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Effect of Long-term Maintenance on Gonad Characteristics and Egg Quality of Collector Sea Urchin *Tripneustes gratilla* (*LINNAEUS 1758*)

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Effect of Long-term Maintenance on Gonad Characteristics and Egg Quality of Collector Sea Urchin *Tripneustes gratilla* *(LINNAEUS 1758)*

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Abstract. The gonad characteristics and number of eggs of the collector sea urchin *Tripneustes gratilla* (Linnaeus 1758) are thought to be determined by the accumulated results of the nutrients consumed during feeding. Long-term maintenance in captivity has been suspected to strongly influence the gonad characteristics and egg quality of the collector sea urchin *T. gratilla*. This research aimed to determine the proper length of maintenance for the collector sea urchin through studying the influence of maintenance time on gonad characteristics and number of eggs produced. This research was conducted at the Laboratory of Marine Aquaculture - Research Center for Deep Sea, Indonesia Institute of Science Ambon. Collector sea urchin (*T. gratilla*) samples with a test diameter of ± 50mm were obtained from Hative Besar, Ambon, Indonesia. The urchins were maintained for periods of 30 days, 60 days and 90 days. The observations indicate that the most appropriate maintenance time for collector sea urchin (*T. gratilla*) is 60 days, because after this maintenance period the gonads were orange (dark and light) in colour, with a firm texture. Moreover, the *T. gratilla* urchins maintained for 60 days produced a greater number of eggs with a larger egg diameter.

1. Introduction

Sea urchins are marine invertebrates commonly found in tropical shallow water (to a depth of 30 meters) coastal ecosystems including seagrass beds and coral reefs. Sea urchins obtain food from their habitat including seagrasses, algae, turf algae and periphyton. Temporal and spatial variations in urchin growth and gonad development are closely related to differences in vegetation, changes in feeding behaviour and other food sources that influencing growth and gonad development [1]. Currently the gonads of sea urchins are becoming an economically important commodity in many countries. Fresh sea urchin gonads or gonads in the form of processed foods have long been used as a luxury food; for example, sea urchin gonads are commonly known as "*uni*" in Japan where the price can reach AU$ 450/wet weight [2]. Since production peaked in 1995, the global production of sea urchin fisheries has continued to decline. On the other hand, the demand for sea urchin products is always increasing. This condition creates opportunities for aquaculture, especially in countries that still rely on the catch of wild sea urchins to meet export demand.

In Indonesia the management of sea urchin resources is far from optimal; urchins are harvested from the wild using simple traditional methods (principally gleaning) without paying attention to the sustainability of the fishery. Therefore, there is a need to develop sea urchin aquaculture to sustainably increase the production of sea urchins. There are several tropical sea urchins with potential for aquaculture development in Indonesia, one of which is the collector sea urchin *T. gratilla* (Linnaeus, 1758) [3]. Mariculture development cannot be separated from the production of seed of the target organism. The hatchery is the starting point in the mariculture business and development activities because marine aquaculture can only happen if seed are available. The hatchery phase is a limiting factor in the development of sea urchin cultivation in Indonesia.

Hatcheries and hatchery technologies are thus vital; however they can only produce good quality seed from good quality broodstock. In the sea urchin, the characteristics of gonads which desirable for human consumption differ from those desired in broodstock. For consumption, a large gonad, with few ripe gametes, with a firm texture and a bright yellow or orange colour is preferred [4,5]. Meanwhile, one good characteristic for broodstock in a hatchery is gonads containing many gametes. The colour of the gonads is thought to be caused by carotenoids obtained from food and deposited or directly stored in gonad tissues [6]. Nutrients stored in gonads will be used as an energy source in the process of gametogenesis. If nutrients are available in good and adequate quantity and composition then gametogenesis can proceed properly. The process involved in gametogenesis will affect the size of the egg, and are also very influential on the development and survival of larvae, which depend heavily on the nutrients available in the egg.

Previous research has studied ways to improve the quality of gonads and eggs with protein in artificial feed [3]. However, these studies did not provide information on the effect of long-term maintenance in captivity on gonads characteristics and egg quality. The purpose of this study was to evaluate the effect of long term captive maintenance on gonad characteristics and egg quality in the collector sea urchin *T. gratilla*.

2. Methods

2.1. Research site and sample collection

The study was conducted in October 2016, at the Aquaculture Laboratory, Center for Deep Sea Research (P2LD), Indonesian Institute of Sciences (LIPI), Ambon. Experimental animals (*T. gratilla*) were obtained from the coast of Hative Besar village, Ambon (Figure 1). In this study we used 27 collector sea urchins. The sampling was carried out at low tide, and the collected urchins were transferred to the laboratory in a container filled with algae and seagrass that were periodically moistened with sea water to prevent the urchins drying out or spawning.

2.2. Experimental maintenance of collector sea urchins

The laboratory experiments were carried out in a culture container with a recirculation system equipped with mechanical and biological filtration (Recirculation Aquaculture System, RAS). Mechanical filtration comprised foam and gravel as well as a protein skimmer, while biological filtration comprised seaweed and nitrifying bacteria grown using a bioball. During the experiments, water circulation was maintained using a magnetic pump with an aeration pump. Water quality parameters routinely checked every day were pH, temperature, salinity and dissolved oxygen, with 50% water changes at weekly intervals.

The collected sea urchins were cleaned to remove dirt and small animals attached to them, then rinsed with sterile seawater until clean. The cleaned sea urchins were placed in the tanks prepared for them at a stocking rate of 3 individual sea urchins per tank. The treatments were maintenance in captivity for 30 days, 60 days and 90 days.

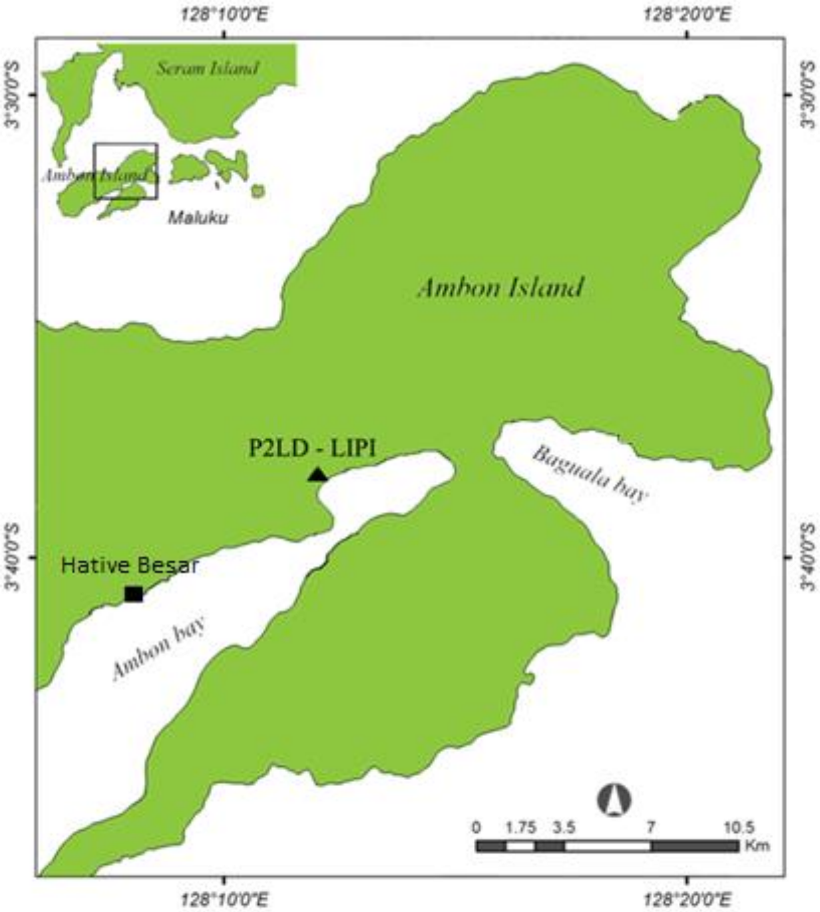

Fig.1. Map of the study area showing the sampling and experimental culture sites

Throughout the experimental period, the collector sea urchins were fed on locally abundant macroalgae collected from the coastal waters of Hative Besar and Tawiri. The sea urchin were fed macroalgae *ad libitum*, however the feed given was weighed in order to calculate the amount of feed consumed by sea urchins during the captive maintenance period by summing the total weight of feed given during that period.

2.3. Experimental parameters

The linear growth rate (LGR) and the specific growth rate (SGR) of collector sea urchins were calculated using the following equations:

$$LGR=(Lf-La)/t$$
$$SGR=[(Wf/Wa)^{(1/t)}-1] \times 100\%$$

where

La = initial test diameter (mm)
Lf = final test diameter (mm)
Wa = initial body weight;
Wf = final body weight (g); t = time (day)

Gonad colour assessment (qualitative) was performed using the PENTONETM colour card. The PENTONETM colour card was printed based on the Adobe Photoshop CS5 Extended libraries program (Figure 2). Gonad colour determination was performed under natural daylight conditions by the same observer throughout the experiment.

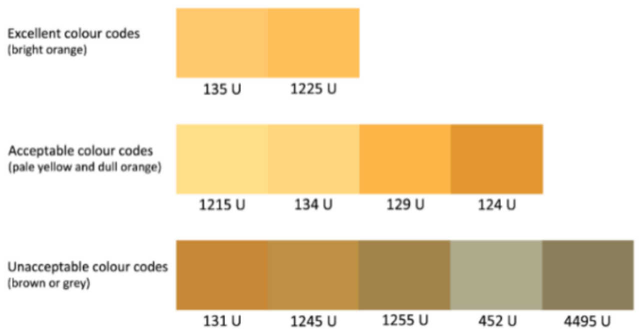

Fig.2. Color classification PENTONETM colour card printed based colour libraries program Adobe Photoshop CS Extended

Legend:
Bright orange = 135 U and 1225 U
Dark orange = 129 U and 124 U
Yellow/pale yellow = 1215 U and 134 U
Brown = 131 U, 1245 U and 1255 U
Gray = 452 U and 4495 U

Gonad degree of firmness was observed and grouped using the gonad firmness scale in Figure 3. The gonad index (GI) was calculated using the following equation:

$$\text{Gonad Index (GI)} = (W_g / W_t) \times 100\%.$$

where
W_g = gonad weight (g)
W_t = total urchin body weight (g)

Fig.3. Classification of gonad degree of firmness.

The eggs were counted after the spawning process. Before the spawning process, all equipment and containers to be used in the spawning process were sterilized. Spawning was induced by injecting 0.5 M KCl of 1-3 ml through the peristomal membrane. The individual sea urchins injected with KCl were then rocked slowly before being placed on top of a beaker containing sterile sea water for 30 minutes with the apical face facing down. Gamete collection was continued for 30 minutes, keeping the test and spines wet. Sea urchin spawn was placed in 1000 ml of sterile seawater and the colour estimated using a 1 ml Calcewick Rafter Chambers column. The eggs were observed at 100x magnification under an optical microscope. Egg diameter was measured while under the microscope.

2.4. Data Analysis
Data related to morphometric characteristics were analysed descriptively. Growth (LGR and SGR), gonad index (GI), egg number and egg diameter were analysed using a One Way ANOVA with Duncan test at the 5% error level ($\alpha = 0.05$, $p < 0.05$).

3. Results and Discussion

3.1. Results

3.1.1. Feed consumption. Feed consumption during the maintenance periods (Table 1) showed that, on average, collector sea urchins consumed 389 g of feed over 30 days. During the 60 and 90 day treatments, the average total quantities of feed consumed were 777 g and 1056 g, respectively.

Table 1. The amount of feed consumed by collector sea urchins during the study

Maintenance time (days)	Feed consumption (g, wet weight, 3 urchins)			Mean feed per urchin
	Replicate 1	Replicate2	Replicate 3	
30	1203	1203	1173	389
60	2409	2277	2307	777
90	3165	3123	3219	1056

3.1.2. Growth. The indicators of collector sea urchin growth were test diameter (TD) and total body weight (BW). Mean collector sea urchin test diameter before the experiment was close to 60 mm. Initial and final mean sea urchin diameters (Table 2) show that mean initial test diameter increased, associated with a noticeable change in total body weight, with the largest increase over 90 days.

Table 2. Test diameter (TD), body weight (BW), linear growth rate (LGR) and specific growth rate (SGR) of collector sea urchins during the experimental treatments

Parameters	Treatment (days)		
	30	60	90
		Initial	
TD(mm)	60.66±1.19	62.18±0.61	58.30±2.61
BW (g)	85.01±2.18	91.65±4.81	78.95±8.61
		Final	
TD (mm)	61.92±0.93	65.28±0.61	64.42±2.45
BW (g)	91.32±3.62	104.83±2.54	103.95±8.72
LGR (mm/day)	0.04±0.01	0.10±0.00	0.20±0.02
SGR (%/day)	0.24±0.10	0.23±0.05	0.31±0.05

3.1.3. Gonad characteristics. The characteristics of collector sea urchin gonads were assessed separately for male and female sea urchins, The data on gonad colour in Table 3 (male urchins) and Table 4 (female urchins) show noticeable changes over time in both sexes.

Table 3. Gonad colour of male collector sea urchins maintained in captivity before spawning for different lengths of time

Maintenance period (days)	Colour composition (%)				
	Bright orange	Dark orange	Yellow/ pale yellow	Brown	Gray
30	-	60	20	20	-
60	50	-	50	-	-
90	-	33	33	33	-

Table 4. Gonad colour of female collector sea urchins maintained in captivity before spawning for different lengths of time

Length of maintenance time (days)	Colour composition (%)				
	Bright orange	Dark orange	Yellow/ pale yellow	Brown	Gray
30	25	50	-	25	-
60	71	29	-	-	-
90	33	50	-	17	-

The optimum colour for male sea urchin gonads is dark yellow or bright orange. The percentage in this category increased from 30-60 days but had decreased by 90 days. For female sea urchin gonads, the optimal colour is bright orange, again increasing at 60 days but declining by 90 days. These data indicate that gonad quality based on colour was relatively poor and highly variable at both 30 and 90 days, with the highest proportion of high quality gonads in both male and female urchins under the 60 day treatment.

The degree of collector sea urchin gonad firmness was determined according to a scale ranging from very soft, mushy, not soft or firm, firm and very firm (Figure 3). For male urchins, at 30 days gonad texture was not soft or firm in 40% of sampled urchins and firm in the remaining 60%. For female urchins, at 30 days gonad texture was firm in 25% of urchins and very firm in the remaining 75% texture. After both 60 days and 90 days, both male and female gonads were 100% very firm textured. The mean shell diameter, shell height, total weight and gonad weight of male (Table 5) and female (Table 6).sea urchins were analysed by using ANOVA one-way with Duncan test at 5% error

Table 5. Mean morphometric characteristics and gonad weight of boiled male urchin.

Maintenance period (days)	Mean morphometric characteristics ± SD			Gonad weight (g)
	Shell diameter (mm)	Shell height (mm)	Total weight (g)	
30	63.14±11.93	39.04±20.16	98.31±08.58	6.86±10.82
60	67.79±11.74	39.46±00.66	109.34±10.27	11.92±11.20
90	62.71±11.46	37.46±20.85	96.05±10.68	9.57±12.65

Table 6. Mean morphometric characteristics and gonad weight of boiled female urchins.

Maintenance period (days)	Mean morphometric characteristics ± SD			Gonad weight (g)
	Shell diameter (mm)	Shell height (mm)	Total weight (g)	
30	62.98±20.87	38.47±40.11	95.96±10.54	4.36±10.99
60	66.31±10.62	40.48±10.56	110.57±70.71	8.31±30.03
90	67.41±10.81	42.20±10.65	116.29±90.39	7.41±10.56

The mean diameters of male and female collector sea urchins shells were similar, but males tended to have heavier gonads than females. The differences in collector sea urchin gonad weight indicate that both male and female urchins maintained for 60 days produced heavier gonads those maintained for 30 or 90 days (Figure 4). This was relected in the gonad index (GI) data which were significanlty different between all three treatments for both males and females (Duncan advanced test, $p < 0.05$). At 30 days, mean GI values for male and female urchins were 6.98 and 4.58 respectively. At 60 days male and female mean GI values had risen to 11.02 and 7.59 respectively, while at 90 days the mean gonad indice were 10.05 and 6.34.

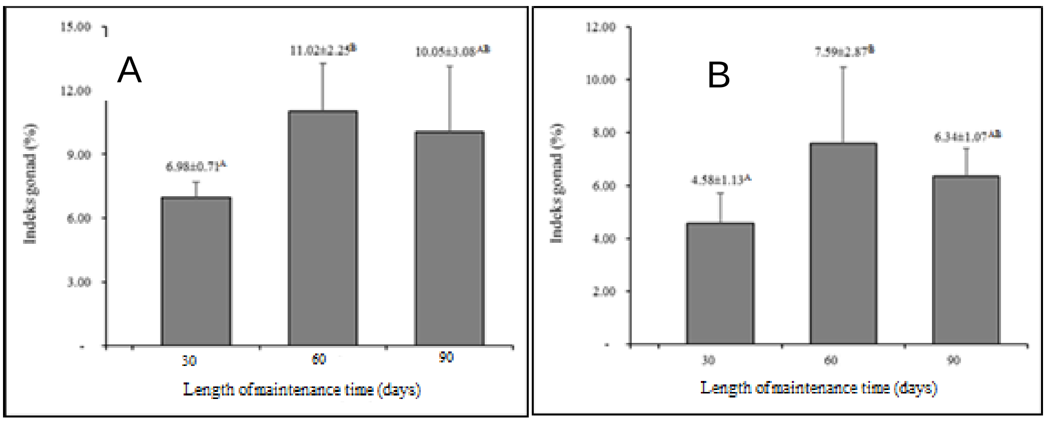

Figure 4. Gonad index (GI) of male (A) and female (B) collector sea urchins induced to spawn after different periods of captive maintenance

3.1.4. Egg Quality . The egg quality was assessed by observing the number of eggs produced (Figure 5) as well as the diameter and colour of the eggs. The average number of eggs produced by female collector sea urchins kept for 30 days was 3.07 × 106; for 60 days was 15.25 × 106; and for 90 days was 2.50 × 106 (Figure 5). The largest number of eggs was produced after 60 days captive maintenance.

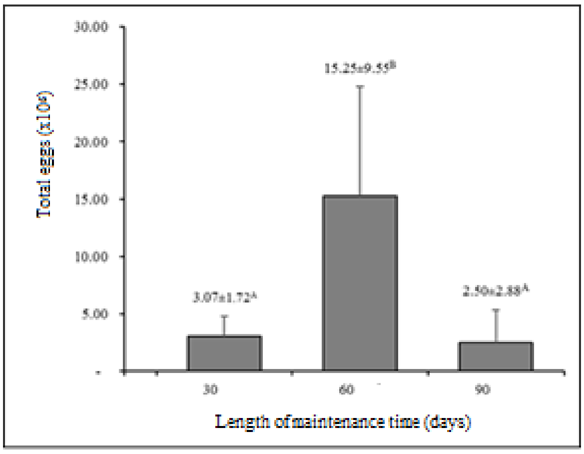

Fig. 5. Number of female sea urchin eggs collected during induced spawning after different periods of captive maintenance

The mean egg diameter produced by female sea urchins was higher at 60 days captive maintenance compared to 30 and 90 days (Table 7). The Duncan advanced test showed that this difference was significant.

Table 7. Mean diameter and color of eggs of female collector urchins induced to spawn after different periods of captive maintenance

Maintenance period (days)	Mean egg diameter (µm)	Egg colour
30	79.80	Orange
60	106.63	Orange
90	70.13	Orange

4. Discussion

The average amount of feed consumed daily by collector sea urchins was similar for 30 days and 60 days, but decreased in the urchins kept for 90 days. The average feed consumed indicates a limitation in eating ability. According to [3], the amount of feed consumed by sea urchins is also influenced by physical and chemical characteristics of food, while the feed consumption of the green sea urchin (*Lytechinus variegatus*) may be determined by perseverance to eat with little regard to food choice [7]. Furthermore, the growth pattern of *T. gratilla* has been reported as allometric positive, meaning the growth in test diameter is not as fast as growth in weight [8].

Gonad colour varies by gender and maturity/gametogenesis stage. Besides color, gender and maturity level/gametogenesis also affects texture, including the level of gonad firmness. Mature gonads are soft-textured, while during the recovery phase the gonad of the is solid (compact), softening throughout the process of gamete ripening [8]. Gonad histology tests show that mature gonads and spawning occur every month. Therefore, the level of gonad maturity can not be determined by looking at body size. In addition, the color of gonads is also influenced by the amount of β-carotene contained in the feed. The feed consumed by the collector sea urchins during this study was predominantly *Gracilaria lichinoides*, a macroalga with a high chlorophyll content. Carotenoids are red to orange pigments found in chlorophyll within chloroplasts. Carotenoids are found in all species of algae [9]. The colour of the collecter sea urchin gonads in this study improved from 30 to 60 days, but had declined by 90 days. The predominance of dark orange and brown (collectively 67%)

after 90 days in both male and female urchin gonads indicates even lower quality than at 30 days. These results indicate that gonad quality peaks around 60 days, and are supported by the data on gonad texture.

The difference in weight of the collector sea urchins gonads, both male and female, indicates that the male and female collected sea urchins kept for 60 days produced heavier gonads than the male and female collector sea urchins kept for 30 days and 90 days. An increase in the gonad index was also seen. It is likely that the decline in the gonad index at 90 days was related to the decrease in feed consumption. Feed volume and quality can greatly affect the gonad index because more feed means more nutrients that could accumulate in the gonad. In *T. gratilla,* it has been found that gonads develop at around 50 mm test diameter at an age of less than one year, with a maximum gonad index of around 10-15% of net weight at a test diameter of around 70 mm and does not decrease therafter up to a maximum diameter of around 100 mm [10]. The increase in gonadal weight of *L. variegatus* depends on dietary protein and long periods of accumulation of diet, protein and lipids are required for gonadal development [11]. The energy used during the process of gonad development comes from carbohydrates contained in nutritive phagocytes [12]. During gonad developmen, gonad weight will increase continuously; however, when gonads are mature but not yet spawning, the nutrients accumulated in the gonads can be used as an energy source for metabolic processes in the body.

The largest number of eggs (15.25 x 10^6) was produced by collector sea urchins kept for 60 days; these eggs also had a larger mean diameter (106.63 μm). Reported egg diameters of *T. gratilla* range from 85 to 90 μm, depending on temperature, food availability and maintenance duration [13]. Egg size represents the amount of nutrients contained, and can vary among individuals [14]. Some studies have found that diet and season also affect the number and size of eggs produced due to temporal and spatial variations the availability of food or nutrients in their habitat [15]. Furthermore, it was found that sea urchin with diet containing xantophil, lutein, and zeaxanthin produce more eggs than diet containing β-carotene. This is because the content of β-carotene is more influential on egg and gonad color [16].

5. Conclusion

Based on the results obtained in this study, it can be concluded that good maintenance duration for *T. gratilla* collector's sea urchin is for 60 days. This is because the resulting gonads are bright orange and dark orange with a very firm texture and the most severe gonad weight. In addition, the number of eggs produced is also the most with the largest diameter.

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