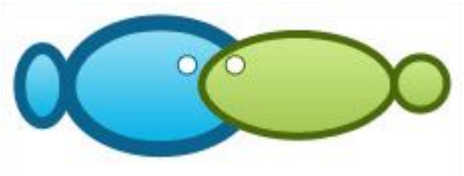

Reproductive aspects of areolate grouper, *Epinephelus areolatus* and six-barred grouper, *E. sexfasciatus* from Terengganu waters, Malaysia

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Abstract. *Epinephelus areolatus* and *E. sexfasciatus* are the most dominant grouper species landed in Terengganu waters and exploited by various type of fishing gears but knowledge on their population characteristics is still limited. This study aims to investigate the spawning season, fecundity and length at first maturity of these two commercially important species in Terengganu waters, Malaysia. To achieve that, macroscopic and microscopic analysis of the gonads are carried out. This study found that both species have extended spawning season from January to May. They also have high fecundity with the mean of 24646 to 26104 eggs per mature female of *E. areolatus*. For *E. sexfasciatus*, the mean fecundity ranges from 15850 eggs to 23961 eggs per mature female. The total length at maturity (Lm) for *E. areolatus* from Pulau Kambing and Kuala Dungun were 32.60 cm and 35.70 cm, respectively. While Lm for *E. sexfasciatus* from Pulau Kambing and Kuala Dungun were estimated at 24.00 cm and 22.80 cm, respectively. The findings from this study can be used to develop suitable management practice for groupers such as establish close season and formulating suitable mesh size regulation.
Key Words: spawning season, fecundity, groupers, fisheries management, length at first maturity.

Introduction. Grouper or locally known as kerapu are in family Serranidae and subfamily Epinephelinae (Mackie 2000) with more than 400 species and widely distributed in tropical and subtropical areas all over the world, inhibit in coral reef areas, rocky bottom environment, seagrass bed or silty sand in depth of range 2 to 200 meters (Mehanna et al 2013; Ambak et al 2012). Groupers are ubiquitous predators, demersal mesocarnivores, stalk and ambush type predators, have unique burrowing and excavation behavior (Sadovy de Mitcheson et al 2013). These characteristics may contribute to ecological stability in complex tropical hard bottom communities, balancing the abundance of prey and significantly alter the other community component (Sadovy de Mitcheson et al 2013; Agembe at al 2010; Ranjeet et al 2015; Kandula et al 2015).

Grouper is one of the most targeted species in Malaysia due to its high market demand. The annual landing of groupers are steadily increased, influenced by several factors such as the increase of licensed fishing vessels and the frequency of fishing trip (Department of Fisheries 2014). The characteristics of reef fishes such as groupers that are demersal, protogynous hermaphrodite, long-live and have aggregation behaviour will makes them highly vulnerable to overfishing (Tuz-Suluz & Brule 2015). The reduction in the total biomass will affect predators, prey and competitor of the target fish and indirectly to the seafloor community structure and will affect the process of spawning and successfulness of fertility among population as well (Darwin Reef Fish Project 2011). Russel et al (2008) considered *Epinephelus areolatus* as a Least Concern species in the IUCN Red List Threatened Species with the assumption that this is not a major target species even though they agree that this species is decreasing due to the increase of fishing pressure.

Grouper population is vulnerable and should be effectively managed and conserved for their sustainability. The management on grouper population should be conducted at species level because their characteristics are different between species with regard to their natural abundance and vulnerability to fishing pressure (Darwin Reef Fish Project 2011). The information about behaviour, reproductive biology and life history data are essential to determine fish vulnerability to fishing (Kandula et al 2015) and also important to provide proper fisheries management to ensure the fishery is sustainably and responsibly exploited (Lamin 2001).

E. areolatus and *E. sexfasciatus* are the most dominant species landed at fish landing ports in Terengganu and they are exploited by various fishing gear such as hook and lines, portable traps as well as trawl nets. Despite this, their population characteristics however are still unknown. In order to manage their population, the reproductive aspects of these two commercially important species should be well understood. This study aims to investigate the reproductive characteristics of *E. areolatus* and *E. sexfasciatus* in Terengganu waters, in particular 1) spawning seasons, 2) fecundity and 3) length at first maturity for both species. The data would be beneficial for the future management plan of resources.

Material and Method. Samples of *E. areolatus* and *E. sexfasciatus* were collected monthly from commercial fish trap fishermen from Pulau Kambing (PK) and Kuala Dungun (KD) fish landing ports in Terengganu from January to December 2014. These two landing ports are the biggest fish landing ports that landed most of the catch from Terengganu waters. The total length measurement of fish was obtained using measuring board and recorded to the nearest 0.01 cm. The body weight was measured using an electronic balance (model AnD GF-3000 by A&D Company Ltd) and recorded to nearest 0.01 g. Gonads were dissected and measured by using electronic balance (model AnD GF-3000 by A&D Company Ltd) and recorded to the nearest 0.01 g. The maturity stages of fish were investigated by macroscopic investigation and microscopic analysis of the gonads following the guidelines by Brown-Peterson et al (2011), Erisman et al (2010) and Cushion et al (2008). For microscopic analysis, the gonads were cut into appropriate size, put in the cassette then fixed in 10% buffered formalin solution for 24 hours before proceed to tissue processing for 18 hours. Then the samples were embedded in paraffin wax and sectioned at 6µm by using automated rotary microtome from Leica. The sections were stained with haematoxylin and eosin and observed under microscope to identify the maturity stages.

Data analyses. The spawning seasons for each species were obtained by calculating the ratio of ovary weight to somatic weight or also called Gonadosomatic Index (GSI) by using computer package MS-Excel 2010. The GSI was calculated for each month using formula (Dorostghol et al 2008; El-Sayed & Abdel-Bary 1999):

Gonadosomatic Index (GSI) = (gonad weight / somatic weight) x 100

Fecundity was determined was determined following the method by Murua et al (2003), based on the relation between ovary weight and the oocyte density in the ovary. To determine fecundity, 3 parts subsamples ovaries (anterior, middle and posterior) of mature fish were extracted and weighed to the nearest 0.01 g. Each subsample was put on the petri dish and dispersed in saline water with fine brush and vitellogenic oocytes were counted by repeatedly sucking in and out by using pipette. The fecundity was estimated by multiplying the number of mature oocytes in the samples taken from the anterior, middle and posterior region of ovaries by the ratio of ovary weight to sample weight. Fecundity was determined by simple equation as below (Murua et al 2003; El-Sayed & Abdel-Bary 1999):

Fecundity (F) = (total weight of ovary / weight of sub-samples) x numbers of eggs in mature fish.

Length at maturity (L_m) was determined as the length at which 50 percent of individuals are mature, during spawning season following the method by Erisman (2008) and Erisman et al (2010). Length at 50% sexual maturity was estimated by fitting a logistic model to the combined percentage of maturing and mature fish. Individual at stage III–V

were considered as mature in order to determine the length at first maturity. The equation to estimate length at first maturity was (Erisman 2008; Erisman et al 2010):

$$P = 1 / (1 + \exp [-r (L_t - L_m)])$$

Where, P is the proportion or ratio of reproductive females for each size class; r is a rate parameter related the speed of size change from non-reproductive to reproductive status; L_t is the total length (cm); and L_m is the size at first maturity (cm).

Results and Discussion. A total of 207 and 399 individuals of *E. areolatus*, and 153 and 82 individuals of *E. sexfasciatus* were collected from Pulau Kambing and Kuala Dungun fish landing ports, respectively. The total length of *E. areolatus* at Pulau Kambing and Kuala Dungun were from 14.70 to 45.20 cm, and 13.70 to 43.80 cm, respectively. Meanwhile, range size of *E. sexfasciatus* from Pulau Kambing and Kuala Dungun were in between 17.30 to 34.00 cm and 17.70 to 32.40 cm, respectively.

Microscopic maturity stages of gonads. Microscopic analysis showed the presence of six sexual maturity stages of female and four maturity stages of male for *E. areolatus* and *E. sexfasciatus* in both landing ports. For female, stage I and II were determined as not mature and developing process. Stage III, IV and V were mature and spawning stages, while stage VI was regressed stage (Figure 1).

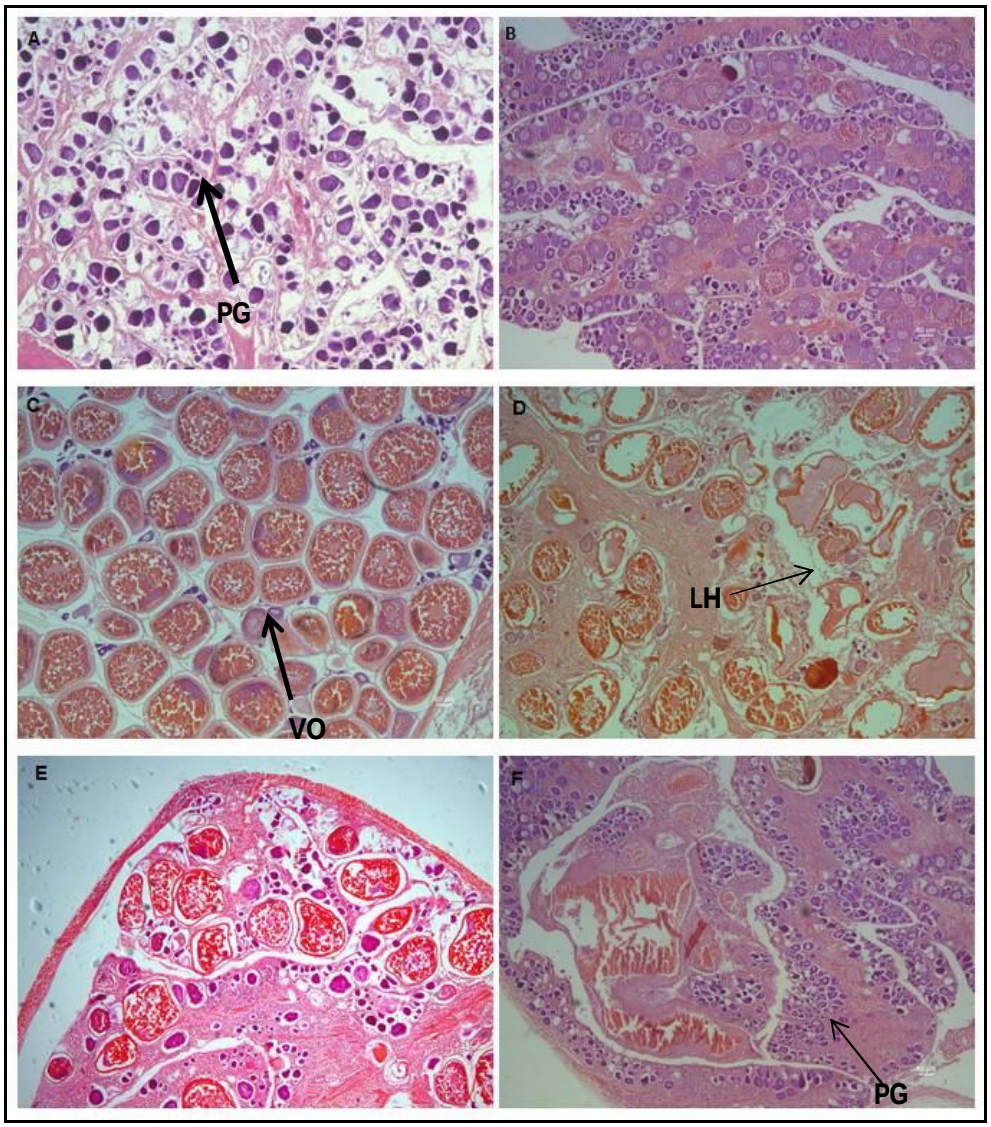

Figure 1. Histological images for maturity stages of *E. areolatus* and *E. sexfasciatus* sp. (female) from Pulau Kambing and Kuala Dungun landing ports. A: Stage 1, B: Stage 2, C: Stage 3, D: Stage 4, E: Stage 5, F: Stage 6. Note: PG – Primary growth; VO – Vitellogenic oocytes; LH – Late hydrated.

For male, stage I and II were immature and developing stages. Stage III and IV were ripe and regressed stages but capable to spawn (Figure 2).

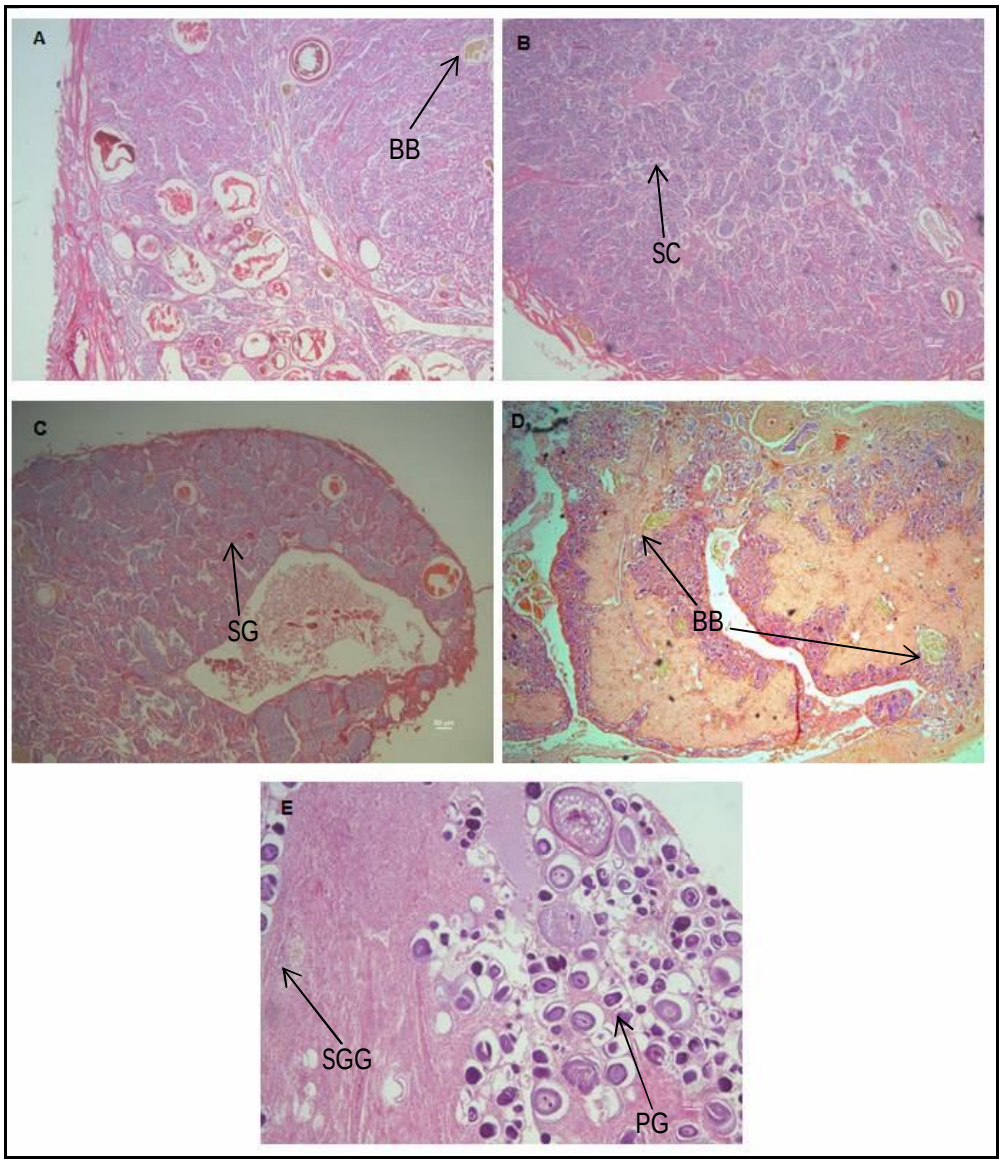

Figure 2. Histological images for maturity stages of *E. areolatus* and *E. sexfasciatus* sp. (male and transitional) from Pulau Kambing and Kuala Dungun landing ports. A: Stage 1, B: Stage 2, C: Stage 3, D: Stage 4, E: Transitional.Note: BB – Brown bodies; SC – Spermatocytes; SG – Spermatogenesis; SGG – Spermatogonia; PG – Primary growth.

Spawning season. GSI was used to explain the degree of ripeness of ovary and indicates the stage and readiness of the ovary for spawning (Chandrasekhara Rao & Krishnan 2009). To estimate the spawning season of both species, the combination of data of GSI, macroscopic observation of maturity stages and microscopic analysis of gonads were carried out.

The GSI of *E. areolatus* from Pulau Kambing and Kuala Dungun as shown in Figure 3 revealed that this species has almost identical trend of GSI which showed that they have similar spawning season even though the value of GSI of *E. areolatus* from Pulau Kambing is lower than Kuala Dungun. Mature *E. areolatus* females were found mostly throughout the year except during August, October and December for Pulau Kambing, while for Kuala Dungun during December and January only. Meanwhile mature *E. areolatus* males were found during January to May for Pulau Kambing and for Kuala Dungun during February, March, May and September. By using the combination of data, the spawning season of *E. areolatus* in Terengganu waters can be observed from January

to May. The spawning season for *E. sexfasciatus* in Pulau Kambing is harder to conclude since no fish was landed at Pulau Kambing in June to October. But, using limited data available on GSI and maturity stages, the spawning season for *E. sexfasciatus* in Kuala Dungun was estimated to occur in January to May as shown in Figure 4.

This finding is similar with the finding by other researches on groupers. Most groupers tend to spawn over 1-5 months interval as mention by Collins et al (2002), Mishina et al (2006) and Kadison et al (2010). Mahmoud (2009) found that GSI peak values for *E. areolatus* from Egypt were occurs in May and June.

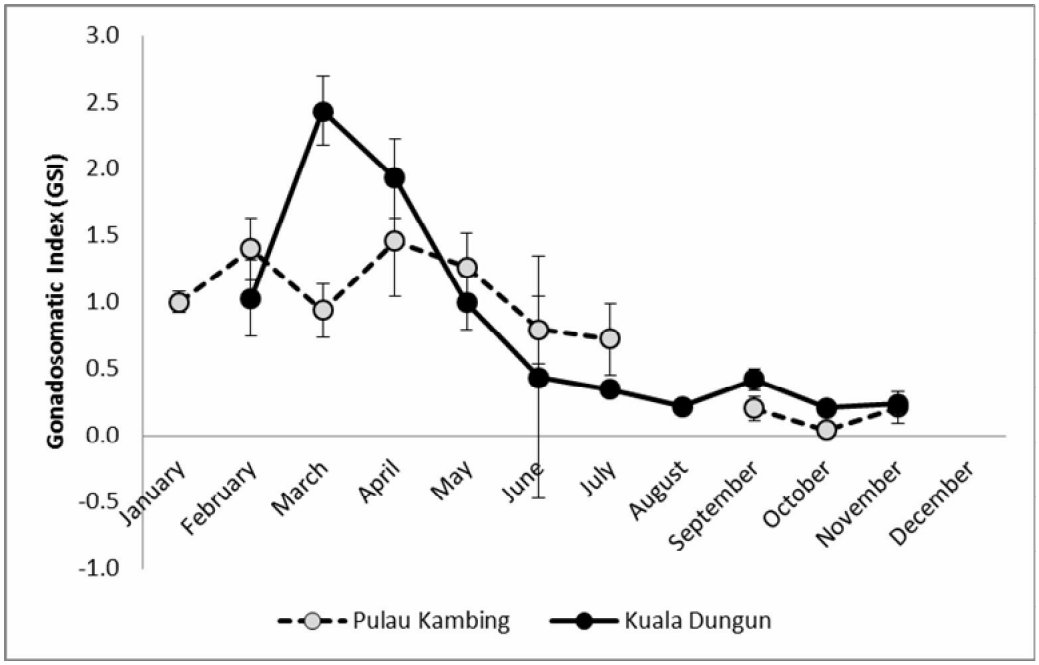

Figure 3. Monthly variation of GSI (mean ± standard error) of *E. areolatus* during study period at Pulau Kambing (N = 207) and Kuala Dungun (N = 399) landing port.

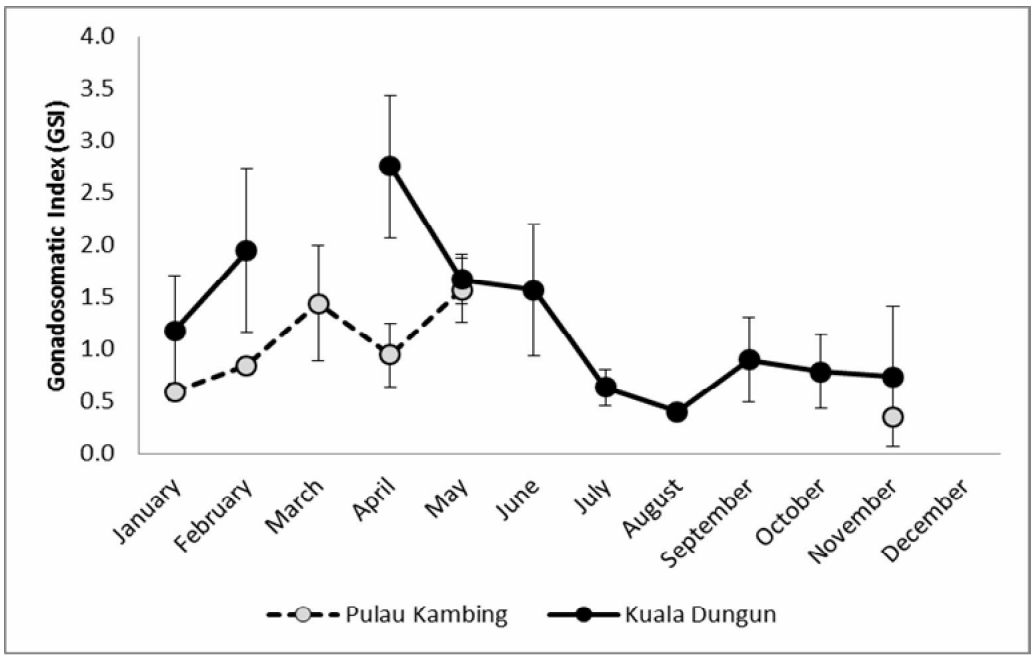

Figure 4. Monthly variation of GSI (mean ± standard error) of *E. sexfasciatus* during study period at Pulau Kambing (N = 153) and Kuala Dungun (N = 82) landing port.

Fecundity. Fecundity information of a species is essential for estimating seed production capacity, spawning population of the species and characterizing specific population, sub-

population and/or stock of fishes (Chandrasekhara Rao & Krishnan 2009; Miller & Kendall 2009; Kandula et al 2015). The fecundity for *E. areolatus* at Pulau Kambing ranges from 1721-68221 eggs per mature female with mean 24646 eggs per mature female. Fecundity for *E. areolatus* at Kuala Dungun ranges 785-84258 eggs per mature female with mean 26104 eggs per mature female (Table 1). For *E. sexfasciatus*, the fecundity ranged between 1771-65688 eggs per mature female for fish from Pulau Kambing with the mean of 15850 eggs per mature female. Meanwhile, fecundity for *E. sexfasciatus* from Kuala Dungun ranges from 9595 to 53194 eggs per mature female with mean of 23961 eggs per mature female (Table 2).

Table 1

Fecundity of *E. areolatus* from Pulau Kambing and Kuala Dungun fish landing port

Ports	*Number of samples, N*	*Range of fecundity*	*Mean*
Pulau Kambing (PK)	48	1721-68221	24646±116
Kuala Dungun (KD)	88	785-84258	26104±124

Table 2

Fecundity of *E. sexfasciatus* from Pulau Kambing and Kuala Dungun fish landing port

Ports	*Number of samples, N*	*Range of fecundity*	*Mean*
Pulau Kambing (PK)	29	1771-65688	15850±111
Kuala Dungun (KD)	25	9 595-53194	23961±62

This study found that fecundity of *E. areolatus* and *E. sexfasciatus* was high, slightly lower than reported by Kandula et al (2015) where they reported that the fecundity of *E. areolatus* in eastern India was ranged between 22128-93607 eggs. Abu-Hakima (1987) estimated the fecundity of *E. tauvina* was in between 850156 to 2904921 eggs for total length between 35.1-62.3 cm. Meanwhile, the fecundity of *E. diacanthus* was ranged 75547-145755 eggs (Chandrasekhara Rao & Krishnan 2009). Kandula et al (2015) also conclude that groupers are among fecund demersal and reef fishes. Differences in fecundity among species reflect different reproductive strategies, and within species fecundity may vary due to the different adaptation to environment habitats (Murua et al 2003). Variations of eggs productions were affected by the nutritional status among the female and selective pressures from fisheries may also be compensatory mechanism regulating fecundity (Morgan 2008; Miller & Kendall 2009).

Length at first maturity. The length at first maturity (L_m or L_{50}) was estimated for female of *E. areolatus* and *E. sexfasciatus* in both landing ports. L_m for *E. areolatus* from Pulau Kambing and Kuala Dungun were 32.60 cm and 35.70 cm, respectively. While L_m for *E. sexfasciatus* from Pulau Kambing and Kuala Dungun were estimated at 24.00 cm and 22.80 cm, respectively. From the result, it shows that *E. areolatus* from Pulau Kambing landing port mature earlier than Kuala Dungun landing port. While *E. sexfasciatus* from Kuala Dungun landing port mature earlier than the fish from Pulau Kambing fish landing port. Mahmoud (2009) reported that female *E. areolatus* from Red Sea, Egypt attained their first maturity at 23.50 cm in total length. However, there are no data found for *E. sexfasciatus*.

The size at maturity may be affected by available resources in a population where increased of resources in low density of population may led to better growth and indirectly result in maturation at a younger age (Morgan 2008). Thus, they need to maintain the population by mature at young or smaller size because delay maturation might decrease probability of surviving (Morgan, 2008). The information on the length at first maturity is important to prevent detrimental effects by overharvesting fish population (Kandula et al 2015).

Conclusions. This study aims to describe the reproductive aspects of the commercially important grouper species landed in Terengganu waters, Malaysia. Results shows that *E. areolatus* and *E. sexfasciatus* have an extended spawning season from January to May. They reach sexual maturity in between 32.00-36.00 cm for *E. areolatus* and in between

22.00 to 24.00 cm for *E. sexfasciatus*. The findings from this study can be used to develop suitable management practice for groupers such as establish close season during spawning and formulate the suitable mesh size regulation especially for trawl net to protect fish below the size at first maturity.

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