

The gonad maturity development and spawning season of orange-spotted grouper (*Epinephelus coioides*) at Kwandang Bay, Gorontalo Province, Indonesia

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Abstract. Biological information of *E. coioides* in Kwandang Bay has been studied to formulate responsible management policies. A quantitative descriptive analysis based on a change in the percentage of fish maturity stage and gonadosomatic index of *E. coioides* population every month were performed. A total of 141 fish was collected for analysis of gonad development, which of 40 individuals among them was randomly prepared for histology analysis. Generally *E. coioides* caught in the Kwandang Bay was ripe and spawn stage category. The average size at sexes change of *E. coioides* was 79 cm in length and 6,500 g in weight. The peak spawning season of *E. coioides* is in the period of May to July which coincides with the dry season. Management strategies proposed is the temporary stopping the capture of the fish in the peak of spawning season of the species.

Key Words: gonado somatic index, biology, management, ripe, responsible management.

Introduction. Grouper fish are an important economically valuable reef fish that are continuously exploited in Kwandang Bay by various types of fishing gear, therefore, caused a high pressure to the resources which can interfere with the sustainability of the species (Achmad et al 2017; Achmad & Nurdin 2017). Decreasing population of grouper every year was detected which increasing catching effort units, which did not considered the decreasing tendency of fish population. The decline production of grouper population occurred when the catching effort unit was increased in the 2009, 2010, 2012, and 2015 respectively (Achmad et al 2018), so that sustainable management is needed.

One aspect that needs to be known in the sustainable management of fisheries is the reproduction biology (Mariskha & Abdulgani 2012; Al-Marzouqi et al 2015). Knowledge of the reproductive biology of fish is needed in the species' breeding, both for purposes of cultivation and conservation as well as supporting the development of fisheries management (Begossi & Silvano 2008; Williams et al 2009; Condini et al 2013). Study of the reproduction biology of *Epinephelus coioides* can be considered in the gonadal maturity and spawning season aspects.

The development of gonad maturity can be determined by morphological observations (Williams et al 2009; Alamsyah et al 2013) and gonadal histology examination (Mujimin 2008; Andamari & Suwirya 2010; Slamet et al 2010; Özen & Balci 2012). Histologically, the development of gonads and eggs can be identified more clearly, whereas the morphological maturity is known through a change of shapes, size, weight, and the color of the gonad. Index of maturity or gonadosomatic index is recognized as a way to explain the gonadal activity and readiness of grouper to spawn (Kadir et al 2016).

Up to date, there is no scientific study that examines the reproductive biology of *E. coioides* as the basis of fisheries management policies in the Kwandang bay. Accordingly, the purpose of this research was to study the reproductive biology of *E. coioides* in the Kwandang bay covering the maturity of the gonads, the initial size to

spawn, and the peak of the spawning season, which as the basis for the formulation of a responsible management policy of fisheries.

Material and Method

Description of the study sites. The research was performed in the Kwandang Bay, North Gorontalo District, Indonesia. The sample was taken by the two largest gatherers, namely CV. Ruslan and CV. Nurluthfi. The selection of the sampling sites was based on the consideration that the place: (1) Has the largest *E. coioides* in the bay Kwandang; (2) easy to acces, near the fish landing site; and (3) An abundance of *E. coioides* in the area.

Sampling and measurement. Samples were harvested twice a month for a year. The obtained samples were preserved in boxes containing iced water and brought to laboratory of Fish Quarantine, Quality Control and Safety Laboratory, Gorontalo for further observation. The total sample of *E. coioides* used to analyze the fish maturity stage and gonadosomatic index consisted of 141 individuals while for the histology analysis served 40 individuals. The body weight was appreciated using a digital scale with 0.1 g accuracy and total length with a precision of 0.1 mm.

Observable parameters were: total weight, weight of the gonad, gonad maturity, and gonad maturity index. Gonad level maturity was determined based on histological observation. The process of histological preparation comprised: (1) Fixation in a solution of Bouin Holland for 24 hours at room temperature; (2) Dehydration in level of alcohol 50%, 70%, 80%, 95%, and 100%; (3) Inclusions and blocking in the paraffin using the 'L' incline block; (4) Cutting with a microtome equipment and the thickness used is 3-5 µm which each piece placed on a transparent slide; (5) Rehydration in alcohol of 100%, 95%, 80%, 70%, and 50%; (6) Coloration in dye Hemotoxylin and Eosin; (7) Dehydration like the second stage; (8) Drying and storage, preparation at every glass slide drained are then stored in dry flask.

Macroscopically, the maturity level of the gonads was determined according to the classification of Tan & Tan (1974) based on the value of the gonad maturity index (Table 1) following the formula:

$$GI = \frac{gw}{L^3} \times 10^7$$

Which, GI = Gonad index, Gw= the weight of gonad (g) and L = the length of the body.

Table 1
Relationship between the gonad index and maturity stages (Tan & Tan 1974)

| Gonad index | | Maturity stage | |
|----------------|-----------------------|----------------|-----|
| Lower than 1 | Immature gonad | | I |
| 1.0-5.0 | Maturing gonad | | II |
| 5.0-10.0 | Maturing gonad | | III |
| 10.0-20.0 | Mature gonad | | IV |
| Higher than 20 | Advanced mature gonad | | V |

Gonad maturity index is calculated based on the ratio between gonad weight in grams and the total body weight of fish in grams multiplied with 100% (King 1995; Andrade et al 2003). The development of gonads and spawning season was analyzed quantitatively descriptive based on changes in the percentage of the amount of fish in certain maturity stage and gonad maturity index of *E. coioides* every month.

Results

Stage of gonad maturity. Stage of gonad maturity of *E. coioides* for each month is presented in Figure 1. The level of gonad maturity is composed of 19.15% young phase (maturity stage I), of 28.37% initial maturity phase (maturity stage II), of 13.48%

mature phase (maturity stage III), of 4.26% spawn phase (maturity stage IV), and a 34.75% post-spawning phase (maturity stage V). The percentage distribution of the gonad maturity stage monthly showed that the young and mature phases were found year-around with the highest percentage in March (55.55%) and December (75%) respectively. The mature phase was found to October (14.28%), the spawning phase was only found for four months with the highest percentage in October (14.28%), while the post-spawning phase peaked in June (53.33%). The peak of mature gonads was found in May, June, and July.

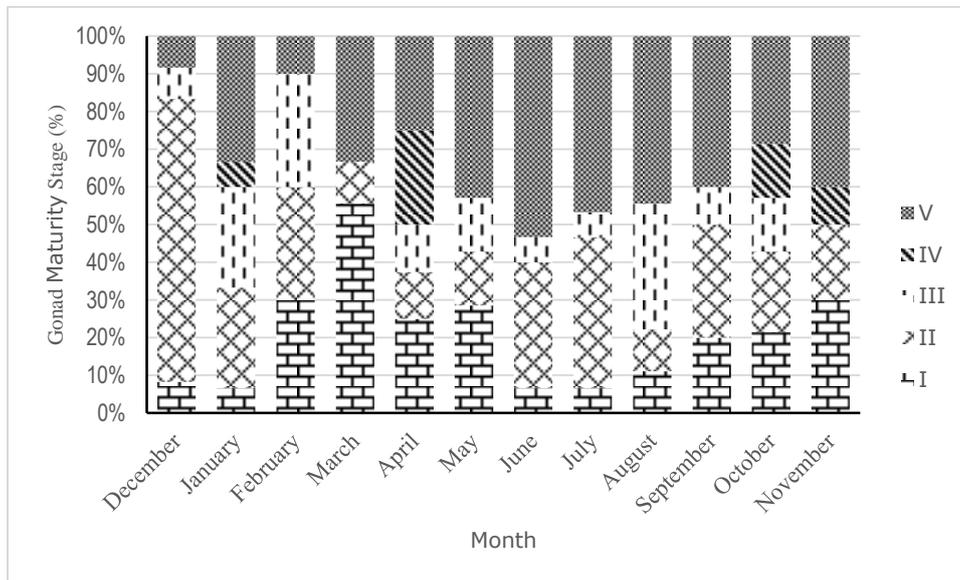


Figure 1. Monthly percentage distribution of the gonad maturity stage of *Epinephelus coioides* in Kwandang Bay, Gorontalo Province.

Most of the caught *E. coioides* in Kwandang Bay were mature and at spawning stage reaching 80.85% and only a small proportion was young fish with 19.15% (Figure 2).

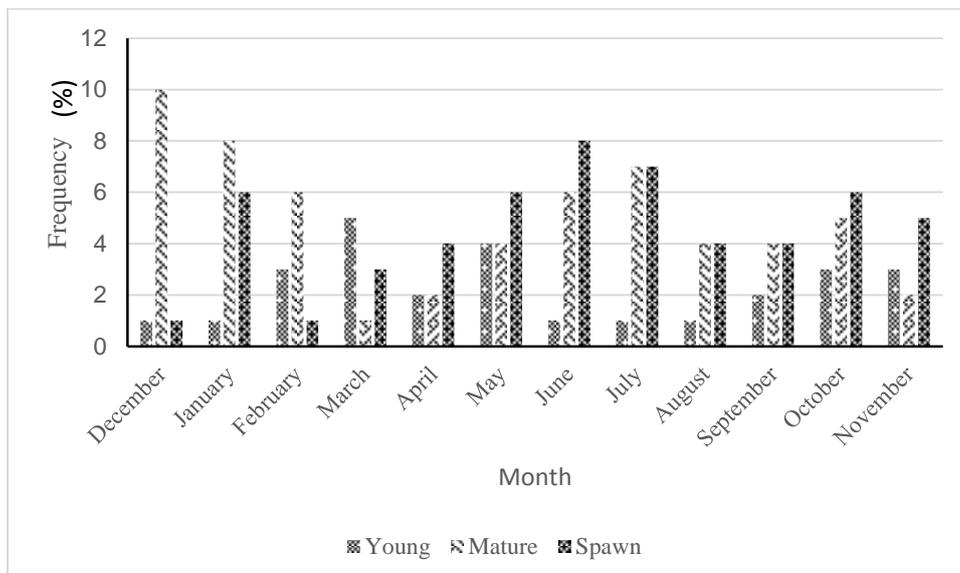


Figure 2. Monthly frequency of young, mature and spawn *Epinephelus coioides* in Kwandang Bay, Gorontalo Province.

Histological observations of gonadal development showed that *E. coioides* at the mature gonadal stage has 42 cm in length and 1,800 g in weight (Figure 3). The transition phase

(changing sex from female to male) in *E. coioides* occurs at a length of 79 ± 4.76 cm and a weight of $6,500 \pm 0.87$ g.

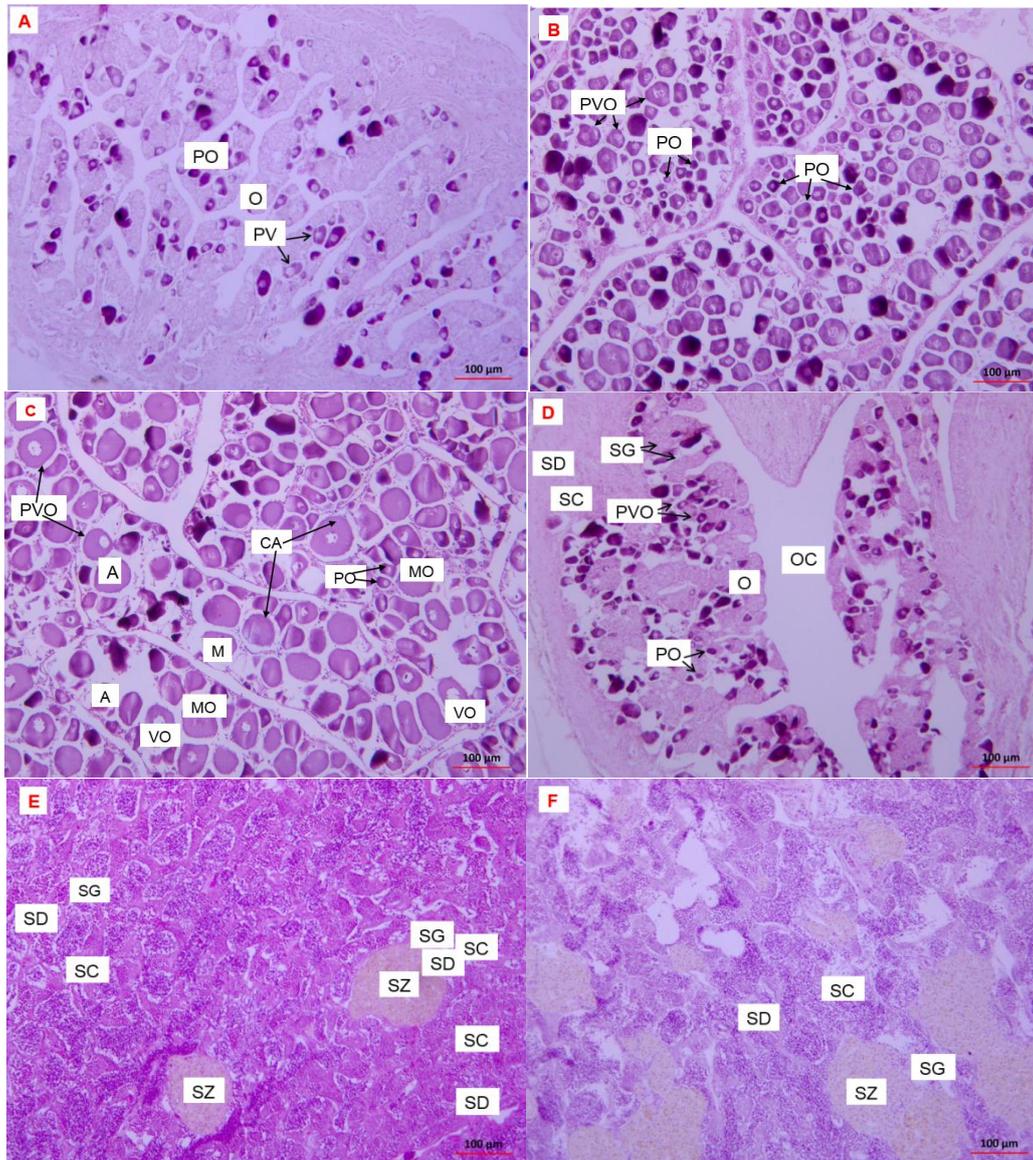


Figure 3. Microscopic structure of *Epinephelus coioides* gonads in Kwandang Bay, Gorontalo Province. (a) Immature females, length of 33 cm, weight 386 g; (b) Mature developing female, length of 42 cm, weight of 1,800 g; (c) Mature active female, length of 60 cm, weight of 2,900 g; (d) Transitional length of 79 cm, weight of 6500 g; (e) Immature male, length of 80 cm, weight of 7000 g; and (f) Mature developing male, length of 83 cm, weight of 12,000 g. PO = primary oocyte; PVO = previtellogenic oocyte; AO = atresia oocyte; MO = mature oocyte; CA = cortical alveoli oocyte; O = oogonium; OC = ovarian cavity; SC = spermatocyte; SG = spermatogonium; SD = spermatid; SZ = spermatozoa.

Gonad somatic index (GSI). The monthly average of the gonadosomatic index (GSI) of *E. coioides* is presented in Figure 4. The changing value of GSI was relatively slow during the study conducted. Based on the development of GSI, the spawning peak may occur for three months, namely May, June, and July. The GSI was increased started in May ($0.1476 \pm 0.1219\%$) and achieve a peak in Juni ($0.1788 \pm 0.1709\%$) before declining in July ($0.1646 \pm 0.0828\%$). In June, when the GSI average has the highest value, many of caught grouper had a maximum gonad weight of 63 g and many of them just finished spawning.

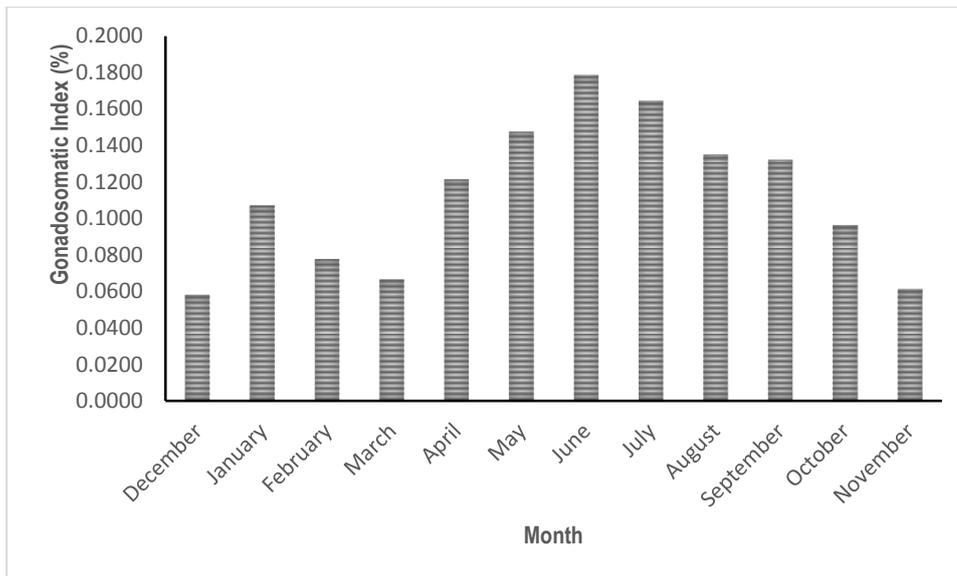


Figure 4. The monthly development of gonadosomatic index (GSI) of *Epinephelus coioides* in Kwandang Bay, Gorontalo Province.

Discussion. Most of caught *E. coioides* in Kwandang Bay were mature and at spawning stage (Figure 2) which was confirmed by the histological analysis related to the size of fish (Figure 3), indicating that the grouper fishing coincides with the spawning season. The development of gonad histology that determine sexes and maturity stage of the fish can be considered and help for determining the size of the fish in the mature stage which is ready to spawn. For hatchery businesses, it is necessary to pay attention to the sex ratio (Andamari 2005). The weight of the male brood stock supply was above of 7,000 g and of females' above 3,000 g.

In the early phase of ovarian development of *E. coioides*, gonad contains oogonia, primary oocyte, and previtellogenic oocytes which are located on the periphery of the oocyte lamella. In the sexual transition phase, the gonad lamellae consist mainly of developing primary oocyte and undeveloped spermatogenic tissue spreads in the area consisting of oogonia and early spermatocyte cysts. Ovary tissue and testes are not physically separated because the spermatogenic tissue is along the lamella and mixed with the oocytes. Gonads do not show morphological evidence of previous sexual maturation as male or female (Erisman et al 2008).

The gonads in the transition phase contain follicle of atresia and/or muscle bundle, both of these characteristics indicate a previously female function (Sadovy & Shapiro 1987) mixed with the proliferation of testicular tissue including spermatocytes, spermatids, and spermatozoa (Brulé et al 2016). Early sex changes are characterized by primary oocyte degeneration and simultaneous proliferation of spermatogonia in the germinal epithelium of the lumen of the ovary. When degeneration progresses, the oocyte is absorbed and proliferation of spermatogenic cells increases (Bhandari et al 2003). In the final phase of the transition, only a small amount of previtellogenic oocyte degeneration is left over in the lamellae and there is a tendency to increase spermatogenic cells, which comprise nearly 75% of ovarian lamellae. At the end of this phase, almost all absorbed atresia oocytes and gonads are transformed into testes containing spermatogenic cells in various stages of development from spermatogonia to spermatids (Bhandari et al 2003).

The peak of GSI occurred from May to July (Figure 4) which coincides with the dry season, which indicates that the spawning of the grouper occurs in the dry season and influenced by temperature (Cushion et al 2008; Teruya et al 2008; McIlwain et al 2016). Peak spawning of *E. coioides* in North Oman waters also occurred for three months, namely March, April and May (McIlwain et al 2016). The increase in sea surface temperature coincides with the increase of GSI of grouper fish (Ohta & Ebisawa 2015) which triggers fish spawning. When the temperature was low, there was not male

grouper found in the spawning ground (Gaspare & Bryceson 2013; Nanami et al 2017) so that spawning is not expected to occur.

Generally, sea surface temperature has an important role in stimulating the endocrine organ system and reproductive activities such as gonadotropin hormone secretion by pituitary cells that support the development of eggs and sperm and stimulate the production of male androgen steroids and female estrogen steroids that will control the activities and reproduction behavior (Ali 2005; Nanami et al 2017). Supporting temperature conditions induces a good photosynthesis process as a primary production and food provider. The availability of food is very essential to support the process of gonad maturity. The more abundant available food will accelerate the process of gonad maturity (Kantun et al 2011). Spawning peaks of grouper in summer, as higher is the temperature of sea surface, was also found in *Epinephelus itajara* in southern Brazil (Gerhardinger et al 2006; Bueno et al 2013), *Epinephelus marginatus* in southern Brazil (Condini et al 2011), and *Epinephelus areolatus* in the Gulf of Suez (Osman et al 2018).

Management and conservation of *E. coioides* resources policy in Kwandang Bay Gorontalo Province need attention and regulate fishing operation during the spawning season. The policy will provide an opportunity fish to population to regenerate before the harvesting season by temporarily stopping capture when the fish are at the peak of spawning (May-July), then re-catching continues after passing the spawning peak.

Conclusions. *E. coioides* caught in Kwandang Bay, in general, were gonad ripe and at spawning stage. Sex change from female to male as a transition phase of *E. coioides* occurs at a size of 79 cm and a weight of 6,500 g. The spawning peak season of the species is from May to July that coincides with the dry season.

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