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# Research Article
# Morphology of the Female Gonads of the Long-legged Spiny Lobster

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## Abstract

**Background and Objective:** Spiny lobster is an economically valuable fishery commodity. For the development of its management, a further study on various biology aspects is necessary. The objective of this research was to study some aspects of long-legged spiny lobster reproduction particularly the gonad development based on morphology and microscopic examination of histological preparations of ovaries and the relationship between body and gonad weight with the aim of contributing to the basic knowledge of reproductive system and introducing this species into aquaculture. **Materials and Methods:** Lobsters (*Panulirus longipes*) were caught in South East Sulawesi, Indonesia coastal water. The lobsters were sampled to check for gonad development and maturity stages. The other lobsters were continued to be maintained under culture. Samples were dissected and characterized and the gonads were used for morphology and histological studies. Gonad developmental stages were classified according to the difference and developmental progress of gonad was observed. Photomicrographs of all stages were made and observed. Diameter sizes of mostly ova were measured in 3 separated parts: Anterior, middle and posterior. This was to determine whether this specie is total or partial spawner. **Results:** Gonadosomatic index (GSI) were also counted to determine the maturity of lobster sample. Maturity level 1 had an average GSI of 0.2%, while lobsters with a maturity level 2 and 3 had GSI of 2.97 and 4.19%, respectively. Gonad pigmentation was not specific between the maturity levels of gonads 2 and 3. **Conclusion:** Results showed that the ovaries contained gonads in different stages of development in the same ovary, indicating long-legged spiny lobster tend to spawn in different groups (partial spawners).

Key words: Gonad, histology, lobster, morphology, reproductive organ, natural spawning, ovarian modifications

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Panulirus longipes* (Long-legged Spiny Lobster), a member of Palinuridae family, is a commercially important species and is harvested for food. This species has a very widespread distribution, as it is found from East Africa to Thailand, Taiwan, the Philippines and Indonesia[1]. It has a high value but has not been widely cultivated owing to restricted data about their biology, breeding and captive survival.

Many lobsters do not easily breed in captivity because they are not able to exhibit their natural spawning conduct in culture, which is a limiting factor in their culture and mass production. This species accounts for 66% of the total lobster catch in the Philippines[2]. High production capacity of Palinuridae family members in the wild has led many fishery researchers to cultivate them. A lobster study in Japan started in the late 1890s and evolved in the 1900s to research hatchery rearing of Palinurid lobsters for more than a century[3]. In New Zealand, successful *Jasus edwardsii* larval rearing was completed in the 1990's. In India, it is documented that since 1977, a group of juvenile *P. homarus* has been raised to sexual maturity and grown in a laboratory[4]. In 2006, Western Australia generated the first *P. ornatus* post larvae grown in the world[4]. *P. ornatus* is regarded to be the best aquaculture candidate because it has the shortest stage of ocean larval development and the fastest post larval growth rate[5].

Even though the crustaceans have been studied extensively, some of the female reproductive system in *P. longipes* species has not been described so far. Spawning behavior of this lobster has not yet been fully described, especially the exact time of spawning in the waters around Sulawesi, Indonesia.

Maintaining lobsters in captivity enables the development of gonad stages in the female reproductive system. The process of maturation can be studied by periodic checks of morphology-based maturity measures, gonad materials, as well as by conducting a gonad histology[6]. This is the study of tissue and cell microscopic structure. This allows interpretation of the structure and function of tissues or cell components[7]. De Lima and Gesteira[8], studied the morphology of the spiny lobster male gonads. External body metric was used and developed for characterizing body size in morphological and sexual functionality in slipper and spiny Hawaiian lobsters[6]. The low incidence of inactive ovaries and the existence of ovaries at any moment of the year show that this species grows in Calatagan, Batangas in the Philippines throughout the year[2]. Ovarian appearance and/or weight are frequently used in reproductive research to stage the ovarian development cycle of female decapods, however, ovarian appearance or gonadosomatic index (GSI) offers restricted data about real ovarian modifications[9]. Histological examination, based on oocyte growth, could investigate seasonal changes in ovarian growth of *P. inflatus*[10]. However, fewer ovarian maturation phases have been defined for other species of palinurid lobsters. Therefore, research of this species is respected to contribute to the morphological and histological aspects of the reproductive system of crustaceans, particularly ovaries of the lobster *P. longipes* which potentially as aquatic organisms culture.

## MATERIALS AND METHODS

**Study site:** The present study was conducted in coastal waters of South East Sulawesi, Indonesia in 2017-2018.

**Sampling:** A collection of 12 lobsters of variable sizes was done. Total length (TL), Total body weight (TBW) and Gonad weight (GW) of the samples were examined and recorded. Lobsters of higher body sizes were chosen for dissection in order to compare gonads of various sizes and body weight. The reproductive system was dissected by a mid-dorsal incision through the cephalothorax.

**Gonad developmental analysis:** The gonads were removed for determination of developmental stage and for histological study. Gonad weight and developmental stages were determined. Gonad developmental stages were classified according to description[11]. The gonad was divided into 3 parts to determine the consistency of oocyte growth along the ovary length. For histological assessment, a total of 12 ovaries were processed into slides. The fragments representing distinct ovarian regions were excised and immersed in formaldehyde of 10%. The samples were gradually dehydrated in an ethanol series, cleared in xylene and embedded in paraffin. Sections (at 5-6 µm thick using microtome blades) were stained with hematoxylin and eosin (H and E) in order to identify the structure. The slides were displayed at 100-400x magnification under a compound microscope. Photomicrographs were taken with Olympus P.A. 35 Camera fitted to the microscope. Throughout the study period, water quality parameters were evaluated monthly.

## RESULTS

The total length of the collected animals ranged from 15.3-22.55 cm. The weight ranged from 86.69-327.0 g (Table 1).

Table 1: Gonad weight, GSI and maturity stage of some *P. longipes*

| TL (cm) | TBW (g) | GW (g) | GSI | Maturity stages |
|---|---|---|---|---|
| 15.4 | 86.69 | 0.39 | 0.45 | 2 |
| 16.8 | 103.46 | 0.29 | 0.28 | 1 |
| 16.0 | 117.22 | 3.04 | 2.59 | 3 |
| 15.3 | 110.68 | 0.12 | 0.11 | 1 |
| 15.5 | 116.45 | 0.42 | 0.36 | 1 |
| 22.55 | 327.00 | 18.94 | 5.79 | 3 |
| 20.5 | 270.00 | 7.62 | 2.82 | 2 |
| 18.0 | 155.8 | 0.21 | 0.13 | 1 |
| 15.6 | 122.1 | 0.16 | 0.13 | 1 |
| 17.8 | 189.4 | 1.07 | 5.63 | 2 |
| 17.8 | 181.5 | 0.41 | 0.23 | 1 |
| 16.0 | 121.8 | 0.21 | 0.17 | 1 |

TL: Total length, TBW: Total body weight, GW: Gonad weight, GSI: Gonadosomatic index

Table 2: Classification of gonad maturity stages in lobster

| Stages | Characteristic |
|---|---|
| 1 (Immature) | Slight development of ovaries confined to the body cavity with slender anterior and posterior lobes. Ovaries are transparent and difficult to differentiate from surrounding muscles |
| 2 (Prematuration) | The volume and extension of ovaries has increased and the color is pink or light yellow. The organ appears distended and rigid to the touch, reflecting germ cell multiplication and suggesting vitellogenesis in the early stages |
| 3 (Mature) | The gonad is now completely developed in the cavity of the body and will fill all available space. It becomes more sinuous and the second abdominal segment may be extended. The color is now orange or reddish and nodes or cysts give the surface a nubbly look. The ovary produces a large number of mature cells when stressed, even if only lightly |
| 4 (Spawning or resorption) | The ovaries may resorb oocytes and other cells after ovulation. The gonad is internally flaccid with pigmented areas and empty spaces as observed on transparency. At this level, the ovary displays coloring and size much like in the immature or premature stage, although the gonad of a spawned lobster is never entirely restored to the condition of original immaturity |

Source: Silva and da cruz-Landim[11]

**Macroscopic description of ovaries:** Lobster ovaries are paired organs located mainly in the cephalothorax, dorsally or dorsolaterally to the digestive tract and often surrounded by hepatopancreatic lobes (Fig. 1). Each ovary is coupled to a thin, tubular oviduct linked to a gonopore opening onto the sternite of the appendage of the 6th thoracic segment, suiting to the base of the third pair of pereiopods. Gonads of female varied slightly in shape, location, size and coloration as they matured (Fig. 2).

Table 1 shows gonad weight, GSI and gonad stages of development of some female samples. The size of the lobster showing well-developed gonads and the gonads were among developmental stages 1, 2 and 3 (Stages have been categorized on t he basis of the features of Table 2). The ovaries at both ends are elongated, round, unlobed, tapering and coated with a soft membrane. The gonads end with a duct forming the urogenital duct. Immature ovaries are whitish and transparent, while mature ones, with noticeable yellowish eggs, appear orange-reddish. Specifically, for the mature stage, there was no difference in the level of maturity in gonads among different pigmentation.

**Microscopic description of ovaries:** Figure 2-3 shows the gonads histological analysis. Fig. 2 shows that there is a range of development stages in an individual gonad and it is indicated that this species is partial (heterochronal) spawners. Figure 3a shows a cross section of an ovary in stage 1 of gonad development. It comprises of lots of secondary oocytes or early vitellogenic oocytes (VO). Eggs were visible in batches surrounded by follicular wall or egg membrane (EM). Table 1 shows gonad weight, GSI and maturity stage of samples. Then, data were supported by microscopic descriptions. Stage 1 ovaries with gonad weighed of 0.12-0.42 g (immature female lobster, n = 7 samples) contained only early vitellogenic oocytes (Fig. 3a). Late vitellogenesis stages of vitellogenic oocytes were dominated stage 2 ovaries weighing 0.39-7.62 g (mature resting, n = 3 lobster samples) but cortical alveolar oocytes were also present (Fig. 3b). Stage 3 ovaries contained large numbers of ovarian cavity (OC) with fully grown but unripe oocytes present with gonad weighed of 3.04-18.94 g (Fig. 3c, n = 2 lobsters). The ovarian cavity (OC) comprised of degenerated the egg membrane or follicular wall. The lipid droplet (LP) egg yolk granules (YG) are visible.

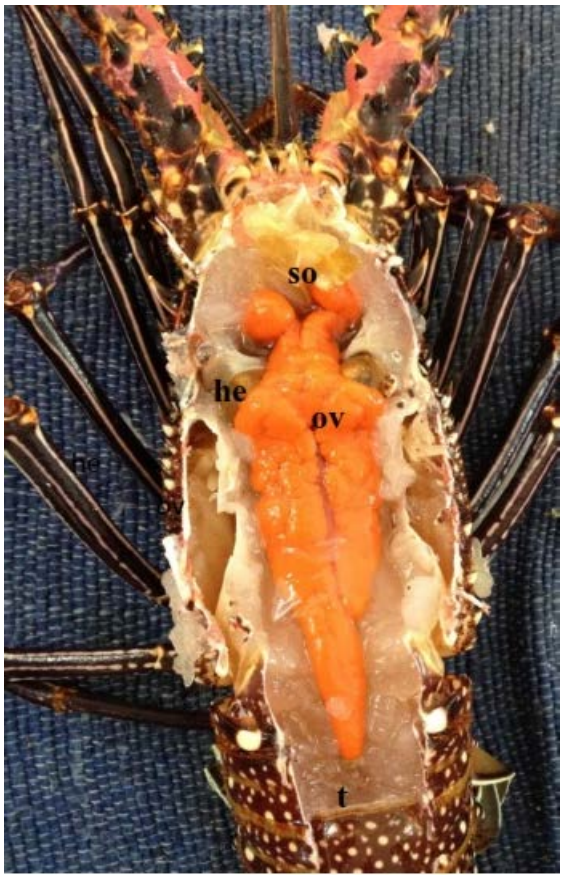


Fig. 1: Dorsolateral view of cephalothorax of *P. longipes* showing some external distinguishing features
so: Stomach, he: Hepatopancreas, ov: Ovary, t: Posterior digestive tract

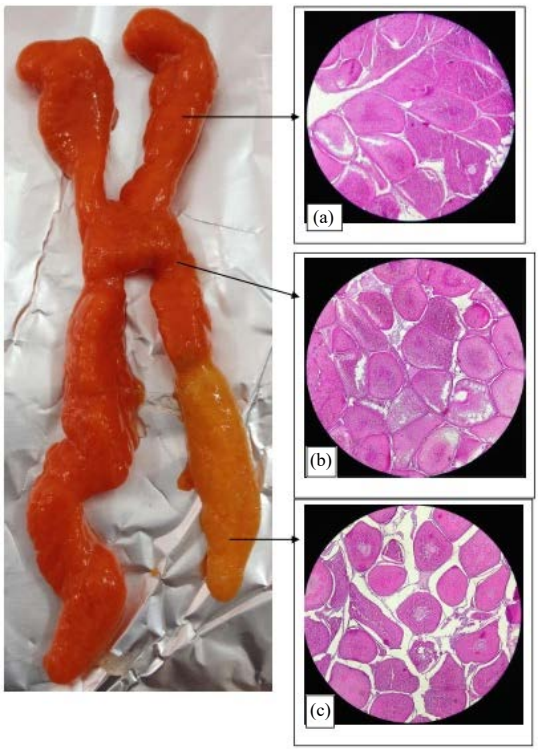


Fig. 2: Orange-yellow colored, mature ovary in *P. longipes* lobes developed. Cross section of gonad in 3 parts: (a) anterior, (b) mid and (c) posterior
Histological magnification 400x

## DISCUSSION

The development of lobsters is very dependent on their cultivation techniques. During this time, various cultivation techniques have been applied to obtain maximum results. However, certain obstacles are always found that hinder hatchery activities. Limited information about the process of reproduction and life cycle is one of the main obstacles in the cultivation of this species. The reproductive process is an important part of the study of species biology[12]. Determination of the sex of some fish species can only be distinguished by examining the gonads if the species does not show clear sexual dimorphism. Differentiating the sexes and determining the maturity level of lobster gonads is rather difficult and requires certain techniques. More reliable microscopic techniques are needed.

In the present study, lobster reproduction was typically based on the macroscopic and microscopic examination. We compared both of them in order to complete information. As with crabs and other species of lobster, the spiny lobster ovary is basically H-shaped with the lobes at the posterior extending over the abdomen. The male gonads of *P. laevicauda* was H-shaped also especially was described by De Lima and Gesteira[8]. Practically, macroscopic staging is sometimes used because it is conventionally reliable for analysis of sex ratios and gonad growth patterns broadly. In addition, it has velocity benefit and is most economically important, making it ideal for routine surveillance of the fish stocks exploited[12]. However, some errors with the use of the macroscopic staging system are unavoidable because, at times, there is difficulty distinguishing between oocyte maturation stages, in particular between late cortical alveoli and early yolk globular development stage[13]. There may also be mistakes in distinguishing between immature and mature females, especially when mature ovaries have lost evidence of spawning before reproductive activity begins.

Histology is the standard method to determine sex and reproductive condition. Gonadal histology can provide insights into the effects of multiple environmental stressors on reproductive health in combination with hormone and vitellogenin measurements, morphological and fecundity research[14]. Through this technique the

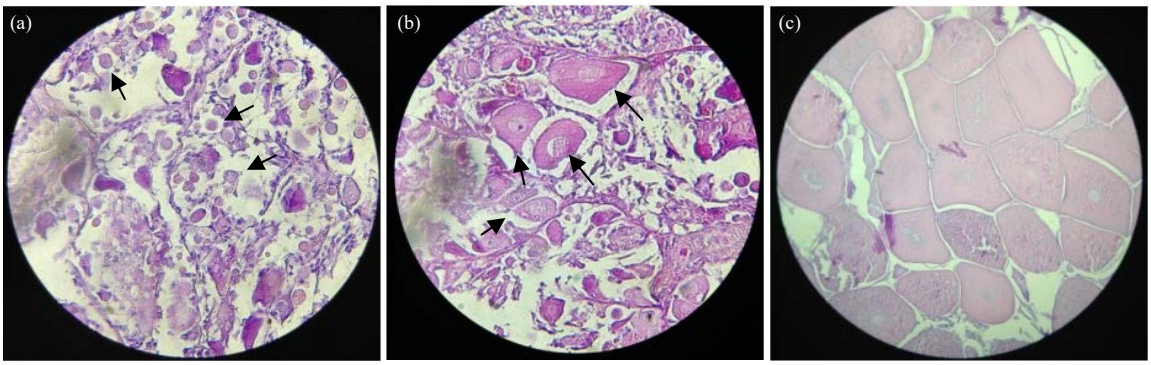


Fig. 3(a-c): Cross section of *P. longipes* in (a) Stage 1 gonad development, arrows showing batches of early vitellogenic Oocytes, (b) Stage 2 of gonad development, arrows showing late vitellogenesis stages of vitellogenic Oocytes and (c) Stage 3 of gonad development showing ovarian cavity with fully grown but unripe Oocytes
Mgx400, Mgx100

information obtained from the gonad is more complete and more detailed.

Microscopic staging systems, however are expensive, time consuming and sometimes too simplistic and do not permit the collection of all the data available in histological slides[13]. Based on the comparison of oocyte diameter measured from the lobe's anterior, middle and posterior areas (Fig. 2) and between left and right lobes showing that ovarian growth throughout the gonad was almost distinct, it could be concluded that the spiny lobster as ovarian species in which oocytes grow and spawn as a few groups (asynchronous development). It is in line with the research result[10], that every month mature female were present, suggesting a consistent pattern of reproduction. The size of the egg diameter varies according to the level of development[12]. This results showed that egg development is consistent throughout the ovary, depending on the degree of maturity of the ovary. The frequency distribution of oocyte diameter shows stock reserves at all levels of maturity but oocytes of larger size are only found in ovaries with higher maturity levels.

GSI value is the ratio value between gonad weight and body weight. GSI values provide helpful data to effectively differentiate mature oocytes and lead to oocyte maturation process research. GSI values also provide estimates of ovarian development stages[10].

This study discovered that the lobsters with a maturity level I have a GSI of 0.2%, while lobsters with a maturity level 2 and 3, have a GSI of 2.97 and 4.19%, respectively (Table 1). Whereas in *P. japonicus* females with immature ovaries had gonadosomatic indexes[15] were <1%. Like others researches, GSI values of *P. longipes* also have been effective in distinguishing between inactive, developing and mature stages.

In this research, it was found that mature gonad pigmentation was either orange or reddish. The color of the gonads seems to differ very little between the species of spiny lobster examined, although in the literature there is no other macroscopic description of the gonadal stage of *P. echinatus*[11]. In some mature fish, changes in body color and gonads occur due to atresia and tissue breakdown during and at the end of the reproductive season[16].

Precise data on aspects of lobster reproduction can be used as a reference and additional information in the management of marine water resources so that lobster resources can be used appropriately and as efficiently as possible for the benefit of the community without neglecting the conservation of these lobsters and the further cultivation of this lobster in the ocean waters.

## CONCLUSION

Morphologically, the female gonads of *P. longipes* as H-shaped, specifically for the mature stage, there was no distinction in the level of maturity of gonads with distinct pigmentation. Their ovaries histologically contained gonads in different stages of development, therefore they tend to be spawned in different groups (partial spawner).

## SIGNIFICANT STATEMENT

This study found the gonad development of female lobster using macroscopic and microscopic methods that can be beneficial for reproductive health and selective

breeding. This study will help the researchers to uncover broodstock selection used in aquaculture for lobster breeding that many researchers were not able to explore. Thus an efficient instrument for fishery biologists and aquaculturist on reproductive system on lobster may be arrived at.

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