

RESEARCH ARTICLE

The Ovaries and Testes: A Gaze Towards the First Record of Gonad Morphogenesis through Macrostructure and Histoanatomy of Frigate Tuna (*Auxis thazard*) in Southern Philippines

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ABSTRACT

The structural configuration of oogonial and spermatogonial models in oviparous species are key determinants of reproductive biological parameters, as these factors also determine gonadogenesis and spawning lengths. This study systematically characterized frigate tuna (*Auxis thazard*) collected from the southern Philippine waters, including Sarangani Bay, Davao Gulf, Moro Gulf, and the Sulawesi Sea. Characterization involved a comprehensive analysis of gross gonadal features, aided by gross morphological identification and histological profiling utilizing Hematoxylin and Eosin (H&E) staining. Samples were caught and categorized into four size classes: Size 1 (≤ 20 cm), Size 2 (20.1–30 cm), Size 3 (30.1–40 cm), and Size 4 (>40 cm). Histological evaluation of gonads indicated asynchronous ovarian strategy, which undergoes six stages: Immature, Onset Maturation, Developing or Maturing, Spawning (during which active release of gametes occurs), Spent, and Recovering. At the spawning stage, ovaries were characterized by their prominent blood arteries, orange or pink color, and large, transparent ovum; testes were characterized as creamy-white full lobes prone to discharge under pressure. Histoanatomy of this stage showed yolk granules in the cytoplasm and mature follicles (in females); for males, the occurrence of the sperm duct, main sperm duct, and spermatozoa. Females at the spawning stage were 25.10–42.00 cm (FL), while males were 42.00–48.70 cm (FL). The size of sexual maturation of *A. thazard* occurred at 30.10–40.0 cm (Size 3). Notably, our study represents the first association between gonadal development and fish sexual maturity length in *A. thazard* within the Philippines.

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1. INTRODUCTION

Scombrids constitute the world's most important fisheries due to their large catches, frequently high commercial value, and vast global trade (Juan-Jordá et al. 2013). Frigate tuna *(Auxis thazard)* is a species of epipelagic and neritic fish (Collette and Nauen 1983) and thus far a scarcely migratory fish species (Cayré et al. 1988). Fish species from this group are significant socio-economic assets for trade fleets and artisanal fisheries, notwithstanding the decreased commercial value when equated to larger tunas (Arrizabalaga et al. 2011; Pons et al. 2017). Since tunas are predominately dioecious, their exterior physical characteristics do not exhibit sexual dimorphism. As oviparous fish with asynchronous oocyte development, tunas are categorized as multiple or batch spawners because they release their gametes into the ocean, where the eggs fertilize them. A stock's reproductive traits and those related to growth and death are some of the most crucial elements in defining a population's capacity for regeneration (Schaefer 2001).

Reproductive biology is the key field with which captured and commercialized fish species' sexual patterns can be identified, as it classifies reproductive and spawning patterns, which are the typical manifestation of sexuality during the lifetime of an individual fish in a population (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008; Sadovy and Domeier 2005). Information on fish population dynamics can be gathered from this as well. The development of breeding technologies for aquaculture and conservation or restocking reasons depend heavily on this information (Muchlisin 2014). Furthermore, collecting fishing data and knowledge regarding tuna reproduction is crucial for effective fishery management. Despite this, however, one of the least researched aspects of tunas is reproduction, and information on this in light of fishery management is either deficient or lacking at all in some regions of the world (Deepti and Sujatha 2012). Existing literature on the gonadal growth and development in tunas, meant to illustrate maturation and spawning distributions, have been established on ovaries and have employed several interpretations of the gonadosomatic index for categorizing condition regeneration (Schaefer 2001). Reports on the length at first maturity (Lm_{50}) of *A. thazard* vary during its distribution (269.0–349.0 mm), and the spawning period is prolonged, with indeterminate fecundity typically linked to warm months of the year (Herath et al. 2019; Schaefer 2001; Jude et al. 2002; Ghosh et al. 2010; Tao et al. 2012; Calicdan-Villarao et al. 2017; Bahou et al. 2016b; Tampubolon et al. 2016; Zapadaeva 2021).

The effectiveness and significance of histological techniques in reproductive biological profiling have been broadly demonstrated for fish species (West 1990; Tyler and Sumpter 1996; Blazer 2002), as histotechnique is an instrument for reproductive studies and is consistently utilized for sex confirmation, evaluation of the reproductive stage, or quantification of atresia (Blazer 2002). Locally, although there are numerous studies describing tuna in the Philippines (Floyd and Pauly 1984; Aprieto 1991; Dickson and Natividad 2000; Llanto et al. 2018), such studies mostly explore the fishing practices and the tuna fishing industry in general, and literature on their reproductive biology is deficient.

This study, therefore, ultimately: (1) describes the gonadal morphogenesis at the gross and histological levels of frigate tuna (*Auxis thazard*) off the waters of southern Philippines—Moro Gulf, Davao Gulf, Sulawesi Sea, and Sarangani Bay, and (2) utilizes histological data to establish the reproductive stages of *Auxis thazard* and identify the size of sexual maturity indices based on fish sizes by pinpointing at which size ranges reproduction (i.e., the active release of gametes) occurs. Furthermore, the data presented in our study may offer valuable insights and serve as a fundamental reference for formulating policies related to the conservation, management, and protection of tuna resources to ensure their sustainability for future generations.

2. MATERIALS AND METHODS

2.1 Field sampling

Data collection was conducted in collaboration with the National Stock Assessment Program of the Bureau of Aquatic Resources (NSAP-BFAR) Region XI and XII, private tuna industries using their vessels, and local fisherfolk. A total of 596 female and 513 male specimens were collected monthly for one year from April 2021 to March 2022 from around four fishing areas in Mindanao: Sarangani Bay, Davao Gulf, Moro Gulf, and Sulawesi Sea, as strategically identified by the regional offices of BFAR XI and XII (Figure 1). Sampling collection utilized a fishery-dependent sampling technique. If sampling becomes impractical or impossible due to adverse weather conditions, we opted to utilize fishery-independent sampling methods. Fishing gears used by the private tuna company's ships, such as handlines and seine nets used by local fisherfolks, were noted while on board, describing the time of the day, the time elapsed for each catch, and the actual location of the fishing activity in the study and their corresponding Global Positioning System (GPS) latitude and longitude coordinates written in three different formats: degrees, minutes, and seconds (DMS), degree and decimal minutes (DMM), plus code or Alphanumeric Code. Samples were then brought to the Histopathology Laboratory, Regional Science Research Center, Mindanao State University, Fatima, General Santos City, for further processing and analyses.

2.2 Morphological diagnostic of *A. thazard*

The morphological characteristics of frigate tuna *(A. thazard)* were identified using the description given by Collette and Nauen 1983 (Figure 2).

2.3 Morphometry

Standard length (SL), total length (TL), body girth (BG), and gonad length were measured with a ruler with 0.1 mm precision. Total body weight (TBW)

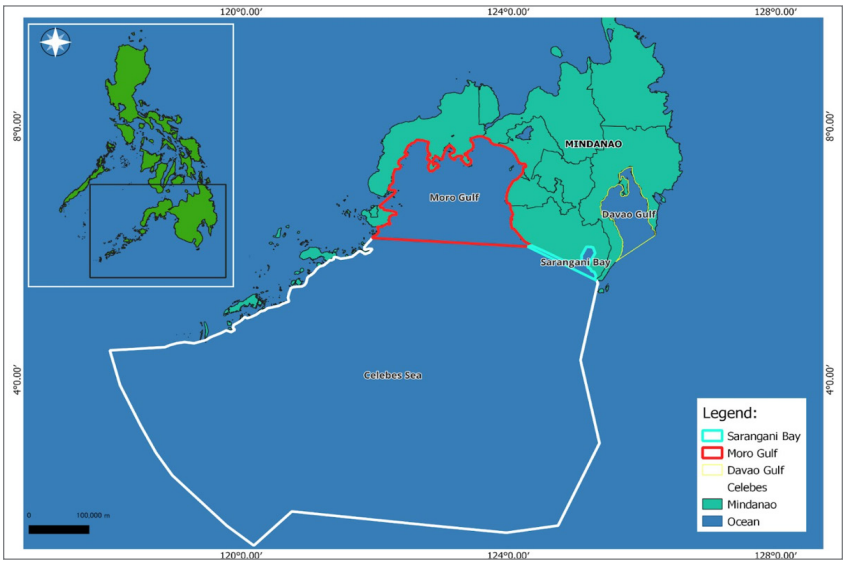

Figure 1. Frigate tuna (*Auxis thazard*) collection sites have been strategically identified in prominent fishing grounds, namely Sarangani Bay (5.9656° N, 125.1929° E), Davao Gulf (6813° N, 125.8280° E), Moro Gulf (6.8014° N, 123.4384° E), and Sulawesi Sea (3.6121° N, 122.2998° E). A legend accompanying the data denotes the specific locations where sampling was conducted, with the content generated through QGIS software.

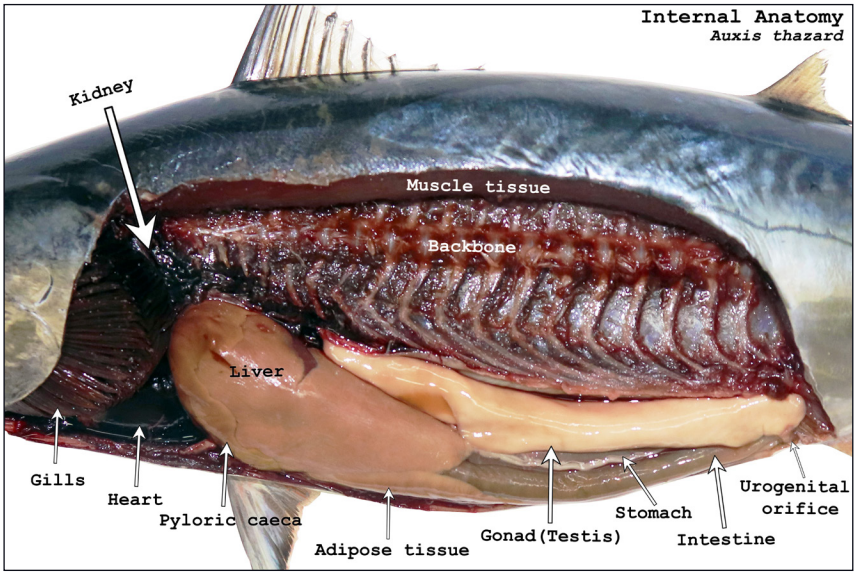

Figure 2. Internal anatomy of frigate tuna *(Auxis thazard)* as represented by the male. Photo by Entia JCD and Nabre NMB.

and gonad weight were measured using a digital scale balanced to the nearest 0.001 g accuracy (Akter et al. 2019)

2.4 Sexual maturity

Determination and classification of maturity stages were done primarily through visual assessment of ovaries (yellowish, granular) and the testis (whitish, smooth), complemented with histological methods for a more precise description of the reproductive condition of fish. General criteria described by McBride et al. (2004) were followed in identifying the reproductive organ. Phases of sexual maturity based on the gonads were identified according to the scheme developed by the General Fisheries Commission for the Mediterranean (GFCM) macroscopic maturity scale for bony fish in the 2018 Report of the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF) of the International Council for the Exploration of the Sea.

Before exposing the fish's gonads for ocular inspection, we size-classified them based on their Fork Length (FL). The gonads were then grouped according to their described visual estimates by looking at their gross morphology according to the development of their sexual maturity. Afterward, we selected random gonad samples from each sexual stage for histological profiling. Due to the bias and vagueness of the ocular gross classification technique, which results in a serious misclassification of the fish's reproductive status, we separated gonadal development (macro) and histological (micro) assessments. In contrast, histology is more sophisticated and accurate and allows for the identification of changes in cellular and tissue structures**.**

We stress that our paper categorized two fork lengths: (1) fork length of the fish sample based on the stage of the (macro) gross gonad external characteristics and (2) fork length of the fish sample based on the (micro) histoanatomical photomicrographs and configuration existing within its gonad at a cellular and tissue levels. These fork lengths were due to the approach employed: after the gonad samples were characterized based on their morphology (macro), random samples were then selected from the same stock of each maturity stage for histological (micro) profiling—as can be observed by the FL range for micro still within the range of the FL for macro.

2.5 Histology

We identified at least three (3) samples for histological exams per stage in gross gonadal development and incorporated serial sectioning. Cross-section of samples about 1 cm in thickness were taken from the middle portion of the gonads and fixed in 10% formaldehyde solution, which were then dehydrated in increasing ethanol concentrations, cleaned in xylene, and embedded in paraffin wax. 5–10 μm sections were taken using an automated microtome and were stained with hematoxylin and eosin. Histological variations were described following the descriptions presented by Bahou et al. (2016b) and Bahou et al. (2017).

3. RESULTS

3.1 Biological characteristics of *A. thazard*

Auxis thazard were differentiated by the dark spots on its operculum and a pattern of 15 or more narrow, oblique-to-horizontal, dark wavy lines on the scaleless area above the lateral line, its white belly, and purple pectoral and pelvic fins. The present study followed data provided by the BFAR-NSAP XI and XII in classifying fish sizes in the Philippine setting, patterned after the scheme developed by Collete and Nauen (1983). Size class and range of each class for *A. thazard* in this study are as follows: Size 1: ≤20 cm; Size 2: 20.1 - 30 cm; Size 3: 30.1 - 40 cm; and Size 4: >40 cm (Figure 3).

Furthermore, gonads of male and female *A. thazard* were similar: bi-lobed symmetrical shapes that, at the posterior region, create a channel leading to the urogenital orifice, where the release of gametes during reproduction occurs. The location of the gonads was at the dorsal-posterior region of the coelom next to the kidneys and swim bladder (Figure 4). Histoarchiture, which refers to the structural organization or arrangement of tissues, cells, or cellular components within the gonad sample, was also described.

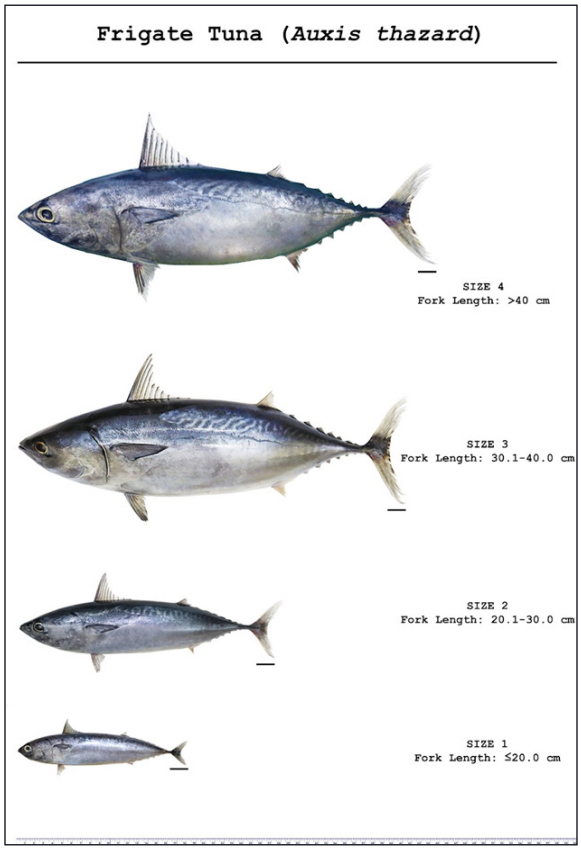

Figure 3. Size classes determined for *A. thazard*. Size 1: ≤ 20.0 cm; Size 2: 20.1–30.0 cm; Size 3: 30.1–40.0 cm; Size 4: > 40.0 cm; Bar = 2.0 cm. Photo by Entia JCD and Nabre NMB.

3.2 Reproductive maturity: gonad morphology, histological characteristics, and corresponding fish size

Gonad maturity of *A. thazard* was classified into six stages, with landmark characteristics of each stage based on the external morphology of the testis and ovary and the presence of tissue structures that differentiate one reproductive stage from its predecessor. Reproductive maturity based on histological qualities was carried out in comparison with the study by Bahou et al. (2016a, 2016b). While certain characteristics were shared between stages–as reproductive maturity occurs in transition–the landmark characteristic of the stage was noted in the profiling of the stages. Nomenclature for both morphological and histological profiling was the same: Stage 1 - Immature; Stage 2 - Onset Maturation; Stage 3 - Developing or Maturing; 4 - Spawning; 5 - Spent; and 6 - Recovering or Resting.

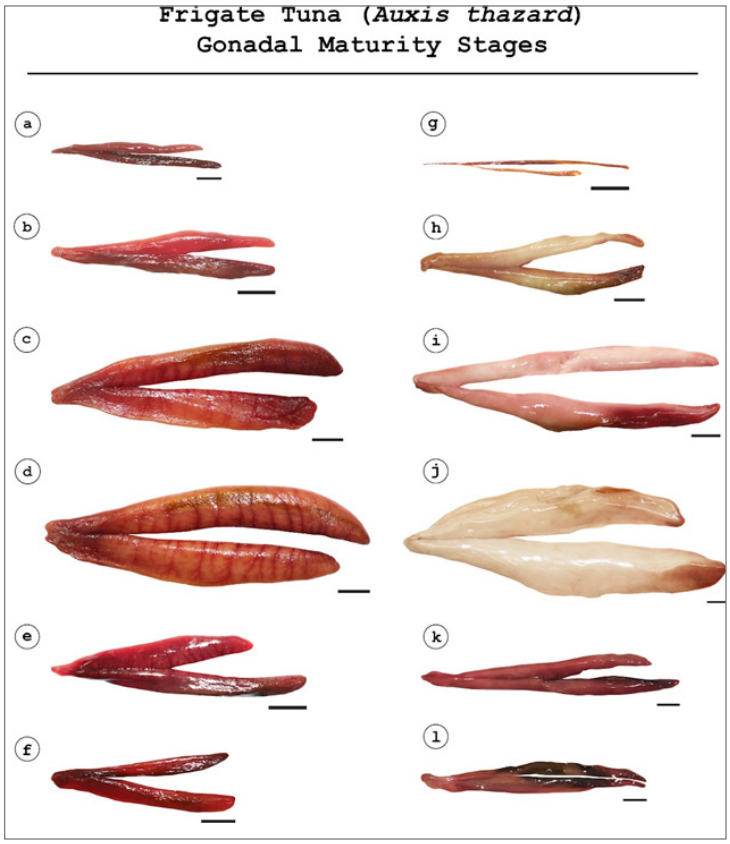

Figure 4. Ovarian (left) and Testicular (right) development of frigate tuna *(A. thazard)*. (a) Stage 1 - Immature ovary; (b) Stage 2 - Onset Maturation ovary; (c) Stage 3 - Developing/Maturing ovary; (d) Stage 4 - Spawning ovary; (e) Stage 5 - Spent ovary; (f) Stage 6 - Recovering/Resting ovary; (g)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

At Stage 1 (Immature), the lobes of the ovary were thin and dark red in color, while the gonadal lobes of the testes at this stage were even thinner, almost string-like, and creamy white in color. The length of ovary samples at this stage was 2.80–12.70 cm with fish FL of 15.50–35.0 cm (min-max). Assessment of histoarchitecture of this stage showed in females the predominance of previtellogenic oocytes (pr)—the rudimentary form of what would later become the viable female. The tissues were also rich in interstitial connective tissues in this stage. In some instances, the enlarged center of some cells, the nucleus (N), could be seen. In males, Stage 1 (Immature), the testicular lobes were more opaque than in Stage 0 and were slender, pale, and covered in red-to-brown pigmentation with testis samples at 2.00–13.90 cm length with corresponding fish size at 14.50–36.10 (min-max) FL. In its microstructure, we observed no high contrast among structures except for the higher concentration of eosin at the cortical (peripheral) region and more purple (hematoxylin) medullary or central region. Tubules (Tb) and lobules (Lb) were observed as early as this stage; however, they were in lower occurrence. Fork length (FL) of female samples was determined at 14.50–36.10 cm, while male samples were 37.10–40.50 cm.

A preliminary increase in mass and length of lobes was observed in Stage 2 (Onset Maturation). At this stage, the lobes of the ovaries have elongated (ranging from 3.65 cm to 10.80 cm for females with fish sizes ranging from 20.0 cm to 35.0 cm FL) and thickened, occupying about one-half of the body cavity and virtually symmetrical. The color of ovaries at this stage was still red, although relatively lighter in hue; for testes, lobes were mostly white, with dark brown pigmentation at the tips. Evaluation of histoarchitecture revealed that the landmark characteristic for females at this stage was the primary vitellogenic oocytes (Ost-I), differentiated by its enlarged nucleus (N). While previtellogenic oocytes (pr) were still present in this stage, primary vitellogenic oocytes (Ost-I) proliferated and dominated the space. In the Stage 2 male (Onset Maturation), the red coloring was

still discernible, despite the lobes' development into elongated, symmetrical structures that occupied over half of the body cavity (ranging from 4.00 cm to 12.65 cm for males with fish sizes FL ranging from 20.50 cm to 35.50 cm). Of the testis, cortex, and medulla were better differentiated, with the medulla much more saturated with hematoxylin. There was an increased occurrence of tubules (Tb) and lobules (Lb). The fork length of female *A. thazard* was determined at 20.50–35.50 cm, while males were 37.50–42.70 cm according to its microanatomy profile.

Stage 3 (Developing or Maturing): gonads at this stage occupied about two-thirds of the body cavity. Ovaries had increased to twice the size (ranging from 3.56 cm to 11.40 cm with fish length from 20.70 cm to 32.50 cm FL) of the first stage. Eggs were visible through the ovarian tunica (which was not translucent), even when examined with the naked eye. Meanwhile, the color of the testis at this stage was opaque white, slightly yellowish, with the posterior tip more pigmented, with testis length ranging from 6.70 cm to 15.45 cm (min-max) with corresponding fish fork length at 23.80–40.30 cm. For both gonads, despite this increase in size, contents would not be easily expelled under light pressure. Of histoarchitecture in Stage 3 females, the successor to Ost-I, the secondary vitellogenic oocytes (Ost-II) began to appear. Ost-II is characterized by the presence of the zona radiata (ZR), functioning as its outer boundary. Notably, the theca (TQ) and yolk granules (YG) within Ost-II are integral structures facilitating the provision of nutrients. ZR, TQ, and YG are released along with the release of the oocyte in the event of reproduction. Testicular histoarchitecture of Stage 3 introduced better-defined, further-developed structures, with the appearance of the sperm duct (SPD), spermatids (Sptd), primary spermatocytes (Sp-I), and simultaneously, secondary spermatocytes (Sp-II). Female fish samples were 23.80–40.30 cm in fork length, and males were 44.70–46.0 cm based on histology (micro).

Maximum fullness of ovaries occurred in Stage 4 (Spawning), during which they were fully plump, and their contents freely spilled, whether under light pressure or without force. The vascularization of the ovarian tunica was the most noticeable feature of the ovary at this stage, looking dark red in contrast to the pink surface of the lobes. The testis, meanwhile, was fully opaque and white, with no vascularization visible. Ovary length at this stage was 7.05–17.95 cm with fish size ranging from 25.0 cm to 42.0 cm FL; testis length was 4.80–17.25 cm with fish FL of 25.10–43.0 cm. Tissue-wise, ovaries also showed yolk granules (YG) were also present in this stage, although now mixed up and scattered within the cytoplasm (Cyt). Oocytes were mostly secondary oocytes (Ost-II); the follicular epithelium (FE), a layer of cells that surrounds and encases the developing oocyte within the ovarian follicle, and mature follicle (FO) were the landmark characteristics of this reproductive stage, with observable instances of vacuoles (vac) in the post-ovulatory follicles (POF)—the shared feature between this stage and the next. For testicular histoarchitecture, although features in the previous stage are still present, such as the Sp-II and the Sptd, spermatozoa (Spz), and lobular lamina (LL) with the connective tissue structure that divides the testes into lobules that each lobule typically contains seminiferous tubules, which are the site of sperm production, and the main sperm duct (MSD) also appeared. The fork length of female *A. thazard* samples that were subjected to histoanatomical (micro) profiling were 25.10–42.80 cm, while male samples were 42.0–48.70 cm.

The ovarian lobes were atrophied at Stage 5 (Spent), as the contents were expended in the previous stage. In this post-spawning phase, the lobes of the ovary were significantly shrunk and flaccid when handled, a key differentiating characteristic from the earlier premature stages despite their shared qualities of reduced length and mass. Ovaries appeared emaciated; vascularization at this stage had disappeared, granulation of oocytes unapparent, and the color reverted to a dark red. Testes darkened to a brownish-red shade as well, with the surface of the lobes wrinkled. Histoarchitecture of ovaries of this stage showed that there was a higher occurrence of vacuoles (vac) and invagination of the follicular epithelium (invaFE). Atretic follicles (AF), empty follicles (EF), and post-ovulatory follicles (POF) were also observable in this stage, structures left behind by the previous stage. Interestingly, previtellogenic oocytes (pr) appeared as early as this stage, hinting at the regeneration of the ovaries in preparation for the next ovulation. In males, the characteristic feature of the tissue matrix in this stage for the testis was the diminished presence of spermatozoa (Spz(less)) and the degeneration of the tissues—loss of mass brought about by the release of the male gametes. At this stage, the length of ovaries was at 5.30–10.20 cm with fish sizes ranging from 22.50 cm to 30.60 cm, while testes were 8.25–10.75 cm long with fish FL of 23.90–33.0 cm. As for *A. thazard* samples, females were 40.80–42.0 cm in fork length; males were 50.20–60.40 cm (FL) based on microstructural observation on histology.

Stage 6 (Recovering or Resting) gonads were roughly of the same length as those of in the preceding stage (5.50–7.30 cm in females with fish FL

of 22.10–30.90cm), although there was an observable increase in mass, with the lobes relatively less flaccid and more translucent. Redness of the ovary was lighter (similar to Stage 3), as well as the pigmentation of the testis, which was gradually lightening in color. Stage 6 testicular color was orange rather than the intense red of Stage 5 instead of being similarly lighter (testicular length of 5.80–9.80 cm with fish body size ranging from 30.20 cm to 30.50 cm). The lobes at this stage were seen to be growing in size as the regeneration of sperm occurred, similar to Stage 3's process. The lobes' texture was flaccid and atrophied, in contrast to Stage 3, and only one-third of the body cavity was occupied by their length. Upon investigation of histoarchitecture, ovarian tissues were marked by the repopulation of the tissue space by the previtellogenic oocytes (pr), although this stage was distinguishable from Stage 1 due to the presence of the reconstituted connective tissue (recICT) surrounding the interspersed, degenerated structures; follicles in resorption state (FR) were observed in this stage. Testicular tissues at this stage, meanwhile, were landmarked by the presence of residual spermatozoa (RSpz). Fork length of female *A. thazard* samples in this resting stage were at 30.20–38.20 cm; males were determined to be between 52.0 cm and 63.80 cm according to micro histoarchitectural configuration. A summary of the histology photomicrographs of female (Figure 5) and male (Figure 6) and reproductive stages, landmark characteristics of each stage, testis length, and corresponding fork lengths for these can be found in Figure 7, Tables 1 and 2.

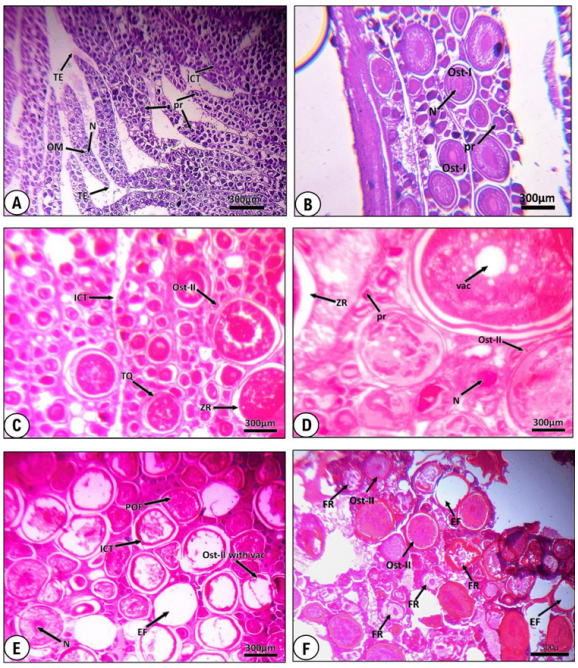

Figure 5. Histoarchitectural structure of ovary of frigate tuna (*A. thazard*). (A) Stage 1 Immature: pr = previtellogenic oocytes, ICT = interstitial connective tissue, OM = oocyte membrane, TE = tapering end; (B) Stage 2 Onset Maturation: Ost-I = primary vitellogenic oocyte, N = nucleus; (C) Stage 3 Onset Maturation: 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 × 400). (Hematoxylin and Eosin). Bar = 300 µm. Histomicrograph by Entia JCD and Nabre NMB.

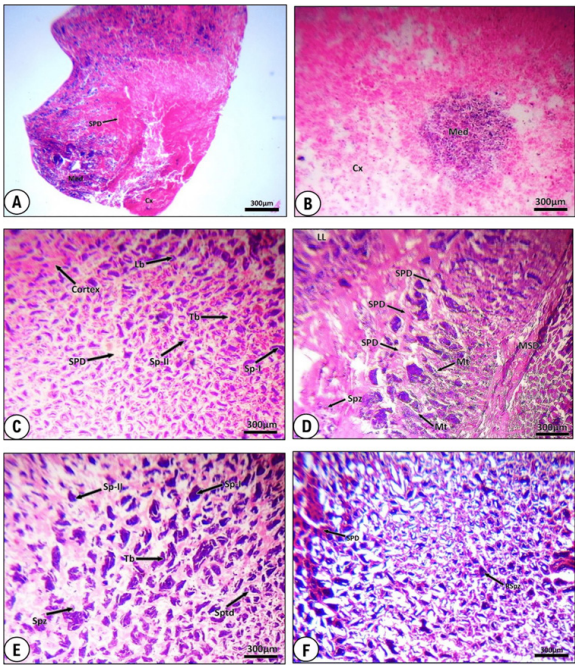

Figure 6. Histoarchitectural structure of testis of male frigate tuna (*A. thazard*). (A) Stage 1 Immature: Med = medulla, Cx = cortex; (B) Stage 2 Onset Maturation: Med = Medulla; Cx = Cortex; Tb = tubules; Lb = lobules (C) Stage 3 Onset Maturation: SPD = sperm duct, Sp-I = primary spermatocyte, Sp-II = secondary spermatocyte, Lb = lobule; (D) Stage 4 Spawning: MSD = main sperm duct, Spz = spermatozoa, SPD = sperm duct; (E) Stage 5 Spent: Sptd = spermatid, Spz = spermatozoa; (F) Stage 6 Recovering/Resting: RSpz = residual spermatozoa. (M × 400). (Hematoxylin and Eosin). Bar = 300 µm. Histomicrograph by Entia JCD and Nabre NMB.

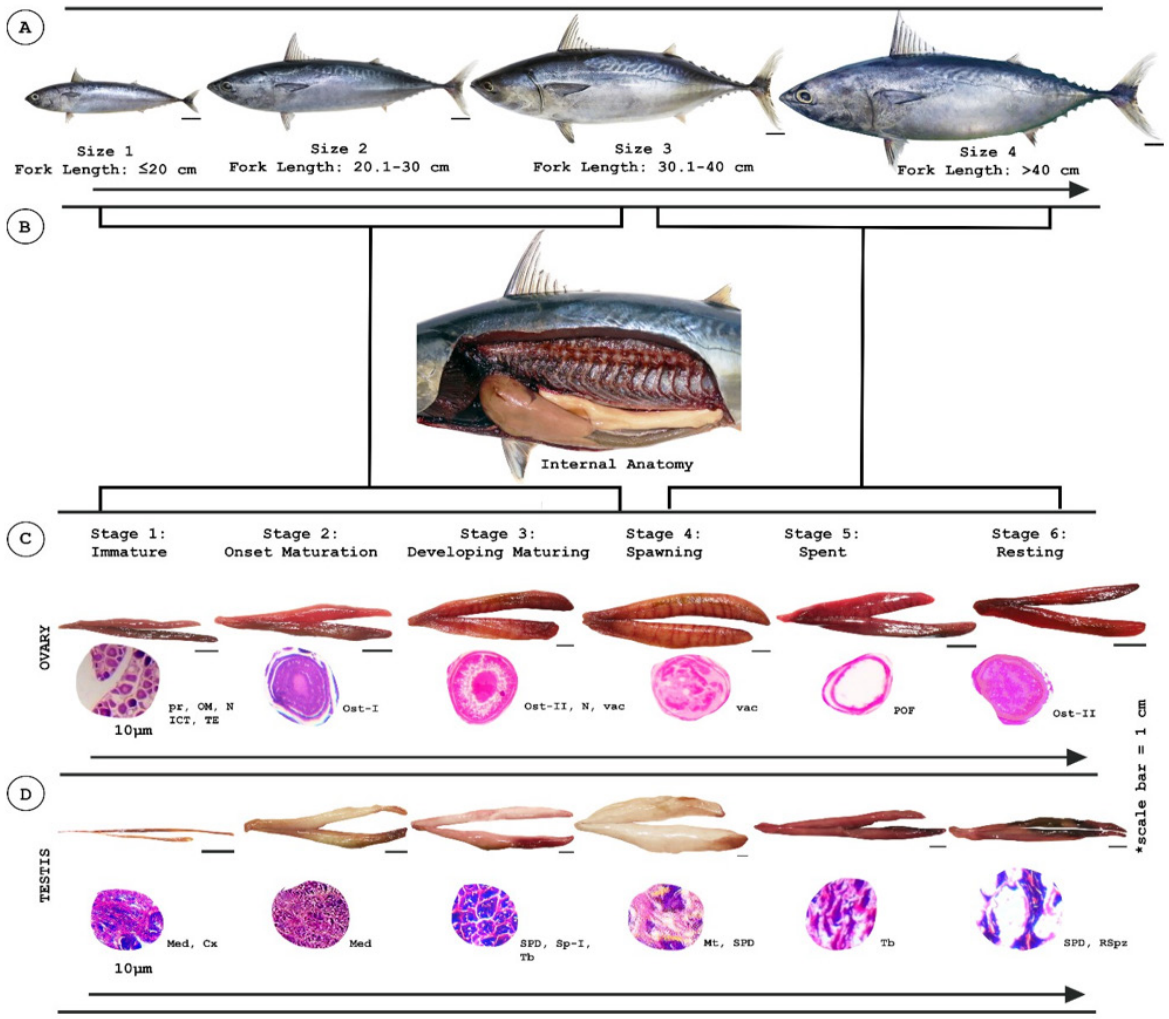

Fig. 7. Direct relationship between fish sizes to macrostructure and histoanatomy of male and female gonads. (A) Fish sizes: Size 1: ≤ 20 cm; Size 2: 20.1–30 cm; Size 3: 30.1–40 cm; and Size 4: > 40 cm, (B) Fish Internal anatomy, (C) Ovarian development through gonad macrostructure and its histoanatomy: 1 (Immature), 2 (Onset Maturation), 3 (Developing/Maturing), 4 (Spawning), 5 (Spent), 6 (Recovering/Resting) and (D) Testicular development gonad macrostructure and its histoanatomy. Bar = 2 cm (fish); 1 cm (gonad); 10 µm (histomicrographs). Photo by Entia JCD and Nabre NMB, and Dela Cruz NA.

Table 1. Female reproductive maturity stages based on gonad appearance (macro) and histological characteristics (micro) in relation to fork length (FL) of *A. thazard*. Size classes based on FL were as follows: Size 1: ≤ 20 cm; Size 2: 20.1–30 cm; Size 3: 30.1–40 cm; and Size 4: > 40 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 (30.1–40 cm), female *A. thazard* is spawning or participating in reproduction, characterized by 30.1–40 cm in FL based on (a) microstructures: follicular epithelium (FE), mature follicle (FO), yolk granules (YG)); of its macrostructure, ovary length is between 7.05–17.95 cm, fullness of the gonads advanced and the contents freely escaping under light pressure; blood vessels conspicuous on the surface. FL based on microstructure is 25.10–42.00 cm, while FL based on (b) macrostructure is 25.0–42.0 cm. Size classes in relation to FL overlap due to the asynchronous reproductive strategy of tuna species.

FISH BODY SIZE	STAGE	PHASE	HISTOLOG Y LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	SIZE CLASS
Size 1: ≤ 20 cm;	1	Immature	previtellogenic oocytes (pr)	14.50–36.10	lobes of the ovary are thin and dark red in color, length at 2.80–12.70 cm (max-min)	15.50–35.0	Size 1, 2, 3
Size 2: 20.1–30 cm	2	Onset Maturation	primary vitellogenic oocyte (Ost-I)	20.50–35.50	lobes of the gonads were elongated and thickened, occupying about one-half of the body cavity and virtually symmetrical, color still red, although relatively lighter in hue;3.65–10.80 cm	20.0–35.0	Size 3
Size 3: 30.1–40 cm	3	Developing/ Maturing	secondary vitellogenic oocyte (Ost-II)	23.80–40.30	occupied about two-thirds of the body cavity, had increased to twice the size, eggs are visible through the ovarian tunica even by just examining with the naked eye, length at 3.56–11.40 cm	20.70–32.50	Size 2, 3, 4
	4	Spawning	follicular epithelium (FE), mature follicle (FO), yolk granules (YG)	25.10–42.00	maximum fullness of lobes occurred, fully plump, their contents freely spilled whether under light pressure or without force, venation of the ovarian tunica is the most noticeable feature of the ovary at this stage, looking dark red in contrast to the pink surface of the lobes, length at 7.05–17.95 cm	25.0–42.0	Size 2, 3, 4
Size 4: > 40 cm	5	Spent	vacuoles (vac), empty follicle (EF), post-ovulatory follicles (POF)	40.80–42.00	gonadal lobes are atrophied, ovaries appeared emaciated; venation and granulation of oocytes unapparent, and the color reverted to a dark red, length at 5.30–10.20 cm	22.50–30.60	Size 4
	6	Recovering/ Resting	follicle in resorption state (FR)	30.20–38.20	observable increase in mass, lobes relatively less flaccid and more translucent, redness of the ovary is lighter, length at 5.50–7.30 cm	22.10–30.90	Size 3

Table 2. Male reproductive maturity stages based on gonad appearance (macro) and histological characteristics (micro) in relation to fork length (FL) of *A. thazard*. Size classes based on FL were as follows: Size 1: ≤ 20 cm; Size 2: 20.1–30 cm; Size 3: 30.1–40 cm; and Size 4: > 40 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). The highlighted row shows that at Size 3 (30.1–40 cm), male *A. thazard* is spawning or participating in reproduction, characterized by 30.1–40 cm in FL based on (a) microstructures: spermatid (Sptd), spermatozoa (Spz); of its macrostructure, testis length is between 4.80–17.25 cm, fullness of lobes, fully opaque and white, with no venation visible. FL based on microstructure is 42.00–48.70 cm, while FL based on (b) macrostructure is 25.10–43.0 cm. Size classes in relation to FL overlap due to the asynchronous reproductive strategy of tuna species.

FISH BODY SIZE	STAGE	PHASE	HISTOLOG Y LANDMARK (MICRO)	FL OF FISH BASED ON MICRO (cm)	GROSS MORPHOLOGY LANDMARK (MACRO)	FL OF FISH BASED ON MACRO (cm)	SIZE CLASS
Size 1: ≤ 20 cm;	1	Immature	medulla (Med), cortex (Cx)	37.10–40.50	thinner, almost string-like, and creamy white in color with a length at 2.00–13.90 cm (min-max)	14.50–36.10	Size 1, 2, 3
Size 2: 20.1–30 cm	2	Onset Maturation	tubules (Tb), lobule (Lb)	37.50–42.70	lobes are mostly white, with dark brown pigmentation at the tips, length at 4.00–12.65 cm	20.0–35.0	Size 2, 3, 4
Size 3: 30.1–40 cm	3	Developing/ Maturing	primary spermatocyte (Sp-I), secondary spermatocyte (Sp-II), spermatid (Sptd)	44.70–46.00	color of the testis at this stage is opaque white, slightly yellowish, with the posterior tip more pigmented, length at 6.70–15.45 cm	23.80–40.30	Size 2, 3, 4
	4	Spawning	spermatid (Sptd), spermatozoa (Spz)	42.00–48.70	maximum fullness of lobes, fully opaque and white, with no venation visible, length at 4.80–17.25 cm	25.10–43.0	Size 2, 3, 4
Size 4: > 40 cm	5	Spent	less spermatozoa (Spz)	50.20–60.40	darkened to a brownish-red shade as well, with the surface of the lobes wrinkled, length at 8.25–10.75 cm	23.90–33.0	Size 2, 3, 4
	6	Recovering/ Resting	residual spermatozoa (RSpz)	52.00–63.80	pigmentation of the testis, gradually lightening in color, orange rather than the intense red, growing in size as the regeneration of sperm occurred, length at 5.80–9.80 cm	30.20–30.50	Size 3, 4

4. DISCUSSION

This paper is the first report on the gonad morphogenesis, macrostructure, and subsequent assessment of histoanatomy of the reproductive of frigate tuna *(Auxis thazard)* in the Southern Philippines.

The findings of this study indicate that the fork length of *A. thazard* for spawning is different between males and females. Spawning occurs between 25.10 cm and 42.00 cm for females, while the fork length measures 42.0–48.70 cm for males. Of size classes, spawning occurs at Size 2 and Size 3 for females, while it occurs at Size 4 in males (Table 3 and Table 4). Per the findings of this study, *A. thazard* was found around the sampling areas—Sarangani Bay, Davao Gulf, Moro Gulf, and Sulawesi Sea—and males are larger than females.

A recent 2019 study by Saber et al. focused on the macroscopic development of small tuna and observed similar characteristics in both spawning and mature phases. The ovaries showed significant enlargement, appearing orange to red with visible superficial blood vessels. Similarly, the testes underwent

marked enlargement with noticeable superficial blood vessels. Notably, a substantial amount of sperm was easily released under minimal pressure. Histologically, Vieira et al.'s (2022) investigative examination of *A. thazard* aligns with these findings, confirming analogous characteristics in mature gonads. Mature oocytes (MO) in "spawning capable" ovaries were identified by their larger sizes, while mature testes displayed spermatozoid cells in the testicular lobules and ducts.

As studied by Jalabert (2005), teleost reproduction exhibits various unique characteristics: different species' reproductive methods have evolved into various adaptations as deemed necessary by their aquatic habitat. This variety may relate to sexuality, behavior during spawning and parenting, sensitivity to environmental influences, and particular aspects of gametogenesis, such as the length of vitellogenesis and egg morphology. Natural modes of sexuality range from hermaphroditism to gonochorism— a reproductive system where an individual fish is either distinctly male or female. In *A. thazard* and tuna in general, they are gonochoristic and homogametic.

The present study's findings coincide with the findings of teleosts by Selman et al. in 1993. In their study, the maturing stage was also known as "initial vitellogenesis," which occurs close to the Golgi complexes, which take part in synthesizing their contents. The oocyte's cytoplasm is filled with cortical alveoli as the oocyte develops. Due to the centripetal buildup of yolk proteins during the later stages of oocyte development, cortical alveoli then move to the periphery of the oocyte. As part of the "cortical reaction" after fertilization, the contents of the cortical alveoli are discharged to the egg surface. This discharge indicates the restructuring of egg proteins developing the chorion.

Lubzens et al. (2010) found that even as recently as 2010, there are still numerous gaps in the knowledge available about oogenesis in teleosts. Recent advances in teleost reproductive research have defined the functions of primordial germ cells, yolk protein precursors and their processing inside the maturing oocyte, vitamin deposition in eggs, the function and structure of egg envelopes, and oocyte maturation mechanisms. In the same paper, Lubzens et al. (2010) stated that there are similar characteristics in the processes leading to the creation of a mature egg, even though egg formation and spawning techniques may vary among fish species. The oocyte growing inside the ovarian follicle gains the ability to generate a viable embryo following fertilization. Massive structural and functional changes are associated with this process. Only recently have genomic and proteomic studies begun to shed light on how broad, complicated, and interconnected these changes are (Cerdà et al. 2008a; Cerdà et al. 2008b).

In 2007, Nagahama et al. published a paper on the male testicular process by which cell differentiation, known as spermatogenesis, which results in the production of mature male gametes, entails spermatogonial growth, meiosis, and spermiogenesis. Although the significance of gonadotropins and androgens in this process is widely acknowledged in vertebrates, what exactly each hormone performs remains unknown. A recent review by Hatef and Unniappan (2019) discussed that metabolic hormones are critical regulating agents in many biological processes, including morphogenesis. Nevertheless, the physiological functions of these metabolic hormones in controlling reproduction in fish, particularly in males, are largely unclear. The review attempts to summarize the key findings on the effects of metabolic peptides on the male fish reproductive system, focusing on testicular development and spermatogenesis, growth, and reproduction through maintaining energy balance despite the limited available information. Reproductive outcomes involving puberty, spermatogenesis, spawning, and cellular developments containing steroidogenesis and sperm development are primarily controlled by the hypothalamus-pituitary-gonadal (HPG) axis (Weltzien 2004). A comparative study led by Uribe et al. (2014) on the testicular composition and spermatogenesis of bony fishes shares similar descriptive findings to our results on the teleost testes examined histologically. They reported that microstructure demonstrates that vertebrate testes are produced from germ cells and somatic cells, including interstitial and germinal cell compartments. A basement membrane separates the two chambers. Spermatogonia, meiotic spermatocytes, and haploid spermatids that differentiate into spermatozoa are some examples of germ cells. Spermatogenesis is a series of morphological and physiological alterations that occur in germ cells and start with developing spermatogonia into meiotic spermatocytes. These develop into spermatozoa through spermiogenesis following the second meiotic division. In creating spermatocytes or cysts, spermatogonia connects with Sertoli cells.

5. CONCLUSION AND RECOMMENDATIONS

While there are studies covering tuna in the Philippines, most of these cover the fishing practices of tuna and the tuna industry in general; none cover the reproductive biology on a histological and gonadal level, much less those of neritic tuna. There are still significant knowledge gaps about the dynamics involved in the oogenesis and spermatogenesis in *A. thazard* in the Philippines, especially in Mindanao. This paper is the first report on gonadal development in relation to the fish sexual maturity length of *A. thazard* in the Philippines.

Results indicate that at Size 3 (40.1–50.0 cm), male and female *A. thazard* are sexually mature and prepared to spawn. The fourth stage of gonadal development (Spawning) and subsequent histological profiling revealed that the structures needed for reproduction are present at this stage—mature follicles for females and spermatozoa for males—mature forms of the gametes possessing the necessary characteristics for fertilization and, eventually, a viable embryo, to be possible. Per our data, spawning in females occurs when they are 25.10–42.0 cm long, while males are 42.0–48.70 cm long. As such, we recommend that policy recommendations going toward the conservation of *A. thazard* should include restricting the collection of *A. thazard* under 42.0 cm, or Size 3 (30.1–40.0 cm), as this size is the state at which they are actively contributing to the population.

As our study considers only the months of collection, the high percentage of immature individuals, opportunistic sampling, and the natural decline of the local fish species, further study, especially the relation of the reproductive biology of *A. thazard* to stocks, is recommended.

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AUTHOR CONTRIBUTIONS

Entia JCD: Writing, Visualization, Investigation, Data and Sample Processing. **Nabre NMB**: Data curation, Data and Sample Processing. **Castrence GA**: Reviewing and Data Validation. **Arellano BJ**: Revisions and Proofreading. **Amoncio RAD**: Data and Sample Processing. **Wetzel JT**: Data Validation, Final Editing. **Guevarra EP**: Conceptualization, Supervision.

CONFLICTS OF INTEREST

The researchers claim to not know conflicting personal or financial interests that could have impacted the research described in this study. We, therefore, assert no conflict of interest.

ETHICS STATEMENT

Approval and partnership 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 the municipalities of Sarangani Province (Alabel, Malapatan, Glan, Maasim, Kiamba, and Maitum). Our research adhered to significant institutional and national guidelines and used independent and dependent sampling techniques.

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