

## Original Article

# Aspects of the reproductive biology of the marine ornamental Vagabond Butterflyfish *Chaetodon vagabundus* Linnaeus, 1758 (Pisces, Chaetodontidae) from Iligan Bay, Southern Philippines

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**Abstract:** The reproductive biology of ornamental fish species from coral reefs is poorly studied despite increasing demand in the aquarium trade industry. Aspects of the reproductive biology of the Vagabond butterflyfish, *Chaetodon vagabundus* Linnaeus, 1758, from Iligan Bay, Southern Philippines were investigated from August 2021 to August 2022. Monthly samples of 30 to 45 individuals per class size with a range of 9 to 14.1 cm (TL) were collected to examine the sex ratio, maturity stages and spawning period, gonadosomatic index (GSI), length at first maturity ( $L_{50}$ ) and the batch fecundity (BF) of the species. The sex ratio showed female dominance (1:1.42) among size classes and across months. The spawning period indicates a peak during the inter-monsoon or warm months (April and May) and extends beginning of the Southwest monsoon period (June and July). These were supported by the relatively higher gonadosomatic index (GSI) recorded during these months. Histological examination showed that the ovaries during this period had the presence of numerous tertiary vitellogenic and hydrated oocytes and post-ovulatory follicles. At the same time for testes, there are occurrences of dense spermatozoa in the lumen of tubules in testes. This served as confirmatory evidence in support of the spawning period. The length at first sexual maturity of males (10.58 cm TL) was slightly shorter than females (10.91 cm TL). Fecundity increased with the fish's total length and weight showing a non-linear relationship that was best described by a power function. This study highlighted the valuable data produced that are required for the management of the population stock of this species.

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## Introduction

The marine ornamental fish trade is promising but still largely depends on the wild fish stocks from tropical coral reef ecosystems (Pouil et al., 2020), particularly in the Philippines. The proportion of marine ornamental fish produced in captivity and trade accounts for only 1-2% of the marine ornamental trade (Wabnitz et al., 2003; Bruckner, 2005). There are mysteries in the wild harvest so the sustainability of the marine ornamental fish trade can be questioned with aquaculture being perceived as a responsible alternative for the procurement of these ornamental marine fish (Stickney, 2022). The abundance and stock size of these ornamental fish species are

ultimately determined by their ability to reproduce (Chen et al., 2020). The study of the reproduction of fish is an important topic in fish biology; so far, it has practical meaning in solving some fishery management questions. Reproductive timing in coral reef fishes is highly variable and diverse at several temporal scales (Lowerre-Barbieri et al., 2011; Palla and Sotto, 2021). Information related to reproduction aspects such as ecological conditions influencing gonadal-maturity, size at first maturity, duration of spawning season and fecundity, sex ratios, etc. have enormous applications for the conservation and management of fish stocks (Ambily and Nandan, 2017). Studies on reproduction in fishes, such as

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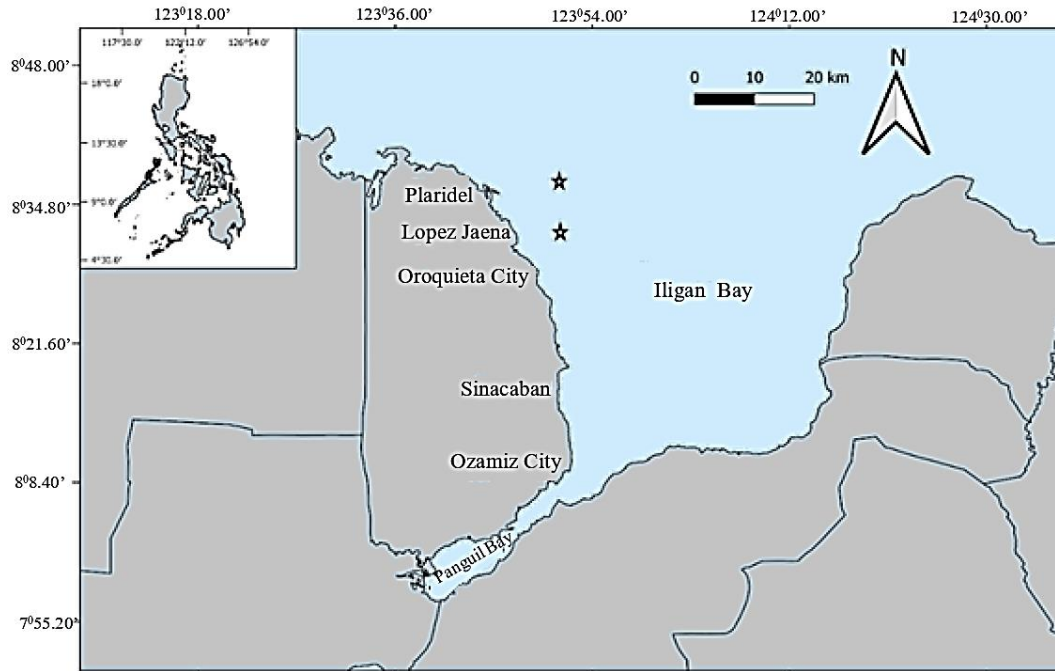


Figure 1. Map of the study area in Northern Mindanao.

duration of spawning season and fecundity, require knowledge of the stage of gonad development in individual fish (Lowerre-Barbieri et al., 2017; Tan and Arai, 2020).

The butterflyfishes (Chaetodontidae) are among the best-studied families of marine ornamental coral reef fishes (Motta, 1989) probably because of their appearance being like terrestrial butterflies. However, information on reproduction habits exists only for a few species (Ralston, 1981; Lobel, 1989, Tricas and Hiramoto, 1989, Yabuta and Kawashima, 1997). Most of these studies were conducted in Japan (Zekeria and Videler, 2000) and the Red Sea (Brokovich and Baranes, 2005). However, there is no information in the literature on the reproduction of butterflyfishes in the Philippine marine waters.

The vagabond butterflyfish, *Chaetodon vagabundus*, also known as the crisscross butterflyfish, is a ray-finned fish, found in reef areas, including coastal reef flats, lagoons, and more exposed, outer reef slopes in the Indian and Pacific Oceans (Bray, 2020). This beautiful fish is currently traded in the Philippines. They are easily maintained in tanks, but fishers who catch them in the wild have limited knowledge of their reproductive biology. Accordingly, overfishing has been reported in the

Philippines due to ineffective fisheries management (Dalzell and Ganaden, 1987; Pomeroy, 2012). Thus, urgent management interventions for this species are needed but would also require knowledge input on reproductive biology apart from other fundamental fisheries parameters. The present study aims to provide some biological information, including sex ratio, maturity stages and spawning period, gonadosomatic index (GSI), length at first sexual maturity ( $L_{50}$ ), and batch fecundity (BF) of *C. vagabundus*. These biological parameters are vital for evaluating its commercial potential in tropical waters, particularly in Iligan Bay, Southern Philippines.

### Materials and Methods

Thirty to forty-five samples of *C. vagabundus* with a class size of 9 to 14.1 cm (TL) were collected monthly from August 2021 to August 2022 from the coastal waters of Iligan Bay. The Bay is located in the southern part of Mindanao Sea, east of Panguil Bay (Fig. 1) and west of Macajalar Bay. It lies approximately between  $123^{\circ}43'15''$  east longitude, and  $8^{\circ}30'31''$  north latitude. It has an estimated coastline of 170 km with an area of about 2,390 km<sup>2</sup> (Quiñones et al., 2002). The Philippine Bureau of

Fisheries and Aquatic Resources (BFAR) identifies Iligan Bay as a main fishing ground for its rich fishery resources and living space for wildlife assemblages and serves as a vital food producer (Lacuna and Alviro, 2014). The samples were procured from the catch landings of the bottom-set longline, bottom-set gill net, and spear artisanal fisheries who went fishing near the coral reef sanctuaries in Capayas Island Marine Sanctuary (CIMS) and Mansabay Bajo Reef in Lopez Jaena, and Baobaon Fish and Marine Sanctuary in Plaridel, in the province of Misamis Occidental. These sites are endowed with rich marine life that has sustained the livelihood and subsistence of its populace for decades (de Guzman et al., 2009).

Each individual fish was measured in terms of total length (TL) to the nearest 0.1 cm, and weight of 1.0 g. Their gonad's weight was measured to the nearest 0.01 g using digital analytical balance JT1003B. The ratio between males and females was determined and tested using the Chi-square goodness of fit test (Zar, 1984). Sex was recognized based on gonad color and shape (Palla and Sotto, 2021). The sex ratio was calculated based on the total number of males and females documented and the ratio expressed as male: female. The spawning period was determined from the highest percentage of spawning capable individuals (hydrated tertiary vitellogenic oocytes) and then further confirmed using the results of GSI analysis. The gonadosomatic index (GSI) was computed using the formula (De Vlaming and Chapman, 1982) of  $GSI = 100 \times G/W$ , where, ( $G$ ) is the gonad weight and ( $W$ ) is the body weight.

For histological analysis, gonads were fixed in Bouin's solution for 24 h and stored in 90% ethanol for succeeding analysis. Histological processing was done by taking gonad sub-samples of 3-5 mm cut transversely from the mid-section, embedded in paraffin, and sectioned transversely into 5-7  $\mu$  thickness. Four to six replicates for each sectioned gonad were mounted on glass slides, stained, and counterstained with hematoxylin and eosin, respectively (Eagderi et al., 2013). The maturity of gonads was determined based on the criteria of Nikolsky (1963) and Amin et al. (2016) as follows:

Stage I: immature, II: maturing, III: nearly ripe, IV: ripe, V: spawning running, and VI: Spent. Maturity stages were identified based on microscopic and macroscopic examination of gonads for use in identifying the size at first maturity.

The size at first maturity ( $L_{50}$ ) was derived from the proportion of matured individuals (Stage III and IV) individuals into each 1.0 cm size class. It was calculated using linear regression and fitted to the logistic model (King, 2007) using the formula of  $P = 1/(1 + \exp[r(L - L_{50})])$ , where:  $r$  is the slope and  $L$  is the length of the fish. Additionally, the batch fecundity (BF) was assessed for 45 females using the gravimetric method (Hunter and Macewicz, 1985; Murua et al., 2003) which was done by counting the oocytes from a known weight in triplicate sub-samples. Only the tertiary vitellogenic and hydrated oocytes were considered. The expected number of oocytes was calculated using the formula of  $BF = \{[\sum i(o_i/w_i)]/n\} * W_o$ , where:  $o_i$  is the oocyte count of the sub-sample,  $w_i$  is the gonad weight of the sub-sample,  $n$  is the number of sub-samples, and  $W_o$  is the weight of the ovary. The relationships between BF and total length and weight were derived from the regression function in MS Excel 2016.

A nonparametric chi-square test was applied to determine the statistical significance of the sex ratio in size groups and in sampling months. Also, linear regression analysis using the Statistical Package for Social Sciences (SPSS, Chicago, Illinois, USA) version 16.0 was used to measure the size at first sexual maturity ( $L_{50}$ ) of individuals. A  $P$ -value less than 0.05 was considered statistically significant.

## Results

**Sex ratio:** A total of 413 (171 males, 242 females) vagabond butterflyfish *C. vagabundus* were examined. The size class ranges from 9 to 14.1 cm TL for both sexes (Table 1). Accordingly, the most frequently recorded size class was from 12-12.9 cm ( $P < 0.05$ ) dominated by females. The overall sex ratio based on size groups was statistically significant ( $P < 0.05$ ) with females outnumbering males (Table 1).

Over the 13 months of sampling, the value of Chi-

Table 1. Variations of sex ratio with length for *Chaetodon vagabundus* from Western Iligan Bay, Southern Philippines during August 2021-August 2022.

Length group (cm)	Fish No.	Male	Female	M:F Ratio	P- value	X <sup>2</sup>
9-9.9	34	16	18	1:1.13	0.73	0.12
10-10.9	81	38	43	1:1.13	0.58	0.31
11-11.9	94	44	50	1:1.14	0.54	0.38
12-12.9	171	59	112	1:1.90	0.00	16.42**
13-13.9	33	13	17	1:1.31	0.47	0.53
14-14.9	1	1	2	1:2.0	0.56	0.33
TOTAL	413	171	242	1:1.42	0.00	12.20**

Level of significance  $P < **0.01$

Table 2. Monthly sex ratio of *Chaetodon vagabundus* with corresponding Chi-square (X<sup>2</sup>) values from August 2021 to August 2022 from Western Iligan Bay, Southern Philippines.

Month/s	Fish No.	Male	Female	M:F Ratio	P value	X <sup>2</sup>
August 2021	31	8	23	1:2.87	0.00	7.25**
September	30	14	16	1:1.14	0.71	0.13
October	30	15	15	1:1	1.00	0
November	31	16	15	1:0.94	0.86	0.03
December	32	15	17	1:1.13	0.72	0.13
January	30	12	18	1:1.5	0.27	1.20
February	32	11	21	1:1.91	0.08	3.13
March	33	12	21	1:1.75	0.12	2.45
April	33	14	19	1:1.36	0.38	0.76
May	35	15	20	1:1.33	0.40	0.71
June	31	9	22	1:2.44	0.02	5.45*
July	35	18	17	1:0.9	0.87	0.03
August 2022	30	12	18	1:1.5	0.27	1.20
Total	413	171	242	1:1.42	0.00	12.21**

Level of significance  $P < 0.05^*$ ,  $0.01^{**}$

Square revealed that the overall sex ratio (MF = 1:1.42) was highly significant ( $P < 0.05$ ) suggesting the dominance of females (Table 2). The highest number of males (18) was observed in July, while the lowest number (8) was in August 2021. On the contrary, the highest percentage of females (23) occurred in August 2021, and the lowest value (15) in October and November. The number of males and females was similar during September, December, and July when the immature and maturing stages were recorded for both sexes.

**Monthly distribution of maturity stages and spawning period:** The results showed that the spawning period of females harmonized with the spawning period of males. It reached its peak during intermonsoon or the warm months (April and May) and at the beginning of the Southwest (SW) monsoon

(June and July). Furthermore, some spawners were still present for the rest of the SW monsoon (August to October), and remnants were observed in the early Northeast monsoon (NE) (November) (Fig. 2). Both sexes have recorded no spawning activity in the midst of NE monsoon (December to January). In addition, the female mature stage was not observed during the intermonsoon months (April and May) when the spawning and ripe stages were high. The higher percentages of mature individuals were in September (37.5%) and February (35%) and the highest percentage (40.9%) of the ripe stage was recorded in May. The spent stage occurred almost all year round except for the month of April and July where spawning was observed to be the highest. On the other hand, the mature stage of males was not observed during the beginning of SW monsoon (June) where the

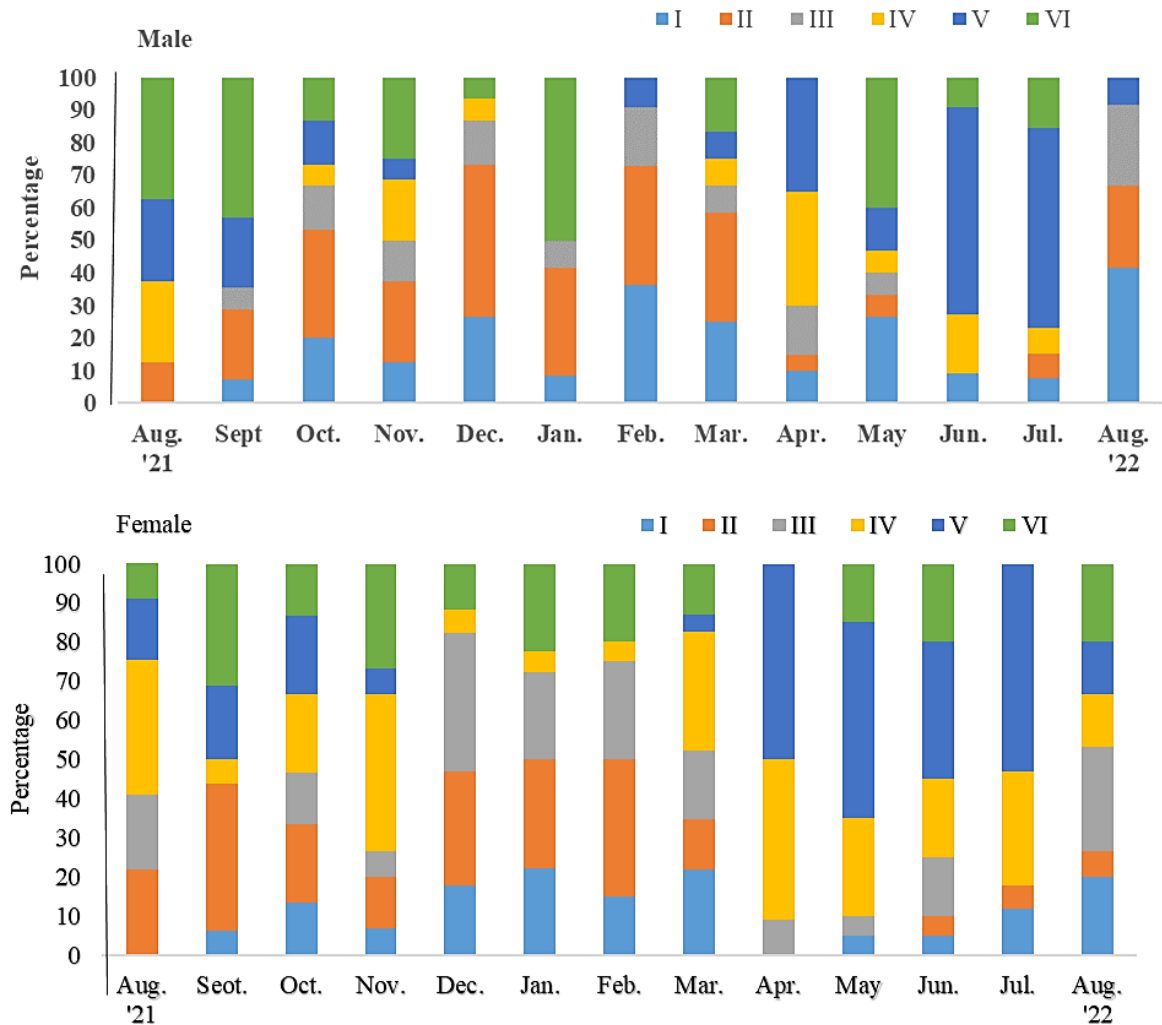


Figure 2. Monthly variation of maturity stages *Chaetodon vagabundus* following criteria of Nikolsky (1963) and Amin et al., 2016 (August 2021 to August 2022, pooled data).

spawning period was at its highest percentage. The maximum value (46.7%) was recorded in December. The individuals with nearly ripe ova were high (25%) in August 2022 but not observed during the months of August 2021, and June to July 2022. The highest percentage (35%) of the ripe ova stage was observed in April but not represented in September, January, February, and August 2022. There was no immature stage recorded in August 2021 for both sexes.

The frequency of maturity stages of males and females based on sectioned gonads is presented in Figures 3 and 4. The existence of hydrated oocytes and tertiary vitellogenic ovaries was an indication of an approaching and recent spawning, respectively. Also, the occurrence of post-ovulatory follicles (POF) is an indication of the recent spawning event. Likewise, in

males, spawning is obvious when spermatozoa are numerous as well as with the occurrence of remaining spermatozoa in the lumen of tubules in the sperm duct. **Gonadosomatic index:** Monthly variations in the gonadosomatic index (GSI) for both sexes of *C. vagabundus* are depicted in Figure 5. Relatively higher GSI values were recorded from April to July for males and females and the GSI reached its peak value in July and June (1.21 and 2.10) for males and females, respectively. These results are consistent with a high percentage of spawning-capable individuals in the gonadal maturity stages (Fig. 2). The lower values were observed from September to February and the minimum value was in January (0.32 and 0.50) for males and females, respectively.

**Length at 50% sexual maturity:** The  $L_{50}$  for

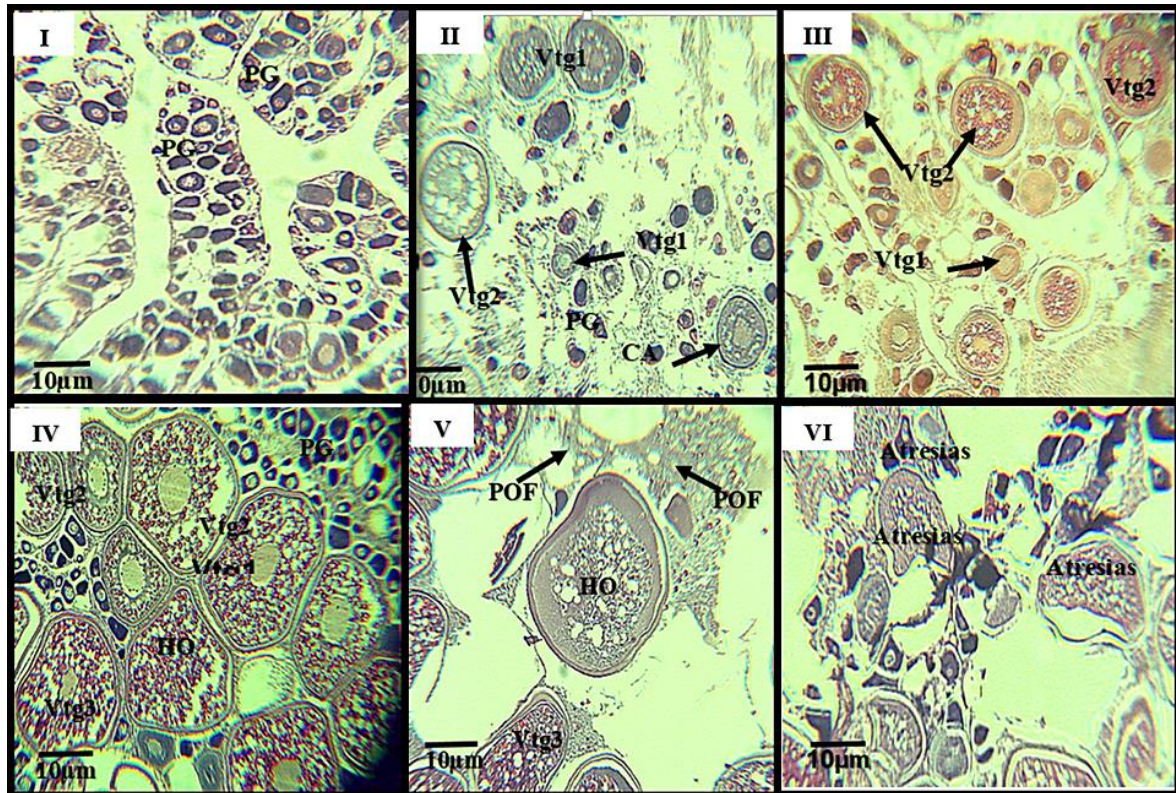


Figure 3. Sectioned gonads of *Chaetodon vagabundus*. Female I: immature, II: maturing, III: nearly ripe, IV: ripe, V: spawning/running, and VI: spent (Abbreviations: PG-primary growth, OW- ovarian wall, Vtg1-primary vitellogenetic, Vtg2-secondary vitellogenetic, Vtg3-tertiary vitellogenetic, CA- cortical alveolar oocyte, HO-hydrated oocyte, and POF-post-ovulatory follicle).

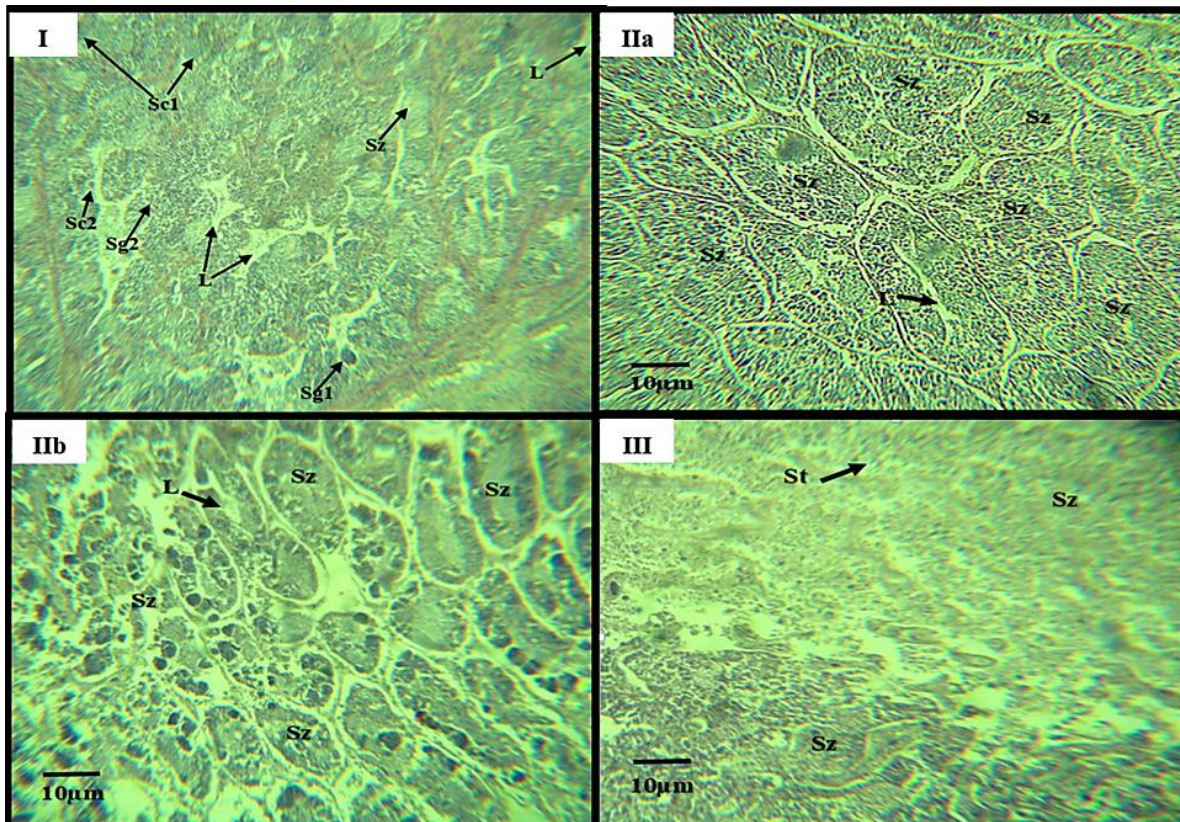


Figure 4. Sectioned gonads of *Chaetodon vagabundus*. Male I: maturing, II: a & b –spawning/running, and III: spent (Abbreviations: Sg1-primary spermatogonia, Sc1-primary spermatocyte, Sc2-secondary spermatocyte, St-spermatid, Sz-spermatozoa, and L-lumen).

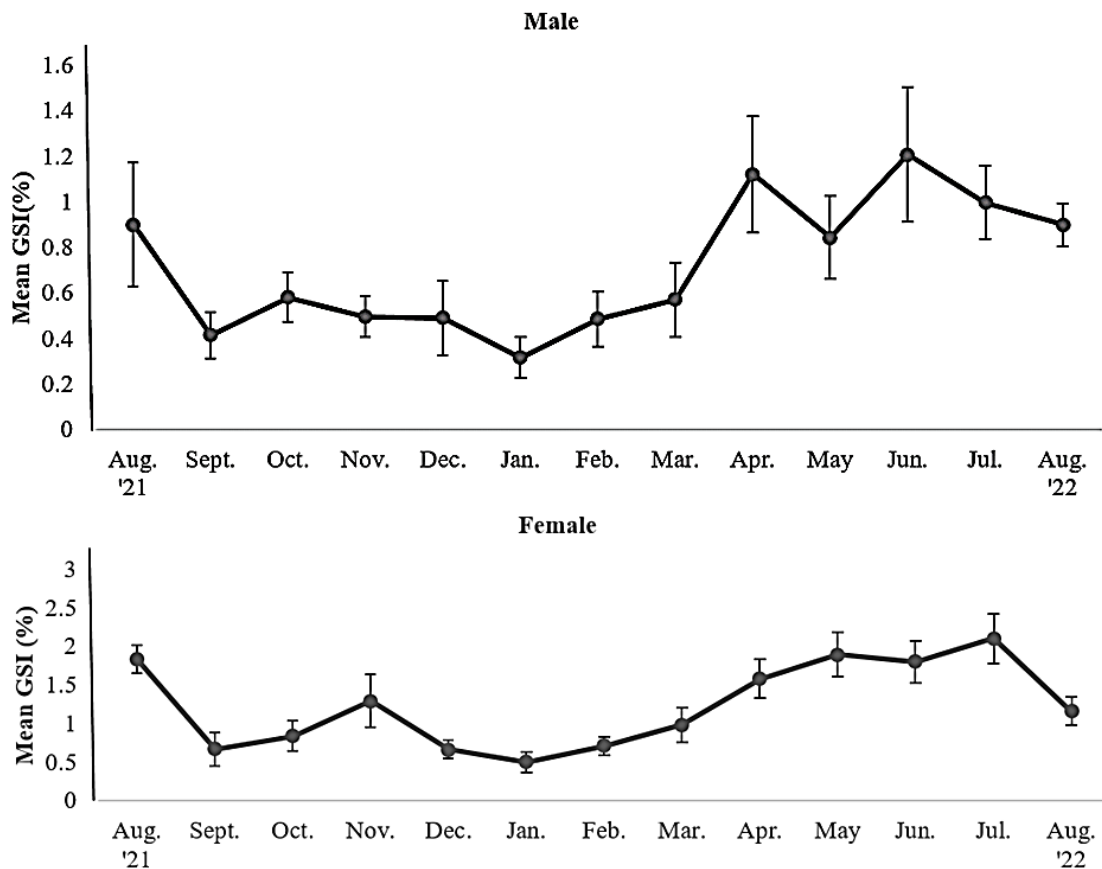


Figure 5. Gonado-somatic index of *Chaetodon vagabundus* (Error bars = standard error).

*C. vagabundus* differed slightly between sexes, with females slightly longer than males. The logistic curves showing the relationship between sexes and the proportion of 50% maturity was estimated and reached 10.58 cm in males whereas the female having 50% maturity was observed at 10.91 cm (Fig. 6). Also, the male vagabond butterflyfish exhibited a somewhat steady rate of maturity as it reaches longer length.

**Batch fecundity:** Batch fecundity (BF) varies from 21,987 to 60,720 oocytes (10.2-13.00 cm, TL) with a mean of  $41,932 \pm 1459$  oocytes (mean  $\pm$  SE) (12.11 cm, TL). The results showed positive correlations between BF against length and weight. Although there was individual variation, batch fecundity significantly increased ( $P$ -value = 0.00) with body length and weight respectively (Fig. 7).

## Discussions

Marine ornamental reef fish are a key component of the marine aquarium trade industry that supports thousands of fishers in developing countries and

provide aquarium hobbyists with over 1400 species of marine fishes (Bruckner, 2005; Monteiro-Neto et al., 2003; Dey, 2010). Studies on the reproductive biology of coral reef fish species are important since there are variabilities in recruitment between these species (Sale, 1990). The current study provides first-hand information on the reproductive biology of *C. vagabundus* in the Philippines.

In the present study, female *C. vagabundus* outnumbered males in almost all sampling months and size groups displaying the existence of female bias in the population in the sampling area. This is similar to the work of Vijay Anand and Pillai (2002), highlighting that in some common coral reef fish species the sex ratio indicates dominance of females. In this study, *C. vagabundus* is gonochoristic but displayed sexual monomorphism. This is true for the species that form heterosexual pairs like the pair-forming coral-reef fish *C. vagabundus* (Yabuta and Berumen, 2013; Nowicki et al., 2018; Shiratsuchi et al., 2020). However, the current study showed a

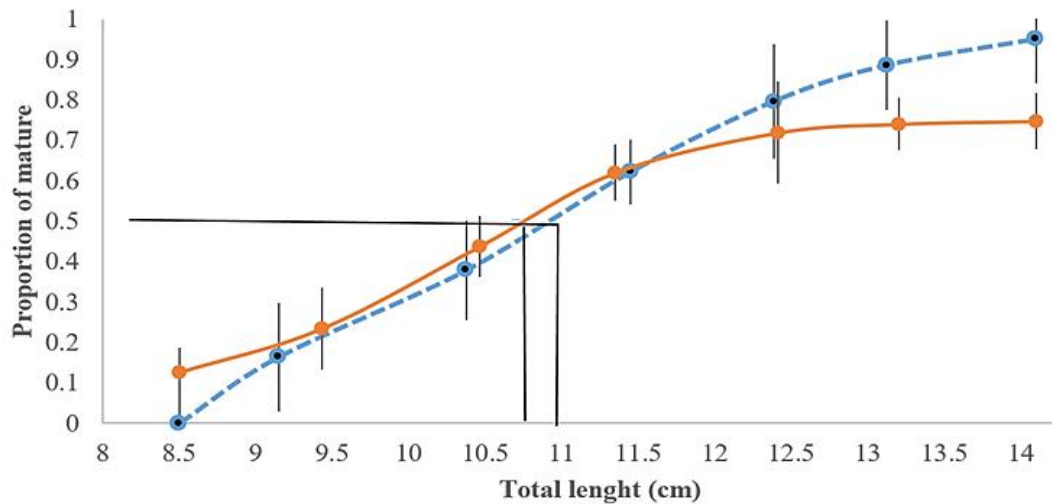


Figure 6. Length at 50% sexual maturity (L50) of *Chaetodon vagabundus*. Solid orange line with circular marker represents males, while blue broken line represents females.

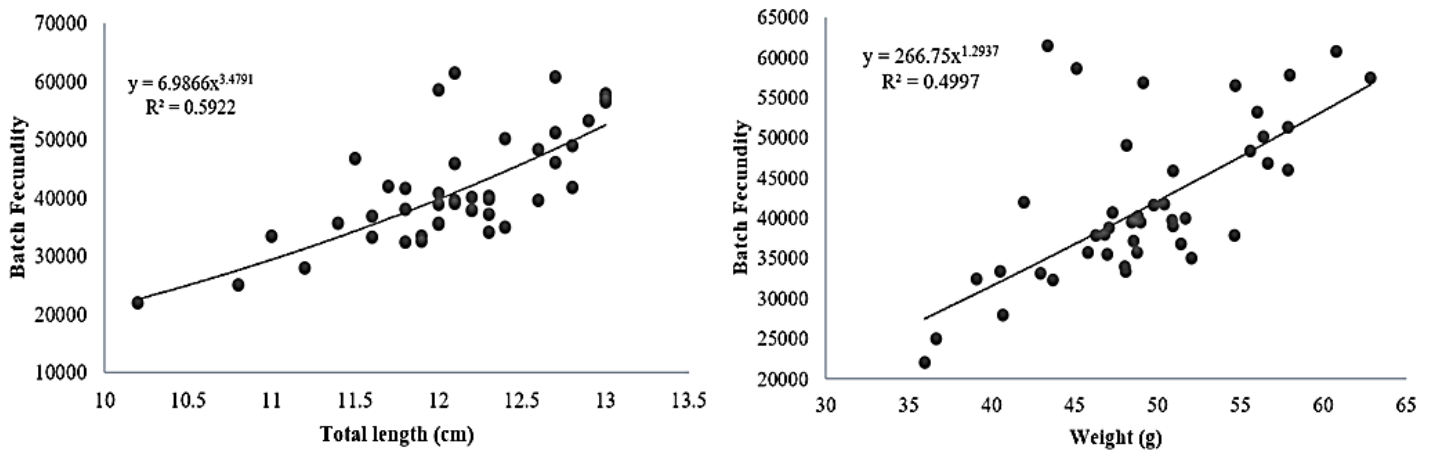


Figure 7. Relationship between total length and batch fecundity ( $P$ -value = 0.00\*\*), and relationship between weight and batch fecundity ( $P$ -value = 0.00\*\*).

significant predominant group size of 12 to 12.9 cm. This might be due to mortality rates, longevity, and differential growth characteristics between the sexes (Nanami et al., 2010; Palla et al., 2016; Palla and Sotto 2021). Grimes (1987) stressed that the size bias in sex is also site and species-dependent. Moreover, territorial size and behaviour vary with the density of preferred corals among Chaetodontids, as predicted by optimal foraging theory (Berumen and Pratchett 2006). In addition, Zekeria et al. (2006) and Ralston (1975) reported that there was no difference in the growth rates of males and females of *C. larvatus* and *C. miliaris*, and no size variation was observed between the two sexes. It was also recognized that this size bias was attributable to the effects of gear

selectivity (Grandcourt et al., 2006) and heavy fishing pressure (Kamukuru and Mgaya, 2004).

Seasonal spawning has been observed in many reef fishes over a wide range of latitudes (Robertson, 1991; Claydon et al., 2014; Asch and Erisman, 2018). Several studies on spawning in butterflyfish were performed in the western Atlantic (Colin, 1989), in Hawaii (Lobel, 1989), in the Red Sea (Fricke, 1986), and in Japan (Yabuta, 1997; Yabuta and Kawashima, 1997), but limited studies are found in the Philippine coastal waters. Vijay Anand and Pillai (2002) pointed out that the exact timing of spawning in reef fishes can only be obtained by tracking the occurrence of mature individuals. In the present study, the spawning period for both sexes of *C. vagabundus* reached its peak



during intermonsoon or the warm months (April and May) and at the beginning of Southwest (SW) monsoon (June and July). This result was also confirmed by the relatively higher GSI values recorded during these months. Females attained higher mean GSI than males because the size of the ovary was bigger than that of the testes at the same maturity stage. Both male and female GSI started to increase in April. Similarly, most of the gonads attained maturity when investigated histologically. The presence of tertiary vitellogenic (Vtg3), post-ovulatory follicles, and hydrated oocyte (HO) in the gonadal section of females and the occurrence of several spermatozoa in the lumen of tubules and spermatocytes in the testis of males proved that there is an imminent and current spawning (Palla and Sotto, 2016). Histologically, the presence of numerous tertiary vitellogenic, hydrated oocytes and post-ovulatory follicles in females and the occurrence of several spermatozoa in the lumen of tubules and spermatocytes in the testis confirmed that spawning is evident.

However, a short break in spawning was evident in the midst of the NE monsoon (December-February). This might be due to the variation in seasonal environmental conditions that seem to alter spawning patterns. In addition, the absence of immature stages in some of the months would indicate that spawning is not all year round. According to Bryan et al. (2011), the frequent appearance of immature and mature ovaries manifests the year-round spawning. Similar results were observed on the three species of chaetodontids from the Gulf of Mannar namely; *C. collare*, *C. melannotus*, and *C. octofasciatus* which seem to be affected by the two monsoons and mature fish were either absent during the period (Vijay Anand and Pillai, 2002). The characteristic avoidance of NE monsoon seasons by spawning individuals is perhaps to sustain the unusual weather conditions which would transport eggs and larvae to unfavorable destinations (Vijay Anand and Pillai, 2002). Unfavorable weather patterns may affect the seasonal timing of the spawning of a reef fish (Johannes, 1980). In the present study, during the midst of NE monsoon

months, the sampling area experienced torrential rains, storm surges, and violent wind patterns brought by typhoon Rai (local name Odette) which might be the reason for this short break in spawning.

The length at first sexual maturity  $L_{50}$  is a very important parameter in fisheries research. It helps to determine the minimum legal size and the optimum mesh size that may be required to preserve the appropriate spawning stock. It also ensures at least one spawning for the mature fish. In the present study, it seems that there is a slight variation in maturity between the two sexes. The male reached 50% first sexual maturity at a slightly shorter length than the female. These variations in the  $L_{50}$  ratio were suggested to be inherent in environmental and biological conditions across locations (Grimes, 1987). The estimation of length at first sexual maturity in this study showed some variation from the other studies. Vijay Anand and Pillai (2002) reported that male *C. collare* matured longer than females. However, the size at first maturity for male and female in some chaetodontids species such as *C. melannotus*, *C. octofasciatus*, and *C. trifasciatus* documented to mature synchronously (Ralston, 1976; Vijay Anand and Pillai, 2002; Zekeria et al., 2006). Under diverse environmental conditions, the onset of sexual maturity differs in different species even among congeners, so this enlightens the differences between all the previous results.

The information on the fecundity of fish is very important in fisheries, population dynamics, and food item availability (Amen et al., 2016). Based on the results, the fecundity estimates of *C. vagabundus* vary from 21,987 to 60,720 oocytes (10.2-13.00 cm, TL). Batch fecundity estimates vary in some chaetodontid species such as in *C. octofasciatus* (1060 to 2879 oocytes: Tan and Arai, 2020), *C. capistratus* (2,900 to 12,900: Colin, 1989), *C. miliaris* (17,800-171,000: Ralston, 1975), and in *C. uriga* (10,368 to 38,400: Vijay Anand, 1990). However, fecundities of 1984 to 21,975 (*C. trifasciatus*), 2,763 to 31,065 (*C. collare*), and 1,492 to 24,532 (*C. melannotus*) were comparable to the higher values of the range (Vijay Anand and Pillai, 2002). These differences in fecundity estimate

might be due to the mature fish selected, size differences in the gonads, and the species. A unit increase in weight will have varying effects on fecundity depending on the weight (Ralston, 1975). There are coral reef species even of the same genus that produce small numbers and those with great numbers (Tan and Arai, 2020), and such variation in fecundity is a consequence of diverse adaptations to environmental habitats (Murua et al., 2003). Even within a stock, fecundity is renowned for experiencing long-term changes that differ annually and has been shown to be proportional to fish size and condition (Murua et al., 2003). In this study, batch fecundity was correlated with total length and weight following an exponential relationship. This result demonstrates that the bigger the fish is, the larger the increase in fecundity and the greater the proportion of available resources that are devoted to reproduction. Ralston (1975) suggests that there is a trade-off between gamete production and growth. Similar results were found in most of the studies on coral reef fish species (Murua, 2003; Carbonara et al., 2015; Palla and Sotto, 2021).

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