

**FISH GILL HISTOPATHOLOGY OF SAPSAP (*Secutor insidiator*) ON
BITO-ON-JARO-MOUTH OF THE FLOODWAY, ILOILO FLOOD CONTROL**

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Fish Gill Histopathology of Sapsap (*Secutor insidiator*) on Bito-on-Jaro-Mouth of Floodway, Iloilo Flood Control

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ABSTRACT

Floodwater carries materials, like sediments, which pose a significant threat to the marine ecosystems around the world. This study aimed to gather baseline information on the fish gill histopathology of *Secutor insidiator* and the water quality of the Bito-on, Jaro-mouth of Iloilo Flood Control. Total suspended solids, salinity, water temperature and pH of the water were measured. There were 3 sampling areas with 3 replicates. Five-hundred mL water samples were collected and were placed in empty distilled water bottles. The gill histopathology was described. There were 5 samples of fish in which 5 replicates of the fishes were used for gill histopathology tests. The amount of total suspended solid in the water is from 0.0402 g to 0.0744g. The salinity of the water ranged from 25ppm to 28ppm. The pH levels ranged from 7.83 to 7.93. The water has temperature that range from 28.6°C to 29.1 °C. Salinity and TSS levels were below the normal range but pH and temperature of the water were normal. The most common gill abnormality is the clumping of the cells or hyperplasia in the secondary lamellae. Thickening of primary lamellae, swelling of gill arch, curling and thickening of secondary lamellae were also observed. The present study has shown that *Secutor insidiator* exhibit gill lesions even before the implementation of the Iloilo Flood Control Project. Water quality is not the only factor that could affect fish gills. Further studies are needed to establish what specifically caused the gill lesions aside. Factors like ammonia, nitrite, dissolved oxygen and other pollutants should be further investigated.

Keywords: gill histopathology, *Secutor insidiator*, Iloilo flood control project, water quality, stress

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CHAPTER 1

INTRODUCTION

A. Background of the Study

The Philippines has an average of 19 typhoons every year, causing intense rainfall, overflowing of waterways, inundations, and deposition of sediment in the flood plain and extensive flood damages in the form of flashfloods, mud slides and soil erosion (Villalorenza and others 2004).

An aggregate of about 1,316,260 ha nationwide is susceptible to flooding. About 62% of the area is located in Region 6. With the present rate of forest destruction and denudation, the Philippines will experience frequent devastating floods during the rainy season and scorching heat during the dry season (Villalorenza and others 2004).

The Iloilo flood control project was created in response to the recurring flooding problem in Iloilo city, which usually affects around 80 percent of the barangays, three to four times yearly. The project also includes the raising of river banks, stretching the alignment of rivers, and excavation of connecting river channels to ensure smooth flow of water. The floodway, one aspect of the Flood Control Project, will be carrying floodwater down the Iloilo-Guimaras Strait. With this floodwater, the aquatic environment can be affected.

Flood water is composed of water that overflowed from ponds, rivers, canals and other bodies of water. It also carries human wastes and a wide range of toxic substances. Sediments occur naturally and are normal components of aquatic systems. Furthermore, sediments can lead to a decline in the water quality and biodiversity of aquatic organisms. These materials that the flood water carries pose a significant threat to marine ecosystems around the world.

For the past decades, there had been a diverse range of studies on the effects of sediments, turbidity, and fresh water to fishes. All of which are factors that altered their natural habitat thus contributing to stress.

There are many reasons why stress in fishes must be determined. Stress in fishes act as a bio-indicator if there are certain changes in the environment that affects their tolerance level. The presence of stress, associated as a result of changes in water quality, can lower the resistance of certain fish species to pathogens (Redding and others 1987).

Suspended sediments can affect fishes in different ways. Exposure to suspended sediments causes gill damage as stated by Noggle(1978) in his study. Sediments can also bind with chemical contaminants which may affect the organisms in the area. Too much sediment can also make the water cloudy and may not allow some of the sunlight to pass through it, which may affect the organisms found in it.

A study conducted by Gregory(1994) stated that smaller fishes(49-55 mm, in fish length) prefer to forage in clearer water than bigger individuals(57-69 mm FL). This implies that an increase in the turbidity of an environment could affect the kind and/or size of fishes that can be found in that particular area. Several studies conducted by Lloyd and others(1987)that salmonid fishes exposed to turbidities of 25-70 NTU can impair their ability to find and capture food.

Due to the growing awareness of the environmental issues today, there is a need to conduct researches to know the effect of implementations of human innovations which may damage the natural resources. The study aimed to describe gill histopathology of fishes and assess the water quality in the Bitoon, Jaro,-mouth of Iloilo Flood Control Project.

B. Statement of the Problem

The study aimed to gather baseline information on the fish gill histopathology of Sapsap(*Secutor insidiator*) found on the the Bito-on-Jaro-mouth of Iloilo Flood Control.

C. Objectives

General Objectives

To gather information on the gill histopathology of Sapsap (*Secutor insidiator*) found in the mouth of the Iloilo Flood Control

Specific Objectives

1. To evaluate the gill histopathology of fish by describing the presence of gill lesions
2. To describe the water quality parameters of three water sampling stations by measuring the:
 - a. total suspended solids
 - b. salinity
 - c. water temperature
 - d. water pH

D. Significance of the Study

The opening of the Iloilo Flood Control Project can bring different impacts to the environment and the aquatic organisms. Water from streams and rivers is used for drinking, irrigation, waste dilution, power generation, transportation and recreation and provides habitat for fish and other aquatic organisms. This water may contain sediment (e.g., eroded soil particles), which can be either suspended in the water or deposited on the bottom (Huggins and others, ND).

Sediments poses a significant threat to stream ecosystems around the world, and increases in bedload can be especially detrimental to benthic communities (Schofield 2004). Sediments can cause lesions to the gills of fishes. These fishes are one of the sources of food by the people living in the area. Sediment and turbidity can result in a variety of harmful impacts to fish. Some of the negative effects of turbid water are: abrasion of gill membranes, impairment of feeding, fatal impacts to small aquatic animals that are food for fish (FOC 2009).

The objectives of the study can provide benefits to the fisherman in the area as well as the people living there. The government could be warned once the results would show that the implementation and opening of the Iloilo Flood Control pose the possible threats to the environment.

D. Scope and Delimitation

The study was conducted at the mouth of Iloilo Flood Control, Bitoon, Jaro. Data gathering was done during the months of September and October. The study was conducted on the mouth of the Bito-on River for species diversity. Only few water quality assessment parameters were chosen because of the lack of instruments and time. Parameters such as fecal organic content, and others were not included. Fish gill samples was sent to the BFAR (Bureau of Fisheries and Aquatic Resouces) and SEAFDEC laboratories for further analysis because the school (PSHSWV) can not provide all the materials needed for the tests. In determining the salinity, a refractometer, provided by the school, was used. It is cheaper than other salinity meters.

In this study, the time of sampling is very important because parameters like temperature are affected by the sun's rays. The water becomes warmer as the sun rose higher in the sky and so its rays strike the water directly thus making the water warmer.

Thus, it is critical to establish well the baseline for the species and condition in which the assessment is being made. A changing baseline can have significant implications in determining whether a state of stress exists. Thus, stress can exist when the measured variable does not change, or have patterns that are not obvious without this baseline reference.

F. Definition of Terms

Gill - the respiratory organ of most aquatic animals that breathe water to obtain oxygen, consisting of a filamentous structure of vascular membranes across which dissolved gases are exchanged (Answers 2011)

Gill Trauma - caused by the increased concentration of the suspended sediments. The primary mechanisms of the action are through physical abrasion of the tissue and particle adsorption onto the gill.

The types of tissue changes observed include swelling of secondary lamella and hypertrophy of the epithelial cells, hyperplasia of gill tissue and the tissue necrosis (Wikipedia 2010)

Hyperplasia - general term referring to the proliferation of cells within an organ or tissue beyond that which is ordinarily seen. Hyperplasia may result in the gross enlargement of an organ and the term is sometimes mixed with benign neoplasia/ benign tumor (Wikipedia 2010)

Iloilo Flood Control Project - the billion-peso project funded by the Japan Bank of International Cooperation (JBIC) and is expected to be completed in 2010. The project comes in two packages, the first package being undertaken by the China Waters and the second one by the Hanjin Industries Inc. The package includes the construction of Jaro Floodway which is now 50.64 percent completed. The floodway covers construction of the Pagsanga-an, Anilao, Tacas, Balabago, Buhang and Bitoon Bridges and the Jaro floodway itself. Also included in the package are the improvements of the Aganan River, which was 6.020 percent completed and the Tigum River improvement pegged at 4.97 percent. Projects under Package I are set to be completed on July 11, 2010 (News Today 2009).

Lamella - a thin plate-like structure in the fish gills, often one amongst many lamellae very close to one another, with open space between. It is for filter feeding and serves as chloroplast membranes (Wikipedia 2010)

Lesions - any pathological or traumatic discontinuity of tissue or loss of function of a part (Dorland's Medical Dictionary for Health Consumers 2007)

Salinity - the presence of soluble salts in soils or waters. It is a general term used to describe the presence of elevated levels of different salts such as sodium chloride, magnesium and calcium sulfates and bicarbonates, in soil and water. It usually results from water tables rising to, or close to, the ground surface (Lewis and others 1980)

Stress - includes conditions that force an organism's body organs to work harder to keep it alive (Wikipedia 2011)

Total Suspended Solids - a water quality measurement usually abbreviated TSS. This parameter was at one time called non-filterable residue (NFR), a term that refers to the identical measurement: the dry-weight of particles trapped by a filter, typically of a specified pore size (Wikipedia 2010).

Water temperature - measures the temperature of water or other medium (Ambient Weather 2011).

CHAPTER 2

REVIEW OF RELATED LITERATURE

A. Flood, Erosion and Sediment Transport

The adding up of sediment into water has been known to be a threat to the aquatic organisms. While natural seasonal variations in sediment levels occur, the input of sediment through catastrophic events such as volcanic eruptions and floods, and human activities such as road building, mining etc., have the potential to harm aquatic organisms and reduce biological productivity (Goldman and others, 1986).

Erosion is a natural process but man's activities greatly increase the amount and rate of soil erosion. Erosion and the resultant input of soil particles (sedimentation) to streams is the main concern for the fish farmers. Soil high in silt and fine sand and low in clay and organic matter are generally the easiest to be eroded, while the drained sandy and rocky soils are the least to be eroded. The rate of erosion depends on climate, geology, exposure, slope, soil type and vegetation cover.

Rivers naturally carry soil, sand and sediment along with them as they flow. These particles that the rivers transport play an important role in the estuarine environment.

The US Geological Survey (2009) stated in their study that when there is rain soil and debris from the surrounding land are eroded and washed into streams and rivers. From there, sediment particles form as small as clay to as large as boulder flow along with the water. They further stated that fast-moving water can pick up, suspend, and move larger particles more easily than slow-moving water. This is why rivers are muddy-looking during storms for they carry a lot more sediments than when they are during their low-flow period. In fact, so much sediment is carried during storms that well over one-half of all the sediment moved during a year might be transported during a single storm period.

The Water and Rivers Commission of Australia (2000) added that the stream or river channel can be considered to be relatively stable when its water flow and sediment flux are in balance over a period of time. If there is a change in either of these two factors, then the channel will adjust its slope, depth, width, meander pattern, bed composition and vegetation density accordingly.

They also described that the transport of surface water in streams as occurring within a two-stage "channel" system. These include the "bankfull" channel, which has the capacity to carry flood flows that occur, on average, once every one to two years and the low flow channel which carries minor flows between floods that are generated by groundwater seepage along the river valley. There is also the floodplain, onto which floodwaters spill out when a flow exceeds bankfull capacity. Changes to streams predominantly occur when the channel is full of water. This is the level at which the water has the

maximum power to move a sediment. When this volume exceeded, the water rises above the level of the channel and spills onto the flood plain. It dramatically reduces the average stream flow velocity and consequently its power. As the power of the water is reduced, so too is its capacity to carry sediment and as consequence large quantities of sediment are often deposited on the floodplain during flood events.

B. Sediments

According to the Ontario Ministry of Natural Resources (1992), sediments are generally classified by particle size. Its three major classes are sand, silt and clay. The size of the particle and the water velocity determine how the particle is transported. In rivers and creeks, sediment exists in two forms, either as suspended or deposited material (WRCA 2000). Coarse particles, like sand and gravel, usually move by rolling along the stream bottom as part of the bedload. Thus their concentrations are low in the water column. On the other hand, silt and clay are usually transported in suspension, and their concentrations are usually uniformly distributed throughout the water column. Fast-flowing water usually carries fine silt or clay particles to greater distances as suspended sediments before settling.

If the water velocity is less than the soil particle's settling velocity, then the particle becomes bedload sediment as it slides, rolls or bounces along the bottom. Bedload sediment may fill in the interstitial spaces or crevices between spawning areas, suffocating the eggs or fry (Hall and Lantz 1969).

The damage done to the aquatic organisms by the increased suspended sediment levels is a function of the concentration of sediments in water (mg/L) times the duration of the organism's exposure to them in hours (Newcombe and MacDonald 1991). A concentration of 25 mg/L of total suspended solids shouldn't harm fishes or its habitat (DFO 1983) but these low concentration must not last for weeks for rubble/gravel areas will rapidly become silted (Carling 1984).

In estuarine waters, a substantial portion of suspended sediments come from the resuspension of fine, unconsolidated sediments and detritus by wave action and currents (Appleby and Scarratt 1989).

C. Water Quality

Estuaries are areas of constant change due to the varying meteorological and hydrographic factors that regulate the physicochemical properties of estuarine waters and sediments (Mark and Hartl 2002). There are many parameters that can be use to assess estuarine water quality. These include pH, turbidity, salinity, dissolved oxygen concentration, temperature etc.

In estuaries, especially those exposed to strong tidal action, parameters such as pH, salinity, oxygen concentration and temperature fluctuate. These are important abiotic factors dictating not only the chemical 'species', but - together with the aqueous solubility of the compound, redox, affinity for

sediments, organic carbon content and sediment mineral constituents- the sorption behavior and in turn the bioavailability of many pollutants (Chapman and Wang 2001).

C.1. Total Suspended Solids

TSS are solid materials, including organic and inorganic, that are suspended in the water. These would include silt, plankton and industrial wastes. High concentrations of suspended solids can lower water quality by absorbing light. Waters then become warmer and lessen the ability of the water to hold oxygen necessary for aquatic life. Because aquatic plants also receive less light, photosynthesis decreases and less oxygen is produced. The combination of warmer water, less light and less oxygen makes it impossible for some forms of life to exist. (Lewis 1973)

Suspended solids affect life in other ways. They can clog fish gills, reduce growth rates, decrease resistance to disease, and prevent egg and larval development. Particles that settle out can smother fish eggs and those of aquatic insects, as well as suffocate newly-hatched larvae. The material that settles also fills the spaces between rocks and makes these microhabitats unsuitable for various aquatic insects, such as mayfly nymphs, stonefly nymphs and caddisfly larva. (Appleby and Scarratt 1989)

Suspended solids can result from erosion from urban runoff and agricultural land, industrial wastes, bank erosion, bottom feeders (such as carp), algae growth or wastewater discharges. Prevention methods include protection of the land in our watershed from erosion by use of conservation tillage measures and giving urban runoff time to settle out before reaching our surface waters. (WRCA 2000).

C.2. Salinity

Salinity is the presence of soluble salts in soils or waters. It is a general term used to describe the presence of elevated levels of different salts such as sodium chloride, magnesium and calcium sulfates and bicarbonates, in soil and water. It usually results from water tables rising to, or close to, the ground surface. (Wikipedia, accessed 2010)

The occurrence of salinity depends on several factors, the most important of which are the characteristics of the landscape, the climate and the effects of human activities. An understanding of water movement associated with the salt-affected area is needed to determine the likely extent of any problem (WRCA 2000).

The sources of these salts are rainfall, which carries low concentrations of salts that have accumulated in our landscapes over thousands of years, weathering and erosion of surface rocks and groundwater that has soaked through sediments and sedimentary rocks that originally formed in salty marine environments. The amount of salts that accumulate in soil depends on the properties of the soil and rainfall, with clay soils generally having the highest levels. (WRCA 2000).

Water salinity impacts on plants and animals to varying degrees depending on their salinity tolerance levels. The use of the water sampled determines the acceptable salt level. An excess of one or more salts can have detrimental impacts. For example, water can be moderately saline, but have a high concentration of specific ions such as chloride, sodium or magnesium. (Wikipedia, accessed 2010)

The normal salinity level of seawater is usually 35 parts per thousand or 35 ppt. This salinity measurement is a total of all the salts that are dissolved in the water. Although 35 parts per thousand is not very concentrated (the same as 3.5 parts per hundred, o/o, or percent) the water in the oceans tastes very salty. The interesting thing about this dissolved salt is that it is always made up of the same types of salts and they are always in the same proportion to each other (even if the salinity is different than average). (Mitsch and Gosselink, 1986)

C.3. Water Temperature

High water temperatures stress aquatic ecosystems by reducing the ability of water to hold essential dissolved gasses like oxygen. The temperature of water has extremely important ecological consequences. Temperature exerts a major influence on aquatic organisms with respect to selection/occurrence and level of activity of the organisms. In general, increasing water temperature results in greater biological activity and more rapid growth. All aquatic organisms have preferred temperature in which they can survive and reproduce optimally. Fish and most aquatic organisms are cold-blooded. Consequently, their metabolism increases as the water warms and decreases as it cools. Each species of aquatic organism has its own optimum (best) water temperature. If the water temperature shifts too far from the optimum, the organism suffers. Cold-blooded animals can't survive temperatures below 0 °C (32 °F), and only rough fish like carp can tolerate temperatures much warmer than about 36°C (97 °F). (Swann, L 2000).

Fish can regulate their environment somewhat by swimming into water where temperatures are close to their requirements. Fish can sense very slight temperature differences. Studies also show that when temperatures exceed what they prefer by 1-3°C, they move elsewhere. (Swann, L 2009)

Fish migration often is linked to water temperature. In early spring, rising water temperatures may cue fish to migrate to a new location or to begin their spawning runs. The autumn drop in temperature spurs baby marine fish and shrimp to move from their nursery grounds in the estuaries out into the ocean, or into rivers, as the case may be. As you can see, all sorts of physiological changes take place in aquatic organisms when water temperatures change. (Wikipedia accessed 2010).

Warm water also makes some substances, such as cyanides, phenol, xylene and zinc, more toxic for aquatic animals. If high water temperatures are combined with low dissolved oxygen levels, the toxicity is increased.

C.4. Water pH

Water pH is a measure of the acidity or basicity of a solution. Most fish can tolerate pH values of about 5.0 to 9.0, but serious anglers look for waters between pH 6.5 and 8.2. When acid waters (waters with low pH values) come into contact with certain chemicals and metals, they often make them more toxic than normal. As an example, fish that usually withstand pH values as low as 4.8 will die at pH 5.5 if the water contains 0.9 mg/L of iron. According to Carver G. and others (1994), it is well-established that levels of pH fluctuate throughout the day and season, and a single pH measure during the day may not draw a very accurate picture of long-term pH conditions in the estuary. Photosynthesis by aquatic plants removes carbon dioxide from the water; this significantly increases pH. A pH reading taken at dawn in an area with many aquatic plants will be different from a reading taken six hours later when the plants are photosynthesizing. Likewise, in waters with plant life (including planktonic algae), an increase in pH can be expected during the growing season. For these reasons, it is important to monitor pH values at the same time of day if you wish to compare your data with previous readings. It is also important to monitor pH values over a long period of time to provide useful data. The actual time to measure pH will depend on local conditions and the monitoring goals of the volunteer program.

The pH of sea (salt) water is not as vulnerable as fresh water's pH to acid wastes. This is because the different salts in sea water tend to buffer the water with Alka-Seltzer-like ingredients. Normal pH values in sea water are about 8.1 at the surface and decrease to about 7.7 in deep water. Many shellfish and algae are more sensitive than fish to large changes in pH, so they need the sea's relatively stable pH environment to survive. (Carver, G. 1994)

Shallow waters in subtropical regions that hold considerable organic matter often vary from pH 9.5 in the daytime to pH 7.3 at night. Organisms living in these waters are able to tolerate these extremes or swim into more neutral waters when the range exceeds their tolerance. (Carver, G. 1994).

Water's pH is affected by the minerals dissolved in the water, aerosols and dust from the air, and human-made wastes as well as by plants and animals through photosynthesis and respiration. Human activities that cause significant, short-term fluctuations in pH or long-term acidification of a waterbody are exceedingly harmful. For instance, algal blooms that are often initiated by an overload of nutrients can cause pH to fluctuate dramatically over a few-hour period, greatly stressing local organisms. Acid precipitation in the upper freshwater reaches of an estuary can diminish the survival considered as indications of industrial pollution or some cataclysmic event. Likewise, a long-term database on alkalinity values provides researchers with the ability to detect trends in the chemical makeup of estuary waters.

Several other factors also determine the pH of the water, including: bacterial activity; water turbulence; chemical constituents in runoff flowing into the waterbody; sewage overflows; and impacts from other human activities both in and outside the drainage basin (e.g., acid drainage from coal mines, accidental spills, and acid precipitation).

According to the study of *Carver and others (1987)*, the pH of water is critical to the survival of most aquatic plants and animals. Many species have trouble surviving if pH levels drop under 5.0 or rise above 9.0. Changes in pH can alter other aspects of the water's chemistry, usually to the detriment of native species. Even small shifts in the water's pH can affect the solubility of some metals such as iron and copper. Such changes can influence aquatic life indirectly; if the pH levels are lowered, toxic metals in the estuary's sediment can be resuspended in the water column. This can have impacts on many aquatic species.

D. Physiology of Fishes

Physiological changes can be measured in fish as a response to the increased stress of suspended sediments. It could be established through the histopathology of fish gills and the alterations in its blood physiology. (Wikipedia accessed 2010)

For the histopathology of gills, the typical measured responses include impaired growth, histological changes to gill tissue, alterations in blood chemistry, and an overall decrease in health and resistance to parasitism and disease. When compared to sediment exposure events that elicit behavioral response, longer exposure periods and/or higher concentrations are generally required before physiological responses are expressed. In this respect, physiological responses are more chronic effect. (Anderson 1996)

A fish's gills are situated one set on either side of the body and near the back of the head. They are open to the gullet at the front, and open to the external environment behind. They are designed so that water can flow continually passed them, coming in through the mouth, and/or the spiracle in sharks and their allies, and passing out through the single external gill opening in fish or through one of the 5 to 7 gill clefts in sharks and rays. In fish there is a bony plate protecting the gills, this is called the operculum, and it is hinged and has muscles attached to it so it can be regularly opened and closed. (Appleby, J and Scarratt, D. 1989)

In sharks and rays the number of gills is usually 5, but there are some species with 6 or 7 sets, in fish the number of gills is 4 on either side of the body. Each gill is supported by a gill arch and protected by gill rakers. Each gill arch supports one set of paired gill filaments. The gill rakers help make sure that no extraneous material gets into the gill filaments to clog them up. Each paired gill filament in turn supports numerous lamellae (sing. lamella), extending out from both sides of the filament body. It's here in the lamellae that the uptake of O^2 actually occurs.

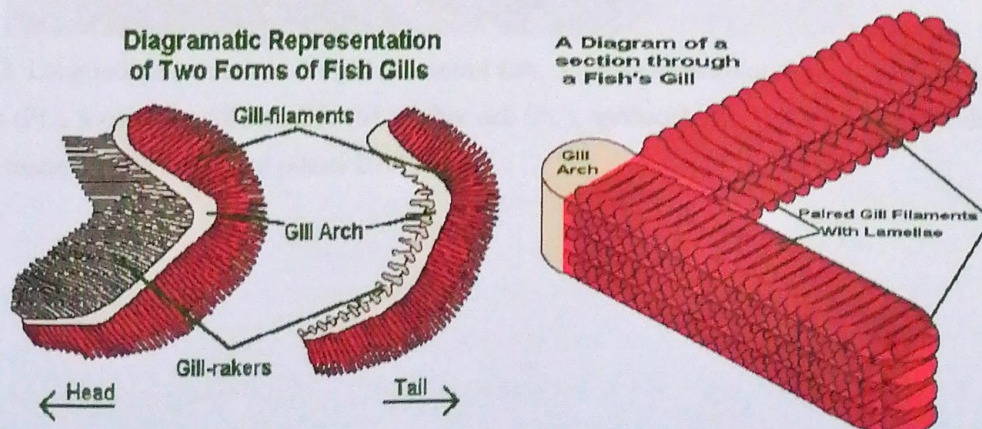


Figure 1. Diagrammatic Representation of Two Forms of Fish Gills (Earthlife 2011)

The lamellae are very fine structures, however their exact dimensions depend on the normal activity levels of the fish in question. The more active the fish the thinner they are and the less distance there is between them. Also the absolute thickness of the individual lamellae walls varies, this is important in considering the facility with which O_2 can diffuse from the water to the fish's blood, the thinner the membrane the more quickly, and easily the O_2 can pass across it.

Increased concentration of suspended sediments is known to physically traumatize gill tissue. The primary mechanisms of the action are through the abrasion of tissue and particle adsorption of the gill. The types of tissue changes observed include swelling of secondary lamella and hypertrophy (cell swelling) of epithelial cells; hyperplasia (increase in cell number) of gill tissue; and tissue necrosis (Servizi and Martens 1987).



Figure 2. Longitudinal section of the gill of control fish, *Rasbora daniconius* showing primary gill lamellae (PL), secondary gill lamellae (SL), pillar cell (PC), epithelial cell (EC), inter lamellar distance, adipose tissue (AT) (Pathan and others 2010)

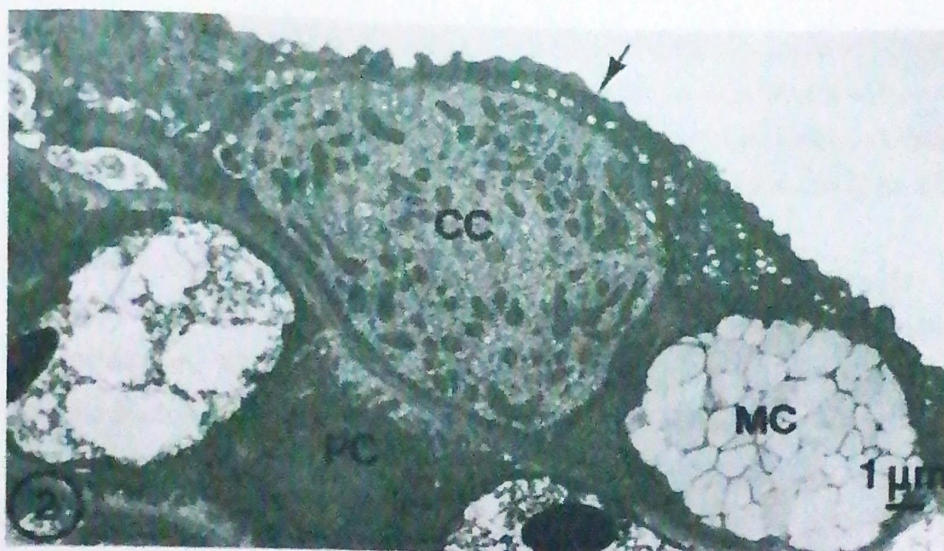


Figure 3. *Pseudopleuronectes*. Secondary lamella with typical mucous cell (MC), chloride cell (CC) and pillar cell (PC). Note location of chloride cell deep to extension (*arrow*) of outer pavement cell (Boyd and others 1980)

D.1. Normal Gills

Normal gills at low magnification shows primary gill filaments with the secondary lamellae branching out from each side (some resemblance with a Christmas tree). The viability of the secondary lamellae is crucial to fish health. In a high magnification of a healthy gill tissue, the red blood cells pass in a single column through the secondary lamellae where they pick up oxygen and dump carbon dioxide and ammonia. Anything that interferes with, damages or obstructs a fish gill delicate structure is clearly going to be extremely damaging to fish health (Johnson 2009).

D.2. Gill Disease

The fish gill has very little protection, other than the bony cover -the operculum - and is susceptible to both physical and chemical damage. A common response of the gill to irritation - whether from parasites, suspended solids or ammonia, or indeed any other irritant - is hyperplasia. Hyperplasia is an abnormal increase in the number of cells in an organ, in this case the gill epithelium. This is similar to calluses that form on hands when subjected to physical work such as digging. Hyperplasia is usually accompanied by increased mucus production. The effects of a gill disease may be swelling and clumping. Extreme hyperplasia where both the secondary lamellae and primary filaments are clumped together in a mass of tissue. No gaseous exchange can take place and the fish literally suffocates (Johnson 2009).

Fish gills are also susceptible to bacterial and fungal disease as a result of poor water quality, stress or parasites. These conditions that cause the disease include chemical irritants such as ammonia and nitrite or high pH, high levels of parasites such as *Trichodina*, *Chilodonella*, flukes or *Costia*, high levels of dissolved or particulate organic solids, and low levels of oxygen or overcrowding (Johnson 2009).

3. *Secutor insidiator*

Secutor insidiator (Bloch 1787) or Pugnose Ponyfish is a schooling species found in shallow waters, usually near the bottom. Occasionally it enters brackish waters. Its body is silvery with a naked head and nuchal spine. Mouth is strongly oblique where protracted mouth points upward. Nostrils are situated above eyes. Tip of maxilla reaching well below level of lower margin of eye. Body depth is twice or slightly more than standard length. Lateral line ending is before dorsal fin. It usually feeds on zooplankton including copepods, mysids, and larval fishes and crustaceans. Its maximum length is 11.3 cm whether male or unsexed. Though its common length is 8.0 cm either male/unsexed.



Figure 4. Pugnose Ponyfish (Encyclopedia of Life 2009)

CHAPTER 3

METHODOLOGY

A. Overview

This study aimed to gather baseline information on the fish gill histopathology of *Secutor insidiator* and the water quality in the Bito-on, Jaro-mouth of Iloilo Flood Control. The gill histopathology of the fish was described. Total suspended solids, salinity, pH, and temperature are the parameters that were measured for the water quality.

There were 5 samples of fish (for physiology) in which 5 replicates of the fishes were used for gill histopathology tests. There were also 3 sampling areas. Then 3 replicates were conducted for the 3 sampling areas.

Five-hundred mL water samples were collected from 3 sample stations and were placed in empty distilled water bottles. Each station has 3 replicates.

A.1. TIME AND PLACE OF STUDY

The study was conducted on the Iloilo Flood Control Project Floodway situated in Brgy. Bito-on, Jaro, Iloilo. Stratified Random Sampling was used in this study. During an interview with a fisherman, it was found out that the fishes caught in the area are (local names): Sapsap (*Secutor insidiator*), Trumpeter sillago or asu-os (*Sillago maculate*) and deep-bodied mojarra or latab (*Gerres abbreviatus*)



Figure 5. The Iloilo Flood Control study site. (Wikimapia)

B. Materials and Equipments

1. Total Dissolved Solids

closed analytical balance

Oven (Binder)

dessicator (Sanplatec Corp Dry Keeper)

Whatman filter paper

500-mL Erlenmeyer flask (Pyrex No. 4980)

Rubber tubing (Pall Vacuum/Pressure Pump Model No. DOA-P730-BN)

Funnel

Deionized water (Wilkins)

Graduated cylinder (50 mL; Pyrex No. 3075)

2. Salinity of the Water

refractometer

measuring bottle

3. Water Temperature

Thermometer

4. Water pH

pH probe

pH meter

5. Histopathology of Fish Gills

clove oil

incubator or refrigerator

10% phosphate-buffered formalin

graded ethanol

spectral confocal microscope

running water

DPX (mounting)

C. METHODS

C.1. Water Quality Determination/ Environmental Procedure

Five-hundred mL water samples were collected from 3 sample stations and were placed in empty distilled water bottles. Each station has 3 replicates. The boats used for collecting the samples were pre-arranged through fishermen near the flood control area, La, Paz Iloilo.

C.1.1. Total Suspended Solids

C.1.1.1 Filter preparation

The erlenmeyer flasks, funnel, and graduated cylinder were washed with Wilkins deionized water which will be bought from Pisay Coop. The 3 equipments were wiped with atissue. Few minutes were waited to dry it up. Nine Whatman filter papers were put it an oven for 4 hours with a temperature of 33 degrees Celsius. After, the filter papers were transferred to a dessicator. Then, the weight of the filter papers was recorded using a closed analytical balance. This was the initial weight.

C.1.1.2 Sample Analysis

Water samples were collected. To avoid contamination, the water samples were placed in a cooler and eventually transferred to a refrigerator, upon reaching PSHSWV. These samples were placed for the determination of the dissolved solids. Each water sample were poured in each filter paper. The filter papers were then placed again inside the dessicator for extraction of moisture and evaporation. The filter papers were weighed again. That was recorded as the final weight. The initial weight of each evaporating dish was subtracted from the final weight of the evaporating dish. The results was averaged. The final result was recorded.

C.1.2. Salinity of Water

A water sample was collected by gently submerging a sampling beaker into the sampling site. The salinity of the water was measured through a Refractometer. After calibration of the refractometer, the daylight plate and pat dry the main prism was opened. Now it was time to measure the salinity concentration of a water sample. To begin, the Refractometer was held horizontally, and the daylight plate was opened to expose the main prism. Using a plastic pipette, a water sample was collected from the plastic container and two drops was placed on the prism. The daylight plate was closed. It was lightly pressed so that the sample spreads across the entire surface of the prism without any air bubbles or dry spots. The sample was remained on the prism for 30 seconds. The front end of the Refractometer was aimed in the direction of a bright light.

Determine the salinity indicated by the boundary between the blue and white portions in the refractometer field. When finish, the prism was rinsed with distilled water and patted dry before putting it away. Salinity was recorded as parts per million (ppm).

C.1.3. Water Temperature

A water sample was collected by gently submerging a sampling beaker into the sampling site. Once it is full, the beaker was removed. The bulb end of the thermometer was carefully placed into the beaker of water for two minutes. Once the thermometer reading is stable, the temperature was determined.

C.1.4. Water pH

The probe and meter was calibrated according to the manufacturer's directions. Sample water was collected in any glass or plastic container. The probe with sample water was rinsed before placing it in the sample. The probe was placed in the sample and wait for the meter to equilibrate. If the meter needs to be manually adjusted to correct for temperature – you'll know it does if it has an extra temperature knob – adjust it to the temperature of the sample before allowing it to equilibrate. The meter will come to equilibrium when the signal becomes steady. If it is taking a long time to equilibrate, the probe was stirred gently. However, the sample must not be agitated since this may cause changes in the pH. The pH was read directly from the meter according to the manufacturer's direction.

C.2. Fish Gill Histopathology of *Secutor insidiator*

C.2.1. Histopathology of Gills

C.2.1.1 Preparation of Fish

The fishes were collected from the catch of a pre-arranged fisherman, Mr. Leo Labrador. Before, 10% buffered formalin was submerged into a bottle with water, the freshly caught fishes were first cut at the back of its dorsal fin so that the formalin will spread although out its body.

C.2.1.2. Fixation and Decalcification

First the operculum was removed so that the fish gill will be exposed. Then the gills were be then removed. After dissecting the fish, the gills were fixed in neutral buffered formalin. Both fixation and decalcification were done with an excess of fluid, approximately 100 mL for two fish, for a solution to tissue ratio of approximately 60:1. All fish were fixed and decalcified at room temperature and were incubated at 4°C. Fixation solutions includes 4% freshly prepared paraformaldehyde, pH 7.2–7.4, 10% neutral buffered formalin, pH 7.0, Bouin's solution (75% picric acid, 10% formalin, and 5% acetic acid, pH 1.0), and Dietrich's solution (30% ethanol, 10% formalin, and 2% acetic acid, pH 2.7) (1,3,4).

Decalcification solutions includes 1.35 M HCl Cal-EXO (Fisher Scientific, Pittsburgh, PA, USA), 10% sodium citrate/22.5% formic acid, pH 2.5, and 0.5 M EDTA, pH 7.8. Decalcified tissue was thoroughly washed in running water and sectioned (5 to 6 μm).

C.2.1.3. Processing of Gill Tissues

The fixed gill tissues was dehydrated in graded ethanol dehydrated through 70, 80, 90, to 95%. Tissue samples from gills was processed in graded levels of alcohol, cleared in xylene, impregnated and embedded in paraffin wax. Trimmed blocks of the embedded hard tissue gills were treated, overnight, with 0.5% trichloroacetic acid (TCA) as a decalcification process. Sections of samples will be stained with haematoxylin and eosin (Bhagwant and Elahee, 2002). Decalcified tissue was thoroughly washed in running water and sectioned (5 to 6 μm). Deparaffinized sections was then dehydrated in graded alcohol, stained with H & E, dehydrated, cleared in xylene and mounted using DPX. Stained sections were observed under a microscope which was then recorded and photograph.

C.2.1.4 Visualization

After rinsing staining, 2–5 filaments was removed from the center of each excised gill arch and was placed on a hydrophobiccoated glass slide. Micrographs of gill lamellae (400 \times magnification) was becreated using compound microscope, with a 40 \times water immersion objective. Optical sectioning of fluorescent gill lamellae was standardized by always capturing the optical section at 50% of lamellar height (i.e., vertical thickness, ± 2 μm). Micrographs of lamellae generated by confocal microscopy were analyzed using Image-ProPlus_ software. Gill thickness (1m) was measured perpendicular to the long axis of each lamella. Fifty gill thickness measurements were taken for each fish using 10–20 lamellae and 1–6 measurements on each lamella. Thickness measurements were taken from the center of lamellae (i.e., the vertical center; not near tip or base). The number of lamellae and measurements per lamella were a function of micrograph quality. Interlamellar area (1m²) was determined as the space between adjacent lamellae for 25 interlamellar regions per fish (Sutherland and Meyer 2006).

D. Handling and Disposal

D.1. Fish transport

The fish was placed in a strong plastic bag with a minimum amount of water (not more than one-third full). It was made sure that the bag will hold enough oxygen; it was tied securely, and placed in another plastic bag and sealed. The double bag was placed in a strong cardboard, plastic or Styrofoam box with insulation.

Collection of just a single fish may not be representative of the fish population and may result in a wrong diagnosis. Some of the fishes were submerged in formaldehyde for gill histopathology analysis.

D.2. Formaldehyde

In the study, 10% buffered formaldehyde was used. The legal airborne permissible exposure limit (PEL) for formaldehyde is 1 parts/million (p/m) in a 8-hour workday. Short-term exposure (15 minutes) is limited to 2 p/m while the action level for formaldehyde is 0.5 p/m.

Formaldehyde was stored in a cool, dry, well-ventilated area and properly labeled. At all times, formaldehyde was only handled, mixed or added to specimen containers with the upmost caution, in ventilated areas such as open air table if in the field.

At all times, disposable gloves were worn to prevent dermal exposure when handling and/or mixing this product. Formaldehyde was transported only in original container, fully labeled and stored properly within the vehicle to prevent shifting, spillage or breakage. Used formaldehyde was stored in a properly labeled hazardous waste container and made available for recycling.

D.3. Water Samples

Nine 500 ml distilled water container were used in the study. Sample bottles were labeled indelibly prior to sampling with a unique sample number, the location, date and analyses required. The mouth of the bottle was held at least 10 cm below the surface. There were 3 sampling stations and 3 trials will be used for each station. The three containers of water samples from each station were put in an ice box and were sent for the lab for water analysis. The samples must be sent within 24 hours after it were collected.

CHAPTER 4

RESULTS AND DISCUSSIONS

This study aimed to gather baseline information on the physiology of *Secutor insidiator* (Pugnose ponyfish) and the water quality of the Iloilo Flood Control, mouth of floodway. The gill histopathology of the fish was described. Total Suspended Solids, salinity, pH, and temperature are the parameters that were measured for the water quality assessment.

There were 5 samples of fish (for physiology) in which 5 replicates of the fishes were used for gill histopathology analysis. There were also 3 sampling areas for the water quality assessment. Five hundred mL empty distilled water bottles were used to collect the water samples. Three water samples were collected from each of the sampling areas.

A. Results

A.1. Water Quality

Water temperature and pH in the water samples are within the normal range while the total suspended solids and salinity level of the water samples were below the normal range of brackish and seawater.

The water has temperature that range from 28.6°C to 29.1 °C. The normal water temperature for tropical areas is above 20°C.

The pH levels ranged from 7.83 to 7.93. These pH levels are near the pH of 8.0 and 8.3 which are suitable for fish growth.

Also, the salinity of the water ranged from 25ppm to 28ppm. This is way below the normal range of brackish water of 500 - 30,000 ppm and that of sea water which is 30,000-50,000 ppm.

The amount of total suspended solid in the water is from 0.0402 g to 0.0744g.

A.2. Fish Gill Histopathology of *Secutor insidiator*

The gill is made up of filaments of primary lamellae arranged in double rows. Secondary lamellae arise from these filaments. The secondary lamellae are lined by

a squamous epithelium. Marked deformities were observed at the fish gills of *Secutor insidiator* (Pugnose ponyfish) found in Iloilo Flood Control, mouth of floodway.



Figure 6. A: Normal gills at LPO magnification showing primary gill filaments with the secondary lamellae branching out from each side. **B-F:** Sections of gill filaments of *Secutor insidiator* in LPO **B:** damaged primary lamellae **C-D:** Clumping of cells at the gill arch. **E:** There is a gap between the cells in the primary lamellae. **F:** Thickening of primary lamellae and clumping of cells in nearby secondary lamellae



Figure 7. G: Clumping of cells in the secondary lamellae. H-I: Extreme clumping and thickening of cells at the secondary lamellae, viewed in LPO in J

The most common gill abnormality is the clumping of the cells in the secondary lamellae. Thickening of primary lamellae, swelling of gill arch, curling and thickening of secondary lamellae were also observed.

B. Discussions

This study described the gill histopathology of *Secutor insidiator* (Pugnose ponyfish) through the presence of lesions. It also presents the water quality of the mouth of floodway, Iloilo Flood Control.

The results of the water quality determination on Iloilo Flood Control showed that the total suspended solids, temperature and pH level fall within the acceptable range suitable for normal fish growth. On the other hand, the salinity levels of the 3 stations fall below the normal range of brackish and seawater. This is because the Iloilo Flood Control is a new body of freshwater which empties out in the sea. When fresh and salt water meet, the two do not readily mix. Warm, fresh water is less dense than cold, salty water and will overlies the wedge of seawater pushing in from the ocean. It can also be noted that in natural settings, salinity levels can fluctuate with tides, season or evaporation from surface waters (Harper).

The histopathology of gills of *Secutor insidiator* showed signs of gill lesions like clumping of the cells in the primary and secondary lamellae and the gill arches and hyperplasia. Although the results show normal water quality parameters, according to Luckhoff, aside from total dissolved solids, temperature, salinity and pH level, some of the most important components of water quality affecting growth and condition are ammonia, nitrite, and dissolved oxygen.

Ammonia, particularly, can cause swelling and inflammation of the gills and other tissues, damages skin and eyes, reduce oxygen transport in the blood, swelling and diminishing of red blood cells, decrease oxygen consumption of tissues and thus increasing susceptibility to disease. Gill hyperplasia is a common effect of ammonia (Luckhoff et. al. 2005). After an investigation of the combined effects of ammonia and elevated pH in Lost River suckers, Lease and colleagues (2003) concluded that structural gill changes were more sensitive the more sensitive than other traditional assays for detecting ammonia toxicity (Harper).

On a study conducted by Daoust and Ferguson (1983), gill tissue responds to injury by epithelial hyperplasia. This type of response has been described in fish chronically exposed to a variety of noxious agents, including pollutants such as heavy metals, pesticides and suspended solids (1,3) and microorganisms such as protozoa, trematodes and filamentous bacteria. Although water quality problems may often be the primary cause of gill lesions, the observations of Daoust and Ferguson demonstrate that bacterial colonization of branchial lamellar surfaces is

the most common manifestation of gill disease. Bacterial gill disease is recognized as a major cause of mortality among artificially raised salmonids.

Capture, transport, and handling are obvious stressors for captive fish, but wild fish may also experience these disturbances, for example through catch and release programs in recreational fisheries. Procedures that can intensify the stress response in aquacultured fish include sorting, grading, and vaccine administration (Burgess and Coss 1982). The gills undergone incision and was traveled for 3 hours from the site to SEAFDEC for the gill histopathology. This may also induce stress the fish thus affecting its gills. Furthermore, the gills have a relative fragility compared to other surface tissues and the fact that they are continually exposed to the fish's external environment.

The damage may be also done due to the long term exposure to the sediment. A concentration of 25 mg/L of total suspended solids shouldn't harm fishes or its habitat (DFO 1983) but these low concentration must not last for weeks for rubble/gravel areas will rapidly become silted (Carling 1984). Also according to the study of Appleby and Scarratt, 1989, a substantial portion suspended sediments come from the resuspension of fine, unconsolidated sediments and detritus by wave action and currents.

The presence of clumping, hyperplasia and other gill lesions may affect the normal function of fishes. According to Johnson, 2009, accumulation of cells in the secondary lamellae may contribute to no gaseous exchange and the fish may suffocate. Presence of lesions may also be an indicator that the fishes are irritated, whether from parasites, suspended solids or ammonia, or any other irritant.

The present study has shown that *Secutor insidiator* exhibit gill lesions even before the implementation of the Iloilo Flood Control Project. However, further studies are needed to establish what specifically caused the gill lesions. Factors like ammonia, nitrite, dissolved oxygen and other pollutants should be further investigated.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study aimed to gather baseline information on the fish gill histopathology of *Secutor insidiator* and the water quality in the Bito-on, Jaro-mouth of floodway, Iloilo Flood Control.

Specifically it aimed:

1. To evaluate the gill histopathology of fish by describing the presence of gill lesions
2. To describe the water quality of three water sampling stations by measuring the:
 - a. total suspended solids
 - b. salinity
 - c. temperature
 - d. pH

A. Summary of Findings

The water quality of the constructed Iloilo Flood Control falls within the suitable range for normal fish growth. Water temperature and pH in the water samples are within the normal range while the total suspended solids and salinity level of the water samples were below the normal range of brackish and seawater.

Secutor insidiator found in the area shows signs of gill diseases like hyperplasia or the clumping of the cells in the primary and secondary lamellae and the gill arches. Other abnormalities were sometimes observed like thickening, swelling, and curling.

B. Conclusion

Therefore, it is concluded that the fish *Secutor insidiator* found on the mouth of floodway, Iloilo Flood Control has hyperplasia. Water temperature and pH in the water samples are within the normal range while the total suspended solids and salinity level of the water samples were below the normal range of brackish and seawater.

C. Recommendations

The following are recommended:

1. Gather data for Dissolved Oxygen Content in the area
2. Conduct another gill analysis for another fish species
3. Use portable pH meter and EC meter to eliminate fluctuations of data due to transportation
4. Include other parameters like the blood physiology and feeding behaviors of the fishes in the area
5. Test the ammonia and nitrate content of the area
6. Presence of bacteria, protozoans and other parasites in the water should be investigated.

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APPENDIX A

Raw Data

		Temperature (°C)	pH	Salinity (ppm)	Total Suspended Solids	
					Weight Before (g)	Weight After (g)
Station 1	1	28.3	7.79	25	1.1359	1.1630
	2	28.7	7.82	25	1.1252	1.2003
	3	28.7	7.90	24	1.1206	1.2040
Station 2	1	28.7	7.77	25	1.1200	1.1851
	2	29.4	8.03	29	1.1290	1.2209
	3	29.2	8.00	30	1.0470	1.1128
Station 3	1	28.9	7.98	26	1.0692	1.1381
	2	28.9	7.92	26	1.1110	1.1494
	3	28.9	7.89	30	1.1047	1.2382

APPENDIX B

Calibration of Refractometer

Distilled water was used to calibrate the Refractometer. During the calibration, the Refractometer was set into a zero reading. It was removed from its protective casing. The Refractometer was held horizontally. The main prism was exposed by opening the daylight plate. Two drops of distilled water was placed using a plastic pipette on the prism.

Now, the plastic pipette was clicked on to place two drops of distilled water on the main prism. The daylight plate was closed and pressed lightly so that the water spreads across the entire surface of the prism without any air bubbles or dry spots. The water sample was allowed to remain on the prism for 30 seconds. The front end of the Refractometer was aimed in the direction of a bright light. The sample was looked through the eyepiece. A circular field with graduations on either side was seen. The upper portion should be blue and the lower portion white. If the field was not in focus, the eyepiece was twisted until the graduations are clearly distinguishable. The boundary between the blue and white portions should fall on the zero mark of the graduations. If not, the calibration screw on top of the Refractometer will be turned until the boundary between the colors reaches the zero mark.

APPENDIX C

PLATES



Plate 1. The fish samples. The average length of sapsap is 52.5 cm



Plate 2. The 9 water samples with their corresponding labels

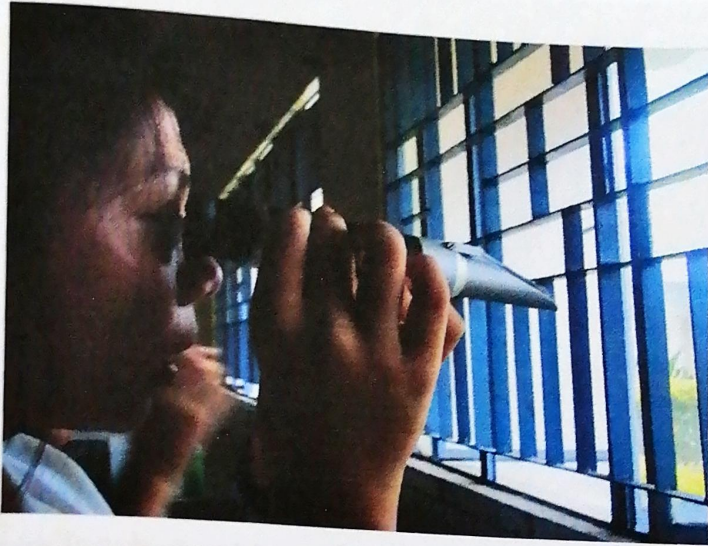


Plate 3. Reading the salinity of the water samples



Plate 4. The filter papers are being placed in the oven at the temperature of 33 degrees Celsius.



Plate 5. The five sapsap placed on the container with 10 % buffered formalin and their corresponding labels.

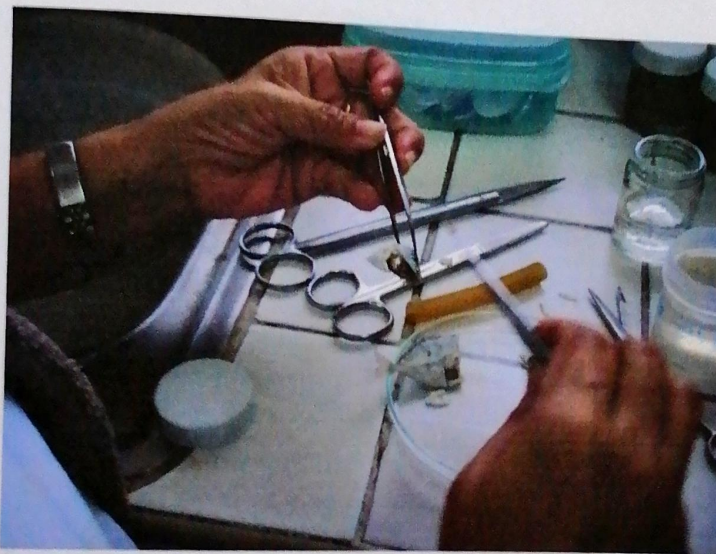


Plate 6. The fish is being dissected for the gills