutritional and growth rate of the fish. The goby fish *P. ocellatus* from the creeks of Mumbai showed good conditional indices in the creeks. This habitat thus seems to be suitable as nursery, feeding and reproductive ground for *P. ocellatus*.

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ARTICLE

Assessment of Ichthyofaunal Diversity in Sasihithlu Estuary of Dakshina Kannada, Karnataka, India

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ABSTRACT

The ichthyofaunal diversity is an excellent bio indicator of the status and health of aquatic ecosystems. The present study elucidates the ichthyofaunal diversity of Sasihithlu estuary in the west coast of Karnataka, India. The study was conducted from January 2019 to January 2020. Field explorations in estuary were undertaken on a monthly basis. A detailed analysis of piscine diversity revealed a total of 63 species of fresh water, estuary and marine fish belonging to 13 orders and 37 families. Perciformes was found to be a predominant order with 20 families and 31 species. Of the recorded species, one is Vulnerable and two are Near Threatened species. The greater diversity of fish was recorded during monsoon and the lesser diversity was recorded during winter.

1. Introduction

Wetlands are some of the most productive ecosystems and an important natural resource [1,2]. Among them, estuaries are the second most productive ecosystems in the world and a significant life support system [3,4]. Estuaries are special transitional zones which connects true freshwater ecosystems with adjacent marine ecosystem. They also host mangroves [5]. They provide various ecological, environmental, economical and scientific services to mankind [6,7] and understanding the biodiversity of these ecosystems is important [8,9,10]. Estuaries, being the special transitional aquatic habitat serve as excellent repositories of ichthyofauna and form a major component of fisheries [11]. India has rich estuarine and brackish water systems along its east and west coast. They provide conducive environment and conditions for breeding, spawning, feeding, nursing grounds and migration routes for several marine and freshwater fish species [12,13]. Estuaries are also called as nurseries of oceans as they provide safe habitat and rich food resources for initial stages of development for fish larvae and juveniles [14,15,16]. About 80% of the world's fisheries are dependent on mangrove [17,18] and majority of marine organisms spend a part of their life in mangroves [19,20]. Concomitantly, fish play an important role in managing the species diversity, its population and ecological balance of an area.

Contrary to this, wetlands and estuaries are severely modified, disturbed and destroyed by humans [21] which has resulted in decreased biodiversity [22,23]. Intense anthropogenic activities have drastically deteriorated and reduced

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estuaries of India [24]. This has led to the rapid decline in ichthyofaunal diversity and population which will in turn cause serious ecological imbalance. Keeping this in view, the study is aimed at cataloguing the ichthyofaunal diversity of Sasihithlu estuary as no comprehensive studies have been conducted so far with this regard. The present investigation, which is part of a larger integrated research, serves as a crucial prerequisite for sustainable management of ichthyofauna and formulation of conservation strategies.

2. Materials and Methods

2.1 Study Area

Sasihithlu is an estuary which is a confluence point of two rivers, Nandini and Shambhavi which originates in Western Ghats (Figure 1). It is located 25 km north of Mangaluru city (13.02° N & 74.47° E) and has an average elevation of 3 m above mean sea level. The region experiences climate and weather pattern which is typical to that of a coastal area. The temperature varies from 26° C to 42° C with an annual precipitation of about 3,500 mm. The depth ranges between 1 to 8 m. The mouth of the estuary is dominated by sand whereas the mid and the upper reaches of the estuary is dominated by silt and clay sediments. The estuary is greatly influenced by tidal inundations exhibiting semidiurnal tides and hence the water is brackish throughout the year. 5 sampling sites were selected (Table 1) with a minimum distance of 500 m from each other to ensure Quasi independence [25].

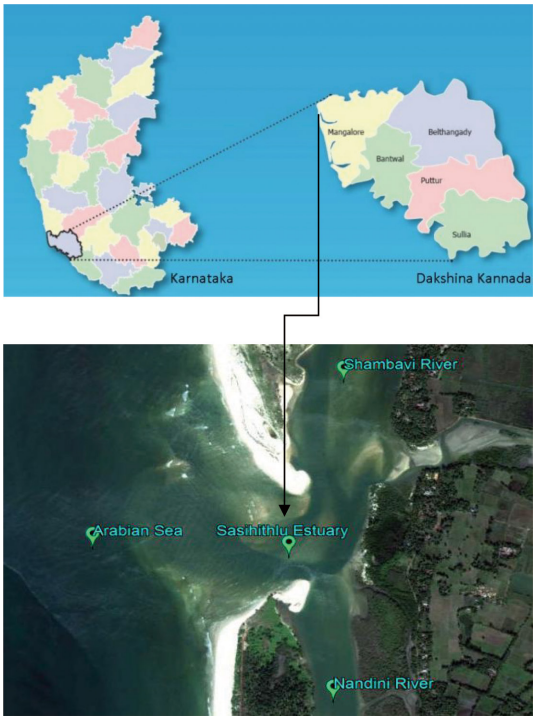

Figure 1. Map and google image of the study area

Periodic field exploration was conducted from January 2019 to January 2020. The study period was divided into 3 distinct phases, viz., the pre-monsoon (March to June), the monsoon (July to October) and the post-monsoon (November to February. Fish samples were collected from the estuary with the help of fishermen through random netting. Seine net, bag net, cast net, gill net, scoop net, drag net, stake net, trap net of varying mesh size and hook and line were used for fishing. Majority of the specimens were identified at the site of collection itself. Unidentified samples were preserved in 10% formalin and brought to the laboratory for identification and experts in the field were also consulted for the same. Standard literature was used for ichthyofaunal identification [26,27,28,29,30,31,32,33,34,35] . Canon EOS 70D and 600D DSLR cameras with 18 - 55 mm and 18 - 135 mm lens were used to photograph the fish and Garmin Etrex 30X GPS machine was used to take the waypoints (latitude and longitude) and altitude of the area.

Table 1. Details of the sampling sites

Sl No.	Study Sites	Latitude	Longitude	Elevation (m)
1.	Site 1	13° 3'5.09"N	74°47'14.65"E	2
2.	Site 2	13° 3'37.23"N	74°46'59.56"E	0
3.	Site 3	13° 4'15.13"N	74°46'41.51"E	0
4.	Site 4	13° 4'46.41"N	74°46'41.38"E	2
5.	Site 5	13° 5'30.01"N	74°46'47.29"E	2

3. Results

The health and ecological status of an estuary can be evaluated by studying its biological assemblages and community. An ecosystem with relatively few species indicates that it is under strain [36]. Sasihithlu estuary harbours a rich ichthyofaunal diversity which reflects its overall health and wellbeing. Perennial supply of freshwater from the rivers and periodic supply of marine water from the tidal activity provides preferable conditions for ichthyofauna. The present study revealed the presence of 63 species belonging to 13 orders and 37 families of class Actinopterygii. The checklist of the documented species along with its conservation status is listed in Table 2. Perciformes was the dominant order with 20 families followed by Beloniformes and Clupeiformes with three families. Pleuronectiformes was represented by two families whereas Anguilliformes, Carangiformes, Cichliformes, Cyprinodontiformes, Gonorynchiformes, Mugiliformes, Scorpaeniformes, Siluriformes and Tetraodontiformes were represented by one family each. Red-tipped Halfbeak (*Hyporhamphus xanthopterus*), a Vulnerable species (Vu) was documented from the estu-

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Table 2. Checklist of ichthyofauna recorded during the study

Sl No.	Order	Family	Scientific Name	Common Name	Conservation Status
1	**Anguilliformes**	Anguillidae	*Anguilla bicolor*	Shortfin Eel	NT
			Anguilla bengalensis	Indian Mottled Eel	NT
2	**Beloniformes**	Belonidae	*Strongylura strongylura*	Spottail Needlefish	LC
			Xenentodon cancila	Freshwater Garfish	LC
		Hemiramphidae	*Hyporhamphus xanthopterus*	Red-tipped Halfbeak	VU
			Hyporhamphus limbatus	Congaturi Halfbeak	LC
		Zenarchopteridae	*Zenarchopterus buffonis*	Buffon's River Garfish	NE
3	**Carangiformes**	Carangidae	*Scomberoides lysan*	Doublespotted Queenfish	LC
			Carangoides praeustus	Brownback Trevally	LC
			Caranx ignobilis	Giant Trevally	LC
			Caranx hippos	Crevalle Jack	LC
			Caranx tille	Tille Trevally	LC
4	**Cichliformes**	Cichlidae	*Etroplus maculatus*	Orange Chromide	LC
			Etroplus suratensis	Green Chromide	LC
5	**Clupeiformes**	Clupeidae	*Tenualosa ilisha*	Hilsa Shad	LC
			Anodontostoma chacunda	Chacunda Gizzard Shad	LC
			Nematalosa nasus	Bloch's Gizzard Shad	LC
			Sardinella longiceps	Indian Oil Sardine	LC
		Engraulidae	*Stolephorus indicus*	Indian Anchovy	LC
			Stolephorus commersonnii	Commerson's Anchovy	LC
		Pristigasteridae	*Opisthopterus tardoore*	Long-finned Herring	LC
6	**Cyprinodontiformes**	Aplocheilidae	*Aplocheilus panchax*	Blue Panchax	LC
7	**Gonorynchiformes**	Chanidae	*Chanos chanos*	Milkfish	LC
8	**Mugiliformes**	Mugilidae	*Mugil cephalus*	Flathead Grey Mullet	LC
			Crenimugil crenilabis	Fringelip Mullet	LC
			Planiliza macrolepis	Largescale Mullet	LC
9	**Perciformes**	Acanthuridae	*Acanthurus gahhm*	Black Surgeonfish	LC
		Ambassidae	*Ambassis natalensis*	Slender Glassy	LC
			Ambassis ambassis	Commerson's Glassy	LC
		Drepaneidae	*Drepane punctata*	Spotted Sicklefish	LC
		Gerreidae	*Gerres erythrourus*	Short Silverbiddy	LC
			Gerres limbatus	Saddleback Silverbiddy	LC
			Gerres filamentosus	Whipfin Silverbiddy	LC
		Gobiidae	*Glossogobius giuris*	Bar-eyed Goby	LC
		Haemulidae	*Diagramma labiosum*	Painted Sweetlips	LC
		Lactariidae	*Lactarius lactarius*	False Trevally	NE
		Latidae	*Lates calcarifer*	Barramundi	LC
		Leiognathidae	*Secutor insidiator*	Pugnose Ponyfish	NE
			Leiognathus equulus	Common Ponyfish	LC
		Lutjanidae	*Lutjanus argentimaculatus*	Mangrove Red Snapper	LC
			Lutjanus fulviflamma	Dory Snapper	LC
			Lutjanus johnii	John's Snapper	LC
			Lutjanus ehrenbergii	Blackspot Snapper	LC
			Lutjanus rivulatus	Blubberlip Snapper	LC
		Monodactylidae	*Monodactylus argenteus*	Silver Moony	LC
		Scatophagidae	*Scatophagus argus*	Spotted Scat	LC
		Sciaenidae	*Otolithes ruber*	Tigertooth Croaker	NE
			Johnius dussumieri	Sin Croaker	NE
		Scombridae	*Rastrelliger kanagurta*	Indian Mackerel	DD
		Serranidae	*Epinephelus malabaricus*	Malabar Grouper	LC
		Siganidae	*Siganus vermiculatus*	Vermiculated Spinefoot	LC
		Sillaginidae	*Sillago sihama*	Silver Sillago	LC
		Sparidae	*Acanthopagrus berda*	Goldsilk Seabream	LC
			Crenidens crenidens	Karanteen Seabream	LC
		Sphyraenidae	*Sphyraena obtusata*	Obtuse Barracuda	NE
			Sphyraena jello	Pickhandle Barracuda	NE
		Terapontidae	*Terapon jarbua*	Tiger Perch	LC

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Sl No.	Order	Family	Scientific Name	Common Name	Conservation Status
10	**Pleuronectiformes**	Cynoglossidae	*Cynoglossus arel*	Largescale Tonguesole	NE
		Soleidae	*Brachirus orientalis*	Oriental Sole	NE
11	**Scorpaeniformes**	Platycephalidae	*Platycephalus indicus*	Bartail Flathead	DD
12	**Siluriformes**	Ariidae	*Arius arius*	Hamilton's Catfish	LC
			Arius maculatus	Spotted Sea Catfish	LC
13	**Tetraodontiformes**	Ostraciidae	*Lactoria cornuta*	Longhorn Cowfish	NE

Note: LC - Least Concern, NT - Near Threatened, VU - Vulnerable, NE - Not Evaluated.

ary. Shortfin Eel (*Anguilla bicolor*) and Indian Mottled Eel (*Anguilla bengalensis*) are the two Near Threatened species (NT) which were documented from the area. 48 species belonged to Least Concern (LC) category, two species belonged Data Deficient (DD) and 10 species belonged to Not Evaluated (NE) category.

Figure 2. Giant Trevally (*Caranx ignobilis*)

Figure 3. Mangrove Red Snapper (*Lutjanus argentimaculatus*)

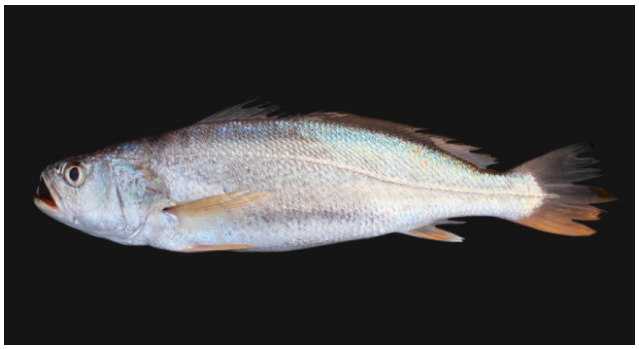

Figure 4. Tigertooth Croaker (*Otolithes ruber*)

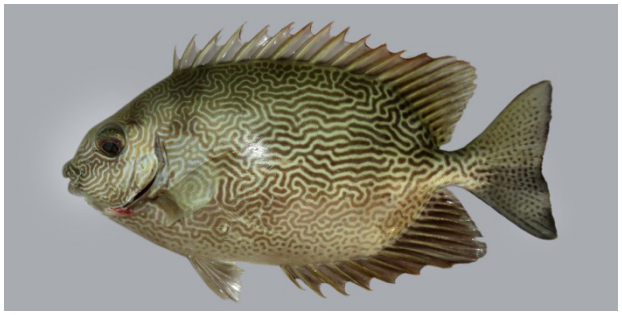

Figure 5. Vermiculated Spinefoot (*Siganus Vermiculatus*)

4. Discussion

Monsoon and post-monsoon were the most productive seasons in terms of abundance and species richness. The maximum ichthyofaunal activity was recorded during the rise and fall of tides. With the rising tides, many marine species would enter the estuary and return back to the marine system with the receding tides. The migration of marine fishes and the overall fish community in the estuary is governed by the suitable hydrobiological, physico-chemical conditions [37,38,39] along with seasonal nutrient variation [40] and other environmental conditions [41]. The young ones and juveniles of Tiger Perch (*Terapon jarbua*), Bar-eyed Goby (*Glossogobius giuris*), Silver Sillago (*Sillago sihama*), Flathead Grey Mullet (*Mugil cephalus*), Giant Trevally (*Caranx ignobilis*) (Figure 2), Tille Trevally (*Caranx tille*), Crevalle Jack (*Caranx hippos*), Indian Mackerel (*Rastrelliger kanagurta*), Indian Anchovy (*Stolephorus indicus*) and Mangrove Red Snapper (*Lutjanus argentimaculatus*) (Figure 3) prove that the estuary is used as breeding and nursing ground by many commercially important species. Presence of catadromous migrants like Largescale Mullet (*Planiliza macrolepis*), Flathead Grey Mullet (*Mugil cephalus*), Tiger Perch (*Terapon jarbua*), Shortfin Eel (*Anguilla bicolor*) and Indian Mottled Eel (*Anguilla bengalensis*) and anadromous migrants like Oriental Sole (*Brachirus orientalis*), Commerson's Anchovy (*Stolephorus commersonnii*), Hilsa Shad (*Tenualosa ilisha*), Chacunda Gizzard Shad (*Anodontostoma chacunda*) and Bloch's Gizzard Shad (*Nematalosa*

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nasus) along with amphidromous migrants like Hamilton's Catfish (*Arius arius*), Milkfish (*Chanos chanos*), Saddleback Silverbiddy (*Gerres limbatus*), Whipfin Silverbiddy (*Gerres filamentosus*), Malabar Grouper (*Epinephelus malabaricus*), Freshwater Garfish (*Xenentodon cancila*), Spotted Sicklefish (*Drepane punctata*), Pugnose Ponyfish (*Secutor insidiator*), Common Ponyfish (*Leiognathus equulus*), Spotted Scat (*Scatophagus argus*), Tigertooth Croaker (Otolithes ruber) (Figure 4), Bar-eyed Goby (*Glossogobius giuris*), Silver Sillago (*Sillago sihama*), Long-finned Herring (*Opisthopterus tardoore*) and Orange Chromide (*Etroplus maculatus*) validate the fact that estuary acts as an imperative corridor for migratory fish species. The present study has unveiled a relatively good ichthyofaunal diversity in Sasihithlu estuary. Contrary to this, the estuary is subjected to ecological degradation caused by intense anthropogenic activities like dredging, overfishing, extraction of shells and water pollution. Other problems like solid waste deposition by rivers and sea, destruction of mangrove patches by riverine and coastal erosion, conversion of mangroves and wetlands into aquaculture ponds for fish and shrimp farming along with siltation and sedimentation issues. This necessitates the systemic and continuous monitoring which is important to ensure the productivity and sustainability of the estuary for future generations. As there were no studies undertaken in this estuary with regards to ichthyofaunal conservation, the present study can be used as baseline data to assess the status of ichthyofauna and to formulate conservation strategies.

5. Conclusions

The present study has unveiled a relatively rich ichthyofaunal diversity in Sasihithlu estuary. On the contrary, the estuary is subjected to ecological degradation caused by intense anthropogenic activities like dredging, overfishing, extraction of shells and water pollution. Other problems like solid waste deposition by rivers and sea, destruction of mangrove patches by riverine and coastal erosion, conversion of mangroves and wetlands into aquaculture ponds for fish and shrimp farming along with siltation and sedimentation issues. This necessitates the systemic and continuous monitoring which is important to ensure the productivity and sustainability of the estuary for future generations. As there were no studies undertaken in this estuary with regards to ichthyofaunal conservation, the present study can be used as baseline data to assess the status of ichthyofauna and to formulate conservation strategies.

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ARTICLE

Can the *Ucides cordatus* Fishing and the *Crassostrea gasar* Creation on the Amazon Coast Make up the Curriculum of Rural Schools

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ABSTRACT

The fishing and ostreiculture activities practiced in the coast of the Eastern Amazon in the state of Pará, Brazil, became important to be inserted in rural education. Thus, this literature review aimed to realize a brief theoretical discussion about the important aspects of rural education in promoting the development of fishing and osteiculture. For the accomplishment of the literary research it was sought to explain the definition in education; rural education and valorization of the rural environment; environmental education as a transversal theme; fishing and aquaculture as a teaching strategy; considerations of fishing and ostreiculture; challenges and perspectives of the teaching of fishing and aquaculture in education; and booklet as a pedagogical tool in education. In conclusion, the use of fishing and ostreiculture as a strategy for rural schools through environmental education is important to foster fishing activity in communities, as well as the sustainable use of natural resources.

1. Introduction

Crab fishing (*Ucides cordatus* Linnaeus, 1763) is one of the oldest and most important activities of state of Pará, Amazon, Brazil [1,2,3]. There are several rural communities in the Amazon region depend on the extraction, processing, transport or commercialization of this crustacean to ensure income and livelihood [4].

Osteiculture (*Crassostrea gasar* Adanson, 1757), in turn, is an aquaculture activity that has been growing mainly in communities closest to mangrove áreas [5,6]. This activity, beyond to generating income for communities,

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contributes to the conservation of estuaries, reducing the pressure on natural stocks and promoting a sustainable exploitation of the environment [7].

Although this local importance, mangroves have been subjected to strong anthropic stresses at increasing levels, caused by the fast and intense degradation process coming from urban occupation, industry and poor land use planning [8]. In addition, the overexploitation of fishing resources, the contamination and pollution of the environment (by chemical substances and urban solid waste) are the main aggressions caused by man [9].

The educational process is configured as an important ally against social determinism [10]. Thus, Environmental Education (EE) emerges as a teaching-learing strategy. This education presents the objective of consolidating in students a social, participatory and permanent pedagogical process. In that way, they acquire responsibilities in the environment in which they live [11,12].

It is worth to mention that experiences in fishing and aquaculture at school have been reported in several studies [13,14,15]. These highlight the purpose of strengthening contextualized learning. Corroborating with the principles of EE, the Rural Education (ER) starts from social, political and cultural interests, taking into account the singularities of their existence, as well as their life contexts [16]. Both, through Gadotti [17], Freire [10] and Brandão [18], defend an education in which subject is respected and heard, where the curricula and contents work the concrete reality experienced by rural people, becoming active agents in their teaching and learning process.

Thus, the purpose of this literature review was to present the importance of rural education in promoting the development of fishing and aquaculture.

2. Definition in Education

Education is the process of integrating the human for self-knowledge and to transmission of moral, cultural and civic values that sustain the society [19]. For Brandão [20] education is a fraction of the way of life of social groups that create and recreate their own culture. Thus, it is a social practice necessary for the development of own life [10].

In Brazil, education is a right for all guaranteed by the Brazilian Federal Constitution of 1988 [21] and regulated by the Law of Directives and Bases of National Education (LDB). Education is the entire responsibility of governments at the federal, state and municipal levels, as well as managing and organizing the respective educational systems [22].

However, according to Marques and Oliveira [19] there is not unique way to do education. According to the same authors, the school is not the only place where it happens and neither the teacher is its only practitioner. Education is present in every people and everywhere in the world, from small tribal societies to large developed and industrialized cities; from small rural farmers to large landowners employers [18].

In this sense, the educatin is divided into modalities to better to serve the different people with their specificities [23]. Among which we highlight the Rural Education which aims to promote the educational process to consolidate values, principles and ways of being and living of those who integrate the field [24].

3. Field Education and Appreciation of the Rural Environment

Rural Education (ER) is constituted as a strategy to transform the Brazilian rural space. Such education rescues not only production, but the territory of sociocutlural relations with nature [16]. According Caldart et al. [24], the ER emerged with greater prominence in the Brazilian scenario, from the 1990, in the combination of the struggles of the Landless for the implantation of public schools in the areas of Agrarian Reform with the resistence struggles of numerous organizations and peasant communities not to lose their schools, their experiences of education, their communities, their territory and their identity.

Since then, the ER has been accumulating a set of legal instruments that recognize and legitimize the necessary conditions for the right to education, respecting the specificities of rural subjects and appreciating the rural environment [23] (Table 1).

Table 1. The rural education in Brazilian legislation.

Legislation	Proposal
Opinion CNE/CEB n°. 36/2001. It provides for the Operational Guidelines for Basic Education in Rural Schools, provides for Rural Education Policy.	It proposes to adapt the institutional project of rural schools for the National Curricular Guidelines in all teaching modalities.
Opinion CNE/CEB n°. 02/2008. It provides guideline for the attendance of rural people and refinement of Rural Education concept.	It guarantees the expansion of care closer to the realities of rural communities.
Decree n°. 7.352/2010. It provides for the Rural Education Policy and the National Program for Education in Agrarian Reform – PRONERA.	It proposes the construction of political and pedagogical projects, the elaboration of training policies for professionals and the effective participation of the community and social movements in the field.
Law n°. 12.695/2012. It provides for the Union's technical or financial support in the ambit of the Articulated Actions Plan and contemplates with FUNDEB resources the comunitary institutions that work in rural education.	It guarantees the registration of rural institutions in the Fund for Maintenance and Development of Basic Education and Valorization of Education Professionals - FUNDEB.

Source: Brazil [25,26,27,28]

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EC carries with it a conception of emancipatory education, which is part of a historical project that cannot be detached from social struggles. It aims to bring education closer to the challenges of building a society without exploitation [24]. According to Freire [10], the educational process should be an ally to counteract social determinism.

The field is constituted as a meaning that incorporates forest, livestock, mining, and agricultural spaces, as well as fishing, caiçara, riverine and extractive spaces [29]. A field that energizes the connection of human with the own production of the conditions of social existence [30].

In this sense, discussing EC in the Amazon implies considering the complexity of the region, the sociocultural diversity and the multiple identity manifestations [31]. According to Cristo et al. [32] the Amazon has a very vast cultural wealth that is significantly expressed in the legends, dances and stories that compose the sociocultural imaginary of the populations.

However, all this wealth is ignored by the urban culture that gradually deconstructs and devalues the imaginary of these populations. This process of devaluation of the knowledge of traditional communities arises as a consequence of a historical process of submission of urban values over rural values [33].

Since colonial times, the education of the Amazonian people as well as their culture has been denied and stereotyped. The problems and particularities of rural education in the Amazon have been occupied a marginal place in the scenario of public educational policies. This resulted in suppressed educational projects that are unable to overcome the existing scholar deficit in the region, nor does it respond to the identity process [31].

Prazeres and Carmo [33] when analysing the Amazonian reality, they point out the insufficiencies of State action in the field, not only related to educational aspects, but also to other constitutional rights. It is observed that the State is incapable of attending to the great diversity and heterogeneity of rural people in the Amazon. This worrying consideration anchors the perspective that the offer of basic rights to the field follows the perspective of offering, as Sammarco et al. [34] emphasize, "a poor education to the rural poor".

According to Hage et al. [35], the reality experienced by the subjects in the existing rural schools in the Amazon depict great challenges to be faced, and one of the first challenges is to enforce the operational milestones in the legislation. Despite the diversity of people and ethnicities in the region with different customs, there is a great precariousness in living, working, health and education conditions, which present a denouncing and worrying reality [33].

In contrast to this, in recent years rural movements have grown in the Amazon. In the state of Pará, the Paraense Movement for an EC, which agglutinates through the Forum Paraense of Rural Education, numerous entities from civil society, social movements, teaching institutions, research, governmental organs of development and educational area [36]. Such movement came to hold debates at state and also regional level, in order to discuss, elaborate and propose inclusive actions for rural people in the Amazon [31].

We emphasize that the people of the Amazon, such as the indigenous, caiçaras, quilombolas, riverside, rubber tappers, fishermen and shellfish gatherers, among others, configure in different ways of life. These actors live and coexist in an intimate relationship with the land, the river and the forest, in floodplain and dryland regions. As well as in settlements, communities, quilombos and indigenous tribes [36].

This result in multiple educational and intercultural processes, new forms of subjectivity and knowledge. This knowledge which is most cases is not valued by the official educational system [31]. For these razons, it is necessary to construct an educational curriculum that is adapted to the identity and reality of each rural people existent in the Amazon.

4. Environmental Education as a Transversal Theme

Environmental Education (EE) emerged in 1972 at the first United Nations Conference on the Human Environment in Stockholm, Sweden. It was considered an international historical-political landmark in the advance of alternatives to minimize the environmental problematic on the planet [8]. An event held by the United Nations (UN), which had representatives from 113 countries, and aimed to establish a global vision on environmental impacts and propose international agreements between countries. This was important in the sense of guiding the humanity on the importance of conservation in the environment for future generations [37].

After this history, EE has become one of the most important contemporary educational demands worldwide. It has been widely discussed with proposals that reinforce the urgency to involve all sectors of society through public policies focused on the environment [38]. In Brazil, the EE was formalized by the Federal Constitution of 1988, in § VI of Chapter VI on Environment, which makes the public authorities responsible for "promoting environmental education at all levels of teaching and public awareness for the preservation of the environment" [21].

From this, was created the National Political of Environmental Education (PNEA), the Federal Law number 9.795/99 which obligatorily insert to the National Curriculum Parameters (PCN's), the EE as a transversal content in Brazilian education. According to Article 2 of this

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law, EE becomes "an essential and permanent component of national education, and must be present in articulated form at all levels and modalities of the educational process in formal and non-formal character" [11].

Corroborating with the PNEA, the Curricular Guidelines for Environmental Education (DCEA) which guide the role of universities in promoting Environmental Education in teacher training. In its article 11, "the environmental dimension must be part of teacher training curriculum at all levels and in all disciplines" [39]. As well as promote its actions of teaching, research and extension focused on the principles and objectives of EE.

When analyzing the inserting of EE in higher education, socio-environmental issues inherent to EE is still little contemplated [34]. Silva and Chelotti [40] affirm that the training of teachers does not give provide subsidies to foster EE, which causes gaps in teacher training. To work with EE, teachers must have skills in several areas of knowledge [16]. This professional must propose a way of working that enables students to be the subjects of their own history, understanding and transforming their world [41].

According to the (PCN's), the teacher must contribute to the formation of conscious citizens, able to decide and act in the socio-environmental reality [25]. In this sense, it is extremely important in the training of teachers the continuing education in EE, which enables to fill gaps left in the graduation [12]. In addition, it fosters improvements in the practices of teachers and, consequently, in the training of students. Such students may experience different challenges proposed by teachers [42].

Therefore, it is up to the teacher to raise awareness and sensitize students about the environmental problems from the local to the global or vice versa. Thus also, develop knowledge, values and actions that promote changes in human behaviors in the space they occupy [8]. Freire [10] states that human being cannot actively participate in history, in society, in the transformation of reality if they are not helped to become aware of reality and of their own capacity to transform it.

Then, working issues such as fishing and aquaculture in rural schools, especially on the northeastern coast of Pará in a transversal way. They make it possible to build practices that solidify the importance of actions aimed at EE, mainly in the sense of discussing possible socio-environmental impacts on mangroves [8].

5. Fishing and Aquaculture as a Teaching Strategy

Fishing and aquaculture are important activities for human nutrition [43,44]. The first is defined as the extraction, collection or capture of aquatic organisms from environments where they live, such as rivers, lakes, oceans, mangroves and beaches [45]. While aquaculture is a science that creates/cultivates as well as reproduces aquatic organism [44]. We emphasize that there are several modalities of fishing and aquaculture with the perspective of insertion in schools (Table 2 and 3).

Table 2. Fishing modalities.

Modality	Definition	Source
Artisanal	It is an activity exercised by autonomous artisanal fishermen, who have their own means of production using small vessels with relatively simple fishing gear.	Zacardi et al. [46]
Subsistence	It is an activity carried out by fishermen who the objective of fishing only for their own consuption, using rowing canoes or motorized canoes (rabetas).	Natividade et al. [47]
Commercial	It is an activity carried out by professional fishermen authorized by the state agency using large vessels, which target aquatic organisms of great commercial value.	Santos et al. [48]
Industrial	In this activity the company is responsible for all the means of production that normally congregates the stages of capture, processing and commercialization. It is performed by professional fishermen on large vessels that employ sophisticated navigation equipment.	Vieira [49]
Sport	It is an activity carried out by amateur fishermen exclusivaly for recreational purposes without commecial purpose and with vessels and equipment properly described.	Rodrigues et al. [50]

Table 3. Aquaculture modalities.

Modality	Definition	Source
Fish farming	Fish farming is a science that creates/cultivates fishes at any stage of its development, in confined and controlled environments.	Baldisserotto et al. [51]
Shrimp farming	Shrimp farming is a science that creates/cultivates crustaceans as shrimp at any stage of its development, in confined and controlled environments.	Maciel and Valenti [52]
Malacoculture	Malacoculture is a science that creates/cultivates (creation of molluscs such as snails and scallops, the creation of mussels is known as mytiliculture and that of oysters as an ostreiculture), at any stage of its development, in confined and controlled environments.	Rodrigues et al. [53] and Lima [5]
Chelonian farming	Chelonian farming is a Science that creates/cultivates chelonians at any stage of its development, in confined and controlled environments.	Magnusson et al. [54]
Frog culture	Frog culture is a science that creates/cultivates frogs at any stage of their development, in confined and controlled environments.	Cribb et al. [55]
Alligator farming	Alligator farming is a science that creates/cultivates alligators at any stage of its development, in confined and controlled environments.	Abrunhosa [56]
Algae culture	Algae culture is a Science that cultivates algae.	Paula et al. [57]

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In Brazil, the fishing and the aquaculture are important economic activities that guarantee employment, income and food for many rural communities [13]. According to data released by the Anuário 2020 of the Brazilian Fish Farming Association (PEIXE BR), 722,560 tons were produced in 2019, with revenues average R$ 5,6 billion. According to the same source, in recent decades there has been a stabilization in extractive fishing and a greater expansion in the production of cultivated aquatic organisms, resulting in a greater volume of supply [58].

In the Amazon, it is common to use these activities for riverside and agricultural communities, where for many they represent the only source of income and familiar maintenance [47]. In this scenario, the state of Pará has become a protagonist in the production of fishing and aquaculture in the Amazon. Currently, is it responsible for 63% of fishing production in the region, with 90 million tons coming from extractive fishing and 30 million tons from aquaculture [58].

From this perspective, working with Environmental Education involving fishing and aquaculture in rural schools in a region like the Amazon is essential in the socio-environmental formation of students [13]. According to the same authors, the role of the school with the teachers is approaching the theme of fishing and aqualculture, taking into account the socioeconomic and local cultural specificities. That is, think of methodologies and pedagogical materials that can be applied in an integral way, to the point that the student knows and undestands how differentiate fishing and aquaculture [59].

Therefore, according to the PCN's, the role of teacher is considering the knowledge that students already have to plan meaningful teaching and learnings situations [22] in order to strengthen the relationship of the theme with the student's experience [8]. Thus, to promote EE through social projects, creating strategies for a more useful socio -environmental education, in order to build a knowledge combination the past and the present to support a more balanced socio-environmental future.

6. Fishing and Oyster Farming Considerations

Among the fishing modalities, the artisinal fishing provides employment, income and food for several communities along the Pará coast, including in São Caetano de Odivelas and other municipalities in the state. It is an economically, culturally and socially important activity in the region [47]. Zacardi [46] points out that artisinal fishing suffer a lack of both biological and socioeconomic information, being important for the management of fishing resources.

Different from what is thought about the dynamics of artisinal fishing, by understanding that it is a rudimentary form of animal extraction, this activity is of paramount importance for present social, environmental and economic relevance in riverside communities that develop such activity [47]. Bonfá Neto [43], points out that artisinal fishing should be thought and understood not only as a direct act of extracting a product from the waters, but as an activity that structures a complex production chain (fishes, mollusks, crustaceans and shellfish) transforms, benefits, distributes, exchanges and markets.

Regarding the main exploitable resource of fishing, there is a high diversity of fish species in the estuary of northeastern Pará, because this region includes the grouping of aquatic ecosystems (rivers, lakes and streams), floodplains, dry land, beaches and magroves [1]. It also highlights the abiotic factors such as tidal regime and rainfall resulting in a dynamic environment, providing the formation of the complex aquatic food chain rich in nutrients [46].

Cruz et al. [3] note that artisinal crab fishing is one of the most important extractive activities in rural communities of the Pará coast, not only for subsistence, but also for marketing. In São Caetano de Odivelas, 20% of the municipality's population depends directly on the extraction, processing, transportation or commercialization of the crustacean market [4].

The uça crab (*Ucides cordatus* Linnaeus, 1763) (Figure 1), is a crustacean that lives in magrove areas burrowing in individual galleries [8]. According to Maciel [1], its capture is carried through different rudimentary techniques, which are subsequently stored and transported for commercialization.

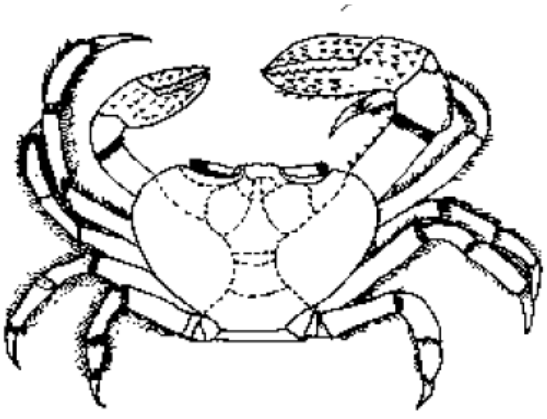

Figure 1. *Ucides cordatus* crab. Source: adaptated by Nascimento [60].

Another activity that has been gaining prominence

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in São Caetano de Odivelas is the creation of bivalve mollusks based on oyster farming or cultivation of mangrove oysters (*Crassostrea gasar*) being developed in the communities of Perurú de Fátima and Alto Perurú [5] (Figure 2). Oyster farming is an activity that generates income and contributes to the conservation of estuaries, reducing the pressure about the natural stocks and promoting a sustainability exploitation of the environment [61].

Currently, oyster farming has been practiced in six communities in the municipalities of Augusto Corrêa, Curuçá, Salinópolis, Maracanã and São Caetano de Odivelas, located in the Northeast Paraense Mesoregion [62]. According to Lima [5] seed collection is carried out in the natural environment. Subsequently, they adopt the suspended system of the fixed type and market the product.

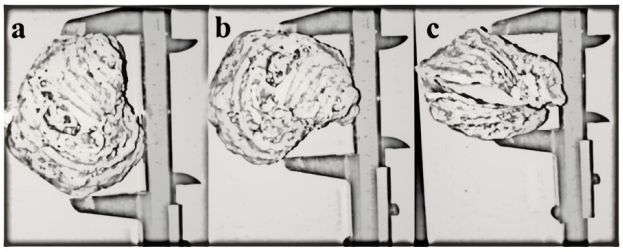

Figure 2. Measurements of the oyster *Crassostrea gasar*: a) height (mm), b) length (mm) e c) width (mm). Source: Macedo et al. [7].

According to data from the Brazilian Institute of Geography and Statistics (IBGE), in Pará, 80 families are benefited to generate income from oyster farming [63]. This same source considers that there is an annual production of 41,802 tons, with emphasis on the municipalities of Augusto Corrêa, Salinópolis, Curuçá and São Caetano de Odivelas, which together represent 79% of the production in the state. In this way, these municipalities contribute to the income of many rural communities and preserve the mangrove ecosystem.

Therefore, the fishing and the oyster farming are human activities that allow greater proximity to the nature. Due to this knowledge, it guarantees fishermen and oyster farmers the interaction of man with nature, being essential this theme in rural schools.

7. Challenges and Perspective of Fishing and Aquaculture Teaching in Education

Working with EE involving fishing and aquaculture in rural schools in our country is a great challenge, since the precariouness of the institutions added to the lack of teaching materials has been influencing the uncouraging performance of teachers and also students [8].

Through this, it is also necessary to remember the responsibility and the share of commitment that refers to public managers to assume more effectively their role in promoting the valorization of education in its totality, bringing tangible and empirical results of improvements in the educational framework at: municipal, state and federal levels [64].

Starting from the assumption that EE favors the socio-environmental awareness of the student to the extent that it provides tools and possibilities for them to realize themselves in nature and their responsibility towards its [8]. Silva and Santos [65], report that teachers must perform a work together with high expectations in relation to teaching-learning, having the mangrove, fishing and aquaculture as a reference to they offer for the students an environmental education that allows them to open paths for their personal fulfillment and social well-being.

In this sense, searching for new techniques and practices of environmental education related to socio-environmental issues in schools, especially in rural areas, is of fundamental importance to develop pedagogical intervention projects aimed at the training of students. According (Table 4) are some works about techniques and practices of environmental education at school.

Table 4. Works about techniques and practices of environmental educational.

Researches	Work title	Author
Fishing	Incentive to learning from student interaction– fisherman as a pedagogical practice.	Silva and Santos [65]
	From fishing to school: an experience of collective construction of knowledges in traditional fishing communities in the Paraense Amazon.	Vieira and Neves (2017) [66]
Aquaculture	Fishing and Aquaculture: environmental education techniques in elementar school, in Marajó (PA).	Miranda et al. [13]
	Elaboration of a booklet as an educational material for the preservation of the green turtle (Cheloniamydas) in Itaipú, Niterói, Rio de Janeiro.	Silva et al. [62]
Mangrove	Environmental education and analysis of mangrove ecosystems with basic education students.	Oliveira et al. [8]
	Effectivenes of practical environmental education actions for the mangrove ecosystem in elementary school.	Silva and Maia [9]

Source: Elaborated by author.

Currently, one of the biggest challenges faced by teachers working in rural schools when working on environmental education is the lack of pedagogical materials [67]. According to Santos [68], it is up to the teacher to seek new teaching strategies that aim to stimulate the students to know the socio-environmental problems of their commu-

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nity. The importance of the construction of pedagogical materials that seek to rescue the student's protagonism is emphasized [69]. A document that encourages them to indignations and concern about the environment, starting from their community/location.

Among the methodologies and educational materials that can be used involving fishing and aquaculture we can highlight: the vídeo lessons, the field trips and the pedagogical booklets [67]. This provides an excellent opportunity for teachers and students to learn in a different fun way about the importance of fishing and mangrove oyster farming in their communities [70].

8. Booklet as a Pedagogical Tool in Education

The booklet is a pedagogical tool with a simple, easy understanding and didactic language that clarifies doubts though explanations and illustrations. It is a way to reflection on a proposed theme [61]. According to Uyeno et al. [70], the thematics booklets have been increasingly used as a pedagogical material especially by basic education teachers in

The use of the booklet becomes an important allied in the teaching and learning process. This material awakes in the student the attention, the curiosity, the interest in what is portrayed in a more relaxed and objective way, thus collaborating in the construction of knowledge [67]. Reis et al. [61] affirm that booklets are able to develop critical thoughts in students, besides illustrating several realities, alerting and sensitizing the reader about the consequences of human actions in nature. For Silva et al. [62], the booklet reminds students a scenario closer to their reality, becoming a great for environmental education.

Finally, the booklet in promoting environmental education in rural schools it can be used with a simple and easy -understanding plot, with the purpose of being applied both in school environments and to general public [62]. In this sense, the elaborating of pedagogical booklet of EE in the mangrove with focus on fishing and oyster farming is essential. This document becomes an important tool to be elaborated and used by teachers in rural schools. Since it guarantees the right to communication. It also assists in several curricular practices [67].

9. Conclusions

This study enables the use of fishing and aquaculture as a teaching strategy for rural school through environment education. The realization of researches that seek to insert themes into the EC curriculum is important to foster fishing activity in the community, as well as the sustainable use of natural resources.

The activities focused on rural education seek to rescue the social role as well as the integration of community to assist and stimulate fishing production in mangrove areas, to improve the quality of basic teaching. We believe that EE should be interdisciplinary and involve the responsibility of everyone.

We suggest the use of pedagogical booklets on environmental education to be used in rural education. Such a pedagogical product will provide an excellent opportunity for teachers and students to learn in a different and fun way, about the importance of fishing and oyster farming in the mangrove in their communities and municipality.

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Declaration of Interest

The authors have no conflict of interest to declare.

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