

# Temporal variation of peanut worm (*Siphonosoma australe-australe*) reproduction in Toronipa Beach of Southeast Sulawesi, Indonesia

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**Abstract.** Bahtiar, Jiwani YE, Findra MN, Ishak E. 2024. Temporal variation of peanut worm (*Siphonosoma australe-australe*) reproduction in Toronipa Beach of Southeast Sulawesi, Indonesia. *Biodiversitas* 25: 2533-2540. The peanut worm *Siphonosoma australe-australe* in Toronipa Beach, Konawe District, Southeast Sulawesi, Indonesia is constantly facing ecological pressure. However, a limited understanding of its reproductive pattern hinders the development of a management plan. This study aimed to determine the sex ratio, GML (Gonad Maturity Level), GSI (Gonad Somatic Index), and the size of the peanut worm at early gonad maturity in Toronipa Beach, Southeast Sulawesi, Indonesia. Worms were collected using a hand spade, and the length and weight were measured using a ruler (accuracy of 0.5 cm) and an analytical balance (accuracy of 0.0001 g), respectively. Dissection was then conducted to observe eggs and sperm in the coelom. Additionally, water quality measurements were taken simultaneously with worm sampling. A total of 467 peanut worms were collected, and data were analyzed using standard formulas. The results showed that during the 11-month duration of this study, the sex ratio of peanut worms was imbalanced, as females were more than males. Aside from being dioecious, these worms were hermaphroditic and parthenogenous; the gonad maturity analysis showed various stages (I-IV) in peanut worms throughout the study. The highest level of gonadal maturity for male and female peanut worms was observed in August and September at Toronipa Beach. The first gonad maturity for male peanut worms occurred at 12.4-13.4 cm, while females matured at 13-13.5 cm. Throughout the year, gonad maturity and spawning were supported by environmental factors, such as temperature and organic matter that ranged from 30-32°C and 2.06-4.31 mg/L, respectively. Reducing harvesting activities during the peak spawning months is recommended to maintain the peanut worm population.

**Keywords:** Dioecious, gonad maturity, peanut worm, reproductive biology, sex ratio

**Abbreviations:** GML: Gonad Maturity Level, GSI: Gonad Somatic Index

## INTRODUCTION

The peanut worm, *Siphonosoma australe-australe* (Kaferstein 1865) is a marine invertebrate classified as one of 62 *Siphonosoma* species in the phylum Annelida (Saiz 2023), although 150 Sipuncula (peanut worm) species were previously recorded (Adrianov and Maiorova 2010; 2014). The geographic distribution of this species is extensive, covering all waters globally (Maiorova and Adrianov 2017) from the intertidal zone to depths of 900 m (Kawauchi and Giribet 2014). It is found in tropical, temperate, and cold environments (Murina 1984), with the highest species diversity in China (40 species) (Li et al. 1992). In Indonesia, *S. australe-australe* is reportedly distributed in eastern waters, including Toronipa Beach in Southeast Sulawesi (Anjarsari 2022; Bahtiar et al. 2024). Morphologically, the peanut worm comprises a long white cylindrical body divided into the main body (trunk) and proboscis (introvert) (Fakhrurrozi 2011) without segmentation (Hsueh and Tan 2016). It was found to occupy intertidal habitats overgrown by several seagrass species (Silaban 2019). Worms burrow below the substrate surface by digging and hiding in sandbars (gosong) or

under seagrass roots (Bahtiar et al. 2024). Although the habitat is water, it is rarely found near river mouths due to sensitivity to waters with low salinity (Açik 2020; Nguyen et al. 2007). This species plays an essential role in coastal aquatic ecosystems assisting in sediment bioturbation (Açik 2011; Li et al. 2015; 2019), adsorption of pollutants in sediments (Wu et al. 2020; Yan and Wang 2002), and being a food source for organisms at higher trophic levels (Kędra and Włodarska-Kowalczyk 2008; Shields and Kedra 2009; Leiwakabessy et al. 2017). *S. australe-australe* distributes organic matter from the surface to the bottom of the sediment, potentially affecting the distribution of decomposing microorganisms (Li et al. 2015; 2019). In addition, it can be used as an indicator in environmental monitoring (Cutler 1994; Shields and Kedra 2009).

Peanut worms have long been food in Eastern Indonesia, particularly in Southeast Sulawesi (Silaban and Nanlohy 2011; Umpain 2022; Rahayu et al. 2019). It is believed to have health benefits due to its rich nutritional content, including macronutrients (protein, fat, carbohydrates) (Leiwakabessy et al. 2017), micronutrients (calcium, phosphorus, iodine) (Silaban and Rieuwpassa

2019), magnesium, vitamins (vitamin B1, B6, B12, and vitamin E), essential fatty acids (arachidonic, linoleic, linolenic), as well as non-essential fatty acids (myristic, stearic, palmitic, and pentadecanoic acids) (Silahooy 2008; Silaban and Nanlohy 2011; Nurhikma et al. 2017). Peanut worm is also an effective fishing bait for various fish species, such as grouper and snapper (Fakhrurrozi 2011).

*S. australe-australe* is utilized in several regions of the world (Lijun et al. 2022), including Southeast Sulawesi, Indonesia (Bahtiar et al. 2024). Local fishermen exploit this species commercially as a desirable food product, particularly among the local ethnic Chinese community (Fishermen interview 2024, pers. com. (personal communication); Anjarsari 2022). Sustained fishing intensity can potentially disrupt the reproductive cycle of *S. australe-australe* in Toronipa Beach, raising concerns about the sustainability and regeneration of its population in nature (Bahtiar et al. 2022a; 2022b). However, there is limited understanding of *S. australe-australe* reproduction, and related studies have never been conducted, particularly in Indonesia. The knowledge of reproductive parameters such as sex ratio, gonad maturity, and size at first gonad maturity is essential for managing its resources in nature (Bahtiar et al. 2023), including planning the construction of conservation facilities for this organism (Ainnoun et al. 2019). This study aimed to determine the sex ratio, Gonad Maturity Level (GML), Gonad Somatic Index (GSI), and size at first gonad maturity of peanut worm *S. australe-australe* in Toronipa Beach, Southeast Sulawesi, Indonesia.

## MATERIALS AND METHODS

### Period and study area

This study was conducted for 11 months, from July 2023 to May 2024. Peanut worm samples were collected at low tide in the intertidal area of Toronipa Beach, Konawe District, Southeast Sulawesi, Indonesia. Reproductive parameters were observed at the Faculty of Fisheries and Marine Science Laboratory, Universitas Halu Oleo, Kendari, Indonesia. Samples of peanut worms were obtained by simple random sampling in all parts of the water at

Toronipa Beach (coordinates 3°53'40.69" S, and 122°39'20.06" E) (Figure 1).

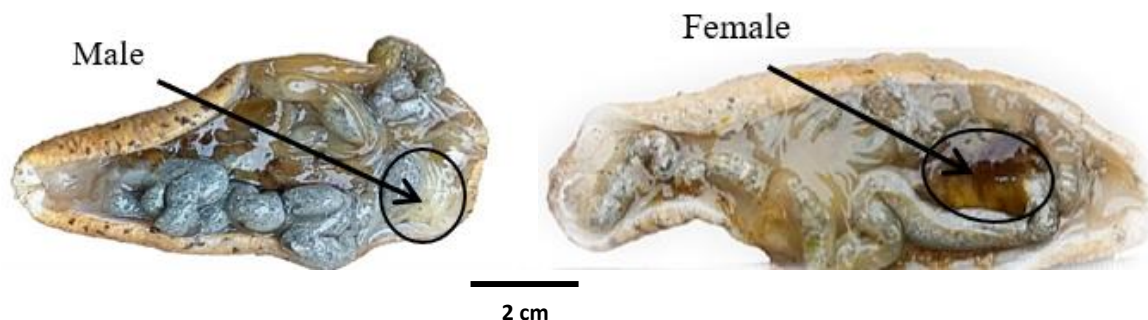
### Sample collection

Peanut worms were identified through feces collected around the hole and obtained using a shovel stuck into the substrate to a depth of 20 cm. Furthermore, peanut worms obtained were counted and measured using a ruler and analytical balance with an accuracy of 0.5 mm and 0.0001 g, respectively (Figure 2). In this study, 467 worms (192 males and 275 females) were obtained and dissected to determine the male and female gonads before weighing using analytical scales with an accuracy of 0.0001 g. The peanut worms were dissected using a cutter along the entire body, from the introvert to the anus. The gonads were located in the region adjacent to the anus (Figure 2).

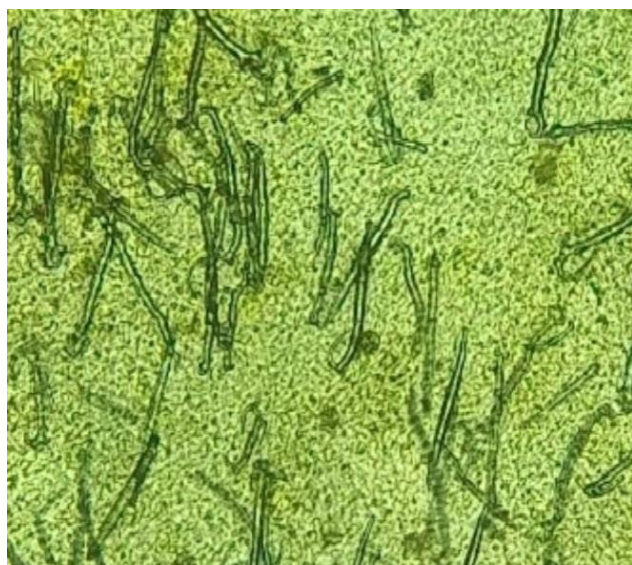
Peanut worm gonads are characterized by shape and color. Male gonads are shaped like fingers and are whitish-clear in color, while female gonads are shaped like grapes and brownish (Towle and Giese 1952). Water quality samples were taken concurrently with worm collection.



**Figure 1.** *Siphonosoma australe-australe* sampling location at Toronipa Beach, Konawe, Southeast Sulawesi, Indonesia



**Figure 2.** Male and female gonads of *Siphonosoma australe-australe*



**Figure 3.** Larvae found around the nephridium in male and female *Siphonosoma australe-australe*

The maturity level of peanut worm gonads was determined by observing the gonad characteristics microscopically under a 10x10 and 10x40 magnification microscope. According to histology preparations conducted by Lijun et al. (2022) and Ying et al. (2009), gonad maturity is divided into four stages (Table 1): early development (GML I), late development (GML II), early maturity (GML III), and late maturity (GML IV), analyzed using image analysis. The IKG was performed by taking the weight of gonads and the body weight of peanut

worms. The size of the first mature gonad was determined by calculating the length frequency of the peanut worm.

### Data analysis

#### Sex ratio

The sex ratio of peanut worms was analyzed by equation and Chi-square test using XLStat software (Mzighani 2005; Kandeel et al. 2010; Bahtiar et al. 2017) as follows:

$$X = \frac{M}{F}$$

Where: X: sex ratio; M: number of male peanut worms; F: number of female peanut worms.

#### GML

The level of maturity of peanut worm gonads was analyzed using descriptive semi-quantitative methods based on observation time (Bahtiar et al. 2023).

#### GSI

The gonad maturity index (gonadosomatic index) was calculated using the following formula (Tuwo and Conand 1992; Bahtiar et al. 2021), namely:

$$GSI = \frac{Wg}{Wt} \times 100 \%$$

Where: GSI: Gonad Somatic Index; Wg: gonad weight; Wt: total weight (body and gonads)

Furthermore, GSI between times was analyzed using the U-Mann Whitney test.

**Table 1.** Stages of *Siphonosoma australe-australe* gonad development based on observation of morphological and microscopic characteristics

Gonad Maturity Level (GML)	Male	Female
I	<ul style="list-style-type: none"> <li>- gonads are creamy white and rod-shaped</li> <li>- spermatogonia are clustered and circular with very high-density</li> </ul>	<ul style="list-style-type: none"> <li>- gonads are yellowish-brown in color and very dense in size</li> <li>- there are fine erythrocytes that develop into oocytes concave on two sides (discs) and clumpy vesiculose with an uneven surface</li> </ul>
II	<ul style="list-style-type: none"> <li>- gonads are clear in color and begin to develop like a thin rod shape, and the cytoplasm increases</li> <li>- morula-shaped spermatogonia gather and develop into a collection of spermatocytes and gather densely</li> </ul>	<ul style="list-style-type: none"> <li>- gonads are brown, begin to develop and thicken</li> <li>- the egg becomes a thick layer with a smooth surface, and the oocyte wrinkle structure disappears and begins to form a vitelline membrane</li> </ul>
III	<ul style="list-style-type: none"> <li>- gonads are milky white and rod-shaped and begin to thicken</li> <li>- sperm are released into the coelomic fluid and begin to develop into spermatocytes; aggregates of spermatocytes develop into spermatids and begin to be released in the coelomic fluid to develop and mature</li> </ul>	<ul style="list-style-type: none"> <li>- gonads are cream brown in color, large and dense</li> <li>- oocytes are developed and round, very dense with varying sizes, membrane pores are concave, and there are two vitelline layers surrounded by Golgi and no channels connecting cell membranes yet</li> </ul>
IV	<ul style="list-style-type: none"> <li>- gonads are milky white</li> <li>- the tail of spermatozoa is clearly visible and interlocked, and the sperm head is gathered; the sperm is around the nephridium</li> <li>- In some circumstances, complete morphology was found (Figure 3).</li> </ul>	<ul style="list-style-type: none"> <li>- gonads are brown-black.</li> <li>- the oocyte is enlarged and contained in the follicle; there is a duct that channels the egg, and extracellular material gradually fills the entire area of the cell nucleus</li> <li>- In some circumstances, larvae with complete morphology were found (Figure 3).</li> </ul>

### Size at first gonad maturity

Size at first gonad maturity was analyzed by non-linear regression using Sigma Plot 12 software (Arocha and Barios 2009) with the following formula:

$$Y = \frac{a}{1 + e^{\frac{-x + x_0}{b}}}$$

Where: Y: chance of peanut worm maturing (%); e: natural exponential number; a: intercept; b: slope; x, x<sub>0</sub>: i-th width measure (cm).

## RESULTS AND DISCUSSION

### Sex ratio

The number of female peanut worms was more than males at all observation times. Chi-square test analysis at  $\alpha=0.05$  on 42 worms collected monthly indicated that the sex ratio of male and female peanut worms in Toronipa Beach was unbalanced or the number of males was smaller than females ( $X^2_{hit} = 31.1 > X^2_{tab(df-1)} = 3.84$ ). In addition, parthenogenesis was observed every month and ranged from 3-9% (Figure 4).

### GML

The GML analysis of peanut worms showed that GML I-IV occurred each month. Meanwhile, GML III and V in males and females were highest in August and September (Figure 5).

### GSI

The results of the GSI analysis of male and female peanut worms during the study were highest in August and September and lowest in April; similarly, low GSI values were also observed in October. The Mann-Whitney test results at  $\alpha=0.05$  (P value = 0.13) showed that the August, September, and October pairs were not significantly different, while April, May, July, and October significantly differed from other months at  $\alpha=0.05$  (P value = 0.025) (Figure 6).

### Size at first gonadal maturity

The minimum length ( $L_{min}$ ) of male peanut worms with mature gonads was 5.5 cm, while the  $L_{min}$  of female peanut worms occurred at 4.9 cm. The first gonadal maturity ( $L_m$ ) in male and female peanut worms occurred at 11.7 cm and 12.8 cm, respectively. The longest peanut worm ( $L_{max}$  = asymptote) found was 35.1 cm (Figure 7).

### Water quality

The water temperature at Toronipa Beach remained relatively unchanged throughout the study, ranging from  $30 \pm 1.50$  to  $32 \pm 1.15^\circ\text{C}$ , while organic matter ranged from  $2.06 \pm 0.32$  to  $4.31 \pm 0.51$  mg/L (Figure 8).

## Discussion

### Sex ratio

Peanut worms are gonochoric organisms, meaning that the male and female sexes have undergone differentiation and generally reside in separate individuals. Additionally, the sexual dimorphism of these worms is undetectable, so sexual identification is determined by observing male and female gametocytes in the coelom (Ferrero-Vicente et al. 2014). Both sexes can be distinguished by the color of the gonads (coelom). The gonads arise from the epithelial cells of the coelom as thin cords located along the base of the ventral retractor muscle. Gamete maturation occurs in the fluid of the coelom, which is filtered through a ciliated funnel into the nephridia excretory sac and released into the environment through the nephridiopores (Adrianov and Maiorova 2010).

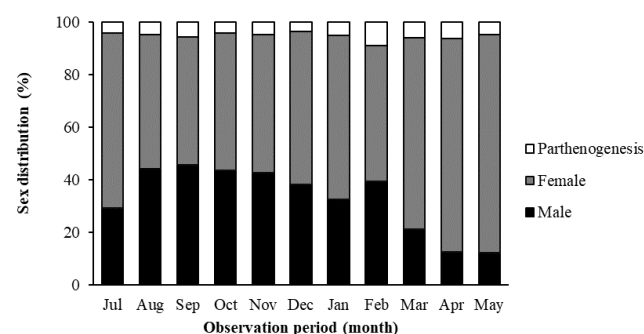


Figure 4. Sex ratio of *Siphonosoma australe-australe*

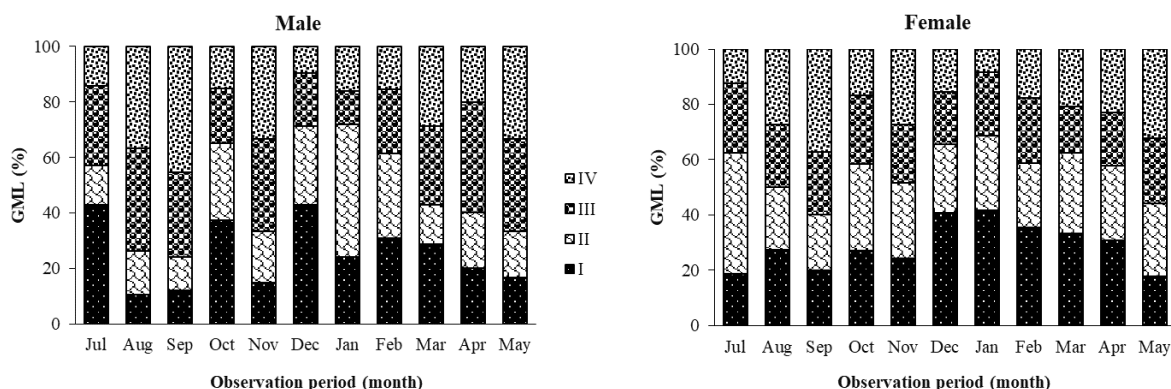
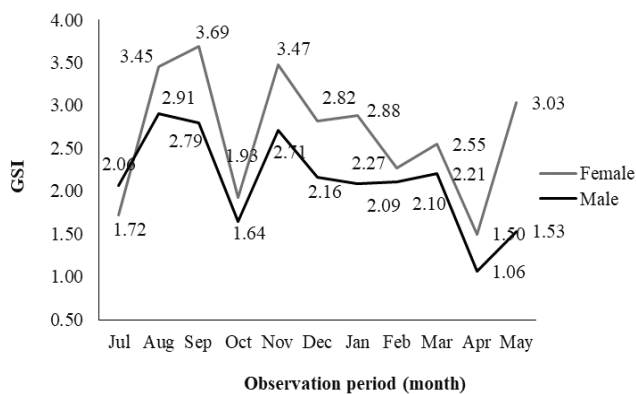
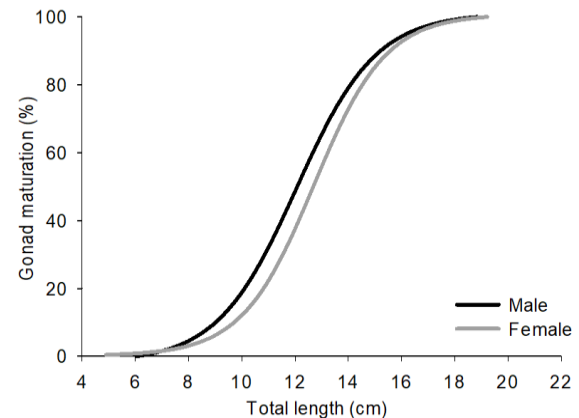


Figure 5. GML of male and female *Siphonosoma australe-australe*

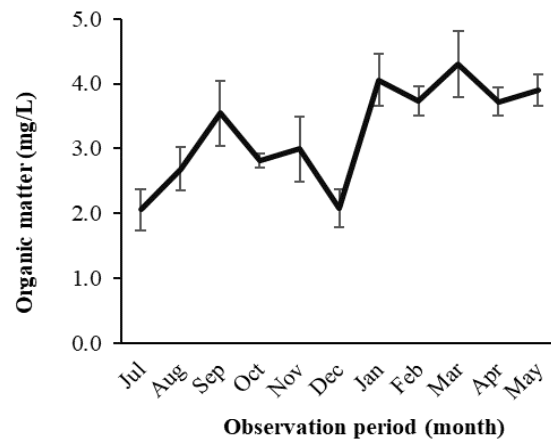
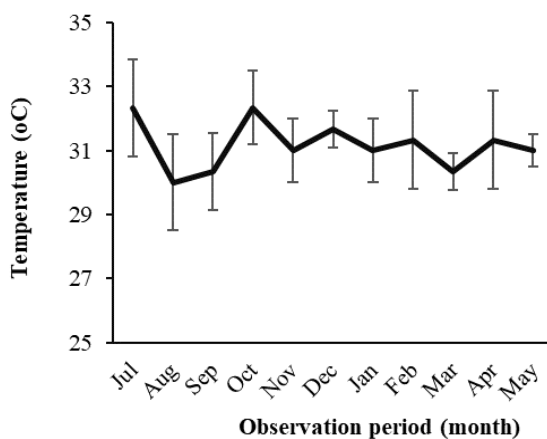




**Figure 6.** GSI of male and female *Siphonosoma australe-australe*



**Figure 7.** Size of *Siphonosoma australe-australe* at first gonadal maturity



**Figure 8.** Water quality at Toronipa Beach, Southeast Sulawesi, during the study

Although male peanut worm gonads are translucent white and females are tanned (Maiorova and Adrianov 2005), this species can exhibit alternative reproductive strategies, including 1) gonads in males and females found in one individual at the same time, 2) parthenogenesis characterized by the presence of larvae in the nephridial ducts that are ready to be expelled through the nephridiopores (Adrianov and Maiorova 2010). Parthenogenesis also occurs in *Themiste lageniformis* by developing unfertilized eggs to become adults (Pilger 1987). In addition, peanut worms have a hermaphroditic form of reproduction, as observed in *Nefasoma menitum* (Paul 1910; Åkesson 1958). Certain varieties of peanut worms also reproduce asexually. Examples include *S. robustus*, which produces new individuals through transverse division and lateral buds (Rajulu and Krishnan 1969; Rajulu 1975), and *Aspidosiphon brocki*, which performs transverse division into two unequal parts (Rice 1970). Peanut worms generally have a dioecious reproductive form and external fertilization (Ferrero-Vicente et al. 2014).

The sex ratio of peanut worms in Toronipa Beach was imbalanced, with females being more dominant than male

peanut worms. In various species, different sex ratios were observed. *S. nudus* in Mod Tanoy Beach, Thailand, had a balanced sex ratio (Ainnoun et al. 2019), and *Aspidosiphon muelleri* in the temperate waters of the Mediterranean had a sex ratio close to 1:1 (54% females and 46% males) (Ferrero-Vicente et al. 2014). *Phascolosoma esculenta* from the Southeast Coast of Zhejiang, China, also had a 1:1 sex ratio throughout the year (Ying et al. 2009). However, the unbalanced sex ratio in the peanut worm *S. australe-australe* population at Toronipa Beach is yet to be determined.

#### GML and GSI

GML and GSI are essential parameters in the reproductive studies of marine organisms, providing information on the stages of gonadal maturity and the species' reproductive cycle. The GML of peanut worms during the 11-month study showed that the gonadal maturity pattern varied from GML I-IV every month. This suggests that *S. australe-australe* is a peanut worm that has partial spawning and spawning patterns that last throughout the year. However, male and female worms have peak spawning seasons, which are observed by the highest GML

(III and IV) and GSI values found in August and September at Toronipa Beach. *S. australe-australe* exhibited a distinct spawning pattern in Wenchang, Hainan; there was no reproductive activity throughout the year. Its reproductive activity occurred from April to August, with peak reproduction in May and July. This was followed by a decline in September and complete inactivity in October (Lijun et al. 2022). The reproductive activity of *S. australe-australe* in Toronipa Beach is similar to several species of peanut worms, including *S. cumanse* which spawns throughout the year with peak spawning in September in Japan (Catalan and Yamamoto 1994). Similarly, *Sipuncula nudus* in Mod Tanoy Beach adjacent to the tropical waters of the Andaman Sea, was found to spawn throughout the year. Some species of peanut worm in China's temperate waters showed strong spawning patterns during spring and summer (Ainnoun et al. 2019), such as *S. australe* in Wenchang Hainan with a peak spawning period associated with rising water temperatures (Lijun et al. 2022). Other bean species, such as *S. nudus* spawn in the Gulf of Tonkin from April to September when the water temperature is between 27 and 34°C (Guobao and Bing 2002; Wang et al. 2005). Some species spawn during summer (July to August) in temperate regions, showing peak reproductive activity for two to three months, depending on latitude (Cutler 1994; Ying et al. 2009). These include *Siphonosoma cumanense*, *Scolops phascolosoma* (Catalan and Yamamoto 1994), *P. esculenta* (Ying et al. 2009), and *Aspidosiphon muelleri* Diesing, 1851 (Ferrero-Vicente et al. 2014). *Golfingia margaritacea* from Ushuaia, Argentina, spawns from late February to April when annual temperatures are highest (Amor 1993). Based on this, an increase in water temperature stimulates the triggering factor for sprinter reproduction (gametocyte development and spawning events) in temperate regions. The spawning patterns of peanut worms in Toronipa Beach waters that occur throughout the year are influenced by food availability and stable water temperatures. Toronipa Beach waters are tropical, with optimum temperatures supporting gonad maturity and spawning throughout the year. In addition, seagrass litter provides abundant natural food and nutrients for peanut worms (Ainnoun et al. 2019). *S. australe-australe* lives symbiotically with seagrass, and the food composition analysis showed a high percentage of seagrass and various sand textures in the stomach (Bahtiar 2024, unpublished data).

*Sipuncula* spawning periods also vary in some locations due to differences in habitat conditions, showing a pattern of extending the spawning period in more stable situations (Rice 1967). Populations can also exhibit different reproductive strategies and spawning times in regions with less pronounced seasons, such as high latitude or equatorial regions (Ainnoun et al. 2019).

#### Size at first gonadal maturity

In aquatic organisms such as the peanut worm, the average length at first gonadal maturity ( $L_m$ ) is taken at 50% gonadal maturity (Pauly 1984, 2021a, 2021b; Pauly and Chu 2021; Meyer and Schill 2020). *S. australe-australe* at Toronipa Beach reached early gonadal maturity

at 124-135 mm. Under natural (unstressed) conditions, the size of a peanut worm at first maturity or adult size varies and is relatively proportional to its maximum size. Some adult sizes of peanut worm reported include *S. nudus* at 150 mm in Trang Thailand (Ainnoun et al. 2019), *T. lageniformis* at 26 mm in Florida Coast, USA, and Hawaii, USA (Williams 1972), and *S. cumanense* at 200 mm in Puerto Rico Coast (Rice 1988). Furthermore, the proportion of early gonadal maturity size to maximum length in peanut worms has management implications. *S. australe-australe* shows maturity when the worm reaches one-fourth ( $1/4$ ) of its maximum size (considered ideal for reproduction). In natural conditions (without fishing pressure), there is a tendency for small organisms to have a higher proportion of  $L_m/L_\infty$  (reproductive load) than large ones (Froese and Pauly 2019). This is observed in peanut worms with smaller sizes and a higher proportion of  $L_m / L_\infty$ , resulting in less time to reach mature gonads and maximum length. However, too high proportions at 0.57-0.61 and 0.80-0.83 indicate over-exploitation of the stock caused by decreasing maximum length (Ragonese and Jereb 2017). From this viewpoint, the fishing pressure of peanut worms in Toronipa Beach was still relatively low (Bahtiar et al. 2024).

In conclusion, *S. australe australe* is a species of peanut worm with high reproductive potential. This is supported by several facts: 1) this species can reproduce through fertilization from dioecious or hermaphroditic individuals, 2) mature gonads were found in this species every month, although peak gonad maturity was observed in August and September, and 3) peanut worms in Toronipa Beach are at an ideal size for reproduction due to low fishing pressure, allowing them to reach reproductive maturity.

#### ACKNOWLEDGEMENTS

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