Implication of Endocrine Disruption in Selected Reef Fishes to Fish Ecology in Iligan City

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Abstract

Iligan City-the industrial city of the south-is home to processing industries believed to be sources of effluents that may contain endocrine disrupting compounds (EDCs). The city is also a fishing community with a number of commercial and non-commercial fish species. These industries and fishing grounds are within the localities of six coastal villages of Iligan City where our samples came from during the June to July 2004 sampling schedules.

This work is a continuing effort to validate sensitive biomarkers for endocrine disruption by looking at male vitellogenin expression in relation to fish ecology in Iligan City. Livers of selected sexually mature male and female reef fishes were homogenized in 63mM Tris-HCl pH 6.8 to obtain crude cytoplasmic extracts and subsequently subjected to electrophoration in 12% Tris-glycine pre-cast gels against standard markers and lyophilized carp vitellogenin to show the comparative vitellogenesis in female and male fish livers, with special concern on male vitellogenin expression.

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Selected fish liver samples were analyzed for expression of putative vitellogenin, distinct high molecular weight (HMW) bands near the 170 - 200kDa range or 97.3 - 110kDa markers. The bands corresponding to three distinct vitellogenin fractions from male livers of fish samples from Iligan City may imply possible disruption of male endocrine function by expressing apparently identical proteins to female samples. Male reproductive anomalies would have direct or indirect effect to fish ecology in the fishing grounds of the city. These findings confirm the previous observations in M. cephalus and implied wider extent of still unidentified EDCs in the surrounding waters of Iligan, and might as well indicate endocrine disruption in other species and may lead to lower chances of fish recruitment in the bay.

Keywords: vitellogenin, male vitellogenesis, endocrine disruption, reef fish, Iligan Bay, Iligan City, Northern Mindanao, Bohol sea, SDS-PAGE

Introduction

Vitellogenesis generally comprises a molecular machinery within the hepatocytes of female vertebrate liver upon sexual maturity by responding to the specific female hormone estrogen (Celius and Walther, 1998). This physiological mechanism can be observed among male individuals that have prior exposure to estrogens or other feminizing agents. Feminizing agents may include chemicals with estrogen properties or those that mimic the inherent functions of estrogens such as promoting the secondary sexual characteristics of female.

Iligan is an industrial city with industrial plants producing cement, chemicals, PVC pipes, coconut edible oil and flour products, which are suspected to be potential sources of effluents containing the feminizing agents or endocrine disrupting compounds (EDCs) that mimic the endogenous estrogen function among vertebrates (Mejstrik, 199-; Cayman Chemical Company, 1998-2001). The city is also home to the two giant industries involved in heavy metal processing and power generation that are also sources of other insults to the biological system such as heavy metal contaminants and heat in the bodies of water, respectively. Pandian and Koteeswaran (1998) specifically looked at the significant role of high temperature to the sexual development of teleost fish.

Wolf (1994) noted many other effects that have been cited in scientific literature which include (1) decreased fertility in fish, birds, mammals, and shellfish; (2) decreased hatching success in fish, turtles, and birds; (3)

demasculinization and feminization of male fish, birds, and mammals; and (4) defeminization and masculinization of female fish, birds, and gastropods. Olsson, et al. (1998) wrote a very comprehensive discussion the reproductive and development impairment among the vertebrate organisms upon exposure to these so-called endocrine disruptors.

EDCs may have deleterious effects on reproduction of fish leading to poor reproductive success (Lee, et al., 1999; MacFarlane and Norton, 1999; Cheek, et al., 2001), which may be caused by low fecundity (Pandian and Sheela, 1995; Pandian and Koteeswaran, 1998) or by unhealthy progeny (MacFarlane and Norton, 1999). This is alarming as reproductive failure is a potential factor in declines or low recruitment of marine fish. Many studies have suggested that disturbed reproduction can be accounted to exposure to environmental chemicals (Lee, et al., 1999; Cheek, et al., 2001) but the relationship between elevated vitellogenin levels and the effects of environmental estrogens on reproductive success are poorly understood. Thus this work is a continuing effort to validate sensitive biomarkers for endocrine disruption and the possible implication of this phenomenon on the ecology of fishes in the coastal areas and fishing grounds of Iligan City. This specifically looked at male vitellogenin expression using three non-target (indicator) and eight target (commercially important) teleost species from the identified fish landing sites in Iligan City to support the previous observation on declining recruitment of commercial fishes. The present findings also tried to verify the previous observations on *Mugil cephalus* and *Stegastes nigricans*, which both showed gonad development irregularities.

Materials and Methods

Sampling Strategies

Fish samples used in this work were supplied by contact fishermen from the selected six coastal villages or fish landing sites of Iligan City (Figure 1) from the north-eastern to the north-western ends of the coastlines during the months of June and July 2004. A total of eight sampling schedules, which fall into a weekly visit, to all the landing sites per month was done.

A total of eight commercial species namely: *Lutjanus campechanus* (red snapper), *Lutjanus monostigma* (onespotted snapper), *Decapterus tabl* (roughear scad), *Selar crumenopthalmus* (purse-eyed scad), *Sphyraena genie* (barracuda), *Mulloidichtys pfluegeri* (goatfish), *Leiognathus fasciatus*, and *Mugil cephalus* (flathead mullet) and three non-target or indicator species such as Abudefduf *sexfasciatus* (sergeant major), *Halichoeres biocellatus* (redlined wrasse), and *Synodus variegatus* (variegated lizardfish) All fish caught by the fishermen were

identified down to the species level whenever possible using Allen and Swainston (1988) and Fish Base 2000 (www.fishbase.org, 2005).

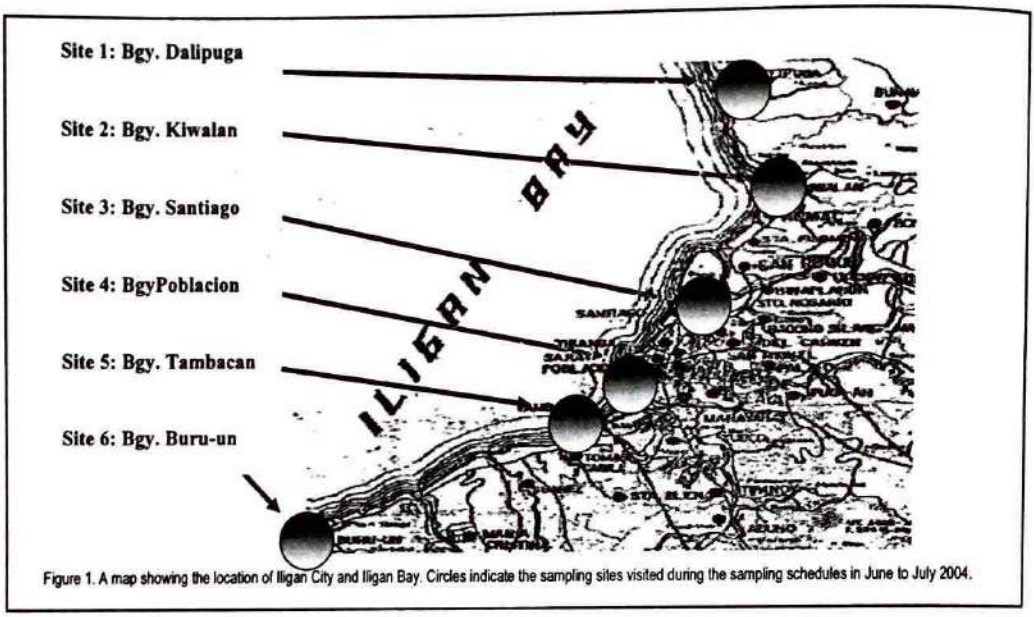

Figure 1. A map showing the location of Iligan City and Iligan Bay. Circles indicate the sampling sites visited during the sampling schedules in June to July 2004.

Sample Preparation

Fish liver tissues were obtained from the fresh samples and were either stored frozen (for biochemical analysis) or preserved in neutral buffered formalin (10% NBF) (for histopathological analysis, which is not included in this paper). Male and female individuals were carefully selected to isolate the livers for biochemical analysis. The protein profiles of the male and female hepatocyte cytoplasmic fractions were compared with the standard protein markers (Mark12™, Novex®, Invitrogen, USA) and with the purified and lyophilized female carp vitellogenins (Japan). Frozen hepatic tissues with weights that ranged from 0.05g (50mg) to 0.08g (80mg) regardless of the whole weight of frozen fresh liver tissues were thawed to room temperature prior to biochemical analysis using the denaturing, non-reducing discontinuous gel electrophoresis (SDS-PAGE) (Novex®), Invitrogen, USA) factory pre-calibrated 1.5mm thickness at 12% consistency.

The liver tissue of each fish species was separately homogenized in 5mL homogenization buffer (mixture of 2.5mL Tris-Cl 63mM, 2mL10% glycerol, 4mL 2% SDS and made to 10mL with deionized water, adjusted to final pH 6.8) or equivalent to approximately 10-16mg of tissue per mL of buffer using either glass to glass homogenizer or Teflon-glass homogenizer. Consequently, 1.5mL of each

of the homogenates were transferred to Eppendorf® tubes and centrifuged at 15,000*g* for 60 min, decanted and the supernatants were re-centrifuged at the same speed and time. The final supernatants constituted the cytoplasmic fractions of hepatocytes of each fish species. The final supernatants in Eppendorf® tubes were placed in ice before the next step and finally, one part (50□L) of the supernatant was added with one part (50□L) of the Tris-Glycine sample buffer, pH 6.8 (Novex® Invitrogen, USA, Inc.) or a total of 100□L was vortexed and heated to 85°C for 2 min. Twenty □L of each heated homogenate in sample buffer were then loaded into the wells and ran at 125V with starting current at 30-40mA and ended at 8-12mA after 90 min. Each gel run was replicated and were stained with SafeStain™ (Novex®, Invitrogen, USA, Inc.) and de-stained with deionized distilled water for 1 hour (at least 4 changes) in a shaker (10 cycles/min).

Documentation and Analysis

The individual fish samples were digitally photographed for documentation and for the database preparation. Their gonad and liver were also documented accordingly and their sexual conditions including the fresh weights of the gonads and their developmental stages. Stained gels of the male and female liver homogenates were photographed and noted the expression of vitellogenin based on the standards used for molecular weight estimates and similarities in the protein band's relative mobility or Rf.

SPSS ver. 10.0 was used to subject the selected values to statistical correlations and comparisons of the mean values of some of the parameters to show how closely or how far these values could be related to each other.

Results and Discussion

Fish Collections

Fish samples used in this study have already been recognized as specifically important to humans and the environment being either non-target (indicator) species or as commercially important (target) species. The name indicator was coined implying their function in the ecosystem as "indicators" for any environmental conditions of the coral communities.

In this work, the name non-target or non-commercially important species was a misnomer. Most, if not all, of the supposedly non-target species from within the 100-200m distance from the shorelines of the coastal villages in Iligan City were at times consumed by fishermen in most *barangays* visited. In this case, only three indicator species from the 18 already identified have been presented in

Figure 2 as specimens. These are the following species namely: *Abudefduf vaigiensis* (n=5), *Synodus variegatus* (n=3), and *Halichoeres biocellatus* (n=3).

Figure 2. Out-of-scale photos of non-target (indicator) reef fish species from Iligan City used in this study. These species were caught from the shallow area of the sea, within 100 to 200 meters from the shore. (A) *A. sexfasciatus*, (B) *S. variegatus*, and (C) *H. biocellatus*.

Among the selected three species of *Abudefduf A. sexfasciatus* mostly represented the collections. *Abudefduf* spp. could be found in any part of the bay throughout the year. Many of these species could be encountered in the piers and in rocky and weedy reefs as has been noted by Allen and Swainston (1988). *S. sexfasciatus* and *H. biocellatus* just like *Abudefduf* are territorial and are usually associated with healthy shallow waters near the intertidal areas.

Only two males from a total of five *A. sexfasciatus* samples collected in June 26, 2004 from *Bgys.* Buru-un/Fuentes were accounted for. *S. variegatus*, on the other hand, was represented by only three individuals, which seemed to belong to a single group as shown by their very similar sizes and wet body weights. There was only one male among the three individuals of *S. sexfasciatus* for the whole duration of sampling activities in Buru-un. *H. biocellatus*, has been represented by only one of each of male and female, and one was sexually immature (unsexed) individual. Using two-tailed Pearson correlation analysis, there was no significant difference on the number of male and female among the samples.

The size and wet weight ranges of the indicator species are presented in Table 1, which also indicates other pertinent information concerning their phenotypic sexes, total length (TL), body (fresh/live) weight (BW in g), gonad (fresh) weight (GW in g) and liver tissue fresh weight (LW in g).

It can be noted that the majority of the captured fish were females (n=6) and there were only four male individuals. These were all set aside for biochemical analysis of liver. Most of the male fishes notably had very small and

delicate liver tissues that weighed only very small fractions of a gram or on the average reached only to 0.25g. However, only a small fraction of the liver tissues were needed for the analysis.

Table 1. Some physical characteristics of three indicator species from Buru-un (Site 6), Iligan City collected in 26 June 2004. Parameters noted were the sex, total length (TL), body weight (BW), gonad weight (GW) and liver weight (LW).

Fish Species	Phenotypic Sex	TL, mm Range	BW, g Range	GW, g Range	LW, g Range
A. saxatilis (n=5)	M (n=2)	121-138	37.4-47.4	0.1-0.6	0.3-0.5
	F (n=3)	92-115	47.5-64.3	0.2-2.5	0.5-0.6
S. variegatus (n=3)	M (n=1)	148	34.4	0.1	0.1
	F (n=2)*	148	33.3-34	0.1	0.1
H. biocellatus (n=2)	M (n=1)	145	45.7	0.1	0.5
	F (n=1)	150	49.1	0.9	0.6

* Same TLs, GWs, and LWs

The commercially important fish species or edible species are basically considered for their market value. The target or commercial fish species collected from most of the six landing sites along the coastline of Iligan City from 26 June to 26 July 2004 consisted of the commonly found fishes in local markets within the city. In Figure 3 are actual photographs of fish samples in the collections made during the aforesaid schedules. These species are the following: (A) *Sphyraena genie*, (B) *Decapterus tabl*, (C) *Mulloidichtys pfluegeri*, (D) *Leiognathus fasciatus*, (E) *Lutjanus campechanus* (red snapper), (F) *Selar crumenopthalmus*, (G) *Lutjanus monostigma* (onespot napper), and (H) the *Mugil cephalus*. Only three species were most represented and these were *M. cephalus* (n=52), *S. genie* (n=40), and *D. tabl* (n=20). These were followed by *L. fasciatus* with only 15 individuals and *S. crumenopthalmus* with only 12 representatives. *Lutjanus* spp. (n=7) was represented by juveniles and adults coming from different species such as *L. monostigma* (n=2) with one female that weighed up to almost 2.0kg and the *L. campechanus* (n=5), which were relatively smaller.

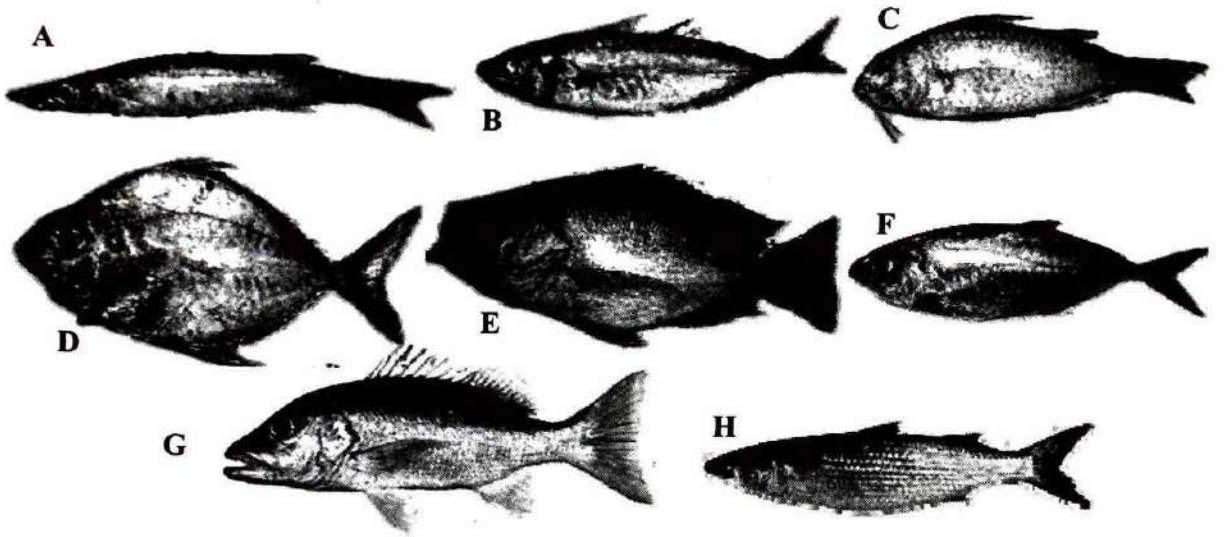

Figure 3. Out-of-scale photographs of the target (commercial) reef fish species from Iligan City used in this study. These species were caught offshore. No distinctions (dimorphism) could be noted between male and female individuals. (A) *Sphyraena genie*, (B) *Decapterus tabl*, (C) *Mulloidichthys pfluegeri*, (D) *Leiognathus fasciatus*, (E) *Lutjanus campechanus* (F) *Selar crumenopthalmus* (G) *Lutjanus monostigma* (photo from Wass, http://www.nps.gov) (H) *Mugil cephalus*.

Open sea fishing reaching a distance of more than 5-km offshore is what our contact fishermen do to gather commercial fishes. It has been impossible for them to gather commercial fishes with considerably marketable sizes within the 100-200m distance or where the non-commercial fishes are caught. During the sampling schedules of the contact fishermen in June to July 2004, only very few representative species were gathered. The fish catch may not reflect the actual catch per unit effort (CPUE) in Iligan City but may help in making an estimate of CPUE per fisherman per *barangay* per unit time.

Table 2 shows the summary of the catch and the parameters used in describing the different species such as their phenotypic sex, total length, body weight, gonad weight, and liver weight. This is only eight of the commonly caught commercial fish species from Iligan City during the June to July sampling period in 2004.

Table 2. Some physical characteristics of eight commercially important species from all landing sites in Iligan City collected in 26 June to 26 July 2004. Parameters noted were the sex, total length (TL), body weight (BW), gonad weight (GW) and liver weight (LW).

Fish Species	Phenotypic Sex	TL mm Range	BW g Range	GW g Range	LW g Range
S. genie	M (n=10)	224-276	62.6-113.5	0.4-4.2	0.4-1.2
(n=40)	F (n=29)	215-280	53.2-120.7	0.5-5.5	0.1-2.2
D. tabl	M (n=10)	229-273	133.5-236.4	0.1-0.9	1.5-4.1
(n=20)	F (n=8)	254-285	211.4-262.2	0.1-4.7	3-4.7
M. pfluegeri	M (n=2)	185-215	194.8-214.6	4.2-5.3	1.7-2.2
	F (n=3)	240-244	82.0-137.6	0.1-0.8	0.4-0.5
(n=5)					
L. fasciatus	M (n=6)	119-169	60-174.5	0.1-1	0.2-1.1
	F (n=3)	129-200	74.2-256	1.8-6.8	0.9-2.7
(n=12)					
L. campechanus	M (n=2)	403-440	558.2-1,096.3	4.0-4.3	7.0-8.4
(n=5)	F (n=3)	285-310	372.2-506.4	6.0-8.7	3.4-4.4
S. crumenopthalmus	M (n=8)	181-225	152.5-186.5	1.1-3.3	1.5-3
(n=12)	F (n=4)	167-195	124.2-202.1	1.5-6.4	1.7-4.3
L. monostigma*	M (n=1)	380	897.5	3.0	6.2
(n=2)	F (n=1)	539	>1,500	67.7	29.3
M. cephalus	M (n=17)	84-118	11.5-18.8	0.1-0.7	0.1-0.2
(n=84)	F (n=62)	104-151	11.4-39.2	0.1-3.5	0.1-0.9
	M=56	84-440	11.5-897.5	0.1-5.3	0.1-8.4
TOTAL (N=169)	F=113	104-539	11.4->1,500	0.1-67.7	0.1-29.3

* only one representative each for male and female

The results from eight sampling schedules in June and July 2004 show that the commercial fishing was apparently difficult for the fishermen, which requires much effort resulting to fluctuating yields, sometimes to no catch at all. The surrounding coastal waters of Iligan City, in this case, the six landing sites identified were observed to have different fish populations. A number of fishermen, based on personal interviews with each representative fisherman from each site, would still choose to fish within the 100 to 200m distance from the shorelines; however, their catch composition would most often include only those

kinds referred to herein as non-commercial that have in these days, already become part of the daily consumption of the local folks.

The barracuda *S. genie* samples had only a total of 40 with one unsexed individual and dominated by females (n=29). The scad *D. tabl* had a total of 20 with two unsexed while the *M. pfluegeri* with only five distributed as three females and two males. The *L. fasciatus* surprisingly had three unsexed from a total of 12 individuals collected representing 25% of the catch. In *S. crumenopthalmus* male individuals dominated in the catch eight (67.0%) and only four (33.0%) females. *L. campechanus* (red snapper) had two and three male and female, respectively, while *L. monostigma* (onespotted snapper) had 1:1 male to female. The female, however, was so big that it reached a TL of 539mm and wet weight of more than 1.5kg.

Based on the total head count of fish samples from all landing sites where Bgy. Santiago/Canaway contributed most of the samples, females (n=113 or 67.0%) dominated against male individuals (n=56 or 33.0%). The total of phenotypically female individuals had highly significant direct relationship with the males based on two-tailed Pearson's correlation (a=0.01, p>0.858) but not male to unsexed or female to unsexed (Table 3)

Table 3. Two-tailed Pearson correlation between the overall counts of male and female sexes in samples collected from the landing sites in Iligan City in June to July 2004.

		Male	Female	Unsexed
Male	Pearson Correlation	1.000	**0.858****	0.629
	Sig. (2-tailed)	.	0.006	0.371
	N	8	8	4
Female	Pearson Correlation	**0.858****	1.000	0.608
	Sig. (2-tailed)	0.006	.	0.392
	N	8	8	4
Unsexed	Pearson Correlation	0.629	0.608	1.000
	Sig. (2-tailed)	0.371	0.392	.
	N	4	4	4

** Correlation is significant at the 0.01 level (2-tailed).

This observation only shows that the number of unsexed adult individuals in commercial fishes in Iligan City may not in any way affect the population structure in the bay. This may also imply the dominance of female individuals playing significant role in the stability of the fish ecology in Iligan bay. However, this female dominated population, if disturbed may have the

tendency to be destabilized or may be sensitive to alteration with the presence of non-sexual development exhibited by other commercial species.

In *S. genie*, one out of the 40 adult individuals remained sexually immature and exhibited low body weight of only 30.9g despite the comparable TL of 178mm. The lone unsexed individual was found to be the smallest among the catch, which may suggest its actual age based on size. Interestingly, the two male and female largest fish had wet weight of more than 1.0kg. *L. campechanus*
In the case of *D. tabl*, there also were two individuals without gonads or were still threadlike and so thin that might have escaped our attention. The TLs of these two unsexed individuals reached a comparably big size of 262 and 284mm, respectively, almost as big as the biggest male and female with recorded size of 285mm and had a fresh body weight of 226.6 and 229.2g, respectively. Both these individuals had liver weight of 4.0g (HSI = 1.7%) also comparable to the biggest males and females in the batch.

In *L. fasciatus* on the other hand, three unsexed individuals had gonads that were still at very early stage of development and were still difficult to identify yet already visible. The liver appeared to be consistent with their gonad state, which was way below 0.1%.

The Hepatic Condition

The liver tissues of the studied specimens were apparently normal by looking at their external appearance as shown in Figure 4. The recorded fresh hepatic weights generally ranged from the lowest of less than 0.1g (e.g., *S. genie* and *M. cephalus*) and a high of up to 4.7g obtained from female D. tabl. The highest weight obtained in liver samples came from the *L. monostigma* but its hepatosomatic index (HSI) remained very low at 1.95% only. The very massive body of female *L. monostigma* showed salient hepatic development against the total body mass. Contradictorily, the smaller fish species exhibited hepatic conditions with higher HSI values recorded from 1.5% to 1.8% not very far from the biggest fish in the collection. *M. cephalus* had HSI from 0.7 to 1.0%. It could be noted from these records that females contributed most of the voluminous livers, but females were also the ones with the lowest hepatic volume recorded to as low as 0.1g.

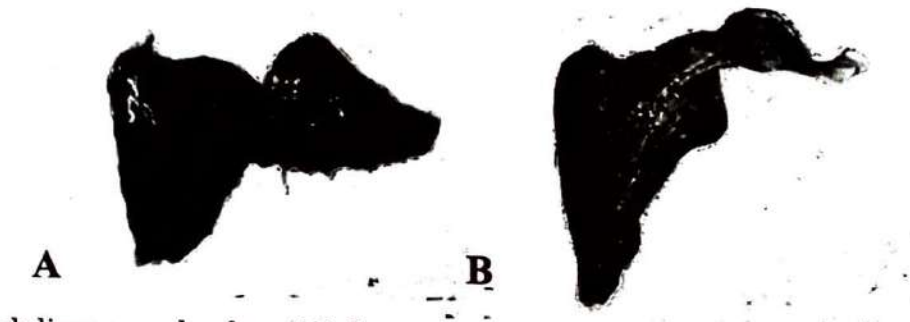

Figure 4. Fresh liver samples from (A) *S. crumenopthalmos* and (B) *L. campechanus*. The color of liver may range from light red (B) to dark brown (A) depending on the fish species. Liver tissues of other species may appear close to either of the two representatives.

The liver is a useful tissue in examining the overall health condition of animals including fish. The hepatocytes are composed of machineries for the manufacture of a variety of molecules doing various functions for their survival. Heavy metal exposure of fish signals the fish to produce metal-binding proteins in the liver and other tissues but basically the liver is the most responsible. The liver also functions as manufacturer of the precursors of yolk and the zona of egg of any oviparous animals (Celius and Walther, 1998; Gundersen, *et al.*, 1999). The next section discusses the details of vitellogenesis and its role in the development of secondary sexual characteristics of fish under investigation in this work.

Vitellogenesis in Hepatocytes

The HSI as an index to the growing and developing liver coincide the gonad maturation. The increasing values of HSIs in either male or female individual reflect progress in gonad and oocyte development. Higher HSI is an indication of increased hepatocytes activities such as protein synthesis thus, the increased in liver volume. Gundersen, *et al.* (1999) gave a clue on the possible relationship between the fecundity of Greenland halibut and their corresponding HSIs. This observation, however, has been suggested to be supported by more statistical measurements and more careful analysis.

The actual vitellogenetic process in the liver tissues is normally expressed by oviparous females to supply the vitellogenin (precursor) that is eventually broken down into final products used by the fish in organizing the egg envelope or chorion. The final products have been identified as lipovitellin (Lv) in the egg to the embryonic stage (Hartling and Kunkel, 1999). Celius and Walther (1998) also noted other proteins upon exposure of Atlantic salmon to estradiol (E2). This work also established the sequential events in the expression of

proteins during the developmental process in oocyte. In their work, zona radiata (zr) protein (outer thicker shell) and the vitellogenin in the plasma increased upon exposure of juvenile salmon to E2 and also suggested that zr organization comes first before the production of vitellogenin. They also found out that male salmon exposed to E2 responds by the synthesis of similar zr protein and vitellogenin resulting to endocrine disruption.

In Figure 5A, the gels show the male and female vitellogenin synthesized in the liver cytoplasmic fractions of eight commercial and three non-commercial species. It was possible to detect the expression of vitellogenin and other fractions of vitellogenin in the liver of male S. genie (Lane 2), *M. cephalus* (Lane 3), M.pfluegeri (Lane 4), *L. fasciatus* (Lane 6), *S. variegatus* (Lane 8), *L. monostigma* (Lane 9) and *L. campechanus* (Lane 10). Vtg fractions indicated by the bands and labeled Vtg1, Vtg2, Vtg3, and Vtg4 are the different polypeptide forms of Vtg in the female carp plasma. These are polypeptides that have been delivered by the liver to the plasma to be transported to the female gonads.

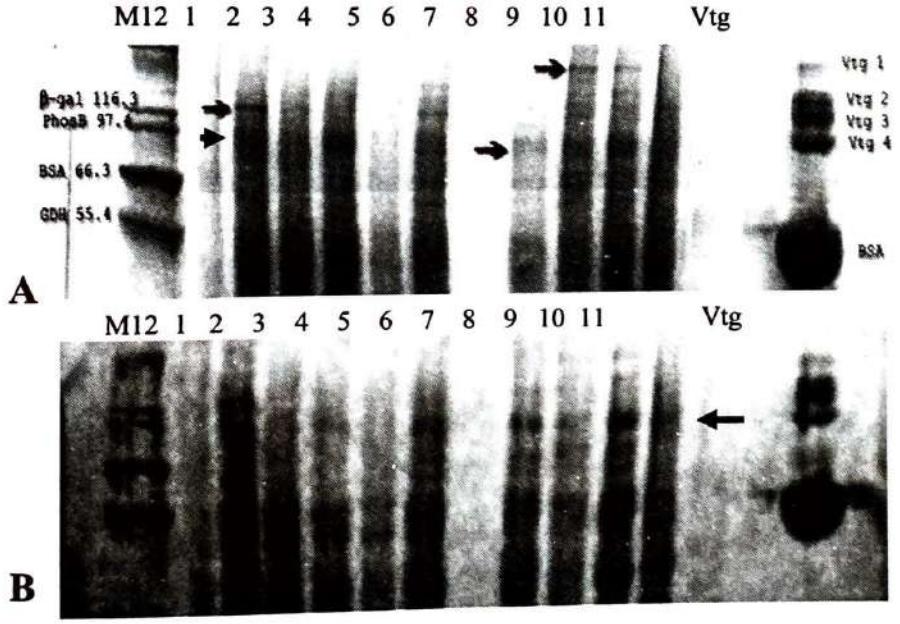

Figure 6. Cytoplasmic fractions of male (A) and female (B) hepatocytes showing or not vitellogenin expression. (A) Male liver cytoplasmic fractions: M12- standard MW, 1-*H. biocellatus*, 2-*S. genie*, 3-*M. cephalus*, 4-*M.pfluegeri*, 5-*S. crumenopthalmos*, 6-*L. fasciatus*, 7-*A. vaigiensis*, 8-*S. variegates*, 9-*L. monostigma*, Lane 10-*L. campechanus*, Lane 11-*D. tabl*, Vtg-carp vitellogenin standard. (B) Female liver cytoplasmic fractions in similar sequence as the males'.

The clear manifestation of high molecular weight vitellogenin fraction (180kDa) in the liver of only the two *Lutjanus* spp. indicated their hepatic condition and probably the gonad status in responding to the environment where they have come from. This anomalous expression gives a warning on the present environmental status of the surrounding waters of Iligan City to as near as less than 5-km distance from the shoreline. Male vitellogenesis is obviously inconsistent with the external appearance of the large fish' liver tissues collected. This only tells us that there are possibilities of losing this particular species or its future recruitment may decline if not given proper attention.

Other stained bands comparable to female fractions could be observed from the male samples. These are the bands above the 116.3kDa and below the 97.4kDa, which may be related to the previously identified vitellogenin fractions by Hartling and Kunkel, 1999 to correspond the 101kDa and 94.4kDa polypeptides.

In the female liver fractions (Figure 5B), the most prominent was the 94.4kDa polypeptide observed clearly in *L. monostigma* and *L. campechanus*. These are more obviously expressed only by *S. genie*, *M. cephalus*, *M. pfluegeri*, *L. fasciatus*, *S. variegatus*, *L. monostigma*, *L. campechanus*, and *D. tabl. S. crumenopthalmus* apparently did not show any of the bands observed in all others for unknown reasons. The clear lanes of *S. biocellatus* and *A. sexfasciatus* in Lanes 1 and 7, respectively, may probably be due to some errors in sample preparations. The livers of these two non-commercial species were too small to be recovered after they were dissected out from the intact fish.

Implications of Male Vitellogenesis to Ecology

Though the observed anomalous expressions of vitellogenins and similar polypeptides in male individual fishes from Iligan City remain under investigation and needing further verification by doing protein characterization to be proven true, the more direct implication of this still unconfirmed possibility is the future ecology of fish in the bay. This particular observation must be re-examined and validated by more elaborate tests using a robust statistical tool before completely accepted and must be done in a multi-generation studies to establish the hypothesis that endocrine disruption may reduce the reproductive fitness of fish as observed by Kime and Nash, (1999) on *D. rerio*. However, this also calls for more focused objective in the next studies to be conducted concerning the environmental health status of Iligan Bay being in the midst of economic progress by industrialization.

Conclusion and Recommendations

Selected coastal villages considered as fish landing sites in Iligan City remain a good nursery and fishing grounds with still good number of commercial fishes namely *Sphyraena genie, Decapterus tabl, Mulloidichtys pfluegeri, Leiognathus fasciatus, Lutjanus campechanus* (red snapper), *Selar crumenopthalmus, Lutjanus monostigma* (onespot napper), and *Mugil cephalus* based on the fish catch composition from June to July 2004 collections. The three representative non-commercial fishes that have already been noted as among the fishes served in the dining table of ordinary fisher folks have shown sturdiness despite the environmental insults mostly observed to be within the near shore areas and may serve as indicators of the present conditions of the coral communities of Iligan City.

Their corresponding liver tissue status and the HSI values were very low indicative of low activity of the liver in relation to their metabolic functions. The male expression of vitellogenins, however, is a clear manifestation of the anomalous development of liver tissues that may reflect the present status of the gonad of male fishes by the mal-expression of female egg protein precursors. This condition if not further checked and verified, may eventually cause the decline in the recruitment of adult fishes in the bay and later result to the more pronounced decline in fish populations in any part of the bay.

It is recommended that elucidation and characterization of the polypeptides from male livers that correspond to the purified female fish Vtg fractions be done and identification of the chemical forms of the still unknown endocrine disruptors in Iligan Bay following the recommendations of United States-Environmental Protection Agency (US-EPA) and United States Geological Services (USGS) be considered in future works to give light to these observations. Moreover, fecundity studies on various commercial fish species in Iligan Bay may be necessary to give a more concrete picture of the endocrine and reproductive status of the commercial fishes. By doing a more comprehensive program that will deal with the overall fish population dynamics and ecological studies in the bay is not too late. When all these things are considered for funding, the life of the fishes and the bay environment will be preserved even in the midst of industrial development.

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