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RESEARCH ARTICLE

Reproductive Biology of Bali Sardines, *Sardinella lemuru* (Bleeker, 1853), in Tayabas Bay, Quezon, Philippines

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ABSTRACT

Fish reproductive biology plays a vital role in fishery management, an essential biological tool that would serve as a basis for formulating policies. In this study, 3,678 *Sardinella lemuru* were sampled to determine its reproductive biology characteristics from January 2014 to December 2016 at Tayabas Bay, Quezon. Fish samples were dissected, and the sex was determined based on the macroscopic features of the gonads. The results showed length sizes ranging from 6.5 cm to 19.6 cm TL. The sex ratio was significantly different from the expected 1:1 distribution. The fish's length and weight indicate allometric growth in both sexes. The length at first capture was 15 cm for females and 14.75 cm for males, which was lower than the length at first maturity of 15.89 cm. The spawning season occurred in the fourth to first quarter from 2014 to 2016, specifically from November to February. In February, the number of mature and spawning fishes increased in number and reached a peak. Likewise, the spawning and recruitment of *S. lemuru* did not vary from other locations like the Sulu Sea and Moro Gulf, which has a spawning period from October to December and December to January in Bali, Indonesia. The computed spawning potential ratio is 24%, near the Limit Reference Point (LRP), indicating that management measures should be directed toward the sustainability of the *S. lemuru* and its productivity in Tayabas Bay.

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1. INTRODUCTION

Philippine sardine biodiversity is among the highest in the world (Willette 2011). This marine resource contributes substantially to the economy, provides cheaper dietary protein alternative, and is a primary commodity for any relief effort during calamities. *Sardinella lemuru*, the country's most abundant sardine, is one of the top ten most important species in commercial fishing (Luceño et al. 2014). It can be distinguished by having a faint golden spot behind the gill opening and mid-lateral line, a distinct black spot at the hind border of the gill cover, and an elongated and sub-cylindrical body (Whitehead et al. 1985). Having an average small size, they inhabit shallow depths of coastal water or, at times, in estuaries. They are widely distributed globally, both in temperate and tropical marine areas, where they feed mainly on plankton and thrive through large schools (Luceño et al. 2014). In the Philippines, they are locally known as "tamban." Sardines in the Philippines form shoals in coastal waters over the continental shelf where depth is less than 200 meters.

Aside from being consumed as fresh fish, *S. lemuru* are also processed for fishmeal, canning, and dried for consumption (Luceño et al. 2014). The ones used for canning are those high-quality, larger-sized fish, while smaller ones, along with spoiled bigger fishes, are processed for fishmeal (Luceño et al. 2014). Ecologically, sardines are basally positioned in a food web that supports pelagic tuna, mackerel, and numerous sea birds and marine mammals (Willete et al. 2011). Despite its economic and ecological importance, the species have been reported to be under heavy fishing pressure in particular, with stocks being overexploited (Luceño et al. 2014). Drastic effects of exploitation can affect the species' reproduction, making it vulnerable to depletion. In other words, fishing not only decreases the abundance of fish but also changes their reproductive biological performance. Moreover, no recent studies have been conducted on the reproductive biology of *S. lemuru* in Tayabas Bay. Hence, this study assessed the reproductive biological performance vital for implementing reliable science-based management policies and conservation.

Specifically, different reproductive parameters such as sex ratio, length at first maturity (L_m), length at first capture (L_c), length-weight relationship, and spawning season were determined. The data used in this study were mainly from the established National Stock Assessment Program (NSAP) landing center located at Dalahican, Quezon, covering Tayabas Bay.

Knowledge about fish reproductive biology (sex ratio, gonadal maturation, length at first maturity, length at first capture, length-weight relationship, and spawning season) plays an important role in fishery management, which is an essential biological tool that would serve as a basis in the formulation of policies in the bay. It is the reproductive success that allows a species to persist. Thus, it is vital to any population (Gervasi 2015).

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out in one of the landing centers established by the National Stock Assessment Program (NSAP) located at Dalahican, Quezon, covering Tayabas Bay (Figure 1). The bay is defined as an area of ocean bounded on the north, northeast, and east by Southern Quezon, on the island of Luzon, and demarcated at the south by two imaginary lines going eastward from 121°28'2" E, 13°41'44" N to 122°03'17" E, 13° 00'09" N (Ramos et al. 2018). It is one of the major fishing grounds in the CALABARZON Region with a total area of 2,213 km^2, covering 16 municipalities, and one city in the provinces of Quezon, Marinduque, and Batangas. In Quezon Province, Lucena City is the only city along Tayabas Bay; the 11 municipalities are Agdangan, Catanauan, General Luna, Macalelon, Mulanay, Padre Burgos, Pagbilao, San Francisco, Pitogo, Sariaya, and Unisan. The bay consists of 121 coastal barangays; the rest are in interior areas. The shelf between Quezon and Marinduque mainland is approximately 518 km^2 of apparently smooth, muddy, and sandy ground with an average depth of 40 fathoms.

2.2 Fish sampling

Data on the catch of *S. lemuru* were gathered twice or thrice a month in the landing site located at Dalahican, Quezon, for 36 months, from March 2014 to February 2017. A total of 3,678 individuals with total lengths ranging from 6.5 to 19.6 cm were sampled and randomly selected from different fishing gears such as purse seine, bag net, and drift gill net. Since purse seine is the most operated fishing gear throughout the year, it has the most catch, which amounts to 90% of the total samples based on the data of NSAP 4A. Whereas other fishing gears catching *S. lemuru* seldom operate throughout the year; bag net (February, October, and December) and drift gill net (September).

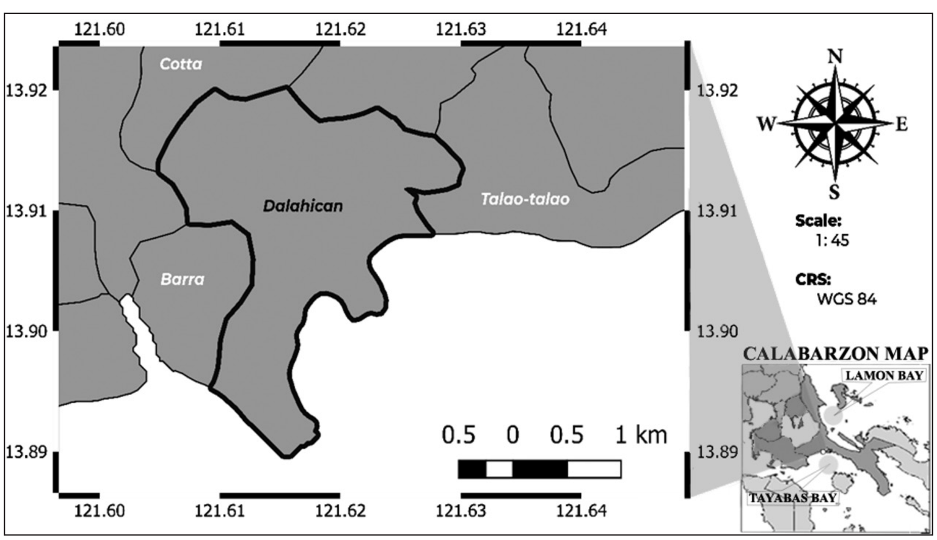

Figure 1. Map of Tayabas Bay showing the landing site where sampling of commercially important fishes was undertaken.

2.3 Fish Gonad Extraction and Identification

Before dissection, each specimen of *S. lemuru* was measured to the nearest gram (g) using an electronic balance with 0.01 g precision and total length measured to the nearest 0.1 cm accuracy measuring from the tip of the mouth to the longest lobe of the caudal fin. Subsequently, the coelomic cavity was carefully opened using forceps to determine the maturity status of gonads through visual inspection following the National Fisheries Research and Development Institute (NFRDI) guidelines. Five maturity stages were allocated based on the shape, color, and appearance of gonads (Nielsen and Johnson 1995) (Table 1). For each sex, the maturity stages were grouped into immature or virgin (1), maturing virgin and recovering spent or inactive (2), developing (active) or developed (3), mature or spawning (4), and spent or resting (5) (Holden and Raitt 1974).

2.4 Statistical Analysis

The length–weight relationship was determined for the male (n = 1,653), female (n = 1,908), and combined sex (n = 3,561) by the exponential equation $W = aL^b$, where W is the weight of the fish in grams, L is the total length of the fish in cm, and variables "a" and "b" are the regression coefficients (Ricker 1975). Sex ratios were expressed as the total number of females to the sum of the total number of males and females. The X^2-test was used to determine whether the sex ratio differed from the expected and theoretical sex ratio of 0.5 (Al-Jufaili 2013). The sexual maturity threshold is set at stage III, which is the beginning of the gonad development phase (FAO 1978; Abderrazik et al. 2016). Therefore, fish gonads in stages III to V were considered mature fish and used to calculate the length at first maturity (L_{m50}). The length at which 50% of all individuals were sexually mature (L_{m50}) was estimated from the proportion of mature

Table 1. Five-point scale used in determining of gonadal maturity stage (Nielsen and Johnson 1995).

Stages of maturity	Oogenesis	Features
Juvenile	Juvenile	Young individuals have not yet engaged in reproduction; fish samples are even small in sizes.
Stage I	Immature/Virgin	Have not yet engaged in reproduction. Very small sexual organ (about 1/3 of the body cavity) close under the vertebral column, usually transparent, colorless to gray.
Stage II	Maturing Virgin and Recovering Spent/Inactive	Maturing Virgin: Male: Testis and ovary thicker, translucent, gray-red. Length is ½ or slightly more than half the length of the ventral cavity. Female: Gonads appear as gelatinous mass. Single eggs cannot be seen with the naked eye (only with a magnifying glass). Recovering spent/Resting: Testis and ovary empty, red. A few eggs in the state of resorption.
Stage III	Developing (Active)/ Developed	Developing: Male: Testes change from transparent to pale rose color. Female: Eggs are distinguishable to the naked eye; very rapid increase in weight of the gonads is in progress. Developed: Male: Testis reddish-white. Female: No eggs are extended on applying pressure to abdomen, eggs clearly discernible and opaque.
Stage IV	Mature/Spawning	Gravid: Sexual organs filling ventral cavity. Male: Testis is white, drops of milt fall with pressure. Female: Eggs completely round, some already translucent and ripe. Spawning: Male: Roe and milt can be extruded with slight pressure of the abdomen. Female: Most eggs are translucent with few opaque eggs left in ovary.
Stage V	Spent or Resting	Spent: Gonads are deflated and shrunken to about ½ length of body cavity. Male: Testis blood shot and flabby. Female: Ovary may contain remnants of disintegrating opaque and ripe ova, darkened or translucent. Resting: Testis and ovary empty, red. No opaque eggs left in the ovary. A few eggs in the state of reabsorption. Begins recovery process by reducing in size and return to stage II.

individuals in each of 0.5 cm length class interval and the fitted logistic curve (Sparre and Venema 1998) as follows:

$$P = \frac{1}{1 + \exp(S1+S2 \times L)}$$

where P is the proportion of mature individuals within a length class, S1 is the intercept, S2 is the slope, and L is the midpoint length. The probability of capture was approximated by backward extrapolation of the regression line of the descending limb of length converted catch curve (Pauly 1987). The probability of capture of sequential length classes was regressed using a logistic curve to estimate L_{c50} (Sen et al. 2014). The Spawning Potential Ratio (SPR) was determined by using the Length Based Spawning Potential Ratio (LBSPR) R Shiny application (Hordyk et al. 2016). To estimate the SPR, the length data of all females was used together with the population parameters (L_{oo} and M/K ratio) obtained from FAO-ICLARM Stock Assessment Tool (FiSAT II) version 1.2.2 (Gayanilo et al. 1996) and descriptions of size at maturity specified as L_{m50} and L_{m95} (the sizes at which 50 percent and 95 percent of the population mature, respectively) as described in the studies of Stergiou (1999), Nurdin et al. (2016), and Lanzuela et al. (2020).

3. RESULTS

A total of 3,678 specimens of *S. lemuru* were collected, wherein 52% were females and 45% were males. The female-to-male ratio was 1.15:1. The monthly sex ratio significantly departed from the expected 1:1 ratio in February, April, June, July, September, October, and December. The chi-square test showed that the overall sex ratio differs statistically

Table 2. Monthly variation in sex ratio of *Sardinella lemuru* caught in Tayabas Bay, Philippines (pooled data C.Y. 2014-2016).

Months	F	M	Total	F:M	Chi-square Value
January	135	155	290	0.87:1	1.38
February	109	66	175	1.65:1	10.57*
March	118	111	229	1.06:1	0.21
April	117	88	205	1.33:1	4.10*
May	198	264	462	0.75:1	9.43*
June	108	92	200	1.17:1	1.28
July	150	107	257	1.4:1	7.19*
August	126	131	257	0.96:1	0.10
September	164	113	277	1.45:1	9.39*
October	326	221	547	1.48:1	20.16*
November	111	125	236	0.89:1	0.83
December	246	180	426	1.37:1	10.23*
Total	1908	1653	3561	1.15:1	18.26*
*P<0.05=3.84					

(p-value = 2E-05) from the expected value of 1:1 (Table 2).

The measured total length and weight for each sex ranged from 10.6 to 19.6 centimeters and 11.9–65.6 grams for females and 12.0–19.0 centimeters and 12–64.5 grams for males. The estimated length at first capture is 15 cm for females and 14.75 cm for males (Figure 4), indicating that males were caught at smaller sizes.

Figure 3 shows the length-weight relationship of *S. lemuru* indicating allometric growth, which has an observed high correlation (r^2 = 0.894 and r^2 = 0.89, for females and males, respectively) and strong significance (P-value =< 2.2e-16) between these

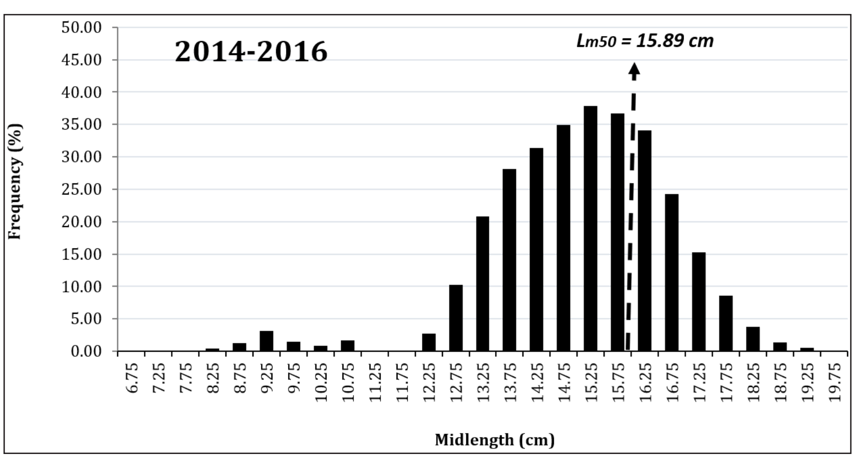

Figure 2. Combined length frequency distribution of *S. lemuru* (2014–2016).

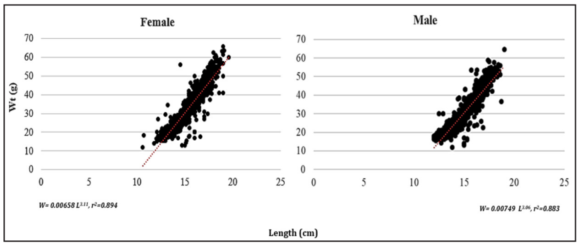

Figure 3. Length and weight relationship for both sexes of *Sardinella lemuru* caught in Tayabas Bay.

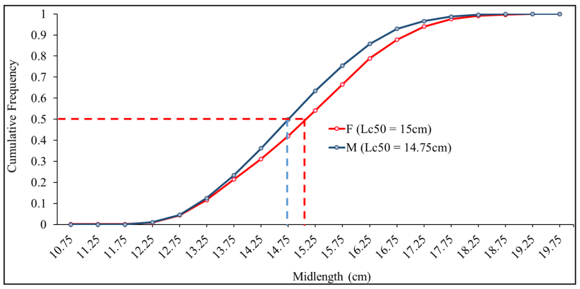

Figure 4. Cumulative size frequency distribution of catches showing the estimated Length at First Capture (L_{c50}) of *Sardinella lemuru* in Tayabas Bay (2014-2016).

variables of both sexes of the species based on the linear regression. The relationship can be summarized as W = 0.00658 $L^{3.11}$ and W = 0.00749 $L^{3.06}$ for females and males, respectively.

Females were dominant in the peak of the spawning season in February. From 2014 to 2016, both male and female *S. lemuru* showed consistent spawning months from November to February (Table 2).

The estimated length at first maturity for females was 15.89 cm (Figure 5). The starting length corresponding to the retained fraction was 12.75–18.25 cm. The collected female fishes with midlength <12.25 cm were immatures (gonads in Stages I and II), as shown in Figure 6. The Stage III developing (active) or developed female *S. lemuru* were at 12.5 cm.

The length frequency distribution of the samples shows that the cohort used has a modal length of 15.75 cm–16.25 cm and a maximum length of 19.75 cm. The LB-SPR assessment model results indicate normal population distribution as the computed SPR was 24% which was near the limit reference point (20%).

4. DISCUSSION

The Bali sardinella (*S. lemuru*) is one of the most important fisheries resources in Tayabas Bay. Reproductive biology provides basic information on the stock, which is necessary for sound management and conservation. In this study, the overall female-to-male sex ratio was 1.15:1. This indicates an overall unbalanced sex ratio. The monthly sex ratio significantly departed from the expected 1:1 ratio in February, April, May, July, September, October, and December. During these months, the females significantly outnumbered the males. According to Trindade-Santos and Freire (2015), this ratio may vary during the life cycle due to successive events acting differently over individuals of each sex. In reproductive success, the significance of the sex ratio has yet to be determined. However, based on the findings in the research of Fryxell et al. (2015), populations that are predominantly female have a greater impact on ecological conditions and lead to significant changes in critical community and ecosystem responses such as zooplankton and phytoplankton abundance, productivity, pH, and temperature through strong pelagic trophic cascades. Sardines reproduce through external fertilization, whereas a higher proportion of females in the sardine population could lead to increased competition for resources and potential implications over mating partners. Thus, this indicates the importance of the sex ratio for mediating the ecological role of *S. lemuru* in Tayabas Bay.

The relationship between the total length (TL) and body weight (BW) shows a highly significant

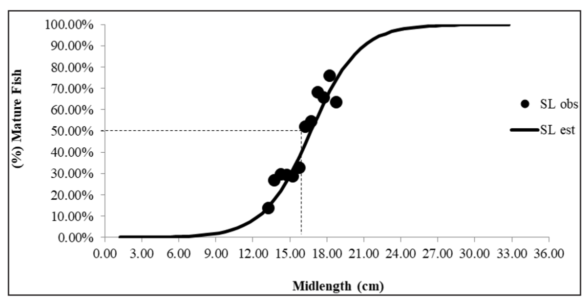

Figure 5. Observed Length at First Maturity (L_{m50}) for female *Sardinella lemuru* caught in Tayabas Bay.

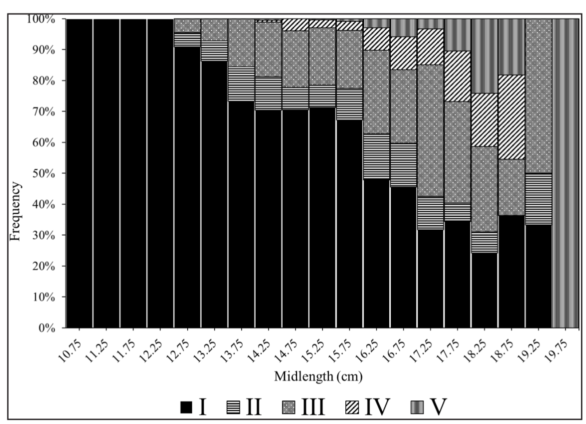

Figure 6. Length distribution by maturity stages of female *Sardinella lemuru* caught in Tayabas Bay.

correlation for both sexes, $R^2 = 0.89$ for females and $R^2 = 0.88$ for males, which indicates an allometric growth. This means that the fish's weight does not increase proportionately with its length. Instead, as the fish grows, its body weight increases faster than its length. A high correlation coefficient value, *r*, validates or explains the quality and reliability of the LWR model or linear regression (Gomez and Gomez 1984). The length-weight relationship is vital in providing reliable biological information in fishery assessments as it helps assess variations from the expected weight for the known length groups. Kuriakose (2017) described that length-weight is particularly useful in determining the biomass of a fish sample based on its length frequency and comparing the parameter estimates of the relationship for a specific fish population to average parameters for the region, estimates from previous years or among various fish groups, to evaluate the relative condition and robustness of the population.

One that is used as a reference in the effort to manage fishery resources to remain sustainable is the estimation of the length at first capture (L_{c50}) of *S. lemuru*. The L_{c50} is a vital parameter used along with length-at-first-maturity to indicate the health status of the resource (Udoidiong et al. 2017). Knowledge of length at maturity and spawning season detects when and at which length the fish should be protected, and therefore, it is crucial for the proper management and conservation of fish stocks (Hunter et al. 1992). As shown in Figure 5, the length at first capture (15 cm for females and 14.75 cm for males) was lower than that at first maturity (15.89 cm) (Figure 2), which shows the possibility of growth overfishing. Growth overfishing is a condition in which young fish are caught before reaching their maturity age (Ben-Hasan et al. 2021). To maintain sustainable fishery exploitation, allowing fish to reproduce at least once during their lifespan is essential to recruit the stock. This could be achieved when the length at first capture (L_{c50}) is bigger than the length at first maturity (L_{m50}) (Al-Qishawe et al. 2014).

Length at first maturity is necessary for the success of fishery management, fundamental to establishing the means that avoid exploitation of young specimens and consequential reduction of spawning stock (Mathialagan and Sivakumar 2017). Following the logistic curve as shown in Figure 5, the length at first maturity was determined to be 15.89 cm, which is relatively higher than the values reported in the previous research conducted by Ramos et al. (2018) at Tayabas Bay, with size at first maturity of 14.0 cm from 2006 to 2013. The computed value was also higher than *S. lemuru* caught in the Sulu Sea with 15.0 cm TL (De Guzman et al. 2018). Stock density, zooplankton distribution, water temperature, and salinity may influence fish growth and further affect the age at 50% maturity (Lee et al. 2005; Metillo et al. 2018). Also, variation in the length at first maturity of fish may occur depending on its location and period.

The gonad development and reproductive strategy have been described in many fish species to understand the time of course and energetic consequences of reproductive effort. The spawning season of *S. lemuru* in Tayabas Bay occurred in the fourth to the first quarter (November to February) from 2014 to 2016 (Figure 7). The same spawning period was reported in the Sulu Sea, Moro Gulf, and Bali, Indonesia (Willette 2011). In February, the number of matured and spawning individuals increased in number and reached a peak. Minor spawning was observed from November to December (Figure 7 and 8). This could be interpreted that seasonal spawning occurs with a single peak during the months around the northeast monsoon with little, if any, mature or older fish for the rest of the year. During this period, cooler water temperatures can increase nutrient levels, stimulating the growth of plankton and other food sources for the fish. This increased availability of food can encourage spawning activity. Also, prevailing winds could blow surface waters offshore (Whitehead et al. 1985), creating favorable conditions for the dispersal and survival of eggs and larvae. However, whether this occurs in the bay has yet to be formally investigated.

Spawning potential ratio (SPR) is the number of eggs that could be produced by an average recruit over its lifetime when the stock is fished divided by the number of eggs that could be produced by an average recruit over its lifetime when the stock is unfished (Fisch and Camp 2022). To calculate SPR, several parameters are needed, such as the computed length at which 50% of the fish are mature ($L_{m50} = 15.89$ cm), the length at which 95% of the fish are mature ($L_{m95} = 17.50$ cm), the asymptotic length ($L_{\infty} = 20.97$ cm), and the ratio of natural mortality to growth (M/K ratio = 1.88). From these parameters, the computed SPR for this species is 24%. The target reference point for SPR is usually set at 40%, which means that the fishery should aim to maintain the stock at a level where 40% of the maximum potential egg production can be achieved. If the SPR falls below this target reference point, it indicates that the stock is being overfished and the population's reproductive capacity is being reduced (Lorenzen et al. 2021). At present, there is no published value for SPR for *S. lemuru* so that no comparison can be made.

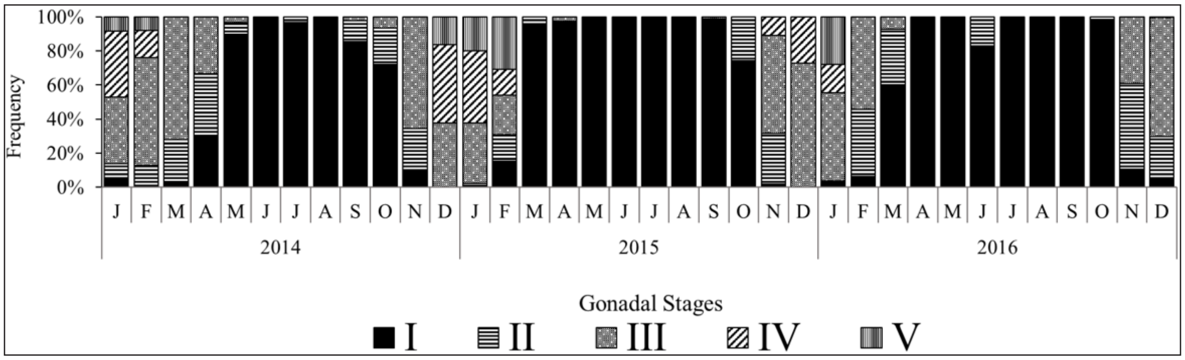

Figure 7. Monthly distribution of gonad maturity stages of female *Sardinella lemuru* caught in Tayabas Bay.

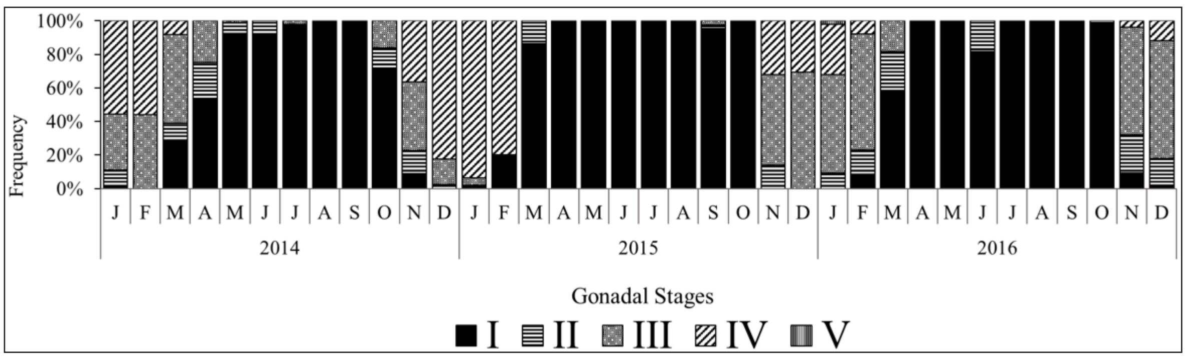

Figure 8. Monthly distribution of gonad maturity stages of male *Sardinella lemuru* caught in Tayabas Bay.

Given the results mentioned above, conservation approaches should be made to maintain the sustainability of sardines and Tayabas Bay as a whole.

5. CONCLUSION

Sardinella lemuru in Tayabas Bay has a distinctive spawning seasonality that occurs in the fourth to the first quarter of the year (November to February), with February as the spawning peak. The seasonality of spawning may affect the unbalanced sex ratio, which was found to be 1.15:1 for females and males, respectively. The length at first capture was 15 cm for females and 14.75 cm for males, which was lower than the length at first maturity of 15.89 cm (Figure 2). The SPR falls below the target reference point of 40%.

Therefore, the present study suggests that for the future conservation of *S. lemuru*, management measures such as the strict implementation of appropriate mesh sizes, regulation of commercial gears during spawning season to obtain the overall goal of high sustainable yield, and imposing seasonal closure for November to February should be considered.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

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