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REVIEW

The Ecotoxicological Effects of Microplastics on Primary Producers in the Marine Environment

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ABSTRACT

Plastic debris is an emerging environmental threat all over the world. But its effect and distribution in the marine ecosystem is barely known. Microplastics abundance in the marine vegetated area is about 2 to 3 times higher than the bare site in the ocean. Although seagrass meadows trap huge amount of microplastics over the ocean floor, a considerable amount of microplastics are also sink incorporating with the marine aggregates from the epipelagic zone of the ocean. Scavenging of microplastics by diatom aggregation decreases the sinking rate of them rather than cryptophyte. As we know, marine snow is the leading carbon source for zoobenthos, but the ubiquitous presence of microplastics damages cell of different microalgae which may alter the food webs of marine ecosystems. Additionally, microplastics releases immense amount of dissolved organic carbons (DOC) in the surrounding seawater that stimulates the growth of heterotrophic microorganisms as well as their functional activity. Plastic debris result in outbreaks of disease in the marine environment and coral reefs are highly affected by it. When coral reef comes in contact with microplastics, the disease infestation rate of the reef increases massively. Three major disease *viz*., skeletal eroding band, white syndrome and black band of coral reef causes approximately 46% of reef mortality due to microplastics consumption. Due to complex structure and size, the corals accumulates huge amount of microplastics that increases growth of pathogens by hampering the coral immune system. Existing scientific evidence presents that exposure of microplastics in aquatic environments triggers a wide variety of toxic insult from feeding disruption to reproductive performance, disturbances in energy metabolism throughout the ocean. The present review focused on the ecotoxicological effect of microplastics on primary producers of ocean, its uptake, accumulation, and excretion, and its probable toxicity with risk assessment approaches.

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1. Introduction

The invention of plastics has achieved a crucial status, with extensive industrial, commercial, municipal and medicinal applications in contemporary society. Marine ecosystems are subjected to peculiar types of anthropogenic pollutants including plastics, nanoparticles, radionuclides, hydrophobic pollutants etc. Plastics incorporate with an enormous variety of polymer types, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamides (PA), polyethylene terephthalate (PET) and so on that contaminate both marine and freshwater ecosystems. Accumulation of these plastic contaminants for time after time creates a risk to the aquatic health and living organisms [1,2]. In last sixty years, plastic production has grown very rapidly, and globally where more than 288 million tonnes of plastic production was recorded in 2012 [3]. Due to its cheap prize, durability and flexibility it is used for making several types of fishing gear that are responsible for microplastics pollution in ocean in different ways [4-6]. Due to indiscriminate waste disposal and effluent of wastewater plastic debris are entering into the oceanic environment and may remain several centuries to mineralize [7]. According to the National Oceanic and Atmospheric Administration (NOAA), particles ignored portion from any kind of plastic debris and smaller than 5mm in size are considered as microplastic [8]. These are microscopic plastic fibres, fragments and beads that comprises 1-5 µm size in diameter [9, 10]. Depending on their origin microplastics has been divided into two categories. Firstly, the primary microplastics that originates from textiles, paints, cosmetics, household wastewater as well as from different plastic industries; and secondly, the secondary microplastics that are departed from the macro plastic (bigger in size >5mm) by dint of physical abrasion [9]. As plastic debris and microplastics had been observed in all over the ocean across the world from epipelagic layer to bottom floor of the ocean and also in the different levels of the trophic web [10-16]. The low density microplastics, polystyrene, polyethylene and polypropylene are usually found in the surface layer of the water column, while the high-density plastics polyamides and polyvinylchloride are tends to sink to the bottom. Sometimes, due to environmental disturbance like storms and currents of water of pelagic region creates vertical mixing of these particles within the oceanic environment [17,18]. Several studies revealed that the concentration of microplastics are very high in the urban coastal area, oceanic gyres and convergence zone of the ocean [19-22]. A study performed by Van-Cauwenberghe et al. [23] showed that a total of 10,000 particles m^{-3} microplastics covered the Belgian coast whereas about 102,000 particles m^{-3} are found in the Swedish coastal water body [24]. Another study on the North Pacific gyre revealed that small plastic particles exceed the phytoplankton in mass [25]. Moreover, Shim et al. [26] found fibers as the most dominant microplastic in the subtidal zone of ocean.

2. Plastics and the Marine Ecosystem

Ecological effects of microplastics on marine ecosystem are well studied [12,27]. Due to high sorption capabilities the microplastics contaminants absorb miscellaneous chemical substances of the epipelagic zone of ocean and transfer into the food webs or marine ecosystems [28]. In many places, microplastics contain high levels of toxic substances [29,30]. For example, at Brazil coast heavy metals adhere to microplastics was found [31,32]. At present, wetland playing an important role to support biodiversity and nutrient cycling, but also a microplastic transmission center of the global ecosystem [33,34]. Mangrove forests, seagrass meadows, saltmarshes and other marine and coastal vegetated area makes blue carbon ecosystem, which stores a significant amount of carbon in plant biomass as well as sediments [35]. The seagrass meadows of the wetland and blue carbon ecosystem plays a significant role in global carbon absorption and mitigate climate change [36]. Seagrass controls the velocity of water flow and enhances particles retention and sedimentation [37-39]. Seagrass meadows trap the particulate things recommend that it may also trap microplastics significantly [40]. The Dissolved Organic Carbon (DOC) pool of the ocean is one of the Earth's biggest, old and refractory [41-44] carbon pool (662Pg C) [45] and almost the same as the atmospheric carbon in size (828Pg C) [46]. Mainly phytoplankton derives DOC which enters the microbial food web and helps micro-heterotrophic organisms to grow [45, 47]. In this review, the term DOC used for biologically available dissolved organic carbon taken by heterotrophic bacteria in the daytime. Plastics release of DOC in the ambient seawater, as well as the biodegradation processes and its effects, are not much studied [48].

Lower plastic concentration in the sea surface than expected [49,50] suggests that majority of the plastics sinks to the bottom [51]. A higher density of the particles is not always true for their vertical fluxes. Because ocean dynamics can mix the bottom and surface particles to the water column [52]. But, sinking occurs because of the particles density most of the time [1] and biofouling (e.g. colonization of microorganisms) process could change the density and reverse their buoyancy [53] and microalgae had found to be attached with microplastics [54]. When

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algae become stressed (e.g. nutrient and light limitation) or get concentrated the release polysaccharides [55-57] to form sticky transparent exopolymer particles (TEP) [56,58] which helps to form cell aggregation. Microplastics incorporates these aggregates [59], which could create a vertical sinking of microplastics [60]. Thus phytoplankton and the upper trophic level grazers are mostly relying on these marine aggregates, this microplastic incorporation could have a considerable impact on the organisms which are ingesting [12,27,61-64]. Occurrence and abundance of plastic litters in marine environment are shown in Table 1.

Table 1. Abundance of plastic litters in marine environment

Saline area	Location	Abundance of microplastics	Methods of detection
North Shore Channel [65]	USA	1.94 items/m^3 at upstream – 17.93 items/m^3 downstream	Scanning Electron Microscope Analysis
Clyde sea [66]	North and West Scotland	Average weight of fibers from lobster gut 0.28–0.68 mg	FTIR Spectroscopy
Southern Ocean [67]	Fremantle to Hobart	44 pieces of microplastic, excluding fibers and expanded polystyrene, were collected over 5 sampling stations; Total particle counts along the sampling stretch is 100,000 pieces km^{-2}	Stereoscopic microscope; visual identification
Goa beaches [68]	India	Total of 3000 pellets	FTIR coupled with attenuated total reflectance (ATR) for polymer composition
Bohai sea [69]	China	0.33 ± 0.34 particles/m^3	Micro-Fourier transform infrared spectroscopy

Additionally, the coral reef is one of the most diversified ecosystems on earth which is threatened by disease [70]. The pathogens containing by plastic continuously trigger the diseases and increases vulnerability to the coral reef [71]. For instance, genus *Vibrio* colonizes on the polypropylene of marine water [54] and this devastating pathogenic bacterium creates white syndrome disease on corals [72]. As microplastic debris sinks frequently to the bottom and the previous studies on microbial rafting [73] suggests that plastic colonization level on coral reef ecosystem may very high.

3. Seagrass Meadows Traps Microplastics

Vegetated area microplastic abundance is much higher in than that in the bare site in both study area [40] (Figure 1). This was the first documentation of seagrass meadows traps and have high particle retention [39]. Seagrass beds create high roughness in the bottom layer and increase boundary friction which reduces the water flow [74]. The water flow is 2-10 times less in the vegetated area, sediments do not resuspend much and results in high sediment and organic particles accumulation [39,75-80].

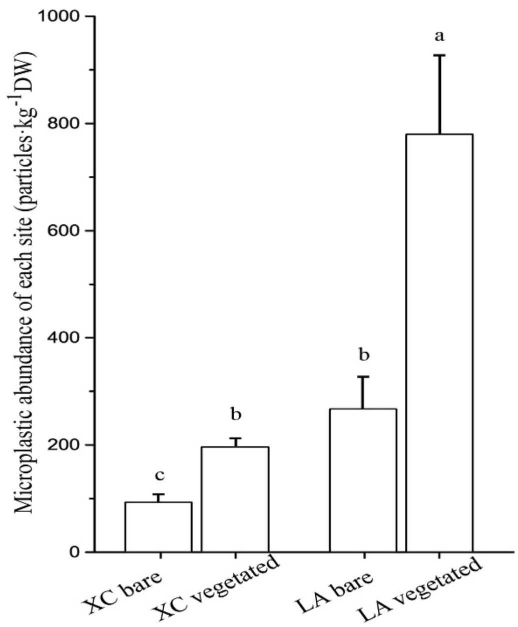

Figure 1. Microplastic abundance in the vegetated and bare site of the study area [40]

Seagrass canopy loss the particle's momentum by trapping particles through leaves and sinks them under the bed [74,81]. In a similar way, the microplastic could be trapped in the vegetated area due to less flow in the bottom and caught by leaves. There could be more accumulation of stacked sediments when leaves are buried [40]. The intertidal mudflats have lower physical turbulence where microplastic abundance is higher than from the exposed area [82] which is the similar findings by Huang et al. [40]. Another fact that the microplastic trapping by blue carbon ecosystem can explain their accumulation, like the mangrove of the Arabian Gulf and Red Sea [83]. Hence, seagrass meadows may be considered as the storage of microplastic of the marine environment [40], the anthropogenic contaminants can affect the living organisms of that ecosystem.

4. Microbial Activity

High- and low-density polyethylene (HDPE and LDPE), polypropylene (PP) and packaging polyethylene (PE) had been used for the experiment shows that these types of plastics leaches major part DOC when first contact with the

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seawater. During the experiment within first 200h almost half of the leached DOC has lost [48] (Figure 2).

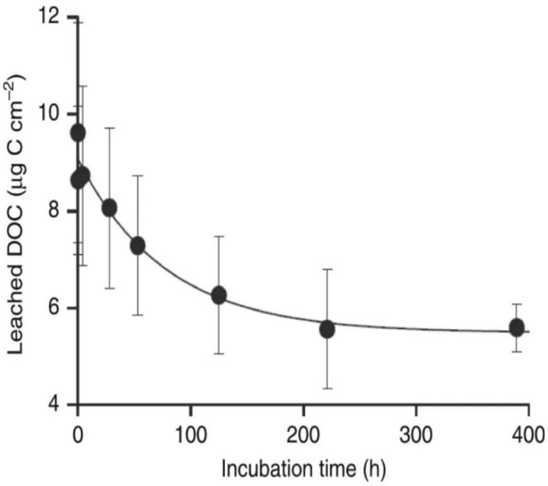

Figure 2. Loss of DOC over time using LDPF [48]

Karapanagioti and Klontza [84], documented the plastics sorption and desorption capacity, thus plastics could reabsorbed the leached DOC until it gets equilibrium [48]. Organic compounds sorption by plastics can be very fast. Because, Bakir et al. [85] found that floating polyethylene reabsorbs organic pollutants and gets equilibrium within 24 to 48 hours. However, other experiments documented more than 20 days [84]. In Romera-Castillo et al.[48] study it reached after 200 hours and may be with including DOC already been in the water. In nature, there could be a competition between bacteria and plastics to absorb natural DOC as bacterial abundance increased over time in the experiment (Figure 3) which may disrupt the other trophic level process [48].

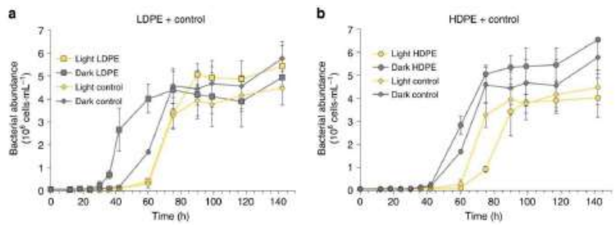

Figure 3. Bacterial abundance during the incubation with plastic leachates (cell mL-1) during the incubation of DOC leached from the plastics [48]

Carbon cycle and microbial activity influences by DOC leaching from plastics. The bioavailable DOC was higher in the dark than in the light (artificial solar radiation). Bacterial community stimulates in the dark condition (Figure 3), indicates they utilizes DOC than in the light condition [48]. Because, the radicles produce by plastics photo degradation [86] which could inhibit the growth of bacteria [87]. But, in the higher plastic concentrated area leaching DOC and their microbial activity could be significant in the near shore area or subtropical gyres [48].

5. Distribution and Impact on Microalgae

The Cryptophyte *Rhodomonus salina* and the diatom Chaetoceros neogracile aggregates were exposed to microplastic. *R. salina* aggregates showed eighteen times more affinity for microplastic beads than *C. neogracile* [60]. One reason could be *R. salina* aggregates are more permeable and small particles get more encountered through the aggreagtes [88]. Again, extracellular polymeric substance (EPS) of algae establishes hydrogen bond with the particles to facilitate hetero aggregation [89,90] with the interaction of the algal cells [91] (Figure 4). This kind of hetero aggregation may cause physical damage, membrane structure may alter [92], even cause damage of cell wall [29]. Besides, micro particles could cause light attenuation and reduce the nutrient uptake and gas exchange ability of algae which may consequent adverse impact on algal photosynthesis and respiration [89].

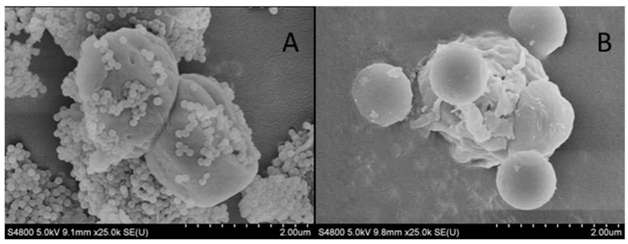

Figure 4. Algae and microplastic aggregation (Hetero aggregation). *Chlorella pyrenoidosa* (A) exposed to 0.1 µm PS microplastics (100 mg/L) and (B) exposed to 1.0 µm PS microplastics (100 mg/L) [91]

Incorporation with the microbeads the density of *R. salina* aggregates get higher and sinking rate increased than *C. neogracile* aggregates. In contrast, *C. neogracile* incorporates with low density microplastics and the overall density of the aggregates reduced [60]. The microbeads incorporating with the phytoplankton aggregates suggests that these aggregates potentially act as microplastic sinking and responsible for distribution [60]. Lack of plastic in the surface ocean [49, 50] and particles higher concentration in the bottom [51] can be explained by this. Phytoplankton aggregates contain microplastics and it may have a significant impact on the marine organisms [60]. Because, microplastics could be more approachable and ingested by the zoobenthos associated with the phytoplankton aggregates [27] as the filter feeders rely mostly on marine snow as carbon source [60]. And this may result in more microplastics access to the food chain [93]. Thus, it poses a serious threat to the biota and eventual ecological niche imbalance. Impacts of microplastics on different marine biota are shown in Table 2.

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Table 2. Observed ecotoxicity of microplastics in marine algal community

Algae	Microplastics size/concentration	Duration of Exposure	End points	Observations
Tetraselmis chuii (Microalgae) [94]	fluorescent red polyethylene microspheres (1–5 μm - 0.046 to 1.472 mg/l), Copper conc. Ranging from 0.02 to 0.64 mg/l,	96h	Growth inhibition	no significant growth rate inhibition
Skeletonema costatum (Diatom) [69]	polyvinyl chloride (PVC) microplastics of 1 μm and 1 mmsize	96h	Growth inhibition	39.7% growth inhibition in 1 μm particle exposure; no effects on algal growth in 1 mm size. Significant adsorption and aggregation
Rhodomonas baltica (Microalgae) [95]	fluorescent polystyrene particles (1–5 μm)	60 min	Uptake and motility	Increased uptake; bio-fouling formation

6. Coral Disease

About 17 genera of reef forming corals were found in contact with the plastics in different Asia-pacific regions [96]. Intensity of causing disease increases from about 4% to about 89% in presence of plastic debris [96] (Figure 5). Altizer et al. [97] demonstrate that terrestrial pollutants outbreak diseases in the marine environment and about 80% of the marine plastic waste comes from land [92]. It is also in consideration that disease effect differs in the different reef building corals in presence of plastic debris (Figure 5).

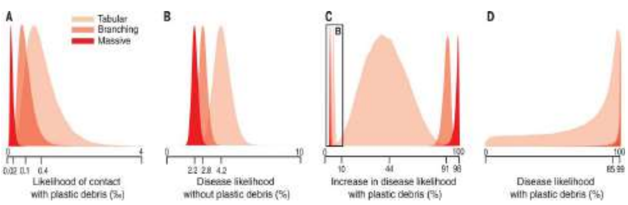

Figure 5. Coral morphological complexity influences the risk of plastic debris and disease. Tabular type corals are more likely to contact with plastic debris and affected by disease [96]

Because, corals with more structural complexity are the microhabitats for the organisms associated with coral reefs forming disease [96]. Lamb et al. [96] found three major diseases which cause rapid coral reef mortality: white syndromes (17% mortality), skeletal eroding band (24% mortality) and black band disease (5% mortality). Plastics not only cause physical damage of the corals, one of the major reasons could be abundance of pathogens and microbial activity in the high plastic concentrated area [48]. Plastic also inhibits light penetration into the water which is essential for some coral forming organisms could be another reason of global coral reef decreasing.

7. Conclusion

The distribution of plastic debris is higher in the vegetated bottom area that at the sea surface in the ocean. Seagrass and other plants act as filters for trapping the plastics. Plastic debris creates shading and triggers a favourable environment for microbial growth in the plastic concentrated area. Additionally, microplastics incorporate with marine snow sinks down and threating the overall biodiversity as it can cause damage of microalgal cell and disease to heterotrophic organisms. At present coral reefs are in very vulnerable to plastic contamination. But, how and what types of diseases causing to the other marine living organisms is not studied at all. It could be very important to do further research on that specific area (e.g. effects of microplastic on marine heterotrophic organisms). Most of the scientists believe that only the proper plastic waste management with strict law can stop this devastating marine pollution.

Authors' Contributions

All authors had searched and contributed to the article writing. Mahibul Islam and Mahmudul Hasan had designed the paper structure and contributed to editing; Bhaskar Chandra Majudmar and Sulav Indra Paul had revised the article. All authors read and approved the final manuscript.

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ARTICLE

The Law of Marine Fishing: Challenges and Coping Strategies for Sustainable Marine Fishing in Ghana

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ABSTRACT

Waters off the coasts of West Africa are very rich with many different types of fish and marine life. Some species like sardines, tuna, mackerel, tilapia and shrimps are important to many local communities and economies in West Africa. However, the current overfishing of these fish affects the sustainability of fishery industry. Despite Ghana ratifying to a number of international marine laws to ensure a sustainable marine fishing, there seem to be some challenges that are associated with marine fishing in Ghana. This paper further examines the strategies that have been employed by the fishing community and the fisheries ministry in Ghana as a response to restrictive measures for the challenges identified and discussed in literature. This paper is fundamentally an extensive review of marine fisheries literature. In general terms, it was found that, Ghana has ratified to a number of international marine laws including the UNCLOS.

1. Introduction

There are a lot of marine waters in West Africa, which are very rich with many different types of marine life. Ghana is one of the countries in West Africa that cannot be overlooked in terms of marine life issues. Ghana is a West Africa country located along the Gulf of Guinea with longitude 7.9465° N, and latitude 1.0232° W [1] and it share borders in the north with Burkina Faso, east with Togo, west with Ivory Coast and south with the Gulf of Guinea. A large number of Ghanaians dwell on fish supplies for their daily animal protein supply [2]. The fishery sector of Ghana over the years has played a significant role in supporting economic growth and the livelihood of the people [3]. In Ghana, fishing engages more than 500,000 people as fishermen, fish traders and fishing boat builders and accounts for about 3.6 per cent of the gross domestic product. [2]

Studies on marine fishing [4-6] suggest that to ensure sustainable marine fishing, there should be a complete un-

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derstanding of the multifaceted relationship between the environment and the laws that regulates them. Fishing in Ghana faces a lot of challenges and without strong and robust laws and regulations in place, its negative impact will continue in the marine sector.

In the second half of the 20th century, there has been a lot of emerging scientific and technological advancements to effectively find a more sustainable way to improve fishing in Ghana not forgetting the existing laws and regulations. [7] However, it is becoming very evident that there is a lot of substantial increase in the challenges that is collapsing the fishing industry in Ghana. In recent time, sustainable fishing in Ghana has been questioned a number of times largely due to the overexploitation. Overexploitation and sustainability have been core concepts in the management of renewable resources since the 1600s. Fishes are not only renewable resources for mankind, but they also provide a variety of benefits, recreational pleasure, as well as supporting livelihoods [8].

Ghana has significant fish stocks and a strong tradition and culture of fishing. Over 440,000 tons of fish are produce every year from the country's marine fisheries. However, more than 2.3 million of the total population depend on the fishery sector for their livelihoods [9]. The fish resources in Ghana are mostly overexploited. Per the sources, the country produces only 50% of its annual requirements. The sector plays an important role by contributing towards the country's economic development relating to employment opportunities, livelihood support, food security, poverty reduction, sustaining resources and foreign exchange earnings. In estimation, the fish sector support the country's total GDP of about 3% and 5% in the agricultural sector [10]. However, more than 10% of the entire population is engaged in the fishing industry, and over 70% of the total fish requirements are undertaken by the artisanal fishing in the country [5]. About 60% of the women population in the country is being employed in the fishing industry. The seabed in Ghana's sea has been destructed by the improper fishing activities such as overfishing and fishing with dynamite and light which has contributed to fish catch reduction rate for over two decades in the country. Currently, the country has no effective management regulations and policies to deal with these problems. Consequently, this paper sought to extensively discuss the laws on marine fishing in Ghana emphasizing on the challenges and effective strategies for sustainable marine fishing in Ghana. It also seeks to find out the current status of these regulations, effectiveness and trends in the marine laws and its application.

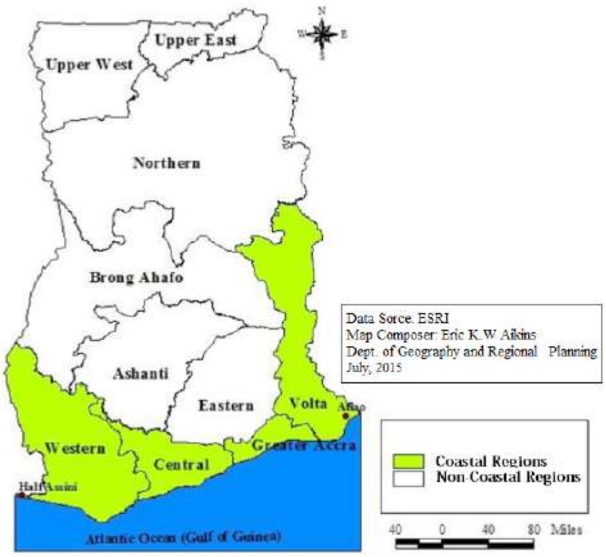

Figure 1. A map of Ghana Showing Coastal and Non Coastal Regions of Ghana

2. Methodological Approaches

In a manner to identify a significant works published to date concerning marine laws and sustainable fishing in Ghana, an extensive literature review was conducted. A range of online scholarly databases, search engines and websites of recognized international as well as national organizations and publishers was searched, to spot out the substantial works carried out in the area of marine laws and sustainable fishing. Varied search terms such as "Fisheries management", "marine laws", "sustainable fishing", "strategies and challenges of fishing" among others were used for retrieving the literature. The researchers further reviewed various laws on fisheries in Ghana; "Fisheries Management Plan 2015-2019", "Sustainable Fisheries Management Project 2018", "Ghana Project under the First Phase of the West Africa Regional Fisheries Program P124775", "National Plan for Action to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing, 2014"

3. Previous Researches

3.1 History of the Fishing Industry in Ghana

During the era of colonization, the fishing industry in Ghana arrived late following the earlier emergence of cocoa among other valuable economic products. In the era of 1930s, the industry received much attention and its first regulatory law in 1946 and the Fisheries Ordinance Cap 165 [11]. The fishing technology had a new face during the early 1950s, and its scope was extended to capture issues

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of safety. However, the industry continues recording more and more casualties, and over 25,000 casualties are being recorded every year. The environmental effects on marine fishing have reduce but have significantly increased the capacity to catch fish [12]. In a very long run, since the early 1970s, growth in employment in the primary sectors of fisheries may be slowing down significantly. Moving forward, fishing in Ghana has grown to employ a lot of people directly or indirectly. Since 1998, fishing is estimated to have primarily employed about 36 million people, comprising about 15 million full-time, 13 million part-time and 8 million occasional workers, of which it is estimated that about 60% are employed in marine fisheries.

Between 1950 and 1970, the ocean ecosystems food supply for direct human consumption doubled practically and stabilized since then at 9.0 to 10 kg of fish per caput, despite world population growth [13]. As total marine capture production stagnates, supply from marine capture fisheries is likely to decrease substantially, unless more effective management of capture fisheries and further development of aquaculture can increase production. Marine fisheries were Carrefour after receiving a rapid geographic expansion and technical advances, and several-fold increases in the annual harvest. The current fishery of sustainability is being questionable as almost all the fishery resources are either overexploited or exploited heavily. The environmental impacts awareness campaign has been to the societies. Browman also contributed that a series of eco-labeling schemes are being proposed and tested [14]. There is an assurance that, an ecosystem-based fisheries management (EBFM) approach might also help to set free some of the impediments that conventional management has experienced.

3.2 Challenges Facing the Fishing Industry in Ghana

Marine fishing in Ghana is one of the important traditional economic activities of the coastal communities and contributes more than 80 per cent of the total fish catch. Mensah and Antwi posit that the challenges of marine fishing in Ghana continue their perilous existence in the coastal environment [7]. In a study on “Challenges of marine fishing in Ghana,” identified some of the continue challenges in the marine fishing industry in Ghana [15]. Such challenges included;

(1) Overfishing: Usually overfishing is described as a fishing activity where fish resources are over exploited and above the fish reproductive capacity of affected marine ecosystem. Over fishing is touted as one of the main challenges of the marine fishing industry in Ghana because it negatively affects the stability and sustainable reproduction of fishes in the marine ecosystem. Friends of Nation added that over fishing does not only affect the stability and reproduction within the ecosystem, but also negatively affects the spatio-economic wellbeing of affected fishing communities that depend on the marine habitat [16]. Food and Agriculture Organization, further suggest that, as a way to control overfishing, in this study highlighted the need for fishing communities to consider having another source of livelihood that will help lessen the overdependence on fishing [17].

(2) There is a limited use of sustainable modern techniques of fishing and overdependence on subsistence fishing: In this fast developing economic era, a lot of fishermen still rely on the use of outdated use of sustainable techniques of fishing and heavily rely on traditional fishing such as DDT and dynamite for fishing. This negatively affects the ecological stability of the marine habitat, sustainable reproduction. Ministerielle adds that, fishermen heavily depend on subsistence fish farming for their livelihood that they can do anything to get a big catch [18]. Some of the techniques that are used in modern day marine fishing include the use of un-prescribed nets (nets with smaller mesh size), light aggregating machines, DDT and dynamites lower the reproductive capacities that affect marine habitats. Also, these bad fishing practices large quantities of fish tend to be destroyed in the process of local fishers extracting fish resources from the affected marine habitats.

(3) There is a limited local knowledge and participation in the maintenance and conservation of marine biodiversity: Basically, a lot of fishermen have low knowledge and participate poorly in what we term as marine conservation fishing. Aikins further unravels in his study that, the general low level of education of fishers and the limited-inclusion of marine fishers in the management and conservation of Ghana’s marine biodiversity explains this [15]. Although the fisheries Act 625 empowers the sector minister on fisheries to open and close the fishing season where necessary for the effective conservation, management and use of marine resources with the sole aim of achieving a sustainable marine biodiversity and conservation to some extent limits the complete observance to effective biodiversity management and conservation policies that are capable of helping Ghana to achieve a sustainable marine biodiversity, conservation and sustainable fish production. The study finalized that, the enforcement policy to marine fishing sustainability is limited. However, the law enforcement agencies are limited to establish, and to handle issues of marine biodiversity in the country. The effort to attain a sustainable marine biodiversity and sustainable fish production is also limited due to the fact that, most of

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the fishermen uses chemical such as DDT and carbides, and the use of explosives such dynamites to harvest more fish. The use of chemicals such as DDT and carbides, and the explosives such as dynamites for more fish harvest limit the country's efforts to attain a sustainable marine biodiversity and sustainable fish production.

(4) Pair Trawling: According to Aikins and Mead pair trawling is a form of fishing where two trawlers move shoulder to shoulder with a net sandwiched between them that scope the marine habitat between the two trawlers for any available fish that could be trapped [15, 19]. The type of fishes caught is directly based on the net mesh size. In reality, pair trawling sinks the productive capacity of affected marine habitats through the damage of fish reproduction and nurturing habitats of affected marine ecosystems that commonly require an unchanging and undisturbed habitat for fish reproduction and production. The researchers further posits that, pair trawling cause the removal of seaweeds which in essence provide food and essential resources for fish growth and production. Correspondingly, Law further adds that smaller fishes that are not mature for consumption are lost from their habitat. This thereby affects the numbers of the available possible mature fish reserves for the fisheries industry [20].

(5) The Use of Unapproved Methods of Fishing: Over the past two decade, the fishing industry in Ghana has seen a lot of unapproved methods of fishing largely including; the use of chemicals (DDT, carbides, dynamite), small mesh net for fishing among others. These have consequently led to the lowering of Ghana's fish productivity. Williams also adds that the mean fish production of Ghana which is about 400,000 metric tons is about 50 percent lower than the estimated annual fish requirement of about 820,000 metric tons [21]. Although fish harvested by this means is less poisonous and relatively healthier for human consumption, the repeated use of this technique in fishing creates an unhealthy habitat for the affected fish that moves farther away from affected habitats to newer and healthy habitats to enjoy a better sleep and a healthier environment.

(6) Lack and Increase of Premix Fuel Price and Fishing Equipment: The fishing industry in Ghana, seasonally see an unexpected increase in premix fuel and prices that generally affect the operational cost and productivity level in the fishing industry in Ghana. The researchers throw more light that, fishermen in Ghana still suffers and incur a lot of operational cost also because they do not have a direct access to the capitals. They however mobilize their own funds from family, friends and self. As a result, most of the fishermen are being prevented to the operational cost to compete the fish scarce and to located in farther distances away from their yet usually not too long a distance habitat. Predominantly, the frequent increase price of premix fuel, turn to set restrictions for the productive capacity of the fishing industry in Ghana.

3.3 International Regulatory Instruments and Policies for the Conservation and Sustainability of Marine Fisheries in Ghana

3.3.1 Marine Fisheries in Ghana before the UNLOS

The era of the pre-UNLOS in Ghana is marked between the years 1960s and the 1970s. The country was in an aggressive state as there was much call for independence. During this period, the government was unwilling to disturb the economic situation as it main focus was to consolidate their power base. They also felt under force to produce speedy economic results and social developments [22]. However, the fisheries sector then had its first regulatory legislation around 1946 as the Fisheries Ordinance (Cap 165) of the Gold Coast to regulate all issues associated with fisheries and marine issues. This ordinance was enacted and enforced by the then colonial government [23]. After the Fisheries Ordinance, the colonial government further enacted other fisheries legislation in the quest of strengthening the already existed Fisheries ordinance in the quest protecting and sustaining marine fishing. These other legislations included Wholesale Fish Marketing Act passed in 1963; Fisheries Act 1964; Fisheries Regulations LI 364 of 1964; NRCD 87 of 1972 (Fisheries Decree 1972); Fisheries (Amendment) Regulations 1977; and AFRD 30 of 1979 and the accompanying regulation, Fisheries Regulation 1979 LI 1235. These legislations did not stand the test of time as they were keen to address issues on importation and operating of fishing crafts. Kwadjosse further expounded that the pre-UNCLOS recorded a rising increase in the number of vessels in all the sectors of the fishing industry. The expansion in the fishing sector made the sector one of the lucrative ventures drawing a lot of investment from both public and private ventures. Hernæs contributed to this argument that, the strategies for developing the fisheries sector were mainly based on assumptions that sea will limit the amount of fish caught with the belief that Ghana had an enormous fishing potential [22,24,25]. The Fisheries sector by then did not have full mandate from the colonial government to enforce the fisheries regulation since the sector was an opened field for investment from anyone.

3.3.2 Marine Fisheries in Ghana after the UNLOS

Prior to the advancement the fisheries sector had made

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earlier, Ghana continued to ratify the UNCLOS in 1983. The post-UNCLOS legislations showed an increasing awareness for the need for conservation. Since Ghana ratified to the UNCLOS in 1983, there have been five (5) fisheries legislations established in Ghana:

(1) Fisheries Regulation 1984, (LI 1294);

(2) Maritime Zones (Delimitation) Law, 1986;

(3) PNDC Law 256 of 1991;

(4) Fisheries Commission Act of 1993;

(5) Fisheries Act 625 of 2002

Consequently, there has been increased awareness for the management marine fisheries as a result; these laws have been legislated to see to it that marine fisheries are conserved and sustained. The initial passing of the Maritime Zone Law established the jurisdiction over the Exclusive Economic Zone (EEZ), making it possible to determine exactly what is to be conserved and in what areas these measures are needed.

Abobi et al., contributed to this discourse that, the PNDC Law (256) of 1991 was then passed to begin the process of the conservation effort through licensing and establishment of fishing zones to control the access of fish stocks in the country. Since the enactment, there has been restrictions placed on some fishing equipment used in fishing and established a Monitoring Control and Surveillance System.

This was also followed by the Fisheries Commission Act of 1993 and defined as "an Act to establish a Commission, provide for its composition and functions relating to the regulation and management of the utilization of the fishery resources of Ghana and for connected purposes." This law can be considered as a major step in the attempt to manage the fisheries resources. The main function of the Fisheries Commission Act, 1993 is responsible for the regulation and management of the utilization of the fisheries resources and co-ordination of policies in relation to them, the Commission had duties that included inter alia the establishing of systems to manage, protect and effectively use the fish resources to achieve the most productive use; foster international co-operation and collaboration in fisheries for the benefit of the nation within the framework of Ghana's foreign policy and international commitments; and advise the Minister on the importation of fish as a supplement to local fish production. Hence, the Commission when established would be the mainstay of the whole fisheries management and conservation effort.

Ghana's Fisheries Act 625 of 2002 is defined an "an ACT to consolidate with the amendments of the law on fisheries; to provide the regulation and management of fisheries; to provide for the development of the fishing industry and the sustainable exploitation of fishery resources and to provide for connected matters."

3.4 Strength and Weaknesses of the International Regulatory Instruments

Despite the efforts made at dealing with the challenges facing the industry, there is ineffective management within the systems in charge of the sector. Also lack of political wills are factors weakening the sector to properly manage it. The industry has been neglected over the years, which have allowed for the influx of foreign vessels daily depleting marine fish resources. Asante et al., adds to literature that, waste is not treated in the country [26]. These wastes are channeled through water bodies which end up in the sea at the end of the day which pollutes and affect the quantity of reproduction of fish stocks. Ntiamoa-Baidu et al., diet, feeding styles and diurnal activity patterns are described for waterbirds using two brackish water lagoon systems in coastal Ghana, the Songor and Keta Lagoons. We project the habitat and activity data on a guild structure defined on the basis of individual feeding style and the sensory mechanism used to detect food. A total of 3 19 9 flocks containing 1 18,648 individuals of 3 6 different waterbird species were examined during October-November 19 94. Feeding habitats varied from dry mudflats to wet mud and shallow water of not more than 20 cm. The depth of water selected by waterbirds for foraging (but not for roosting further adds that's, there are structural developments along wetlands and water bodies which are habitats to many different species of fish. Because of this structural development, habitats for fish are being destroyed [27].

Consequently, the government of Ghana, however, in its effort to conserve and sustain fish stocks have declared an open and close season [28]. This was not taken lightly at first by the fishermen but in the end it has helped restore and restock marine fisheries. The government is also working with local traditional leaders in fishing communities along the coastal areas to enact and implement bye laws for those fisher folks living in those communities to follow and observe them.

3.5 Coping Strategies for Sustainable Marine Fishing in Ghana

According to Agardy there are a number of coping strategies for sustainable marine fishing [29]. This paper outlines the coping strategies for sustainable marine fishing and they are expounded below:

(1) Formulated policies and created regulatory frameworks to enhance other government interventions including finance mobilization and infrastructure development

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Regulatory bodies and government agencies have ensured the compliance to standards and participate in the capacity building of stakeholders in relevant areas (responsible fisheries and proper harvesting). Nunoo employment, livelihood support and socio-economic benefits to the Ghanaian economy. Fishery resourcesof Ghana are under stress from population pressure, increasing demand of fish and fishery products and open-access regime.Formal fisheries management practices have not yielded the desired results. There is an increasing need for traditional fisheriespractices to be incorporated into formal fisheries management practices. The aim of this paper is to conduct an in-depth studyon traditional marine fisheries management systems in Ghana in order to provide information to enhance the management of theartisanal fisheries.Data was collected through document analysis (between May 2014 and January 2015explains that, the government of Ghana, however, in its effort to conserve and sustain fish stocks have declared an open and close season. This was not taken lightly at first by the fishermen but in the end it has helped restore and restock marine fisheries.

(2) There has been an improved Knowledge and Community Participation in the Maintenance and Conservation of Marine Biodiversity: Mutimukuru-Maravanyika explains that, although the subject of marine biodiversity management and conservation requires some level of education, the general appreciation of the need for a sustainable management and conservation of marine fisheries resources could not be daunting to the local marine fisher [30]. There has however been a full addition of marine fishers in the management and conservation of Ghana's marine resources as introduced to by the 2003 Ghana coastal fisheries discussion structured by the "Hɛn Mpoano" initiative could be implemented without delay [29].

(3) There has been an increased and timely supply of subsidized premix fuel timely supply of premix fuel all year round: In Ghana, the fish plenteous season in particular is from August to September. Intended premix fuel shortages, hording and illegal resale of government subsidized premix fuel due to frequent premix shortages, particularly during the plenteous harvest period (August) negatively affect the level of annual seasonal fish production. Short supply or long delays in the supply of premix to affected fishing communities largely frustrates and discourages marine fishers who consider this once a year plenteous harvest period a prime business period within which affected fishers work hard to defray larger portions of their investment cost and generate additional income for the expansion of already existing fishing businesses. An improvement in the quantity and quality of premix fuel supplied to coastal fishing communities in Ghana at the official subsidized prices could to help eliminate negative consequences of perennial premix fuel shortages that often results in the payment of bribes for the timely supply of premix to the needed fishing communities and the payment of unapproved high prices for available premix fuel.

(4) There has been an of improved use of sustainable methods of fishing: According to "The fishing sub-sector and Ghana's economy" by the Bank of Ghana, the use of improved sustainable method of fishing through the use of suitable modern fishing methods that are locally and environmentally responsive (use of fuel economy outboard motors, fishing boats and fast moving big canoes with some form of modern refrigeration facilities) could help Ghana to improve on the productive capacity of its subsistence traditional marine fishing, which is by far the most prevalent type of marine fishing in Ghana. The use of improved method of fishing and fishing technology could contribute substantially to the overall improvement in the quality and quantity of marine fish landed per annum.

4. Discussions and Implication

Fisheries have been one of the major sources of food for a large number of people globally [22]. Till date fisheries continue to provide employment to a lot of people and also economic benefits throughout the world. In many developing countries where Ghana is no exception, there are limited opportunities for employment. A lot of researches [31,32] confirms that, around 59.6 million people are employed globally in the fisheries industry. There is evidence that easier access to fishery resources has not always interpreted on the long term into higher incomes and increased well-being of coastal communities. However, the practice of inappropriate use of marine fish resources has recently been increased and completed in most parts of the globe to prevent sustainability, and contribute to the major causes of overfishing, fish stocks degradation, ecosystem habitats and biological diversity.

Davis and Wagner in their study "*A right to fish for a living? The case for coastal fishing people's determination of access and participation*," explains that sustainable marine fishing implies leaving enough fish in the ocean, respecting habitats and ensuring people who depend solely on fishing can maintain their livelihoods. Undoubtedly, Ghana still has a great potential to increase and sustain its marine fish production. The Ghana ministry of fisheries and Aquaculture Development on in 2019 announced a closed fishing season for inshore and artisanal fishers with the aim of leaving enough fish stock in the sea. Although the Fisheries Act of Ghana, 2002 (Act 625) made it mandatory for the fisheries sector to observe a closed season

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as part of the ratification of the UNCLOS, however, it had been silent and never been implemented. The ministry was of the view that, if fully observed, it would be the first time the Ministry would have implemented a major fisheries policy intervention. The Ministry in collaboration with the Fisheries Enforcement Unit, comprising representatives from the Fisheries Commission, Ghana Navy, Ghana Maritime Authority and Attorney-General's Office, will monitor and ensure compliance of the closed season. A lot of stakeholders were of the views that the closed season was too short to ensure that fishes are restocked. A lot researches [32, 33] have aimed explaining the closed fishing season for artisanal and marine fishing in an effective management measure for restoring the fish stock. Their study confirms that the impacts of the closed fishing season was too short and/or lack of strict supervision to realize any significant change in fish population and sizes.

Notwithstanding, a lot of fishermen in Ghana still hardly adhere to the basic sustainable marine fishing practices despite the regulations and set by authorities. For example, fishermen still use the same old equipment and outdated fishing practices for fishing with some gradual improvements overtime because of new regulations. A study on marine fishing in Ghana. In his study he noted that fishermen use illegal light attraction equipment to improve their catches in the Ghana's marine waters [34]. This negatively affects the sustainability of marine fishing in Ghana. The practice of light fishing has led to overfishing of wild fish stocks. Fish of all kinds are attracted towards the artificial light so that they can be easily harvested. It can be concluded that the overexploitation of the wild fish stocks has the potential to collapse it and reduce future levels of fishing.

In most fishing communities in Ghana, the fishermen have established fishing associations. These Associations help them to better understand some fishing challenges and also pull resources to support their operations. Through these fishing associations, most fishermen have gained sufficient knowledge of traditional sustainable fishing practices in the fishing communities although they need to be empowered to practice them the more. These traditional sustainable fishing practices are simple, yet effective ways to improve marine fishing in Ghana. These Associations act as structures established to ensure that the sustainable marine practices are adhered to. These fishing Associations are established base on the national fisheries rules and regulations. However, some fishermen do not have the desire to obey national fishing rules and regulations. In a similar study on Artisanal Fisheries and Climate Change in Ghana, [35] it was unraveled that a lot of fishermen are always reluctant to adhere to the national policy but are quick to respond and adhere to the traditional fisheries associations rules and regulations. These associations are in the same community with them and will be quick to punish offenders. It can be concluded that, these fisheries management policies must be implemented as a policy hand in hand with the fisheries associations in the various fishing communities and the national fisheries policies to ensure that Ghana derives the maximum benefits from its fisheries resources for the current and future generations.

Since 1983, the marine fishing industry in Ghana and its sustainability has been recognized internationally. Due to this, the agenda for sustainable development has also been fully committed to make certain of an international goal on marine, Oceans and seas. Due to this, the Sustainable Development Goals (SDGs) 14 is solely committed to "conserve the use seas, oceans and the marine resources in a sustainable manner to ensure continue sustainability". The major aim of this goal is to reduce or eliminating marine pollution, ensuring marine and coastal ecosystems safety, reducing ocean acidification, sustainable management of fisheries and ending harmful fisheries subsidies, protecting coastal and marine areas, increasing economic benefits to SIDS and Least Developed Countries (LDCs). In order for Ghana to fully realize and achieve this international goal, there must be more implementation of policies, investment and innovations to restore the productive capacity of the oceans and increase economic benefits to developing countries. There should be an innovation in relation to the policies and laws enacted which will integrate best practices for marine fishing which can benefit greatly the future generation.

The environmental implication of unsustainable marine fishing is significant. The marine waters of Ghana are more likely to see a reduction in the marine fish stock. Fishermen in the future will end up bring dead fishes instead of live one. To a larger extent, fishermen will end up catching immature fishes from the marine waters of Ghana. Despite the numerous policies and marine laws coupled with the fishermen associations, there are still fishermen who flout these policies and laws. It is interesting to note that, fishermen will be the ones to benefit when they adhere accordingly to these marine laws. The will have a bumper catch all year round while protecting and sustaining our marine waters for the future generations.

5. Conclusion

Ghana is struggling to effectively deal with illegal, unreported, and unregulated (IUU) fishing. Even with the ratification of these conventions, there is weak management systems coupled with corrupt government officials

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who refuse to tackle issues of sustainable marine fishing because they themselves are involved in it and they get a share from what the illegal trawlers and foreign vessels harvest from the sea bed off the country's waters. In order to prevent the unregulated growth in the country's fishing canoe fleet, and has been blamed for the fast-falling small oceanic catch in the country, the fisheries ministry in Ghana has launched the first ever Canoe Authorization Card in the sub-region, as Ghana moves to address the influx of new canoes that have flourished under the country's previous open-access policy, which has been linked to the fast diminishing sardinella species and other small pelagic fishery. The Ministry in its efforts to address the issue has made an open and close season for fishing in Ghanaian waters. This declaration was to help prevent the pressure on fish stocks, safeguard marine habitats, and to ensure that fish stocks within the marine waters in the country are exploited within the biologically acceptable levels.

6. Recommendation

To reverse overfishing trends in Ghana, the following recommendations are therefore suggested to effectively achieve sustainable marine fishing. However, when these recommendations are adopted and adhered to, will further strengthen marine laws to ensure a sustainable marine fishing in Ghana.

(1) There should be an adoption of international legal instruments in the field of maritime fisheries and updating national regulations The use of improved sustainable methods for fishing should be encouraged

(2) The fisheries sector should limit the activities of pair trawling

(3) Provision of subsidized supply of premix fuel

(4) There should be participation of community folks in educative programs in the maintenance and conservation of marine biodiversity

(5) There should be a strengthening of research capacities and the scientific opinion to support the decisions of development and rational and sustainable management of fisheries resources

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ARTICLE

Chemical Exposure Hazardous for Fish *Hyphessobrycon eques* through the Incorrect Release of Oil in Amazon Region

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ABSTRACT

Incorrect discard of oil used by fishing ship in the brazilian north region has become a common activity. Despite the possible hazard to the aquatic organisms, still missing scientific data about their toxicity. Thus, this study aimed to evaluate the stress and lethality caused by lubricant oil (FSAOLU) on fish *Hyphessobrycon eques*. Therefore, it was used six different concentration diluted in water (0, 22, 24, 26, 28, 30 and 32% of oil) and three replaces during 96 hours. At the end or during the experiment (with dying fish), it was collected blood samples (cutting the caudal fin) to determine physiological changes. The FSAOLU showed mean lethal dose (LD50-96h) of 27.36%, classified as toxic causing alterations in glucose values from the 26%. The greater dilution of FSAOLU (32%) increased 115% in glucose values when compared to the control. Thus, lubricant oil when discarded in water, it present hazard to the aquatic organisms causing stress and mortality for fish being necessary adequate management to discard of this residue.

1. Introduction

Amazon region has a large biodiversity which exploration of natural resources becomes the main income of population. Among the practiced activity in the region, the fishing suffers two types of exploration (industrial and artisanal) with two types of fishing ship (with and without motor) [1,2]. Currently, 370 thousand anglers have this activity as their main source of income [3,4].

Fishing ships with combustion engine also used to

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freight transport in the region, they use lubricant oil to reduce engine friction or wear [2,5]. However, when it reaches the maximum life time losing its functional characteristics, it become dark and dense named "*burned oil*" commonly discarded [6,7].

With regard to the current legislation [8] all burned oil should be collected and recycled by refining process. Nonetheless, in the brazilian north region, they have 12% of fishing ship discarding oil in water [5] providing hazard to the local biodiversity because still missing knowledge about the toxic effect of this residue for native fish.

These oil derivatives have hydrocarbon compounds linked to others substances as nitrogen and sulfur, being the size of hydrocarbon chain determines their toxicity [6,9,10]. The toxic potential of oil derivatives has direct correlation with its solubility in water, releasing polycyclic hydrocarbon compounds and BTEXs (Benzene, Toluene, Ethylbenzene and Xylene) [11-13]. These compounds has potential to cause bioaccumulation in environment providing hazard to the human and animal health.

Furthermore, the burned oil has greater toxicity when compared with the natural oil which adds heavy metals as lead and benze(α)pirene, affecting the cellular process as well as its use of carbohydrate or glucose [14], increasing the mutagen and carcinogen effect [10].

In this scenario with incorrect discard of burned oil and missing knowledge about the toxic effects, the use of toxicity tests to determine physiological changes or mortality it becomes too necessary for an adequate future management [15].

Among the native fish species, the *Hyphessobrycon eques* stands out as adequate biological model for acute toxicity tests due to their high sensibility [16,17]. Thus, this study aimed to determine the lethal dose (LD50%) of burned oil diluted in water using the biological model *H. eques* evaluating physiological responses with glucose values.

2. Material and Method

For this study, it captured (sisbio license 19515) in the Chumucuí River (01º 08 '40.12" S and 46º 34' 04.8" W) and acclimated in 300 L water tanks species of *H. eques* for a period of seven days. Afterwards, all fish passed by feeding deprivation before the experiment [15,18]. Preliminary tests determined the FSA dilutions of burned oil that would cause 0 and 100% of mortality. As well as the potassium chloride (KCl) sensitivity test was performed as reference substance. For this test, five KCl concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 $g.L^{-1}$) were used with three replications and one control using four fish (1.09±0.12 g) per treatment.

To obtain the concentrations, it prepared a laboratorial solution consisting of 300 mL (burned oil) with 700 mL of distilled water (3:7) homogenized during six hours (QUIMIS® Shaker). Subsequently, the insoluble part was discarded, using only the water-soluble fraction (FSAOLU) for the experiments [19]. For definitive acute toxicity test, it was carried out an experiment in completely randomized design with six FSAOLU dilutions (0, 22, 24, 26, 28, 30 and 32% v/v) with three replicates having 15 fish per treatment (1.19±0.14 g) during 96 hours. During the experimental period, each hour had fish mortality determined and the water quality parameters measured for each 24 hours: dissolved oxygen (6.74±0.29 $mg.L^{-1}$), temperature (28.6±0.62 °C), pH (6.42±0.32), conductivity (119±10.3 $\mu S.cm^{-1}$) and ammonia (0.02±0.01 $mg.L^{-1}$).

The Trimmed Spearman Karber method [20] determined the lethal concentration (LC50) classifying its toxicity according to CETESB [21], which classifies the xenobiotic compound into: very toxic (when LC50 is less than 25%), toxic (25 to 50%), moderately toxic (51 to 75%), slightly toxic (above 75%) and virtually non-toxic (100%).

During the experiment, dead or dying fish with loss of reaction, swimming imbalance and minimal opercula beat, had blood samples collected to determine glucose values (Accu-chek Performance®) and afterwards euthanized by medullar section.

The data blood glucose was submitted to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests. Then, it was applied analysis of variance (ANOVA) with post-hoc Tukey test ($p<0.05$) for comparison of means. Data between mortality rates and FSAOLU concentrations passed by *Pearson* linear regression.

3. Results

The Potassium chloride (KCl) showed lethal concentration ($LC_{50\text{-}96h}$) 1.66 $g.L^{-1}$ with a lower limit of 1.49 $g.L^{-1}$ and upper limit of 1.89 $g.L^{-1}$. The FSAOLU showed lethal concentration ($LC_{50\text{-}96h}$) 26.38% with upper limit of 27.36% and lower of 25.45%, considering toxic chemical according to CETESB [21]. The highest concentration (32%) present 100% of mortality within 12 hours of exposure (Figure 1), generating a positive correlation ($y=10.84x-242.59$ with $r^2=0.96$, $p=0.0012$). Furthermore, during the definitive trial, occasional behavioral changes were observed by the addition of FSAOLU, such as erratic swimming, agitation and fish positioning on the water surface.

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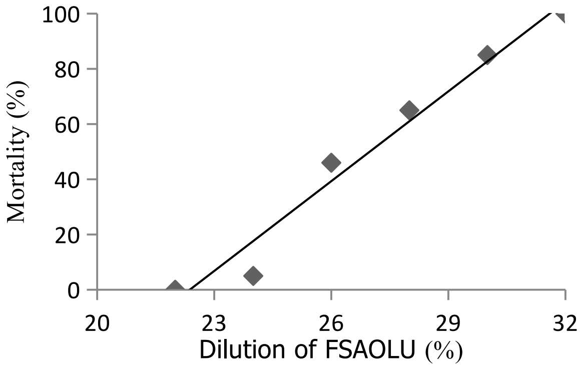

Figure 1. *Pearson* correlation between mortality and different concentration of FSAOLU in *H. eques*

It was observed alterations in glucose values of fish (figure 2), with the highest values in the greater dilution (32%) (p=0.0021) (65.42±2.4 mg.dL^{-1}), showing increases of 115% when compared to the control (30.21±1.11 mg.dL^{-1}).

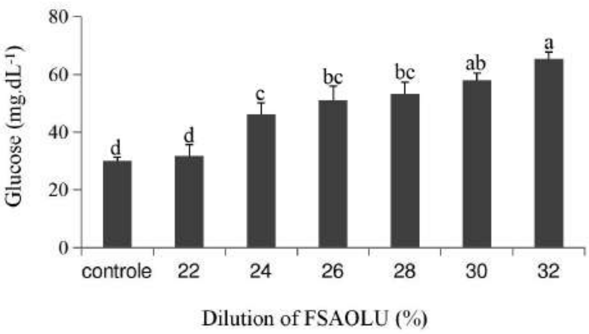

Figure 2. Values (Mean±standard deviation) for glucose values of *H. eques* exposed to different concentrations of FSAOLU

4. Discussion

Fishing ship change the lubricant oil periodically to maintain its properties as viscosity or coloration [5]. Nonetheless, the most of them remain discarding burned oil in aquatic environment due to the missing of information. However, according to legislation, it used oil should be placed in adequate local and collected by specialized companies to refine [8,22].

Nonetheless, incorrect discarding of burned oil in the ground or water body it does exist [22,23]. According to Melo [5], fishing ship discarded approximately 12% lubricant oil in river, lake and ground becoming an environmental problem with regard to the contaminated fish income.

In aquatic environment, this residue can release molecular contaminants, mainly aromatic hydrocarbon, BTEXs (Benzene, Toluene, Ethylbenzene and Xylene), nitrogen and sulfur-linked heterocyclic compounds which through the water-soluble fraction cause toxicity to aquatic organisms [9,10,12,13,24].

In the literature, toxicity studies about the burned lubricant oil and its effects on aquatic organisms are still few. However, this xenobiotic can change the physic-chemical parameters of water making it unfavorable causing mortality of sensible organisms [10,25,26].

Thus, when fishing ship releasing burned oil contaminating the aquatic environment it becomes hazardous because the toxic potential. According to Otitoloju [25] as well as Ayoola and Akaeze [27] determined the lethal dose of 53.89% for *Poecilia reticulata* and 56.20% for *Clarias gariepinus*, respectively, greater values than those observed for this study 26.38%.

Different organisms can show different sensibility with toxicity tests until in several development stages [15,17,18]. According to Rodrigues [12], they observed severe toxicity of brute oil, diesel and gasoline for lavae *Odontesthes argentinensis* which determined lethal dose 70.68, 13.46 e 5.48%, respectively, showing its toxic effect.

Thus, both marine and freshwater fish are subject to the toxic effects of FSA from oil products. These compounds dissolved in water are responsible for the risks to the aquatic environment and the organisms affecting homeostatic imbalance, which results in changes in hematological and tissue parameters [11,13,15,18,24], may cause stress and later fish mortality.

In the present study, changes in glucose values caused by burned oil would be an indicator of stress in fish [18]. This elevation of glucose was probably stimulated by catecholamines, increasing glycogenolysis in order to prepare the animal for rapid action for escape the stressor [18,28]. According to Simonato [11] monitoring the toxicity of diesel FSA dilution of 50% *Prochilodus lineatus* juveniles for 6, 24 and 96 hours, observed a difference in glucose values with an increase of 172% (24h exposure) and 120% (96 h of exposure) in relation to the control (22±1.00 mg.dL^{-1}). For Simonato [24] also evaluating the physiological effects of *P. lineatus* exposed to 5% dilution of the water-soluble fraction of gasoline for 6, 24 and 96 hours, observed an increase of glucose for 45, 52 and 52% when compared to the control (42±2.00 mg.dL^{-1}). Thus, corroborating the present study, it was observed increases 115.8% of glucose values for fish in the trial causing 100% of mortality in less than 24 hours with dilution (32 %).

The effects of these xenobiotic when discarded in water can cause mortality [18,27,29] as observed in the present study. Furthermore, it should be noted that hydrocarbons and heavy metals released from burned oil derivatives

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might be accumulated in fish [29]. According to Milinkovitch [30], they observed that oil containing 3.3±0.6 µg.L^{-1} polycyclic aromatic hydrocarbons (PAHs) in water, showed a reduction for hydrocarbons to 0.5±0.1 µg.L^{-1} after 48 hours of experimentation. This resulted in the accumulation of PAH-derived metabolites in the gallbladder of fish, showing an increase of pyrene (0.2 IU) compared to the gallbladder of the control fish. These pyrene derivatives are toxic, carcinogenic and mutagenic, causing inflammation in animal tissues [31].

Although not being the object of this present study the accumulation of these products in aquatic organisms also becomes a concern mainly regarding public health [18,31]. In this sense, the irresponsible disposal of burned oil is a concern for both the aquatic environment and humans, as the spillage or improper disposal of these products can cause aquatic animal mortality and even bio-concentration affecting health.

5. Conclusion

The water-soluble fraction of the used lubricating oil (FSAOLU) shows an environmental risk to the Amazonian rivers because it is potentially toxic, altering glucose values and leading to the mortality of fish. Therefore, flows the need to adopt socio-educational measures for the correct disposal of this compound.

Declaration of Interest

The authors have no conflict of interest to declare.

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ARTICLE

Exploitation Patterns of Anchovies (*Engraulis encrasicolus*) by Marine Artisanal Fisheries in Togo (West Africa)

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ABSTRACT

Pelagic fish, including sardines and anchovies (Order Clupeiformes), are the most common species taken by artisanal marine fisheries along the Togolese coast. We investigated fisher involvement as well as fish captures over a period of 10 years, particularly of the European anchovy (*Engraulis encrasicolus*). Our results indicate that 60% of fishers operated from the Lomé fishing harbour, most fishers being Ghanaians working seasonally in Togo. 63.7% of all the fishers used canoes with outboards, a higher percentage compared to the previous decades. Seven fishing gear type were identified, with bottom gillnet and surface gillnet being the most commonly used. However, in the most important fishing camp in the country in terms of fish production (Lomé fishing harbour), all fishers used shark nets. Overall, fisheries catches did not change significantly across years, but anchovy fishing effort and catch per unit of effort (CPUE) declined over the study period, suggesting some depletion of the species stocks on the Togolese coast and a demotivation of fishers.

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1. Introduction

Exploitation trends of fisheries in West Africa is little known and the data available on the subject fragmentary and outdated [1-7]. In Togo, around 200 fish species are found along the continental shelf [8-18]. Pelagics, particularly members of the Clupeidae family, the European anchovy (*Engraulis encrasicolus*) and sardinellas (*Sardinella* spp.) are the most exploited (unpublished data of the Fisheries and Aquaculture Office in Lomé). Fishing in Togo is an almost exclusively artisanal activity, supplying up to 80% of the national fish production (unpublished data of the Fisheries and Aquaculture Office in Lomé). However, trends in catches, and in particular pelagic fish, have declined since the 1980s [19], suggesting overexploitation of most fish and in particular these species [14-16].

Here, we first analyse the evolution of artisanal catches along the Togo coastline, then the fishing effort defined as both the number of boats and cumulative power of their engines, to quantify their impact (catch and selectivity) and their respective efficiency measured by the catch per unit of effort (CPUE). Finally, the trends of overall CPUE are computed for artisanal fisheries in the country, to assess possible impacts on the ecosystem.

2. Study area

The country's only shoreline (50 km) is found in the Maritime region (Figure 1). Maritime region is about 6,395 km^2. In the west, it borders the Volta Region of Ghana, and in the east, two departments of Benin [20]. It includes large conurbations, of which Lomé, the capital, alone accounts for 30% of the Togolese population. Our study area extends between 01° 11' and 01° 37' East longitude and between 06° 06' and 06° 14' North latitude (Figure 1).

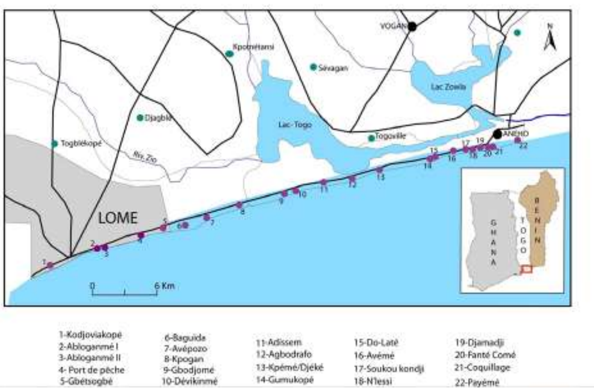

Figure 1. Map of the coastal area of Togo showing the fishing sites surveyed during the present study

Togo's continental shelf, as typical of other Gulf of Benin countries, is separated from the abyssal bottom by the Romanche fracture relative to the "Côte d'Ivoire-Ghana Ride". According to [8], the continental shelf varies from East to West between 20 km at the border with Benin to 31 km at the Ghana border. Its limit is between 85 and 110 m deep. Beyond the 100 m isobath, the continental shelf falls steeply by 15%. There are four bottom types: (1) Hard bottoms, well developed but not very extensive (less than 15 m) and coral reefs (coral reef continues in 50 m and then scattered heads); (2) Muddy sands corresponding to the littoral bottoms in the vicinity of the lagoon outlet and the funds beyond 35 m; (3) Sandy bottoms extending up to 35 m outside the lagoon spill area and (4) deep sandy mudflats that extend from 45 m, scattered with corals after 52-56 m depth [8].

3. Methods

Fisher and fish seller surveys were conducted from 1st to 21st December 2011. The whole Togolese coastline was crossed to identify any existing fishing camps. The position of each camp was recorded by GPS and then mapped using ArcGIS software to establish the extent of the main fishing areas. Interviews covered characteristics of fishers, description of fishing gear and techniques as well as product processing techniques and marketing channels.

Information on catches, CPUE and fishing effort were obtained from the Office of the Fisheries and Aquaculture (Lomé, Togo) for the period 1999-2009. The Fisheries Office identified four sites from which data on landings were collected. At least 5% of landings were recorded at each site during each field day. These data were first analysed by the Fisheries Office Data Manager with the Artfish software. Fishing effort expresses the number of days that fishing has been carried out. Off the line each trip is considered as a fishing day. CPUE expresses the quantity of fish caught per unit of effort and is expressed in kg/day. A 10-year data sequence covering the period 1999 to 2009 was analysed. Curves and graphs were developed from these data and made it possible to establish the variation in CPUE during the year as well as that between 1999 and 2009, the evolution of fishing effort and total production.

Inter-site differences in frequencies of motorised boats were tested by observed-versus-expected χ^2 test. Normality and homoscedasticity of the variables was tested by Shapiro-Wilk W test; when non-normal, the variables were log-transformed to achieve normality and homoscedasticity prior to applying any parametric test. Correlation between variables were done by Pearson's correlation coefficient using raw data or (log) transformed data when appropriate. Statistical analyses were performed with PASW version 15.0 software, with alpha set at 5%.

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4. Results

4.1. Characteristics of Artisanal Marine Fishing

We identified a total of 22 fishing camps (Figure 1) and a total of 125 stakeholders were interviewed (105 fishers and 20 fishmonger women).

We found that 71% of the artisanal marine fishers were Ghanaian in origin, the rest were Togolese. The interviews included the following ethnic groups: Ahloan (62.9% of the interviewees), Guin (7.6%), Fante (0.9%), Mina (4.8%), Ouatchi (3.8%) and Ewé (20%). Some interviewees have been settled along the Togolese coast for centuries (Ewé, Guin and Mina), while Ahloan have only arrived recently.

Most fishers used dugout canoes and boards of 6 - 15 m in length. The percentage of fishing canoes with outboards (25 - 40 hp) was on average 63.7%, but the differences among sites were statistically significant (χ^2= 635, df = 21, P < 0.0001). In particular, 95% of the canoes at the Lomé fishing harbour were with outboards, 75% in Adissem, 7.5% in Kpémé, and none at the other sites.

Seven types of fishing gear were identified, with bottom gillnet and surface gillnet being the most commonly used (Table 1). However, in the area of Lomé fishing harbour, the most important fishing camp in the country in terms of fish production, all fishers used shark nets (Table 1). The number of fishers per crew depended on the type of gear used, with up to 20 persons for those using beach seine and purse seine.

Table 1. Frequency of utilization of the various types of fishing gear used in the surveyed fishing camps along the coast of Togo

Fishing sites	Beach seine	Bottom gillnet	Surface gillnet	Fishing long-lines	Purse seine	Floating gear	Shark net	Total	Canoes	Motorised boats
Kodjoviakope	10	0	0	22	0	0	0	32	10	3
Ablogamé II	3	0	0	0	0	0	0	3	3	0
Ablogamé I	8	6	0	0	0	0	0	14	14	0
Fishing harbour	0	252	207	196	148	720	352	1875	277	277
Gbetsogbe	0	0	0	14	0	0	0	14	9	0
Baguida	0	24	0	0	0	0	0	24	2	2
Avepozo	0	50	5	0	0	0	0	55	25	0
Kpogan	0	84	0	6	0	0	0	90	13	3
Gbodjomé	2	100	0	0	0	0	0	102	8	0
Devikinme	3	30	0	0	0	0	0	33	8	0
Adissem	4	50	2	0	10	0	0	66	20	15
Agbodrafo	1	30	0	0	0	0	0	31	5	0
Kpeme/djeke	2	150	0	0	0	0	0	152	40	3
Goumoukope	3	72	4	0	0	0	0	79	9	5
Do late	2	0	0	0	0	6	0	8	2	0
Aveme	3	400	0	0	0	16	0	419	18	5
Soukou condji	1	0	0	0	0	0	0	1	1	1
N'lessi	3	12	0	0	0	0	0	15	6	0
Djamadji	0	0	0	6	0	0	0	6	3	0
Fante come	3	39	0	0	0	0	0	42	6	0
Coquillage	3	84	0	0	0	0	0	87	8	0
Payeme	0	100	0	0	0	0	0	100	6	0
Total	51	1483	218	244	158	742	352	3248	493	314

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4.2. Exploitation of Fisheries Resources

Monthly CPUE values varied considerably across years (period 1999-2009), with peaks concentrated in July, August, September and October (wet season; see Figure 2). Overall, annual fish catches were fairly stable throughout the study period (log-log relationships; r = -0.043; P = 0.900; Figure 3). CPUE increased by 59.9% from 450 kg/d in 1999 to 720 kg/d in 2009 (r = 0.77, P < 0.001; Figure 4a), whereas fishing effort decreased by 37% (r = - 0.71, P < 0.01; Figure 4b).

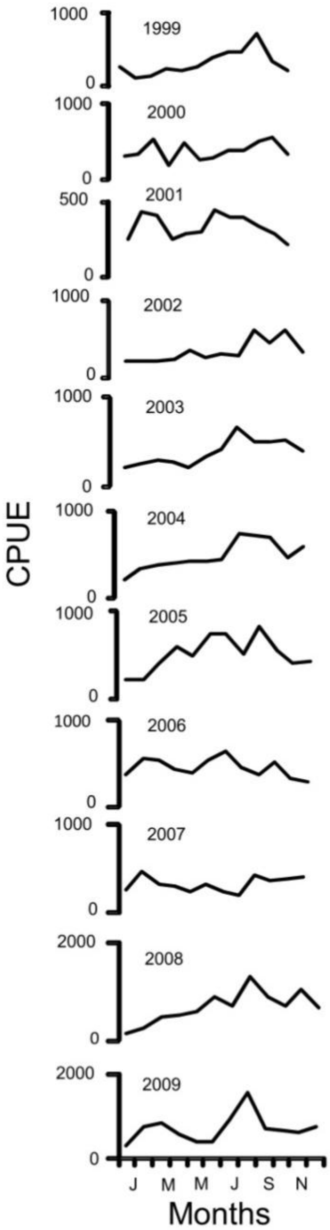

Figure 2. CPUE variation curves by year (period 1999-2009) and in relation to the various months of the year

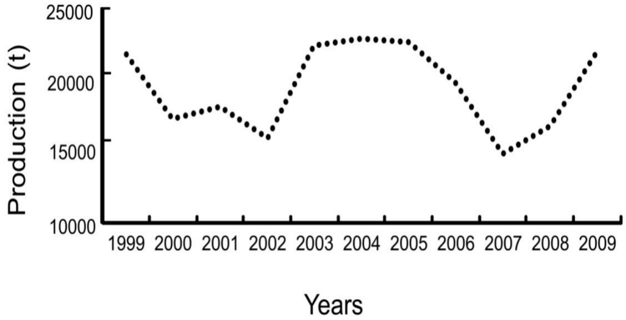

Figure 3. Variation in total production of fish along the coast of Togo during the period 1999-2009

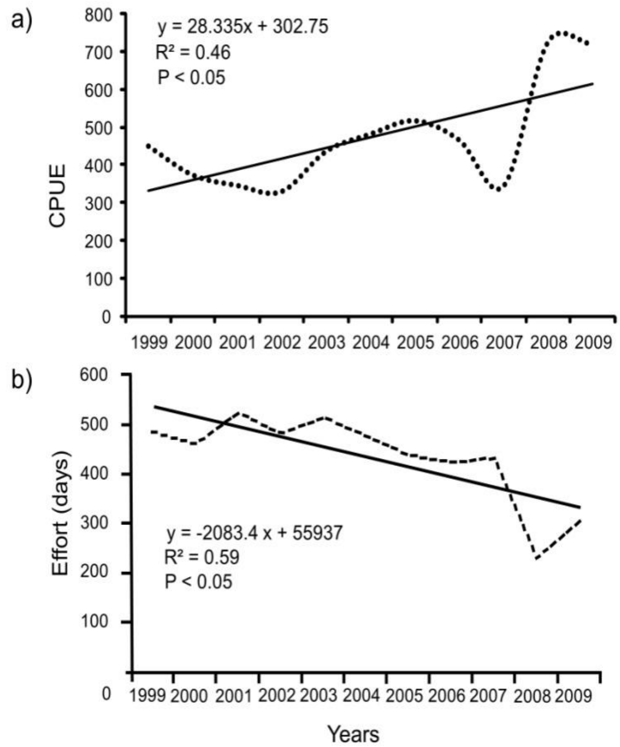

Figure 4. Variation in annual CPUE (graphic (A)) and variation in fishing effort (graphic (B)

Anchovy fishing took place year-round, with several peaks during the year, particularly from July to October (Figure 5). Catches declined by 72.29% from 1999 to 2009 (r = - 0.76, P < 0.0001; Figure 6), with significant drop in CPUE from 287.389 kg/d to 161.898 kg/d (r = - 0.61, P < 0.05; Figure 7a) and fishing effort by 50.8% (r = - 0.79, P < 0.0001; Figure 7b). Concurrently, the percentage of anchovy catches also declined significantly (r = - 0.72, P < 0.001) by about 73.3% during the study period (it accounted for about 45% in 1999 but was only 12% in the year 2009) (Figure 8).

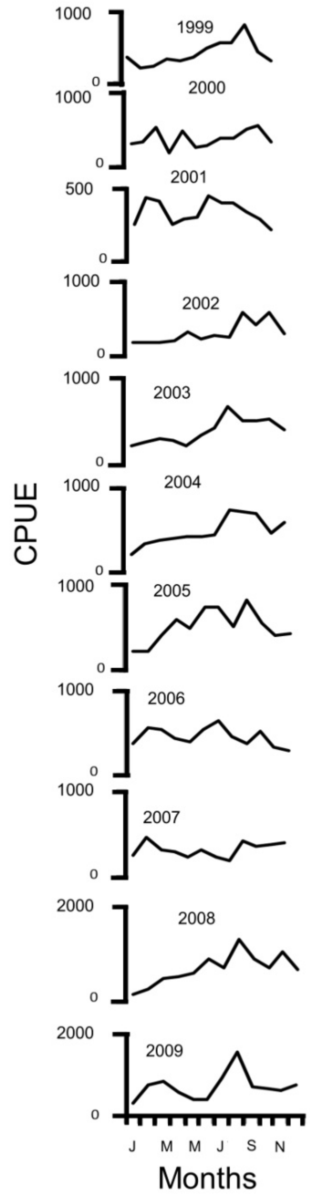

Figure 5. Variation of anchovy production by year and by month along the Togolese coast

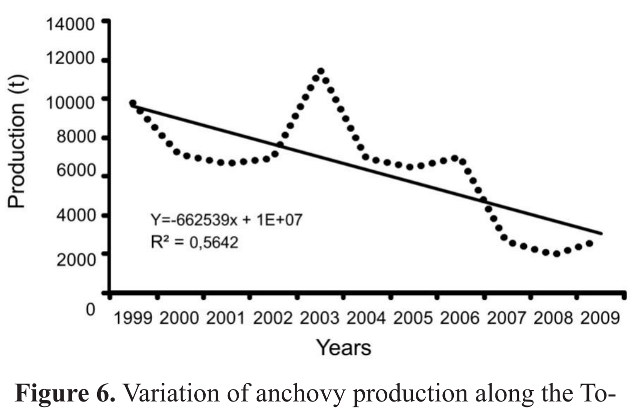

Figure 6. Variation of anchovy production along the Togolese coast between 1999 and 2009

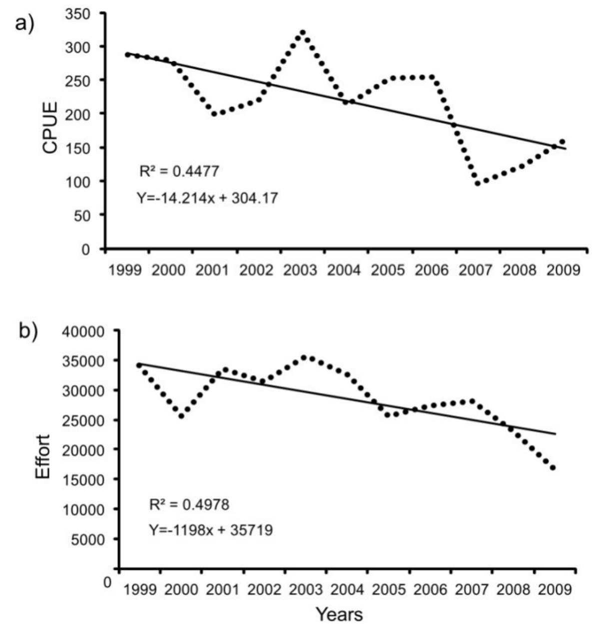

Figure 7. Variation of the CPUE for anchovies (graphic a) and in fishing effort for anchovies (graphic b) along the Togolese coast during the period 1999-2009

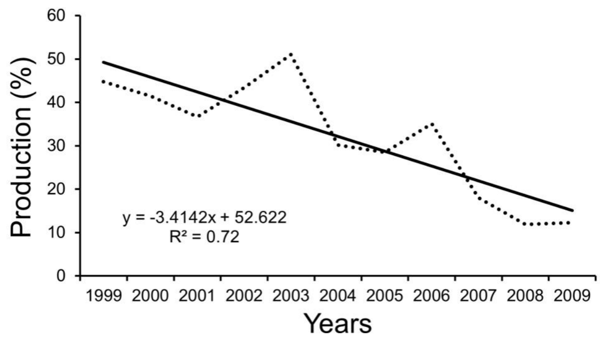

Figure 8. Evolution of the percentage of anchovy production in total production along the Togolese coast during the period 1999-2009

5. Discussion

The total number of fishers along the Togolese coast was estimated to be more than 5,000 in 2002 [7]. However, because fishing is highly seasonal it is difficult to estimate their exact numbers. What is clear is that fishing camps are unevenly distributed with the majority concentrated in the Lomé fishing harbour, which since its creation has facilitated greater access to the sea by reducing the rocky strip that emerges as a result of coastal erosion.

Our surveys revealed a higher percentage of Ghanaian fishers in Togo compared to previous data from [7] where 59.90%, 39.87% and 0.23% of Ghanaian, Togolese and Beninese fishers were reported, respectively. These dif-

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ferences between the two sources can be explained in part by the period of the year during which the surveys were conducted (FAO data being relative to the 1990s and confined to a short period of the year). In fact, although both our data and those of FAO clearly showed that sea fishing in Togo is largely dominated by Ghanaians, they are not permanent residents, so their importance varies from one period to another during the year. Most of them are seasonal fishers who spend around eight months in the country, arriving in Togo in April and returning to Ghana at the beginning of December. Fisher communities along the Togolese coast have been documented as having performed several exoduses and migrations throughout the recent history of the region [19].

We showed that the increase in yield was not correlated with fishing effort, and that the increase in CPUE was mainly due to improved fishing techniques. The latter was due to a rise in the number of bottom and surface gillnets owned by each fisher (up to 16 for an outing of 4 fishers at sea). Additionally, we also observed that a high number of canoes were now motorised; an increase of as much as 18% since 2002 [7]. Perhaps in line with the observed rise in fishing efficiency, our interviewees reported a decline in the number of fishers in some sites. According to some interviewed fishers, this decline was due to the fact that there has been a shift in fish distribution, with stocks now found further offshore. Given the low income typical from fishing, fishers often lack the means to purchase more elaborate equipment (e.g. motors) for boats to move further away from the coast as well as provide for their needs during the off season. Thus, if fishers want to keep up with this changing situation they become insolvent debtors of their consignees. Consignees, mostly women (fish sellers), finance all fishing activities in return for all fishing products. In the 1980s, consignees operated at an estimated interest rate of nearly 70% [21].

Other constraints also weaken the marine fishing sector in Togo. In particular, the lack of adequate regulations and enforcement will not halt the overexploitation of fish stocks, undermining the long-term sustainability of this sector. Hence, without proper guidelines, overexploitation of fish stocks will continue. Moreover, control of the impact of marine pollution from the disposal of phosphate sludge and from sewers needs to be improved.

Our study revealed that the season of highest exploitation of anchovies was July-October. The period coincides with the Ivorian-Ghanaian upwellings that reach the Togolese coasts with the arrival of the cold Benguela sea current (August) [22, 23]. Such upwellings are the main source of enrichment of coastal ecosystems in the Gulf of Guinea apart from the area between Guinea and Liberia where inflows from the rivers also play a significant role in the fishing seasonal cycle [23]. Anchovy productivity is closely related to the coastal upwellings that occur from Cape Palmas to Togo [19].

Various factors are likely to have caused the decline in anchovy production in recent years. These include overexploitation, recruitment processes, environmental and climatic factors. Moreover, since production is dependent on upwellings and the cold Benguela current, any modification of these processes will affect the fish stocks [24]. However, since the overall fish production has not declined, the decline issue is specific to anchovies and possibly to other ecologically similar species. We hypothesize that these massive declines of anchovies across the years may be also due to the sensitivity of these species to changes in the physico-chemical parameters. For instance, [25] believe that the dynamics of pelagic populations in upwelling systems is governed by a series of hydroclimatic processes such as water temperature, environmental richness, ocean circulation which could partly explain the variability of biomass of small pelagics. According to [26], the anchovy can be classified as a species with high reproductive potential but fragile balance. In addition to fishing effort, it is the environmental conditions that can cause a sudden change in its exploitable biomass. It should also be noted that at the global level, negative growth rates were recorded for the main producing countries of Turkey, Ghana, Spain, Morocco, Ukraine and France during the 1995- 2005[27]. According to [27], overexploitation is the main cause for this species' decline, but the drop in the Black Sea production in the 1990s has been attributed to the invasion of *Mnemiopsis* spp.

In economic terms, the production of small-scale maritime fishing in Togo between 1999 and 2009 has fluctuated considerably. The increase in the price of fish is not only the result of the need for more resources (as the population size of Lomé and surroundings has greatly increased in the last decades) but also a stagnant production in the face of increased needs.

The data processed in this study can only briefly explain some of the factors that determine productivity. The sustainability of the exploitation will necessarily involve fishery management mechanisms based on adequate scientific data as well as the development of appropriate monitoring mechanisms for the effective implementation of the policies and regulations put in place. Special attention must be given to this sector not only to ensure the sustainability of the fishing activities but also to preserve regional biological diversity, that may include finding other alternatives such as aquaculture. Unfortunately, the current state of research on exploited species and the lev-

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els of exploitation are not particularly well developed in this part of Africa. If the results of this study indicate that the drop in anchovy production is due to overexploitation, many questions remain unanswered regarding the currently prevailing environmental and climatic conditions that may influence the recruitment of the anchovies.

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ARTICLE

Mitochondrial Haplotypes suggest Genetic Component for Habitat Preference in Blue Crabs

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ABSTRACT

Atlantic blue crabs (*Callinectes sapidus*) are ecologically and commercially fundamental. Life stages are punctuated with migration. Adults and juveniles live in estuaries and sounds. Larval stages develop in the coastal ocean. Juvenile and adult crabs occupy habitats from high salinities to fresh water. We determined whether maturing juvenile and adult blue crab habitat use is reflected in mitochondrial cytochrome oxidase 1 haplotypes. High salinity crabs had lower haplotype diversity (0.7260 ± .03900) compared to spawning crabs (0.9841 ± .00021) and low salinity crabs (0.94154 ± .00118). Significant pairwise differences in haplotypes were found between high salinity and spawning crabs (Nm = 0.26018, $p < 0.001$), and between high salinity and low salinity crabs (Nm = 0.19482, $p < 0.001$) indicating a lack of gene flow. Crabs from high salinity had highly significant genetic differentiation compared to spawning crabs (Fst = 0.11830, $p < 0.001$) and low salinity crabs (Fst = 0.09689, $p < 0.001$). Results support the hypothesis that genetics influence habitat selection. Crab larvae mix in the coastal ocean but occupy specific habitats upon return to sounds and estuaries. These findings have implications for the management of fisheries.

1. Introduction

The Atlantic blue crab (*Callinectes sapidus*) is an ecologically and commercially fundamental species whose life cycle is punctuated with migrations [1]. Juveniles and adults reside throughout estuaries. Mature females store sperm and migrate to higher salinity waters [2-5] to extrude and brood multiple clutches of eggs [6-8]. When eggs hatch around the time of the nocturnal high tide, they release zoea in sounds, mouths of estuaries, and the coastal ocean [9-11]. Zoeal behavior delivers them to the coastal ocean [12] where they spend 4 to 7 weeks [13] or more, while mixing with other larvae, molting multiple times, and metamorphosing to megalopa. Megalopal behavior carries them from the coastal ocean back into sounds and estuaries [14-16].

The presence of odors of vegetation and macroalgae in sounds and estuaries initiates metamorphosis in megalopa. Seagrass and oyster beds serve as critical, structural habitats for juvenile crabs [7,8,17-19]. As they maneuver closer to the mouths of estuaries, environmental cues influence crabs to move upward during nocturnal flood tides and rest near the bottom during other phases allowing for selective tidal-stream transport [8,20,21]. Blue crabs have an expansive range of nursery habitats to select from during their migration inland [1,22]. Most juvenile crabs

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migrate further up estuary [11]. However, some crabs remain throughout sounds and estuaries, including near the mouths of inlets [4,6,,21,23,24].

The physical, chemical, and biological characteristics of estuarine habitats vary greatly. Blue crabs can select from a variety of habitats displaying a wide range of environmental conditions [20]. Crab movement behavior is influenced by environmental cues and ultimately determines where crabs live. Juvenile stage and adult crabs move with the flow [25]. This means that crabs can occupy any habitat where flows go in two directions. In North Carolina, final habitat selection can vary from ~0 to > 35 PSU. Habitat at 0 PSU is ecologically and physiologically different than habitat at 35 PSU. Blue crab juveniles and adults are capable of hyper- and hypo-osmotic regulation [13]. Since genetic diversity within blue crab populations is known to be very high, habitat selection may help explain some of this genetic variation [26,27]. Crabs in our hypothetical scenario would migrate to habitats that suited their genetic make-up. Here we use salinity as a proxy for the differences in habitats.

We chose to use the mitochondrial-encoded cytochrome oxidase I (COI) to compare genetic diversity. Mitochondrial markers are often used to examine population origins and interrelatedness due to their maternal inheritance, rapid rate of evolution, and low rate of recombination [28]. Using COI, Darden reported reduced gene flow along the western Gulf of Mexico [29]. COI was also used to evaluate the variation and genetic structuring of blue crab populations on the east coast of the US [24,30]. These authors reported minimal geographic structuring and provided evidence that there was a dramatic increase in the population after the last ice age.

Blue crabs have a high level of diversity in COI haplotypes [26]. The only concern with using COI is the abnormally high levels of heteroplasmy that have been found in blue crabs [25]. Since there is routinely a dominant mtDNA type, the COI sequences can still be used for studies like the one proposed here [25]. We compared crabs resident in two habitats with differing salinity levels as well as spawning (aka. migratory) crabs representing the entire estuary.

2. Materials and Methods

2.1 Data Collection

Sampling of high salinity crabs was conducted at low tide on Bird Shoal (~29-35 PSU), part of the Rachel Carson Reserve in Beaufort, North Carolina. Only late stage juvenile crabs that were preterminal molt, and mating female-male pairs were used, because they were known to have grown up at high salinity [21,22]. Pressure was applied near the merus-basis joint of the fourth leg on the right side of each crab to trigger limb autotomy. After sampling, crabs were returned to the environment. If a crab was already missing its fourth leg on the right side, it was assumed that it had already been sampled. Each leg was stored individually with 95% ethanol. Tissue was removed from each crab leg for DNA extraction.

We used data collected by the same methods described in this section for samples from 26 female crabs that had grown up in Lake Mattamuskeet (~0-3 PSU) and 28 spawning females from the Rachel Carson Reserve. These crabs were known to be spawning since they carried a visible egg mass. Though they were collected from the same location as the resident crabs, the spawning crabs are representative of the entire watershed (0-35 PSU) and were collected as they were migrating to release clutches of eggs [8,12,21].

2.2 DNA Extraction, PCR, and Sequencing Procedures

DNA was extracted from tissue samples using the Wizard® Genomic DNA Purification Kit (Promega, USA). Broad spectrum primers (LC0I/HCOI) were used to amplify a 710 base pair fragment of the cytochrome c oxidase I (COI) gene encoded in the mitochondrial genome (Folmer et al., 1994). A total volume of 20 µl was used for each polymerase chain reaction (PCR), including 0.2 µl MyTaq™ HS DNA polymerase (Bioline), 2 µl 10x reaction buffer, 1.6ml 25mM $MgCl_2$, 1.6ml dNTPs (2.5mM), 1 µl of each primer (10mM), and 2 µl DNA template. PCR products were analyzed using agarose gel electrophoresis in Tris-acetate-EDTA buffer, stained with GelRed®, and visualized in UV light. Once it was ensured that the COI fragment was successfully amplified, samples were purified using Exonulcease I and Antarctic Phosphatase (New England Biolabs). Further DNA sequencing services were performed by Eurofins Genomics LLC (Kentucky, USA). Processed sequences were edited and aligned using Codon Code Aligner 9.0.1 with MUSCLE algorithms [31].

2.3 Data Analysis

The program DnaSP 5.10.01 was used to calculate the haplotype and nucleotide diversity, variable sites, nucleotide divergence, nucleotide differences, and the net genetic distance between sample groups [32]. Molecular variance was analyzed to examine the population and subdivision structure. Arlequin 3.5.2.2 was used to compute the Fst statistics, conduct neutrality tests, and report shared haplotypes between the sample groups [33,34]. For the Fst statistic, a pairwise Fst significance test was conducted through nonparametric permutation with 1,000 data permutations. PopArt 1.7 was used to create non-parsimonious (TCS) haplotype networks [35].

3. Results

From the high salinity sequences 26 were female and 69 (73%) were male. Though herteroplasmy was common, levels were low and did not interfere with interpretations. These CO1 sequences were compared to 26 female sequences from low salinity (Lake Mattamuskeet) and 28 spawning female sequences (Bird Shoal). Our data set was comprised of a total of 244 COI sequences. After alignment, there was a full overlap of 552 base pairs from all three locations.

3.1 Haplotype Distribution

For all 149 samples, sequence analysis revealed 56 variable sites, 24 singleton variable sites, and 54 haplotypes. The total haplotype diversity was 84.4% and the total nucleotide diversity was 0.55%. Haplotype diversity was 76.62% for high salinity females, 71.70% for high salinity males, 94.25% for low salinity females, and 98.411% for spawning females. Nucleotide diversity was 0.00211% for high salinity females, 0.00288% for high salinity males, 0.01070% for low salinity females, and 0.00933% for spawning females (Table 1). The greatest number of haplotypes was found in spawning females, while the least number of haplotypes was found in the high salinity females.

Table 1. Haplotype statistics for each group sampled. SD = standard deviation

	No. Individuals	No. of Haplotypes	Haplotype Diversity ± SD	Nucleotide Diversity ± SD	Average No. of Nucleotide Differences (k)
High Salinity Females	26	10	0.7662 ± .06700	0.00211 ± .00038	1.163
High Salinity Males	69	21	0.7170 ± .00228	0.00288 ± .00046	1.590
High Salinity (both sexes)	95	26	0.7260 ± .03900	0.00266 ± 0.00035	1.466
Low Salinity Females	26	18	0.94154 ± .00118	0.01070 ± .00277	5.905
Spawning Females	28	23	0.9841 ± .00021	0.00933 ± .00198	5.148

Visual inspection of haplotypes by location is informative (Table 2). Haplotypes H3 and H8 were very common in high salinity residents. Haplotype H3 had a frequency of 42.3% in the high salinity female group and 47.8% in the high salinity male group. Haplotype H8 had a frequency of 26.9% in the high salinity female group and 24.6% in the high salinity male group. Out of the 10 haplotypes found in high salinity females, 4 were unique and not shared with any other group. The high salinity males also had 14 out of 21 haplotypes that were unique and not shared with any other group. In low salinity females, H3 and H8 were 11.5% and 23.1% of the total respectively. Thirteen of the total 16 haplotypes in low salinity were unique to low salinity. For spawning females the H3 haplotype was 8% and H8 was 13%. Spawning females had 15 out of the 21 remaining haplotypes that were unique to their group. Thus, of the 54 haplotypes, 48 were only represented in one specific group and 3 out of 54 haplotypes were found in all groups.

Table 2. Distribution of haplotypes in crabs of varying salinities

Haplotype	High Salinity Females	High Salinity Males	Spawning Females	Low Salinity Females	Total
H1	0	0	2	0	2
H2	0	0	2	0	2
H3	11	33	2	3	49
H4	0	0	1	0	1
H5	0	0	1	0	1
H6	0	0	1	0	1
H7	0	1	1	0	2
H8	7	17	3	6	33
H9	0	0	1	0	1
H10	0	0	1	0	1
H11	0	0	1	0	1
H12	0	0	1	0	1
H13	0	0	1	0	1
H14	0	0	1	0	1
H15	0	0	1	0	1
H16	0	0	1	0	1
H17	1	0	1	0	2
H18	1	1	1	0	3
H19	0	1	1	1	3
H20	1	1	1	1	4
H21	0	0	1	0	1
H22	0	0	1	0	1
H23	0	1	1	0	2
H24	0	1	0	0	1
H25	0	1	0	0	1
H26	0	1	0	0	1

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H27	1	0	0	0	1
H28	0	1	0	0	1
H29	1	0	0	0	1
H30	1	1	0	1	3
H31	0	1	0	0	1
H32	0	1	0	0	1
H33	0	1	0	0	1
H34	0	1	0	0	1
H35	1	0	0	0	1
H36	0	1	0	0	1
H37	0	1	0	0	1
H38	0	1	0	0	1
H39	0	1	0	0	1
H40	1	0	0	0	1
H41	0	1	0	0	1
H42	0	0	0	1	1
H43	0	0	0	1	1
H44	0	0	0	1	1
H45	0	0	0	1	1
H46	0	0	0	1	1
H47	0	0	0	2	2
H48	0	0	0	1	1
H49	0	0	0	1	1
H50	0	0	0	1	1
H51	0	0	0	1	1
H52	0	0	0	1	1
H53	0	0	0	1	1
H54	0	0	0	1	1
Total	26	69	28	26	149

3.2 Genetic Structure

An AMOVA analysis was conducted to analyze genetic structure. Based on the results, 92.24% of the variation occurred within groups, and 7.76% of the variation occurred among the three separate groups (Fst = 0.07762, $p < 0.001$). Significant, genetic differentiation was observed among the sampled groups. The highest Fst value resulted from the comparison of high salinity males and spawning females (Fst = 0.10037, $p < 0.001$), while the lowest Fst value was found between high salinity males and high salinity females (Fst = -0.01753, $p > 0.05$). The corrected pairwise differences between each group also suggested that there was high genetic differentiation (Table 3). Four of six corrected pairwise tests were significant and were comparisons with high salinity crabs and another group. The highest corrected pairwise difference existed between high salinity males and spawning females (Nm = 0.25207, $p < 0.001$). The two comparisons that were not significant had compared high salinity crabs to each other and low salinity females to spawning females.

Table 3. Fst, genetic variance between the two groups; Nm, corrected average pairwise difference; Dxy, nucleotide divergence; Da, net genetic distance

		Haplotypes Shared	**Fst**	**Nm**	**Dxy**	**Da**
High Salinity Males	**Spawning Females**	7	0.10037***	0.25207***	0.00656	0.00046
High Salinity Males	**Low Salinity Females**	5	0.07969**	0.18583**	0.00713	0.00034
High Salinity Females	**Spawning Females**	5	0.07531**	0.26884**	0.0062	0.00049
Low Salinity Females	**High Salinity Females**	4	0.05503*	0.20580*	0.00677	0.00037
Low Salinity Females	**Spawning Females**	4	-0.00512	-0.02913	0.00996	-0.00005
High Salinity Males	**High salinity Females**	5	-0.01563	-0.01753	0.00246	-0.00003

Notes: P-values: * < 0.05; ** < 0.01; *** < 0.001.

An overall comparison from each sample type (Table 4) shows high salinity crabs had highly significant genetic differentiation when compared to spawning females (Fst = 0.11830, $p < 0.001$) as well as low salinity females (Fst = 0.09689, $p < 0.001$). Significant, corrected average pairwise differences were found between high salinity and spawning females (Nm = 0.26018, $p < 0.001$), as well as between high salinity and low salinity crabs (Nm = 0.19482, $p < 0.001$). Taken together, these statistics indicate that there is significant genetic differentiation between high salinity and low salinity crabs, as well as between high salinity and spawning females. Low salinity females were not significantly different from spawning females, and high salinity males and females were similar.

Table 4. Statistical summary for spawning females, high salinity crabs (both sexes), and low salinity crabs. Fst, genetic variance between the two groups; Nm, corrected average pairwise differences; Dxy, nucleotide divergence; Da, net genetic distance

		Haplotypes Shared	**Fst**	**Nm**	**Dxy**	**Da**
Spawning Females	**High Salinity Crabs**	8	0.11830***	0.26018***	0.00646	0.00047
High Salinity Crabs	**Low Salinity Females**	5	0.09689***	0.19482***	0.00703	0.00035
Spawning Females	**Low Salinity Females**	4	-0.00512	-0.02913	0.00996	-0.00005

Notes: P-values: * < 0.05; ** < 0.01; *** < 0.001.

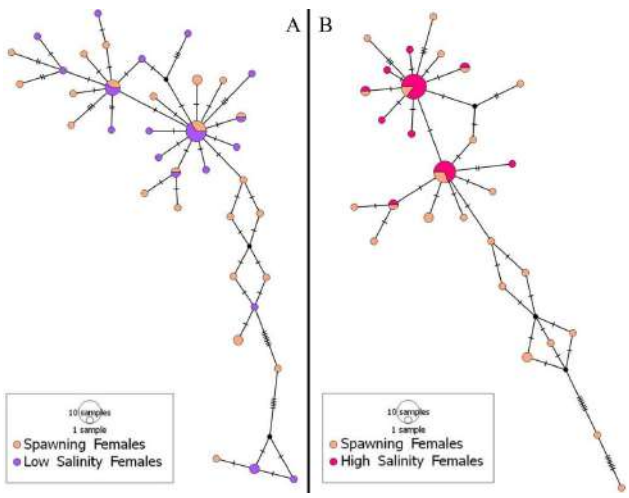

Figure 1. TCS haplotype network demonstrating the relationship between (A) spawning females and low salinity females and (B) spawning females and high salinity females

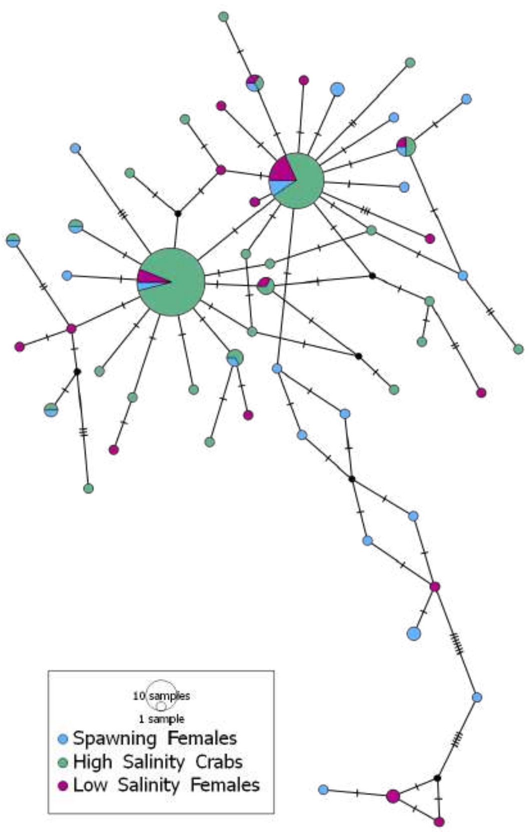

Figure 2. TCS haplotype network demonstrating the relationship between spawning females, high salinity crabs (both sexes), and low salinity females

4. Discussion and Conclusion

This study tested the hypothesis that habitat preference in blue crabs is influenced by genetics. With a knowledge of migration patterns and using salinity as a proxy for habitat, we sampled blue crabs living in different salinities and spawning females that had migrated to high salinity. We sequenced and analyzed a region of the mitochondrial cytochrome c oxidase 1 (COI) gene for genetic differentiation and gene flow. Differences would support our hypothesis.

Spawning females matured in a wide range of habitats and had the highest haplotype and nucleotide diversity. Spawning females arrive at the Rachel Carson Reserve from many watersheds [2]. Many spawning females migrated down the Intracoastal Waterway from the Neuse River. Others are from the Newport River, North River and the southwestern end of the Pamlico Sound. Because of the low number of resident blue crabs where the spawning crabs were sampled, representatives of the local population were rare. Local spawning crabs end up in the coastal ocean when they release their first clutch of eggs because crabs migrate on falling tides with late stage embryos and move offshore through the inlet [1-3]. If our hypothesis is correct, the high haplotype and nucleotide diversity found within the spawning females reflects the large geographic expanse and diversity of habitats. Like the spawning females, the haplotype and nucleotide diversity in the low salinity habitat was high. Juvenile crabs migrate into Lake Mattamuskeet through canals that connect it to the Pamlico Sound and Albemarle Sound [11].

Water control structures on the canals are constructed to only let water out of the lake if it is higher than the water level in the canals. As a management strategy, the refuge slightly opens the gates which enables small organisms such as crabs and elvers to enter the lake in the spring and summer. Once small crabs pass the water control structures into the lake, they are not able to exit whether they prefer the lower salinity or not. The control structures are closed all summer because the lake is used to irrigate crops which keeps the lake significantly lower than the canals. The exception is heavy rains which raise the lake level and cause the structures to open. However, in heavy rains, the crabs become inactive. This setup provides some explanation for why the haplotype and nucleotide diversity is also high within the low salinity lake environment. Though salinity is rarely if ever higher than that 3 PSU, depending upon rainfall, the canal waters can have salinities that range from 0 to over 10 PSU. Once crabs get into the lake they are trapped until fall and are adults by then. In contrast, the haplotype and nucleotide diversity from crabs resident in high salinity were significantly lower. This supports the hypothesis that specific genotypes took up residence less than a mile from the inlet. Thus all the data are consistent.

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Genetic diversity is influenced by population size, immigration rates, and mutation rates. However, the rate of gene flow among populations and the history of ancestral populations directly influence genetic diversity [36]. AMOVA results also support the hypothesis of a pattern of genetic differentiation by habitat origin and significant genetic structuring among the crabs sharing the same habitat location. High genetic differentiation was found between high salinity and spawning crabs, as well as between high salinity and low salinity. Regardless of sex, sequences from high salinity crabs were centered around two major haplotypes. There was a correlation between genetic differentiation and habitat suggesting significant structuring. Structuring is evident within the haplotype maps for the high salinity group. This supports the hypothesis that specific genotypes select high versus low salinity habitats. Significant average pairwise comparisons between high salinity crabs and spawning females, as well as between high salinity crabs and low salinity crabs indicates the lack of strong gene flow among the crabs from those locations. Crabs migrating to these separate locations are acting independently.

This structuring strongly reflects the migration to contrasting environmental conditions since salinity varied greatly per sampling site [11,12]. Thus, a lack of gene flow and high genetic differentiation may be a result of genetic adaptation to different salinity environments. Different salinities pose different kinds of challenges. Previous studies report blue crabs, which are hypersaline regulators [37] expend more energy at lower salinities versus higher salinities. Expending more energy could negatively affect growth rate and result in a decrease in molt increment and/or an increase in intermolt period [38,39]. However, low salinity waters are warmer and nurseries for many prey, offsetting energy losses due to osmotic regulation. Despite the increased energetic costs, crabs from lower salinity waters are routinely larger than those from high salinity [26].

Genetic differentiation by habitat is in stark contrast to the high genetic diversity [24,40] but general lack of strong genetic structuring seen at large regional scales [28] is consistent with our hypothesis. The genetic differentiation found between distinct blue crab habitats supports the hypothesis that there is a genetic component in blue crabs that influences migration and habitat selection and is likely to be reflected in physiological responses. In the future, genome-wide scans should be capable of identifying candidate enzymes responsible for adaptation to the spectrum of habitats found in estuaries and sounds.

5. Implications for Fisheries Management

The high salinity population we sampled was about 73% male, very similar to the low salinity population in Lake Mattamuskeet which was 70% male [25] and very different from the sex ratios in the commercial fishery which is male limited [41-45]. The high salinity population has a minimal recreational fishery as does the low salinity population. Over time, the high salinity crabs should have a reproductive advantage because they are subjected to the least pressure from the fishery. In the long term this habitat isolation could result in sympatric speciation.

Major blue crab fisheries are managed by small and large size cutoffs [42], spawning sanctuaries, migration corridors, restrictions on females brooding eggs and trawling bans [1]. The small size cutoff is accomplished with cull rings which enable small crabs to escape and reduces most the work fishers must do to meet the small size cutoff. Large size cutoffs are ineffective because large crabs are rare and a sufficient percentage of large crabs is allowed. Spawning sanctuaries have become popular and may be effective at increasing the total number of clutches that crabs produce. Deep water migration corridors are an attractive management tool which is hard to implement because of crab foraging behavior. Spawning females migrate in the corridors but stop to forage in shallow water in the midst of the fishery [3,4]. Some states restrict taking of brooding females. Especially in the warm summer months, pot stress causes females to damage or remove their egg masses [3]. Bans on trawling seem effective especially when the trawled crabs are spawning females that have run the gauntlet of the fishery.

If we are correct that blue crabs mix genotypes in the coastal ocean and then occupy different habitats in sounds and estuaries based upon their genotypes, then this could set the stage for sympatric speciation over a long time period. There could be profound impacts due to fisheries pressure over time as well. For example, all spawning crabs must move back to high salinity to hatch eggs and release larvae. Genotypes of crabs migrating to fresh water are at a disadvantage because they have to return through the entire fishery to spawn. Crabs resident in spawning sanctuaries would experience no fishery pressure. This is supported by the 3:1 male to female sex ratio we found in the high salinity crabs which is similar to that of Lake Matamuskeet [25] which also only has a recreational fishery.

Changes in fisheries regulations threaten human sub-cultures and livelihoods. Historically, as fisheries crash due to ineffective management, fishers go out of business. Instead of discarding skilled fishers and eliminating their way of life, it would be an interesting social experiment to support impacted fishers and protect livelihoods until effectiveness of new regulations to the fishery is demonstrated.

6. Conclusions

On the east and Gulf of Mexico coasts of the United States blue crabs are predators, prey, and important to commercial and recreational fisheries. Crabs mature and mate in sounds and estuaries. Larvae develop in the coastal ocean. The last pre-juvenile stage, the megalopa returns to sounds and estuaries and metamorphoses to a juvenile which migrates variable distances from less than a kilometer from an inlet to hundreds of kilometers to fresh water. We hypothesized there that there is a genetic component to habitat selection. We tested the hypothesis using CO1 haplotypes and high salinity, low salinity and spawning crabs. Analysis supports our hypothesis. This finding has potential for fisheries management as fisheries pressure is most intense on females crabs returning from fresh water through the fishery to spawn in high salinity water, next most intense on crabs that comprise the majority of the fishery in mid salinities and least intense on essentially nonmigratory crabs resident near inlets. If our hypothesis is correct fisheries managers should consider practical ways to level the playing field.

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