

RESEARCH ARTICLE

At the Onset of Gonadogenesis: The First Description of Ovarian and Testicular Macro and Microanatomical Maturation of Bullet Tuna (*Auxis rochei*) in Southern Philippines

John Christian D. Entia[1]*, Niña Mae B. Nabre[1], Glennville A. Castrence[2], Blessie Justine G. Arellano[1], Red Arthur Duke Amoncio[1], James T. Wetzel[3], Edna P. Guevarra[4]

[1]*Histopathology Laboratory, Regional Science Research Center, Office of the Vice Chancellor for Research and Extension, Mindanao State University General Santos*
[2]*Department of Aquaculture, College of Fisheries, Mindanao State University General Santos*
[3]*Biology Department, Presbyterian College, South Carolina, USA*
[4]*Biology Department, College of Natural Science and Mathematics, Mindanao State University General Santos*

ABSTRACT

Structural and functional maturation in gametogenesis through comprehensive histoarchitectural and gross morphological analyses can serve as a powerful tool for gauging population health and resilience within gonochoristic fish populations. The Philippines boasts a robust neritic tuna fishery, with Bullet Tuna (*Auxis rochei)* being a significant species. Nonetheless, our understanding of reproductive parameters in neritic tunas remains deficient, hampering sustainable management strategies. In this study, 975 fish samples were collected from Sarangani Bay, Davao Gulf, Moro Gulf, and Sulawesi Sea. Samples were then classified and grouped into four size classes: Size 1 (≤ 15 cm), Size 2 (15.1 – 25 cm), Size 3 (25.1 – 35 cm), and Size 4 (>35 cm). Six reproductive maturity stages were profiled: Immature, Onset Maturation, Developing/Maturing, Spawning/Mature, Spent, and Recovering. In Spawning/Mature, the testis appears opaque white, with full lobes discharging contents.At the same stage, the ovary is orange/pink in color, with noticeably visible venation. Examination of histoanatomy shows viable spermatozoa in testes, along with lobular lamina, secondary and primary spermatocytes, and spermatids. Ovaries at this stage show secondary vitellogenic oocytes and prominent yolk granules, with distinguishable follicular epithelium at the border of the follicle. Our data show that *A. rochei* gonad strategy is asynchronous and spawns at 25.1–35.0cm (Size 3). Our study is the first description of the gonadal maturity on the gross and histological aspects of *Auxis rochei* concerning its fish body length in the waters of Sarangani Bay, Davao Gulf, Moro Gulf, and Sulawesi Sea.

*Corresponding Author: *johnchristianentia12@gmail.com*
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1. INTRODUCTION

The tropical and subtropical waters of the world are home to the *Auxis* genus. Like the frigate tuna *(Auxis thazard*), the bullet tuna *(Auxis rochei*) is closer to the shore compared to its oceanic counterparts and is considered neritic (Collette and Nauen 1983). Being a fishery resource and an economic commodity, especially in the Philippines, neritic tuna has been the subject of numerous scientific investigations, especially regarding fishing and harvesting methods. However, there is little published scientific literature regarding the reproductive biology of neritic tuna (Rey and Cort 1981; Sabatés and Recasens 2001). At present, the basis for determining the sexual maturity of neritic tunas is their physical size. At the 2018 meeting of the International Council for the Exploration of the Sea (ICES), a workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF) proposed morphology as the criteria for determining maturity.

Our study proposes that evaluating the gonadal stages is a crucial element of many studies on fish reproductive biology development. The

techniques used range from thorough histological examinations to quick descriptions of the physical appearance of gonads, but other comparative studies are scarce, and accordingly, interpretations can vary dependability of such descriptions (West 1990). The information on reproductive biology is crucial for efficiently managing fisheries and aquaculture. Large-scale field biology manifestations, such as spawning season, sex ratio, batch fecundity, gonadosomatic index, and gonad histological analysis, have generally been the focus of such research to date (Susca et al. 2001). In *Scombridae*, the lack of knowledge regarding the reproductive physiology of tuna has recently been addressed by researchers (Susca et al. 2001; De Metrio et al. 2010; Chini et al. 2008). Research on fish gonadal histological alterations is essential for identifying a reproductive time frame encompassing developmental stages (Unver and Saraydın 2012). Investigation into the physiological principles driving tuna reproductive biology, such as gonadal differentiation and reproductive stages, is crucial to the management of fisheries and aquaculture (Gardner et al. 2012; Rasmussen et al. 2006).

Gonad histological characterization is a microscopic technique that, while somewhat expensive and considerably time-consuming, can offer an objective evaluation of maturity to confirm at-sea categories and demonstrate a more comprehensive process of oogenesis and development (McBride et al. 2013). A fish's reproductive biological profiling may be described by its reproductive traits, and it also conveys the mixture of the species-specific reproductive approach (Murua and Saborido-Rey 2003; Morgan 2008; Winemiller and Rose 1992). Jahan et al. (2014) stated that information on the reproductive physiology of any fish species is crucial for estimating the viable potencies of its population stock, culture practice, life history, and protection of its fishery.

Although several studies on tuna abounding within waters around the Philippines have been carried out, none focused on their reproductive biology, factors affecting sexual maturity, and their size in relation to their physical characteristics. Failure to produce knowledge on these may affect policymaking in the direction of sustainable consumption. This could lead to a future crisis and, eventually, a collapse in the supply of this valuable species. This study, therefore, aimed to (1) evaluate the gonadal maturity of *A. rochei* both on the macro and micro scale and (2) establish at which size the reproductive maturity of *A. rochei* occurs.

In an attempt to acquire a thorough description of gonadogenesis, this paper provides in-depth evidence of the reproductive maturity indices based on fish body length of A. rochei as seen in the testicular and ovarian gonadogenesis at the (a) gross morphology (micro) as well as the (b) histological levels (micro). The findings of this study may serve as a baseline for developing policy for tuna resource management and its allied sectors.

2. MATERIALS AND METHODS

2.1 Field sampling

The collection of samples was carried out in collaboration with the National Stock Assessment Program of the Bureau of Aquatic Resources (NSAP-BFAR) Region XI and XII, private tuna enterprises, and local fisherfolks. Monthly sampling was conducted for one year, from April 2021 to March 2022, around the waters of Mindanao–Sarangani Bay (5.9656° N, 125.1929° E), Davao Gulf (6813° N, 125.8280° E), Moro Gulf (6.8014° N, 123.4384° E), and Sulawesi Sea (3.6121° N, 122.2998° E), in areas selected by the BFAR XI and XII (Figure 1). Fishery-

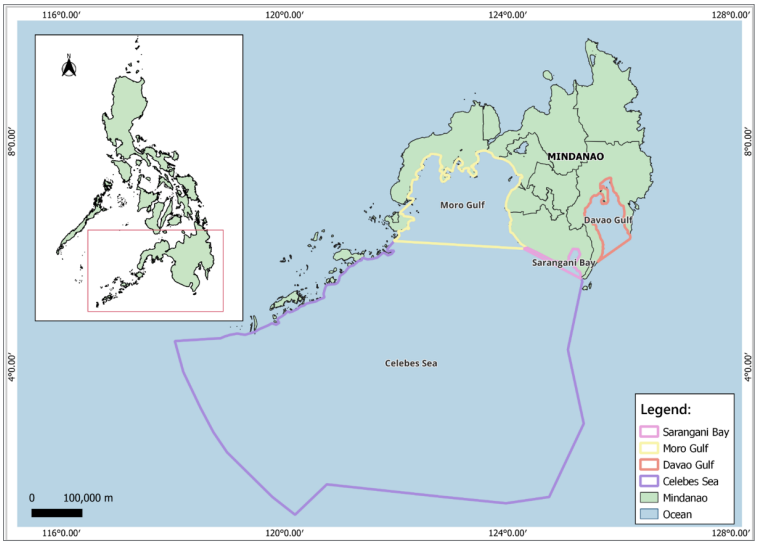

Figure 1. Collection sites for Bullet Tuna *(Auxis rochei)*. Sampling was conducted throughout the main large waters of Mindanao, particularly where fishing grounds for tuna species are well-established, specifically, in Sarangani Bay (5.9656° N, 125.1929° E), Davao Gulf (6813° N, 125.8280° E), Moro Gulf (6.8014° N, 123.4384° E), and Sulawesi Sea (3.6121° N, 122.2998° E), Southern Philippines. The sampling sites' legend identifies the locations of the fish captures (QGIS-created content).

dependent and -independent sampling strategies were used in the collection. In lieu of the event that adverse weather makes sampling impractical or impossible, fishery-independent sampling techniques were opted to be utilized. Fish morphometry, dissection (gonad extraction), tissue processing, and subsequent histological analysis and microscopy were carried out at the Histopathological Laboratory in the Regional Science Research Center of Mindanao State University - General Santos City.

2.2 Morphological Diagnostic of Bullet Tuna and Gonads

Morphological characteristics of *A. rochei* were examined and identified based on the description given by Collette and Nauen (1983) (Figure 2). Evaluation of gonad morphology after dissection was carried out ocularly immediately after extraction from the fish. The color and surface texture of lobes were profiled: ovaries (yellowish, granular) and the testis (whitish, smooth). The General Fisheries Commission for the Mediterranean (GFCM) macroscopic maturity scale for bony fish, as indicated in the 2018 report by the International Council for the Exploration of the Sea for the Workshop for Advancing Sexual Maturity Staging in Fish (ICES WKASMSF), was used to identify the stages of maturity.

2.3 Length and weight measurement

Fork length (FL) of *A. rochei* samples was taken with a ruler (1 mm accuracy) and weight was measured using an electronic weighing scale calibrated to the nearest 0.001g accuracy. The length of gonads was measured using a vernier caliper (0.01 mm accuracy). Standard length (SL), FL, total length (TL), body girth (BG), and gonad length were also measured using a ruler with a precision of 0.1mm (Akter et al. 2019).

2.4 Sexual maturity

The fish samples' FL was measured before exposing the gonads for visual assessment. The gonads were then divided into groups (maturity stages) based on their gross morphology, which was determined by the progression of their sexual development. Afterward, random gonad samples were selected from each sexual stage for histological characterization. The assessment of gonadal maturity (macro) and histological (micro) evaluations were separated due to the bias and ambiguity of the ocular gross classification technique, which could seriously misclassify the fish's reproductive stage. Histology, on the other hand, is more accurate and advanced and enables the detection of alterations at cellular and tissue levels. It should be emphasized that this study classified FL into two categories: (1) FL of the fish sample based on the stage the gonad morphology was in, and (2) FL of the fish sample based on the histological descriptions existing within its gonad at a cellular and tissue levels.

The FL during which the particular gonad maturity occurred and the FL of a specific maturity stage, as identified through histoarchitecture, were both considered in determining at which size class sexual maturity occurred. The approach was as follows: gonad samples for a specific maturity stage underwent gross (macro) morphological analysis; afterward, random samples from the same gonad stock of each stage were then selected for histological (micro) evaluation—it can be seen that the FL range for micro still falls within the FL range of macro. This study used the investigations of *A. rochei* by Bahou et al. in 2016 and 2017 as the basis for characterizing, comparing, and identifying current findings.

2.5 Histology

Cross-sections of gonads, ~1.0cm in thickness, were preserved in a 10% buffered formaldehyde solution. Cuts were washed in xylene, dehydrated in increasing amounts of ethanol, and finally embedded in paraffin wax. Histological sections

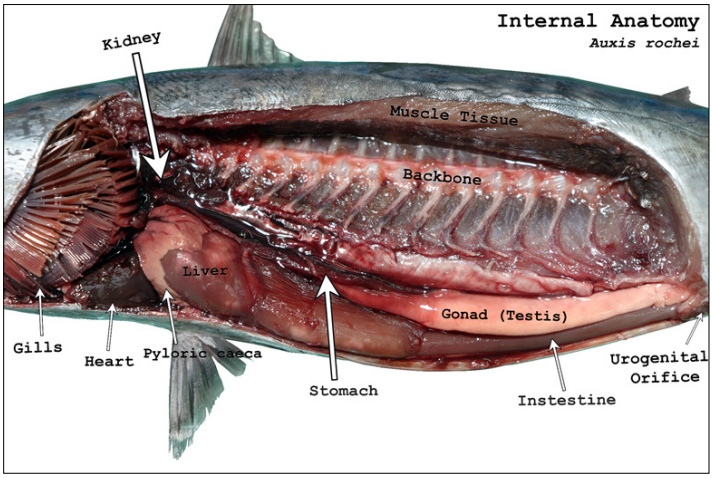

Figure 2. Internal anatomy of *A. rochei* showing no externally visible genital features (without sex dimorphism) that can distinguish males from females. Anatomy represented by male. Photo by Entia JCD and Nabre NMB.

of 5–10 um in thickness were created with the use of an automated microtome. Hematoxylin and eosin were used for differential staining. For histological analysis, we have selected three or more samples for each stage of gross gonadal development and integrated serial sectioning. Descriptions by Bahou et al. 2016 and Bahou et al. 2017 were used as the basis for determining histological variations.

3. RESULTS

3.1 Biological characteristics of *Auxis rochei*

Auxis rochei was differentiated through its broad pattern of 15 or more spots and a black-lined operculum. Dorsally, the *A. rochei* presented diagonal, nearly vertical dark bars, with a well-developed corselet on its rear side visible (more than six scales wide under the second dorsal-fin origin). We report four class sizes of *A. rochei*: Size 1: ≤ 15 cm; Size 2: 15.1–25 cm, Size 3: 25.1 – 35 cm; and Size 4: > 40 cm (Fig. 3).

3.2 Gonad morphology and histological characteristics

Testes and ovaries were bi-lobed structures, with the paired lobes more or less symmetrical, extended, and connected at the posterior to create a channel (duct) that led to the urogenital pore, which is the channel from which gametes are secreted in the event of breeding. Gonads were situated at the dorsal-posterior region of the coelomic cavity next to the kidneys and swim bladder (Figure 4).

A. rochei has six stages of reproductive maturity: Stage 1, Immature; Stage 2, Onset Maturation; Stage 3, Developing/Maturing; Stage 4, Spawning; Stage 5, Spent; Stage 6, Recovering/Resting. Stage 0, called Undetermined, was also considered. At this stage, the gonad was undifferentiated, <10.0 cm in size and translucent in appearance.

3.2.1 Female

Stage 1 (Immature) of *A. rochei* ovary was distinguished mainly through its color: pinkish, almost dark red at the anterior part; translucent, with no granules (eggs) visible to the naked eye. The ovary at this stage occupied less than one-third of the body cavity, measuring 1.85–10.30 cm. The corresponding FL of fish with this size of the ovary was measured at 19.0–26.50 cm. Subsequently, the characterization of histoarchitecture revealed that the intercellular space was mostly occupied by interstitial connective tissue (ICT). With regards to oogenesis, the Immature stage was rich with pre-vitellogenic oocytes, with the

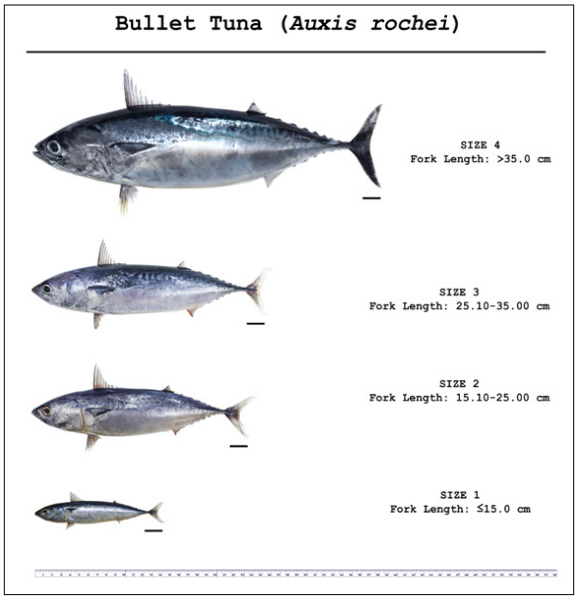

Figure 3. Determined fish size classes of *A. rochei*: Size 1 (≤ 15.0 cm), Size 2 (15.10–25.00 cm), Size 3 (25.10–35.00 cm), Size 4 (>50.0 cm). Bar = 2.0 cm. Photo by Entia JCD and Nabre NMB.

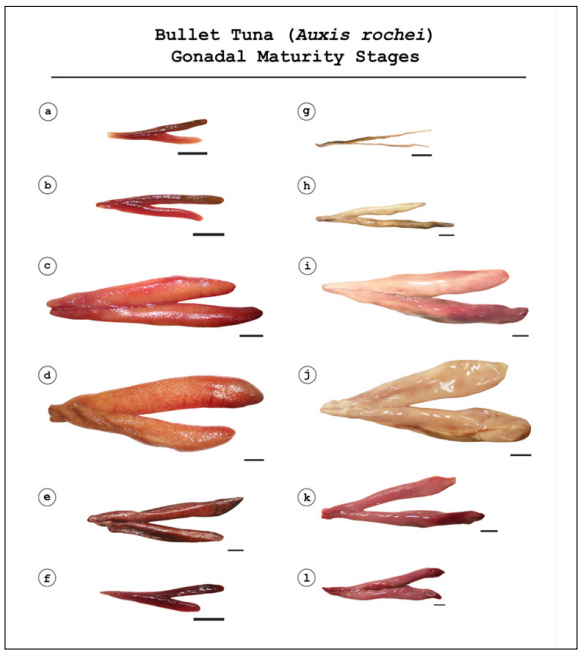

Figure 4. Ovarian (left) and Testicular (right) development of Bullet Tuna *(A. rochei)*. (a) Stage 1 (Immature) ovary; (b) Stage 2 (Onset Maturation) ovary; (c) Stage 3 (Developing/Maturing) ovary; (d) Stage 4 (Spawning) ovary; (e) Stage 5 (Spent) ovary; (f) Stage 6 (Recovering/Resting) ovary; (g) Stage 1 (Immature) testis; (h) Stage 2 (Onset Maturation) testis; (i) Stage 3 (Developing/Maturing) testis; (j) Stage 4 (Spawning) testis; (k) Stage 5 (Spent) testis; (l) Stage 6 (Spent) testis. Bar = 1.0 cm. Photo by Entia JCD and Nabre NMB.

cytoplasm (Cyt) apparent and the oocyte membrane (OM). Female A. *rochei* samples possessing these histological states ranged from 19.00–26.50 cm FL.

Stage 2 (Onset Maturation) was characterized by the preliminary enlargement of the lobes. The color of the gonads at this stage was red to light pink, with the length of the gonads ranging from 3.10–10.90 cm. Female *A. rochei* with this ovary length belonged to samples 21.70–30.10 cm in FL. Histology sections showed that previtellogenic oocytes (pr) were still prominent in this stage, although the landmark feature for this stage was the primary vitellogenic oocytes (Ost-I), which dominated most of the space, signaling the next maturation stage of pr. It must be noted that pr was distinguishable from Ost-I, as Ost-I possessed a prominent, large nucleus (N). Stage 2 fish samples with these histological characteristics were measured at 21.70–27.40 cm (FL).

Oocytes (in the appearance of granules) began to be visible through the ovarial tunica at Stage 3 (Maturing/Developing). The length of the ovary had also increased at this point, occupying two-thirds of the body cavity, precisely at 3.50–10.80 cm, belonging to fish samples 23.30–27.70 cm (FL). Contents were not expelled under light pressure. Furthermore, Stage 3 tissues of the ovary show the secondary vitellogenic oocyte (Ost-II), with the supporting structures zona radiata (ZR), its apparent boundary, the theca (TQ), alveoli, and yolk granules (YG), the last one of which is integral for the nutrition of the future embryo. The FL of *A. rochei* samples with this histoarchitecture was 21.90–29.20 cm.

At Stage 4 (Spawning), with the *A. rochei* actively breeding at this point, the contents of the ovary freely escaped, even under light pressure. The color of the gonad at this point was a bright pink, with the venation apparent with the length of 4.20–12.60 cm. Fish samples with this length of ovary were measured to be at 24.0–33.80 cm (FL). Tissue-wise, a landmark attribute of this stage is the mature follicle (FO), sharing the space with the Ost-II. Yolk granules (YG) are scattered across the cytoplasm (Cyt). The follicular epithelium (FE) was also visible at this stage. Female Stage 4 *A. rochei* were measured to be between 24.0–29.10 cm.

Stage 5 (Spent) was marked by a dramatic decrease in the mass of the gonad. Although diminished, however, what distinguished Stage 5 from earlier stages were the flaccid ovarial walls, with the color of the lobes reverting to a dark red. Discharge of residual contents, specifically disintegrating opaque/translucent eggs, occurred during the evaluation of gonads. The ovary length of this stage was 6.50–9.45 cm, belonging to samples 23.50–26.70 cm in FL. Tissue-wise, vestiges of the reproductive contents exiting were more apparent, with the empty follicles (EF) the major indicator, along with post-ovulatory follicles (POF), nuclei (N) atretic follicles (AF), and the invagination of numerous follicular epithelia (invaFE). Interestingly, though, as the stages are simultaneous with one another, a few pre-vitellogenic oocytes (pr) were also observed at this stage. Samples with these histological characteristics were 25.50–30.20 cm (FL).

By Stage 6 (Recovering/Resting), the fish returns to the schooling population to feed and prepare for the next breeding cycle. The ovarian lobes were pinkish and translucent at this stage, the whole gonad occupying only one-third (6.15–8.60 cm) of the fish's body cavity. Much like Stage 1, eggs were not visible to the naked eye. *A. rochei* samples with this range of ovary length were measured at 24.80–26.50 cm (FL). Histoarchitecture of this stage revealed the repopulation of pre-vitellogenic oocytes (pr), but more importantly, follicles in resorption state (FR), and the reconstitution of the interstitial connective tissue (recICT). The fish size of *A. rochei* falling under Stage 6 (tissue-wise) was determined at 24.80–33.80 cm (Fig. 5).

3.2.2 Male

Stage 1 (Immature) testes occupied one-third of the fish cavity, with the lobes thin, string-like, and dark in color at the anterior area. Stage 1 *A. rochei* testes were identified to be at a minimum of 3.0 cm and a maximum of 17.85 cm in length, while fish sizes with these testes lengths were at 19.0–26.50 cm. For its histological architecture, the male *A. rochei* gonad was easily differentiated from the female in which the gametes themselves were not distinguishable yet, only the regions where they were forming. The medulla (Med) and cortex (Cx), with clumps of cells, either the tubules (Tb) or lobules (Lb). Most of the cells at this point are eosinophilic, as shown by the mostly magenta slides. Male *A. rochei* samples in Stage 1 of reproductive maturity (tissue-wise) were recorded to be at 18.16–22.30 cm (FL).

The lobes of the testes gradually gained mass at Stage 2 (Onset Maturation). At this stage, the color was transitioning progressively to red. The length of the gonad at this stage was 3.05–14.30 cm, with corresponding FLs of 21.70–30.10 cm. Histoarchitecture in this stage was largely similar to the first stage, only with the higher frequency of lobules

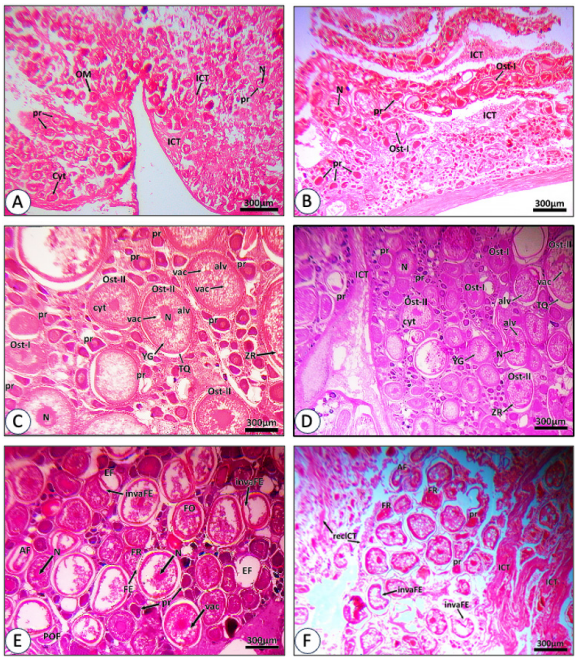

Fig. 5. Histoarchitectural structure of ovary of Bullet Tuna *(Auxis rochei)*. (A) Stage 1 (Immature): pr = previtellogenic oocytes, ICT = interstitial connective tissue, OM = oocyte membrane; (B) Stage 2 (Onset Maturation) Ost-I = primary vitellogenic oocyte, N = nucleus; (C) Stage 3 (Developing/Maturing): Ost-II = secondary vitellogenic oocyte, ZR = zona radiata, alv = alveoli, cyt = cytoplasm, TQ = theca, (D) Stage 4 (Spawning): FE = follicular epithelium, YG = yolk granules, FO = mature follicle; (E) Stage 5 (Spent): invaFE = invagination of the follicular epithelium; POF = post-ovulatory follicle, EF = empty follicle, AF = atretic follicle; (F) Stage 6 (Recovering/Resting): recICT = reconstituting interstitial connective tissue; FR = follicle in resorption state. (M x 400). (H&E). Bar = 300µm. Photo by Entia JCD and Nabre NMB.

(Lb) and tubules (Tb), two structures that interact closely with one another throughout spermatogenesis. Male A. rochei samples possessing these histological characteristics were determined to be at 20.90–24.80 cm (FL).

By Stage 3 (Developing/Maturing), the color of the lobes was fully opaque, ranging from pink to red. The testes occupied about two-thirds of the body cavity as the lobes gained more mass, thickness, and length. However, unlike in the succeeding stage, contents did not freely escape the lobes. The length of the testes at this stage was between 6.10–14.40 cm, representing fish sizes 23.30–27.70 cm (FL). Upon photomicrography, the testicular gonads showed in detail the lobular and testicular arrangements, and the population of intercellular elements was observed to be more diverse in Stage 3 (Developing/Maturing), as the tissues presented primary spermatocytes (Sp-I), secondary spermatocytes (Sp-II), spermatids (Sptd), and the sperm duct (SPD). Collected samples of male *A. rochei* falling under Stage 3 of reproductive development (for its histology) were at 16.70–29.40 cm (FL).

Similar to the female samples, the contents of the gonad in male *A. rochei* at Stage 4 (Spawning) were easily ejected under light pressure. Observed with the fullness in size, the color of the gonad at this stage was fully white with slight yellowish pigmentation at the edges of the lobes. The length of Stage 4 *A. rochei* male gonads was 8.40–13.55 cm, belonging to fish samples 24.10–30.90 cm (FL). Histological examinations showed that Sp-I, Sp-II, and Sptd were still present at this stage, now accompanied by spermatozoa (Spz), lobular lamina (LL), and the main sperm duct (SPD). The apparentness of the main sperm duct (MSD) in Stage 4 indicated that the conduit from the testis to the urogenital orifice actively played a part in the reproductive process. The fish length of male *A. rochei* at this stage (based on its histoarchitecture) was identified at 32.60–41.20 cm (FL).

In Stage 5 (Spent), the atrophy of the testis was evident, with the lobes significantly diminutive and flaccid when handled. Redness of the lobes was observable at this stage as well. The length of the testes at this stage was 5.10–13.65 cm, excised from fish samples measuring 24.0–27.80 cm (FL). Histological attributes of this stage indicate the presence of spermatozoa (Spz), although in lower occurrence, along with the SPD, Sp-I, and Sp-II. Samples with these histological characteristics were at 30.70–39.0 cm (FL).

Lastly, regeneration of the sperm was observed to reoccur in Stage 6 (Recovering/Resting). The testis was also observed to be primarily red. Similar to Stage 3, the lobes in this stage were observed to increase in size. The gonad length of Stage 6 was identified at 4.85–11.70 cm. However, unlike Stage 3, the texture of the lobes was flaccid and visibly shrunken in appearance, with the length occupying only one-third of the body cavity. The most significant histological feature of this stage was the residual spermatozoa (RSpz). The sperm duct (SPD) was also prominent. Fish samples of these histological characteristics were at 36.50–42.70 cm (FL) (Fig. 6).

A summary of the reproductive stages, premier characteristic(s) of each stage, testis length, and corresponding FLs are shown in Figure 7 and Tables 1 and 2.

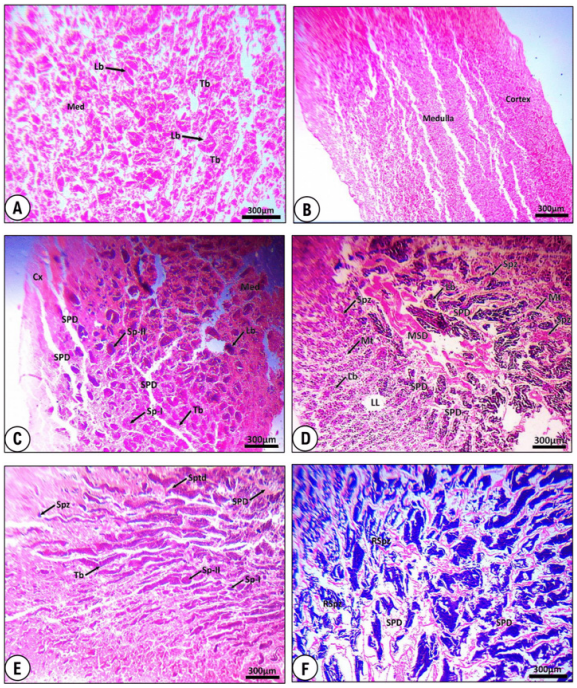

Fig. 6. Histoarchitectural structure of testis of male Bullet Tuna (*A. rochei*). (A) Stage 1 (Immature): Med = medulla, Cx = cortex; (B) Stage 2 (Developing/Maturing): Tb = tubules; (C) Stage 3 (Onset Maturation): SPD = sperm duct, Sp-I = primary spermatocyte, Sp-II = secondary spermatocyte, Lb = lobule; (D) Stage 4 (Spawning): LL = lobular lamina, MSD = main sperm duct, Spz = spermatozoa, Mt = milt; (E) Stage 5 (Spent): Sptd = spermatid, Spz = spermatozoa; (F) Stage 6 (Recovering/Resting): RSpz = residual spermatozoa. (M × 400). (Hematoxylin and Eosin). Bar=300µm. Photo by Entia JCD and Nabre NMB.

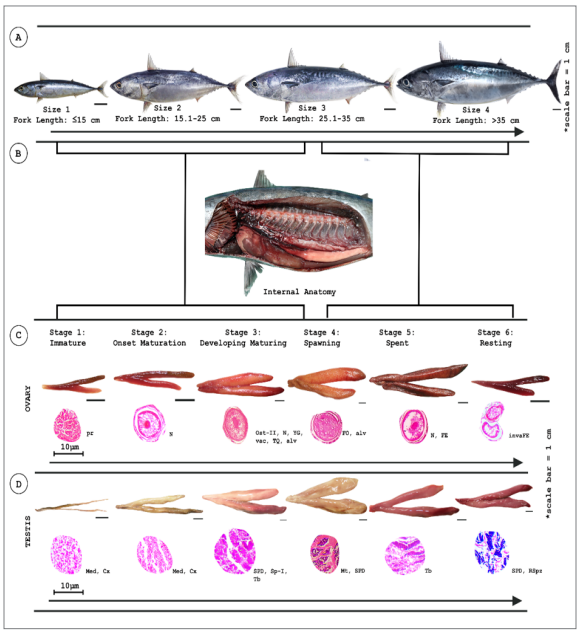

Fig. 7. Direct relationship between fish sizes to macrostructure and histoanatomy of male and female gonads. (A) Fish sizes: Size 1 (≤ 15.0 cm), Size 2 (15.10–25.00 cm), Size 3 (25.10–35.00 cm), Size 4 (>50.0 cm), (B) Fish Internal anatomy, (C) Ovarian development through gonad macrostructure and its histoanatomy: 1 (Immature), 2 (Onset Maturation), 3 (Developing/Maturing), 4 (Spawning), 5 (Spent), 6 (Recovering/Resting) and (D) Testicular development gonad macrostructure and its histoanatomy: 1 (Immature), 2 (Onset Maturation), 3 (Developing/Maturing), 4 (Spawning), 5 (Spent), 6 (Recovering/Resting). Bar= 2cm (fish); 1cm (gonad); 10µm (histomicrographs). Photo by Entia JCD, Nabre NMB, and Dela Cruz NA.

Table 1. Female reproductive maturity stages based on macro (gonad appearance) and micro (histological characteristics) in relation to fork length (FL) of *A. rochei*. Size classes based on FL were as follows: Size 1 (≤ 15.0 cm), Size 2 (15.10–25.00 cm), Size 3 (25.10–35.00 cm), Size 4 (> 50.0 cm). Nomenclature for both macro and micro aspects are the same: Stage 1 (Immature), Stage 2 (Onset Maturation), Stage 3 (Developing/Maturing), Stage 4 (Spawning), Stage 5 (Spent), Stage 6 (Recovering/Resting). Highlighted row shows that at Size 3 (25.10–35.00 cm), female *A. rochei* is spawning/participating in reproduction, characterized by 25.10 - 35.00 cm in FL based on (a) microstructures: follicular epithelium (FE), mature follicle (FO), yolk granules (YG)); of its macrostructure, ovary length is between 4.20–12.60 cm, the contents of the ovary freely escaped, even under light pressure, color of the gonad at this point was a bright pink, with the apparent venation. FL based on (b) microstructure is 24.0–29.10 cm, while FL based on macrostructure is 24.0–33.80 cm. Size classes in relation to FL overlap due to the asynchronous reproductive strategy of tuna species.

FISH BODY SIZE	STAGE	PHASE	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	HISTOLOGY LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	SIZE CLASS
Size 1 ≤ 15.0 cm	1	Immature	pinkish, almost dark red at the anterior part, translucent, with no granules (eggs) visible to the naked eye, the ovary at this stage occupied less than one-third of the body cavity at 1.85 - 10.30 cm (min – max)	18.5 – 27.0	previtellogenic oocytes (pr)	19.0 - 26.50	Sizes 2, 3
Size 2 15.10 - 25.00 cm	2	Onset Maturation	characterized by the preliminary enlargement of the lobes, the color of the gonads at this stage are red to light pink 3.10 - 10.90 cm	21.70 - 30.10	primary vitellogenic oocyte (Ost – I)	21.70 - 27.40	Sizes 2, 3

Continuation of Table 1. Female reproductive maturity stages based on macro (gonad appearance) and micro .....

FISH BODY SIZE	STAGE	PHASE	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	HISTOLOGY LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	SIZE CLASS
Size 3 25.10 - 35.00 cm	3	Developing/ Maturing	oocytes (in the appearance of granules) began to be visible through the ovaric tunica increased at this point, occupying two-thirds of the body cavity 3.50 - 10.80 cm	23.30 - 27.70	secondary vitellogenic oocyte (Ost-II)	21.90 - 29.20	Sizes 2, 3
	4	Spawning	the contents of the ovary freely escaped, even under light pressure, color of the gonad at this point is a bright pink, with the apparent venation, length at 4.20 - 12.60 cm	24.0 - 33.80	follicular epithelium (FE), mature follicle (FO), yolk granules (YG)	24.0 - 29.10	Sizes 2, 3
Size 4 >50.0 cm	5	Spent	marked by a dramatic decrease in the mass of the gonad, flaccid ovaric walls, with the color of the lobes reverting to a dark red, discharged of residual contents, specifically disintegrating opaque/translucent eggs, occurred during the evaluation of gonads, length at 6.50 - 9.45 cm	23.50 - 26.70	vacuoles (vac), empty follicle (EF), post ovulatory follicles (POF)	25.50 -30.20	Sizes 2, 3
	6	Recovering/ Resting	ovarian lobes are pinkish and translucent at this stage, the whole gonad occupying only one-third eggs are not visible to the naked eye, length at 6.15 - 8.60	24.80 - 26.50	follicle in resorption state (FR)	24.80 - 33.80	Sizes 2, 3

Table 2. Male reproductive maturity stages based on macro (gonad appearance) and micro (histological characteristics) in relation to fork length (FL) of *A. rochei*. Size classes based on FL were as follows: Size 1 (≤ 15.0 cm), Size 2 (15.10–25.00 cm), Size 3 (25.10–35.00 cm), Size 4 (> 50.0 cm). Nomenclature for both macro and micro aspects are the same: Stage 1 (Immature), Stage 2 (Onset Maturation), Stage 3 (Developing/Maturing), Stage 4 (Spawning), Stage 5 (Spent), Stage 6 (Recovering/Resting). Highlighted row shows that at Size 3 (25.10–35.00 cm), male *A. rochei* is spawning/participating in reproduction, characterized by 25.10–35.00 cm in FL based on (a) microstructures: spermatid (Sptd), spermatozoa (Spz); of its macrostructure, testis length is between 8.40–13.55 cm, the atrophy of the testis was its prominent characteristic, with the lobes significantly shrunk and flaccid when handled, redness of the lobes was observable at this stage. FL based on microstructure is 32.60–41.20 cm, while FL based on (b) macrostructure is 24.10–30.90 cm. Size classes in relation to FL overlap due to the asynchronous reproductive strategy of tuna species.

FISH BODY SIZE	STAGE	PHASE	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	HISTOLOGY LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	SIZE CLASS
Size 1 ≤ 15.0 cm	1	Immature	testes occupied one-third of the fish cavity, lobes thin and string-like, dark in color at the anterior area, length at 3.00 - 17.85 cm (min - max)	18.50 - 27.00	medulla (Med), cortex (Cx)	18.16 - 22.30	Sizes 2, 3, 4
Size 2 15.10 - 25.00 cm	2	Onset Maturation	lobes of the testis gradually gained mass, color transitioning progressively to red, length at 3.05 - 14.30 cm	20.90 - 27.10	tubules (Tb), lobule (Lb)	20.90 - 24.80	Sizes 2, 3, 4
Size 3 25.10 - 35.00 cm	3	Developing/ Maturing	the color of the lobes is fully opaque, ranging from pink to red, testis occupied about two-thirds of the body cavity as the lobes gained more mass, thickness, and length at 6.10 - 14.40 cm	21.90 - 29.20	primary spermatocyte (Sp-I), secondary spermatocyte (Sp-II), spermatid (Sptd)	16.70 - 29.40	Sizes 2, 3

Continuation of Table 2. Male reproductive maturity stages based on macro (gonad appearance) and micro .....

FISH BODY SIZE	STAGE	PHASE	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	HISTOLOGY LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	SIZE CLASS
Size 3 25.10 - 35.00 cm	4	Spawning	the contents of are quickly ejected under light pressure, observed with the fullness in size, the color of the gonad at this stage is fully white with slight yellowish pigmentation at the edges of the lobes, length at 8.40 - 13.55 cm	24.10 - 30.90	spermatid (Sptd), spermatozoa (Spz)	32.60 - 41.20	Sizes 2, 3
Size 4 >50.0 cm	5	Spent	the atrophy of the testis is its prominent characteristic, with the lobes significantly shrunk and flaccid when handled, redness of the lobes is observable at this stage as well with length at 5.10 -13.65 cm	24.0 - 27.80	less spermatozoa (Spz)	30.70 - 39.0	Sizes 2, 3

4. DISCUSSION

This study examined the reproductive characteristics of *A. rochei* off the waters of the Southern Philippines, both at the gonadal and tissue levels. This study reports that the developmental traits of *A. rochei* are of the asynchronous development strategy: all ovaries throughout the year contain oogonia and perinuclear oocytes, which are regarded as the ovary's overall stock (Macías et al. 2005; Megalofonou et al. 2000; Niiya 2001). Additionally, the continuous production of gametes over time and the overlapping characteristics between reproductive stages indicate that *A. rochei* is a batch spawner. There was a notable progression of oogonial development among the female—from the pre-vitellogenic oocytes becoming fully mature follicles to being vacuole-populated follicles. In contrast, the structures within the testicular matrix became more defined for the males until spermatozoa were differentiated, and eventually, the residual spermatozoa remained. The development, apparent destruction, and regeneration of supporting structures were also observable: at the first few stages of oogenesis, the ovarian space was rich in interstitial connective tissue. This tissue was eventually depreciated during spawning but later reconstituted to serve its purpose to the next batch of oocytes. As for males, at post-spawning (Stage 5), the testicular matrix was comparatively diminished in mass, especially at the medullary region; at the event of reproductive resting, however, primitive spermatogonial structures from the cortex migrated towards the center over the course of their maturity. This study showed that female *A. rochei* around Southern Mindanao is larger than its males: the earliest largest recorded size of the females is at 42.70 cm, while for the males is at 33.80 cm.

Additionally, our findings are consonant with those of Bahou et al. (2016) in their study of the frigate tuna (*Auxis thazard*) off the coast of Cote d'Ivoire in West African waters. In this study, they documented six stages of gonadal development of *A. thazard*: Stage I (Immature), Stage II (Onset Maturation), Stage III (Maturation), Stage IV (Ripening), Stage V (Spent), and Stage VI (Resting).

The bullet tuna's oocyte maturation developmental traits are comparable to those of other species with an asynchronous development strategy. All ovaries throughout the year contain the ovary's overall stock, and weight gain in females reflects the reproductive cycle, as the ovaries' maturation adds to the fish's overall weight (Rodriguez-Roda 1983).

Females' different growth patterns can be a helpful method for responding adaptively phenotypically to changes in tropical coastal habitats. Numerous ecological differences stimulated by the onset of rain manipulate reproductive processes, such as gonad growth and development in tropical fish species (Chellappa et al. 2010).

In the present study, features similar to the description in a study by Timohina and Romanov (1996) were found. The most prominent of these are at Stage 3 of development, during which the ovaries consume a considerable portion of the abdominal cavity: at this point, the eggs lose their transparency, changing from a clear yellow to orange color, and are visible to the naked eye. In addition, ovaries also turn from being colorless to yellow.

Among the numerous reproduction methods in fish teleosts, gonochorism is the most common,

where the sexes are distinct (Hoar 1969). Organic and synthetic steroid hormones induce gonadal sex variation and sex ratios in teleost fish (Segner et al. 2013; Denslow and Sepúlveda 2007 Leet et al. 2011; Borg 1994; and Guiguen et al. 2010). Androgens control testicular growth and development in spermatogenesis, govern the development of male secondary sex attributes, regulate sexual performance, and contribute to the preservation of the male sexual phenotype in teleosts (Borg 1994; Melo et al. 2015; Rajakumar et al. 2015; Amer et al. 2001; and Shi et al. 2017). The sperm of fish is distinct from that of mammals. They are immobile until water is added to the milt during ejaculation, at which point they briefly become motile. They do not penetrate the oocyte wall, instead they enter through a micropyle passage. The male must create viable, motile sperm that can find the eggs and then the micropyle to enter the oocyte for adequate fertilization (Marchand et al. 2008). Ecological conditions, through spawning, trigger the sperm, but not all considerations implicated are well identified. Efforts to modify the environmental conditions, involving water conditions through adding contaminants, may lead to a more robust interpretation of these components (Linhart et al. 1999).

The morphology of testes and ovaries being bilobed longitudinal structures is common among gonochoristic species. For instance, in studies carried out by Gimeno et al. in 1998 *C. carpio's* gonads, also a gonochoristic species, were bilateral, longitudinal structures with multiple seminiferous tubules connected to the dorsal abdominal wall by connective tissues. Gimeno et al. (1998) reported that its lobules were packed with spermatogenic cysts, with the luninae loaded with spermatozoa, while vacuoles and fibrous eosinophilic tissues were detected around or within the seminiferous tubules. In the induced oogenesis, oogonial development was similar, with the previtellogenic oocytes being the first marker of the cycle.

4.1 Shared fish sizes between reproductive stages

The overlap of histological features between stages, specifically among the stages after the fish have spawned (Stages 5 and 6), indicates that the process of developmental maturation for the next round of spawning is immediate and simultaneous, as exhibited by the presence of pre-vitellogenic oocytes (pr) and the reconstitution of the interstitial connective tissue (recICT) (for the females) and primary and secondary spermatocytes (Sp-I and Sp-II) in males in Stage 5 (Post-Spawning).

Interestingly, the increments in which the sizes increase among stages are inconsistent. Class sizes between reproductive stages are shared. In females, Stages 2 and 3 share Size 2 class size, as some fish samples at Stage 3 of development measured as small as 16.70 cm—even smaller than the smallest recorded size for the *A. rochei* in Stage 1. An overlap is observed between Stages 4 and 5 as well: notably, the range of fish length in Stage 5 is smaller than in Stage 4 (Table 1). Also, there is no recorded occurrence of Stage 4 in males; most male *A. rochei* caught were between Size 2 and Size 3, shared amongst Stages 2, 3, and 4 of reproductive development. Stages 2 and 3 overlap the most in FL range, with Stage 2 at 21.70–27.40 cm, and Stage 3 at 21.90–29.20 cm. The smallest recorded specimen for male *A. rochei* at Stage 4 is at 24.0 cm, and the largest is at 29.10 cm. Stages 5 and 6 for males occur at Size Class 3. This frequent overlap may be attributed to the simultaneous occurrence of each stage.

4.2 Gonadal maturity and corresponding size

For the findings of this study, gonadal maturity in *A. rochei* occurs or has already occurred mostly at Size 3 (25.10–35.00 cm) for both males and females. *A. rochei* of Size 3 class have already spawned and thus have contributed to the population. Gonadal maturity at this stage shows that the contents of the gonads are diminished–prominent vacuoles and destructed interstitial connective tissue for the females and residual spermatozoa for the males. Results of this study suggest that the male and female *A. rochei* are sexually mature and have spawned by Size 3 (25.10–25.00 cm). For males, the minimum post-spawning size is 25.50 cm and 30.70 cm for females.

Knowledge of any fish species' reproductive attributes and potential is essential for fisheries resource assessment and management (Zhu et al. 2010). The reproductive system's ultimate goal is the production of sufficient numbers of viable gametes, making gamete quantity and quality the sole factors that matter. Gamete viability testing can, therefore, be used to evaluate reproductive failure and demonstrate effects at doses lower than those that entirely prevent the formation of gametes (Kime 1995; Kime and Nash 1999).

5. CONCLUSION AND RECOMMENDATION

This study is the first to provide primary data that describes gonadal growth pertaining to fish length for *A. rochei* found in the waters of the Southern Philippines. The histological profile of the reproductive system's maturity and ocular tests for gonadal development were the foundation for these inferences. The data show that *A. rochei* gonad strategy is asynchronous with overlapping size ranges and spawns at 25.10–35.0 cm (Size 3). However, the analysis only considers the months of collection, the high percentage of immature individuals, opportunistic sampling, and the local fish species' natural mortality.

It must be noted that this study includes only *A. rochei* off the waters of Sarangani Bay, Moro Gulf, Davao Gulf, and Sulawesi Sea in the Southern Philippines. Further studies must be carried out in other parts of the archipelago's waters in order to either support the *A. rochei* data herein or present variations in its reproductive traits; after all, geographical and climate differences, various epigenetic factors, and even as specific as fishing practices around the municipalities may contribute to similarities or differences in reproductive attributes, whether in their histoarchitecture or FL at sexual maturity. To enhance comprehensiveness, we recommend incorporating complementary techniques such as scanning electron microscopy (SEM) and molecular biological assays. Consequently, we advocate for further investigations on policy provisions while emphasizing sustainable fishing practices for *Auxis rochei*, prioritizing the protection of individuals measuring 35.0 cm and below.

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AUTHOR CONTRIBUTIONS

Entia JCD: Writing - Original Draft, Methodology, Data curation, Formal analysis, Investigation and Visualization. N**abre NMB**: Data curation, Formal Analysis, Investigation, Visualization, and Project Administration. **Castrence GA**: Supervision. **Arellano BJG**: Writing - Review & Editing. **Amoncio RADA**: Data Curation. **Wetzel JT**: Supervision. **Guevarra EP**: Conceptualization, Supervision).

CONFLICTS OF INTEREST

We affirm no conflict of interest.

ETHICS STATEMENT

Authorization and clearance to proceed were granted by the Department of Environment and Natural Resources - Protected Area Management (DENR-PAMB) Board Region XII, Bureau of Fisheries and Aquatic Resources- National Stock Assessment Program (BFAR-NSAP) Region XI and XII, Philippine National Police Maritime Group (PNP-MG) Regional XII, Philippine Coast Guard (PCG) Region XII, Local Government Units (LGUs)of General Santos City and, 6 Municipalities of Sarangani Province (Alabel, Malapatan, Glan, Maasim, Kiamba, and Maitum). Our research followed significant institutional and national guidelines and used independent and dependent sampling techniques.

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