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Reproductive aspects of the native Sharpnose hammer croaker, *Johnius borneensis* (Bleeker, 1850) from Agusan River Estuary, Caraga region, Philippines

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Abstract
Preliminary analysis on the reproductive features of the commercially important *Johnius borneensis* from the Agusan river estuary was studied between the months of February-April, 2017. A total of 223 specimens, of which 117 were females (b=3.356, K=0.5560) and 106 males (b=2.9054, K=0.5560) were examined. A strong coefficient of correlation was observed between the length and weight of fish samples. Overall sex ratio is 3:1 (female dominance) with a mean fecundity of 52487.5. Length at first maturity was 137mm. March to April was considered as one of the breeding seasons for *J. borneensis.* Mature to spawning individuals were collected at the heart of the estuary while immature individuals were observed offshores. Because of the peak fecundity and number of mature to spawning individuals in April it is initially recommended to lessen fish catch for *J. borneensis* within this month especially at lengths 180mm and above (spawning).

Keywords: Length weight relationship, fecundity, maturity stages, sex ratio, estuary

1. Introduction
The physiological plasticity of estuarine fishes stand out among other euryhaline fish because of their response to frequent changes in salinity and other environmental challenges [1]. Estuaries may be used by fish species as nurseries, feeding grounds, spawning grounds or migration pathways [2]. Even though estuarine areas are characterized by complex process of salinity variations diverse biotic communities were still adapted to these fluctuations [3]. Sciaenids were abundant residents in estuaries [4] but studies were less compared to other euryhaline fishes. They are noisy, bottom oriented fishes with high commercial value. The genera *Johnius* was the most speciose genus in the Indo-West Pacific area [5]. One of the most important estuary in Mindanao was at the mouth of Agusan river. Influx of freshwater from the Agusan river meets the seawater of the Butuan Bay in this area. *Johnius borneensis* (locally known as "guama") is one of the most abundant and important food fish in the Agusan river estuary. *J. borneensis* was a marine-estuarine dependent fish [5].
Effluents from the upland areas of Agusan del Norte, Agusan del Sur, Compostella Valley and Davao region coupled with overexploitation pressured the organisms in the area [6].
Because of the alarming increase in population along the mouth of the estuary, and in Butuan City and nearby municipalities, food security to sustain the demands was a big issue [7].
New methods were currently underway to monitor the fish population and their relative robustness not just stocks assessments. Among these were Length-Weight Relationship (LWR) [8] and reproductive biology assessments such as fecundity estimates and maturity schedules [9]. LWR is important in predicting length to weight values and biomass estimation [10]. Fecundity determines the total egg production in fishes [11] which is essential in decision-making, [9] since there is a strong relationship between the size and fecundity [12]. On the other hand, the gonado-somatic index (GSI) reflects the energy allocation by a fish population. It is a measure of the gonad weight to body weight. Large GSI values indicate a favorable status of the organism [13]. GSI also determines the reproductive seasonality of the fish such as spawning time [14]. Study on reproductive biology is essential in assessing commercial potentialities of its stock, life history, culture practice and actual management of its fishery [15]. To have an effective management of fishery and in order to make success in fish culture, it is important to assess the yearly breeding cycle of culturable fishes [16].

Study of sex-ratio, length at first sexual maturity, cycle of maturation and spawning periodicity are important aspects of reproductive biology study of any fish species [17].

To compensate the demands of food and the conservation of species, conservation managers should also look into reproductive accounts not just mere fish stocks assessments [18]. As dependence on aquaculture stocks increases in the Lower Agusan river due to the increase of domestic households along its course, this necessitates the need to study this native fish to develop strategies for effective management such as sustainable harvesting.

The present study was aimed to assess the length-weight relationship of male and female *J. borneensis,* length at first maturity, condition factor, maturity stages, sex ratio, fecundity and be able to mapped out maturity stages and discuss conservation implications.

2. Materials and Methods

2.1 Study Site

The study was conducted at the Agusan River estuary, Agusan del Norte, Philippines (Fig. 1). The freshwater Agusan River drains towards the Butuan Bay forming an estuary at the junction. The estuary was a fishing ground to various brackish water fishes, mud shells and crabs. Local fishermen were tapped to collect fish samples for three consecutive months from the months of February to April, 2017. Method of collection includes the use of hook and line and lodging of fish nets.

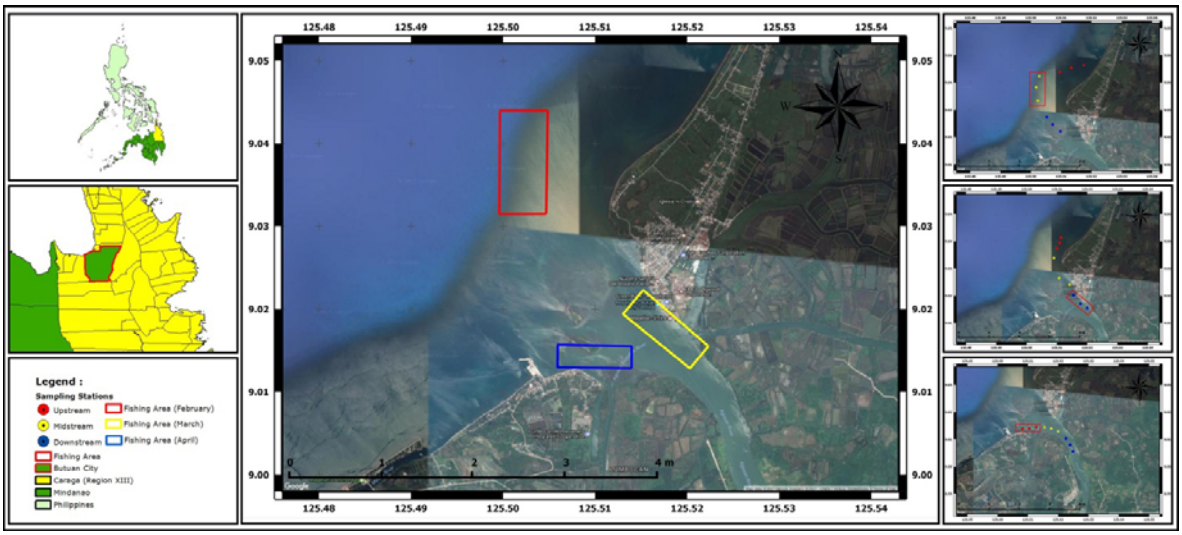


Fig 1: Map of the Agusan River estuary showing the fishing area from February-March, 2017. Inset left: Map of the Philippines and Agusan del Norte.

2.2 Sampling and specimen processing

A total of 223 specimens of *Johnius borneensis* (117 females and 106 males) from the Agusan River estuary were examined for three consecutive months (February-April, 2017). *J. borneensis* (Fig. 2) is a bottom dweller fish (inhabits at about 2inches from the bottom of the substrate) and thrives on clear waters (according to interviews). There were 94 individuals examined in February in which 50 were females and 44 were males, 57 individuals were examined in March (females=27 and males=30) and 72 were examined in April (females=40 and males=32).

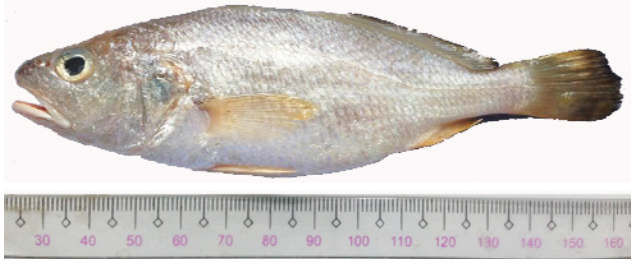


Fig 2: *Johnius borneensis* (Bleeker, 1850) from Agusan River estuary, Philippines.

The number of fish examined depends upon the number of fish caught by the tapped fishermen. The total length (TL) of each fish was measured from the tip of the snout to the caudal fin using a ruler and the weight of the fish sample was measured using a top-loading balance. For Gonad analysis, fish samples were sorted as to male or female and maturity stages was identified (Table 1). The ovary and testis were weighed and measured.

2.3 Fish Analysis

A. Length-weight Analysis

The statistical relationship between the length and weight of fishes will be established using the formula: $\mathbf{W=aL^b}$; where W=weight of the fish (g), L=length of the fish (cm), a=constant, b=exponential expressing relationship between length-weight. An exponent with the value between 2.5 and 3.5 to describe normal growth dimensions of relative well-being [19].

Transformation was made using the natural logarithms of the observed LW:

$$\mathbf{Log\ W = b\ log\ L + log\ a}$$

The correlation r^2 that was the coefficient of determination between the LW was computed from the linear regression analysis.

B. Condition Factor

The condition factor represents the degree of well-being/robustness of fish, are determined by using the equation:

$$K = \frac{100W}{L^b}$$

Interpretation Scale [20]:
K_{TL} value: Description
1.60 Excellent condition, trophy class fish
1.40 A good, well-proportioned fish
1.20 A fair fish, acceptable to many anglers
1.00 A poor fish, long and thin
0.80 Extremely poor fish, resembling a barracuda; big head and narrow, thin body

C. Gonado-somatic Index
Calculated using the formula [21]:
GSI= [(gonad weight)/(body weight)] x 100

D. Fecundity Estimation
Following the volumetric method, ovaries were mixed to 50ml distilled water and stirred repeatedly. Three aliquot samples were taken from the 50ml mixed eggs with the formula:

$$F = \frac{nV}{v}$$

;where n=number of eggs in the subsample, V=volume to which the total number of eggs is made up and v=volume of the subsample.

E. Maturity Stages
Maturity Stages of *J. borneensis* were ranked from stage I (immature) to stage VI (spent), adapted from Hoda and Ajazuddin) [22].

2.4 Statistical Analysis
Analysis of Variance (ANOVA) was computed for the comparison of data sets and Graph Pad Prism version 6 was used for the graphical illustrations.

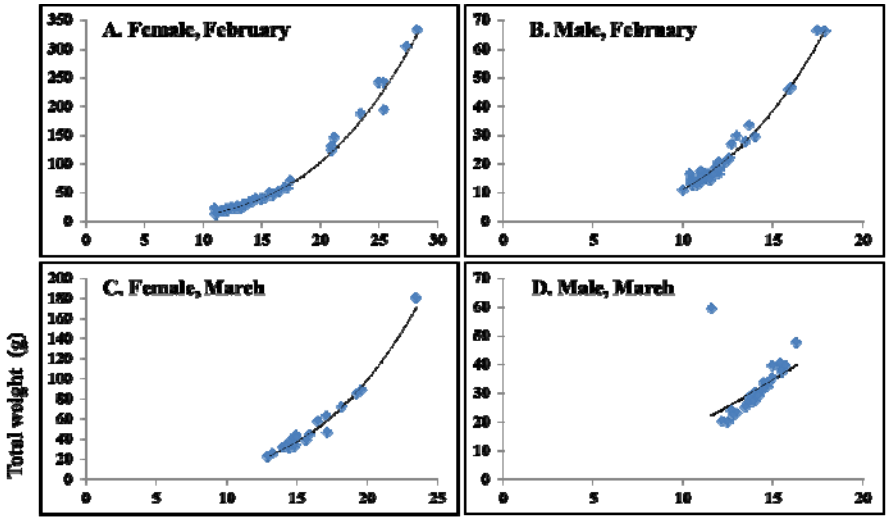
3. Results and Discussion

3.1 Length-Weight Relationship and Condition Factor
223 specimens of *Johnius borneensis* (117 females and 106 males) were examined throughout the study period. Female ranges between 11cm to 28.3cm and a body weight ranged from 15.18g to 333g. While males had a size range between 10cm to 17.9cm with a body weight of 10.74g to 66.30g. The relationship between length and weight of each sex was shown in Table 1 and in the graphical illustrations in fig. 3. A positive allometric growth in all females (b=3.356) was recorded from February-April, 2017. This implies that, as body size (length) increase, its body weight also increases in a geometric fashion. While males growth pattern was accordingly following an overall negative allometric fashion (b=2.9054).

Table 1: Length-weight relationship ($W=aL^b$; g,cm) of male and female of *J. borneensis* collected from February-April, 2017.

Months	Sexes	Total length range (cm)		n	a	b	r^2
February	Female	11	28.3	50	0.0049	3.3256	0.9869
	Male	10	17.9	44	0.0092	3.0784	0.9497
March	Female	12.9	23.5	27	0.0038	3.3949	0.975
	Male	11.6	16.3	30	0.3587	1.6872	0.3006
April	Female	12	22.5	40	0.0032	3.4879	0.9847
	Male	11.8	17	32	0.0063	3.2192	0.9629
Combined	Female	11	28.3	117	0.0044	3.356	0.9828
	Male	10	17.9	106	0.0142	2.9054	0.8825

Regression analysis showed a strong correlation between the length and the weight of *J. borneensis* in all studied months except in the males on the month of March (r^2=0.3006). The female had an overall r^2 value of 0.9828 (98.28%) while males have a 0.8825 (88.25%) correlation. Females were usually larger in size than males and were much heavier because of the presence of reproductive gonads.
LWR and regression analysis of *Otolithes ruber* (a sciaenid) from Tamil Nadu India were recorded to have a length group ranged from 11.2cm to 42.5cm, a 'b' value of 2.8347, which also exhibited an allometric growth pattern and an overall r^2 value of 0.885 [8]. Other sciaenids from Mandapam Region such as *Johneiops sina* (length 73-162mm), *Dendrophysa russelli* (length 66-181mm), *Johnius dussumieri* (length 71-204mm) and *Johnius macropterus* (length 78-167mm) also showed a strong correlation between the length and the weight (r^2 value 0.95-0.98) [23].



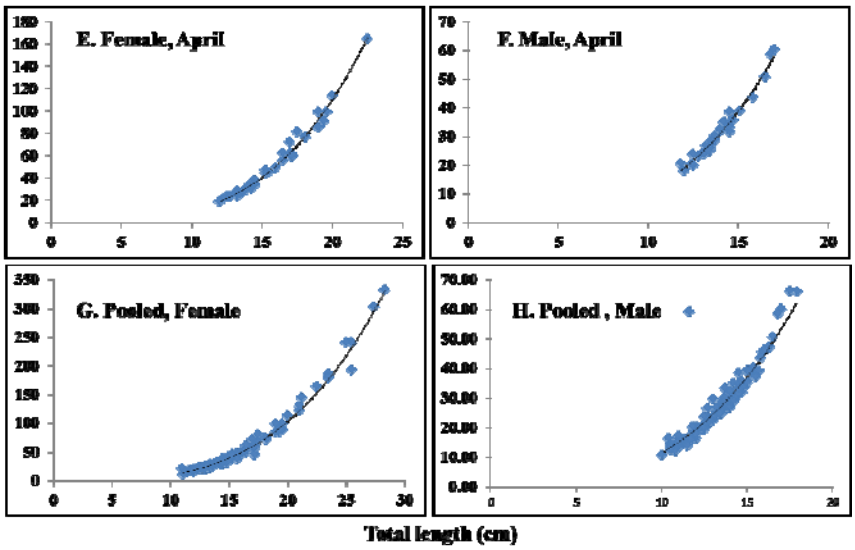


Fig 3: Length-weight relationship of *Johnius borneensis* A. Female, B. Male in the month of February; C. Female, D. Male in the month of March and E. Female, F. Male in the month of April, 2017 at the Agusan River estuary.

The mean condition factor (Table 2) in females was consistently lower in all months studied compared to males. Mean condition factor for all female was 0.5560 which was lower compared to males (K=1.5127). Computed condition factor for female falls within an extremely poor condition category while males was depicted to have a well-proportioned body. Condition factor reflects the physiological state of the fish influenced by both intrinsic and extrinsic factors [24] it is important in monitoring fish feeding intensity, age and growth [25]. Fish samples that had a higher condition factor values are in better condition than with lower condition factor [26]. Males usually had higher condition factor [27] than females. This could be attributed to feeding intensity of males compared to females [28]. According to the scale of Barnham and Baxter [29] overall female condition factor fall into the extremely poor condition category while males fall into a good, well-proportioned fish category.

Table 2: Mean condition factor (K) in both sexes of *J. borneensis* from February-April, 2017.

	Female	Male
February	0.6908	0.9985
March	0.4336	36.8252
April	0.3553	0.6519
Combined	0.5560	1.5127

3.2 Sexual Maturity and Sex Ratio

As shown in Table 3, the month of February had the most number of individual's studied (n=94) with the month of March to be being the least (n=57), the number of fish studied depends upon the scheduled fish catch. The trends produced by the data were of valuable importance. To highlight, there were more immature and developing virgin individuals (stage I and II, respectively) both in female and in male was recorded in the month of February. The trend increases as in the month of March more maturing and mature individuals (stage III and IV) was recorded. April had the highest mature; gravid and spawning individuals (stage IV, V and VI, respectively) recorded both in females and in males. This means that April is one of its spawning seasons. All maturity stages occurred throughout the conduct of the study except in spent stage (stage VII).

Table 3: Maturity stages of *J. borneensis* from the Agusan River estuary between the months of February to April, 2017.

Stage	February		March		April	
	Female	Male	Female	Male	Female	Male
I	11	10	2	5	1	2
II	19	29	2	7	1	1
III	6	2	6	8	5	5
IV	5	2	14	10	13	16
V	5	1	2	0	11	7
VI	4	0	1	0	9	1
VII	0	0	0	0	0	0
Total	50	44	27	30	40	32

Table 4: Sex ratio of *J. borneensis*.

Stage	Counts		Sex Ratio		Total	Ratio %
	Female	Male	Female	Male		
I	14	17	1	1	31	13.90
II	22	37	1	1	59	26.46
III	17	15	1	1	32	14.35
IV	32	28	1	1	60	26.91
V	18	8	2	1	26	11.66
VI	14	1	14	1	15	6.73
VII	0	0	0	0	0	0
Total	117	106	20	6	223	100
Mean			3	1		

An unequal, overall sex ratio of 3:1 in *J. borneensis* in favor of females was recorded (Table 4). Maturation and breeding could be the reason for the predominance of females in large group size [22]. *Johnius elongatus* as recorded by Hoda and Ajazzudin [22] had a sex ratio value of 1:1.20 still in favor of females. Female dominance was also observed in other sciaenids such as in *Dendrophysa russelli, Johnius sina* and *J. dussumieri* [23].

3.3 Length at first maturity (L50)

The percent occurrence of *J. borneensis* in different stages at maturity was calculated at 10mm length interval. Females were only taken into consideration since the population of matured males did not reach 50%. Stage IV to stage VI was treated as mature. Results showed that below 115mm all specimens of *J. borneensis* were immature and 50% were mature at 137mm and all fishes above 180mm were mature. In comparison to other sciaenids, lengths at first maturity of *D. russelli, J. sina, J. dussumieri* and *J. macropterus* from Mandapan region were 144mm, 152mm, 168mm and 136mm respectively [23]. *Pennahia anea* from northern South China Sea size at 50% maturity was 143mm [37]. The values calculated for *J. borneensis* size at 50% maturity were close to other sciaenids species. $L_{50\%}$ is an important relationship between the body size and the number of individuals mature within the size intervals [38].

3.4 Fecundity Estimation and Fecundity-Length, Weight Relationship

Of the 117 females of varying sizes and weight, 64 samples were regarded as fecund with 32 stage IV (mature), 18 stage V (gravid) and 14 stage VI (spawning) individuals. Table 5 shows the estimated fecundity of stage IV-VI females of *J. borneensis*. The highest fecundity in mature female was 51300 eggs; in gravid females was 72450 eggs and 100000 eggs in spawning females. Spawning female count was highest in the month of April with 9 individuals and a fecundity range of 56400-100000 eggs. Gravid females was highest still in the month of April with 11 individuals but highest fecundity was recorded in March (72450 eggs). Mature females were highest in March with 14 individuals and a fecundity range of 20250-51300 eggs. It was observed that spawning females count and the highest fecundity was recorded in April followed by March, leading to the conclusion that March and April is one of the spawning seasons for *J. borneensis*. Investigation on the reproductive biology of *Johnius elongatus* revealed that this species spawns twice a year from January-February to April-May and from August to October [22]. This supports the spawning observation in *J. borneesis* in April.

Table 5: Fecundity estimates of mature and spawning female of *J. borneensis.*

	Stage	Number of individuals	Estimated fecundity range (No. of eggs)
February	IV	5	29400-49900
	V	5	49900-65000
	VI	4	65650-75000
March	IV	14	20250-51300
	V	2	68200-72450
	VI	1	88350
April	IV	13	21350-49900
	V	11	36750-60000
	VI	9	56400-100000
Combined	IV	32	20250-51300
	V	18	36750-72450
	VI	14	56400-100000

The mean fecundity in combined females of *J. borneensis* was 52487.5 ranging from 20250-100000 in fish sample measuring 12.9cm and 26.35cm, respectively. When compared to *J. elongatus, J. elongatus* has mean fecundity of 42818 with a range of 4238-167669 [22].

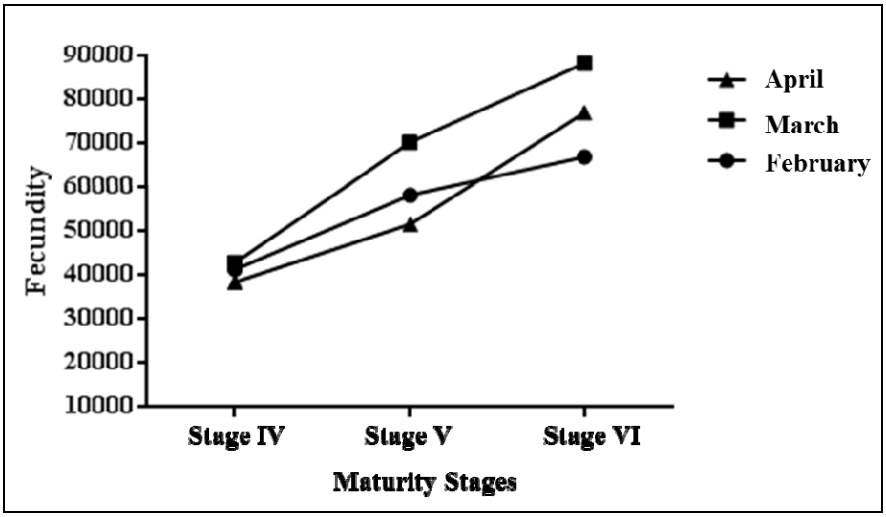


Fig 4: Mean fecundity of *J. borneensis* across sampled months.

Although the month of April had the highest fecundity due to the highest number of spawning and gravid individuals, mean fecundity was highest in March as shown in Fig. 4. Thus, March and April were consistently had the highest fecundity in terms of egg counts and highest number of fecund individuals.

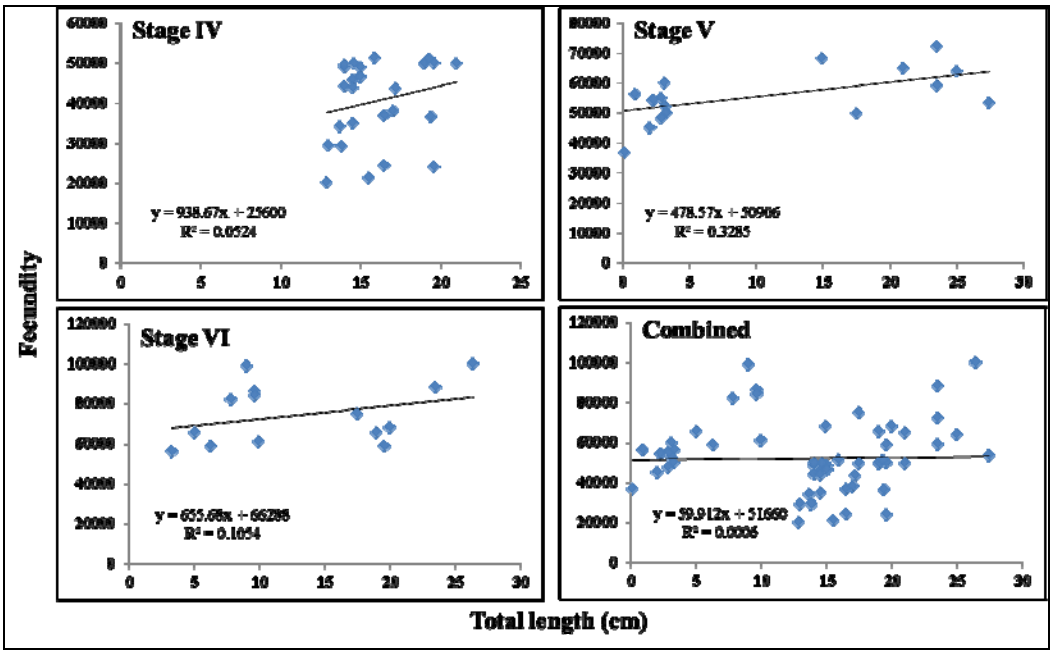


Fig 5: Fecundity-length relationship of fecund females of *J. borneensis* in all sampled months at Agusan River estuary, Philippines.

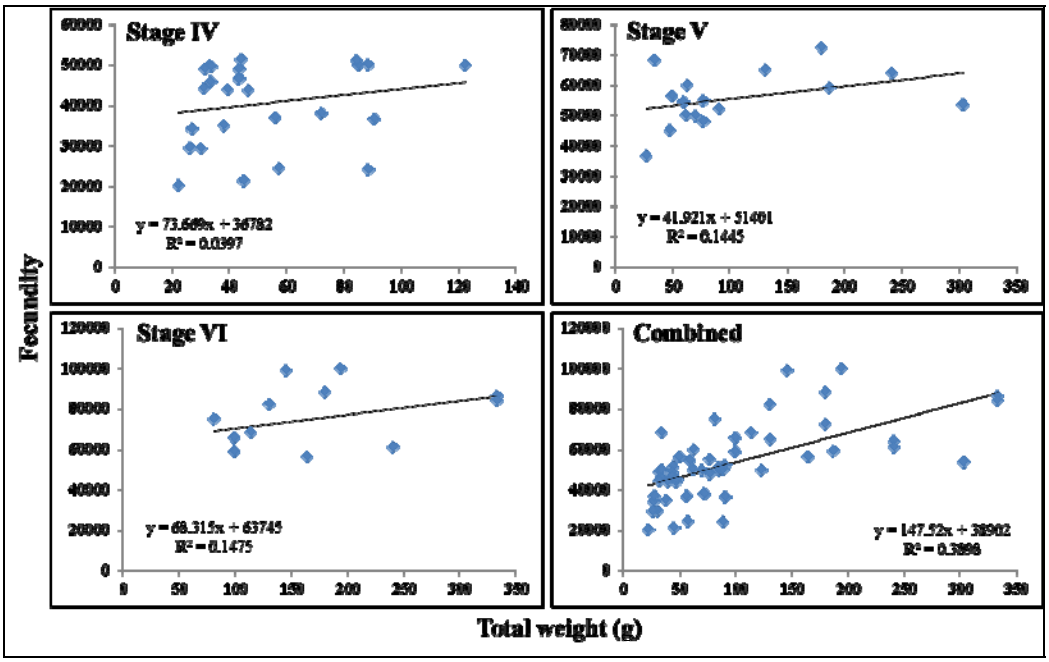


Fig 6: Fecundity-weight relationship of fecund females of *J. borneensis* in all sampled months at Agusan River estuary, Philippines.

The coefficient of correlation in combined females between fecundity and length (r=0.0006) and weight (r=0.3898) was not correlated (Fig. 5-6). Thus, fecundity in female *J. borneensis* was not correlated to its length and weight. Sciaenids spawn only once a year either in single or two batches [22]. The month of March to April is one of the spawning batches for *J. borneensis*.

3.5 Gonado-somatic Index (GSI)

The Gonado-somatic index is a function between the weights of the gonad to the weight of the body [30]. Gonado-somatic index is a common metric to determine the reproductive allocation and condition of fishes [31] and is species specific [32]. Table 6 shows an increase in calculated GSI from gonadal stage 1 and peaked at stage VI both in male and in female. There was no stage VII individuals recorded in the study. The highest GSI value in female was in stage VI (5.56%) and in male (0.77%). The highest GSI value in stage VI is not surprising since body weight also increase with an increase in gonad weight. Between sexes, females had higher GSI values compared to males because of the heavier weight of ovaries which contain the eggs [33].

Table 6: Mean GSI values at different stages of maturity in *J. borneensis* across months.

Stage	Female (%)	Male (%)
I	0.54	0.47
II	0.53	0.48
III	1.96	0.44
IV	2.05	0.61
V	3.20	0.59
VI	5.46	0.77
VII	0	0

3.6 Maturity stages of *J. borneensis* plotted in Agusan River Estuary

Graphical illustrations on dominant maturity stages recorded on each sampling months at Agusan River estuary, Caraga Region was depicted on Figures 7A-C. February with fishing grounds at geographic coordinates: 9° 2' 17.232" N, 125° 30' 10.008" E; 9° 2' 3.804" N, 125° 30' 5.94" E and 9° 1' 47.784" N, 125° 30' 8.316" E was composed dominantly of immature (stage I) and developing virgin (stage II) individuals of *J. borneensis*. This fishing ground was still in brackish water but is offshore (towards the sea already). From the 94 individuals sampled in this area, an amazing 21 individuals were immature and 48 were developing virgin, concluding, that this area was constituted of immature individuals (Fig. 7A).

Fishing grounds in the month of March had the geographic coordinates of 9° 1' 4.224" N, 125° 30' 53.568" E; 9° 0' 54.9" N, 125° 31' 5.052" E and 9° 0' 50.112" N, 125° 31' 13.188" E. This month was characterized mainly by maturing (stage III) and matured (stage IV) individuals. The fishing area was on the upper reach of the brackish water, in the junction of Magallanes and Banza river meeting the Agusan River. Out of the 57 individuals of *J. borneensis* studied in this area, 13 were maturing and 24 were matured both in male and female (Fig. 7B).

Fishing grounds for the month of April was still at the middle of the Agusan River estuary, located offshore of Barangay Lumbocan, Butuan City with geographic coordinates of 9° 0' 46.512" N, 125° 30' 46.116" E; 9° 0' 45.108" N, 125° 30' 37.512" E and 9° 0' 45.108" N, 125° 30' 29.16" E (Fig. 7C). The area was dominantly composed of mature, gravid and spawning individuals. Of the 72 individuals sampled from this fishing ground, 29 individuals were matured, 18 were gravid and 10 were spawning both for male and female *J. borneensis*. The area was at the junction of Masao river draining towards the Agusan River estuary.

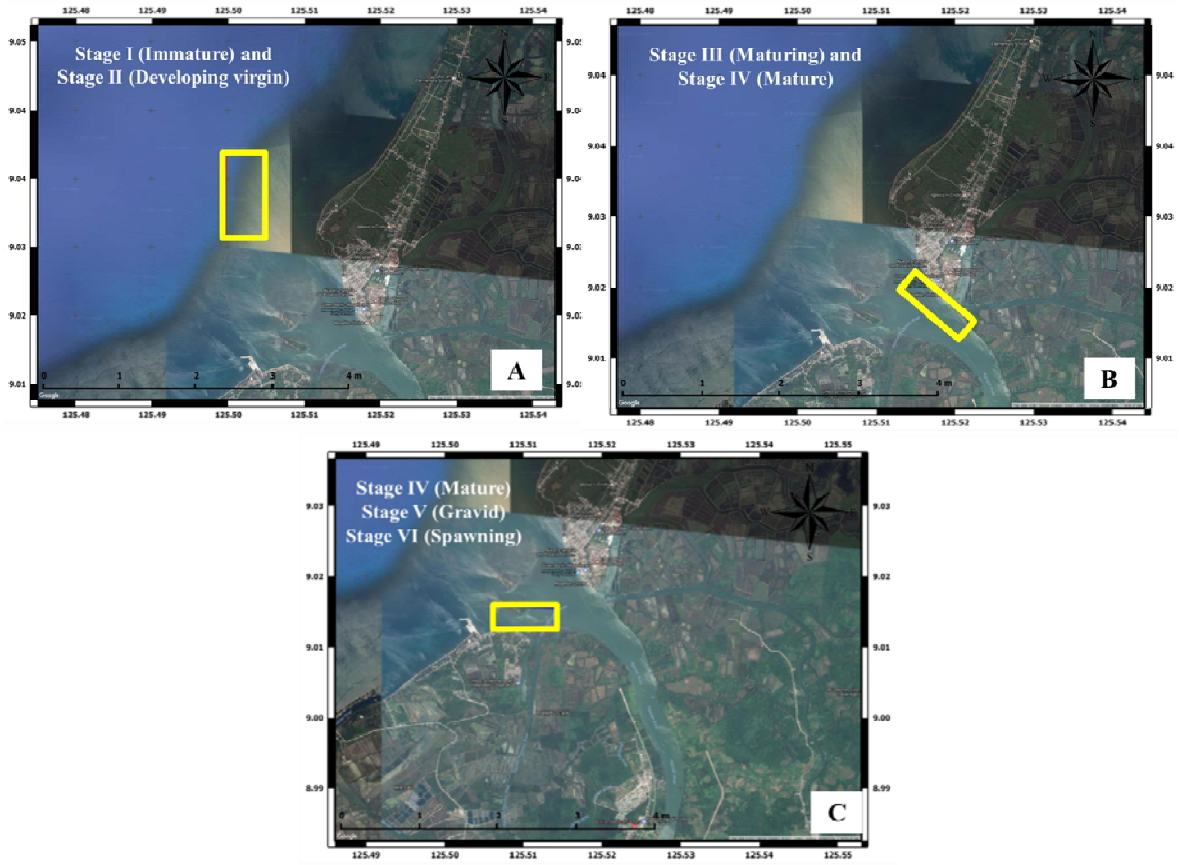


Fig 7: Map of the fishing area at Agusan River estuary during the month of A. February, B. March and C. April, 2017 with the dominant fish maturity stage.

Based on the position in which matured to spawning individuals were located, it was observed that fecund *J. borneensis* for both male and females was present on the heart of the estuary at the junction of freshwater rivers (Masao, Magallanes, and Banza river) as observed in the months of March-April, 2017. As mentioned earlier, March to April had the highest fecundity and GSI values, thus, these areas can be referred to as mating and spawning grounds. Chao *et al*., [34] stated that sciaenids (the family in which *J. borneesis* belongs) utilizes estuaries as spawning and nursery grounds.

While mature to spawning individuals were observed at the heart of the estuary, immature individuals were recorded at the lower reach of the estuary (towards the sea). This support the claims of Kinoshita and Fujita [35] that during the early life stages of sciaenids, they utilized surf zones because of the abundance of food supply and migrate back to estuarine areas

during spawning. Furthermore, estuarine fishes such as *J. borneensis* were indeed not permanent residents but were migrants from marine to brackish waters [36]. A study conducted at the estuary in the Gulf of Mannar in *Dendrophysa russelli* (a sciaenid) recorded that specimens caught at sea were immature and fish catch of few estuaries along Tirunelveli coast of the Gulf of Mannar had fully mature gonads (Stage IV), concluding that *D. russelli* migrate to estuaries during spawning [37]. These support the presence of mature and spawning individuals in the upper reach of the estuary and immature individuals in the lower reach (near surf zones at sea).

3.7 Conservation Implication

Results of the study showed that highest number and fecundity of mature, gravid and spawning individuals were observed concentrating in the heart of the Agusan River estuary while highest counts of immature individuals was observed offshore (near surf zones at sea). Size range analysis for females was 110mm to 283mm and for males 100mm to 179mm. Preliminary results on length at first maturity (L_{50}) was137mm. Meaning, when *J. borneensis* reach this size they were mature. Below 115mm all specimens were immature and above 180mm all specimens were mature. Since all fishes recorded above 180mm were regarded as mature, and these individuals reside at the heart of the estuary for their mating and spawning periods during the months of March to April and peak at the month of April, these individuals were at their breeding season. Literature reviews revealed a single breeding cycle of sciaenids but with a maximum of two batches per year. Because of the peak fecundity and number of mature to spawning individuals in April it is initially recommended to lessen fish catch for *J. borneensis* within this month especially at lengths 180mm and above (spawning). Data on reproductive biology of *J. borneensis* recorded in this study would be essential for successful culture of this native fish in the estuary.

4. Conclusion

Reproductive Biology is important in fishery resources and conservation. Reproductive biology of *Johnius borneensis* locally known as “guama” from Agusan river estuary was studied between the months of February-April, 2017 caught through hook and line and fish net lodging. *J. borneensis* length range between 110mm to 283mm for females and for males was 100mm to 179mm with size of 50% length of maturity at 137mm. All of the fish samples at 180mm and above were mature. Condition factor for female was 0.5560 0.5560 for males. Females dominated males at a ratio of 3:1. Computed fecundity was 52487.5. Most of the immature specimens were observed near surf zones and mature to spawning individuals were observed at the estuary proving the migratory nature of this sciaenid species.

5. Recommendation

Effective sustainable management plans should be anchored in reproductive aspects of the fish. Thus, results from this study could strongly help policy makers to ensure food security in the near future.

6. Acknowledgement

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