SHORT COMMUNICATION:

EFFECT OF CULTURE DENSITIES ON MATURATION AND SPAWNING OF THE PORTUGUESE OYSTER (Crassostrea angulata)

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ABSTRACT

The Portuguese oyster (*Crassostrea angulata*) is a mollusc species with many advantages for culture such as fast growth, and high economic importance. However, spat quality and quantity have not met the needs of farmers, leading to unstable annual oyster yield. To improve the spawning efficiency of oyster, we carried out an experiment to evaluate the effect of rearing densities on the maturation and spawning capacities of the Portuguese oyster broodstock, *C. angulata*. The experiment was evaluated with three rearing densities of 50, 80 and 100 individuals/m³. The results showed that there were no significant changes in the parameters of the environmental factors among three rearing densities (P > 0.05). At a density of 50–80 individuals/m³, survival after 30 days was > 84% and the percentage of oyster matured and spawned was 76–78%. Therefore, this is an essential reference basis for commercial spat production or mass selection program of *C. angulata* where there are more males and females involved in mating.

Keywords: Portuguese oyster, *Crassostrea angulata*, rearing density, fertility, environmental factors.

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INTRODUCTION

Portuguese oyster (Crassostrea angulata) are among the most important mollusc species around the world. Portuguese oyster and Pacific oyster (Crassostrea gigas) have similar morphology, therefore, it is hard differentiate without molecular tools (Hsiao et al., 2016; Van In et al., 2017). C. angulata has been distinguished from Crassostrea gigas by genetic structure (Boudry et al., 1998; Van In et al., 2017). C. angulata grows well in warmer environments compared to C. gigas, that is why C. angulata aquaculture sector is relatively developed in Vietnam, China, Taiwan (Le et al., 2023; Vu et al., 2020a). Global aquaculture contributes substantially to the quantity and quality of food for humans, especially in Asia, which accounts for 85% of aquaculture world's production. the C. angulata, is a bivalve species with many outstanding characteristics such as fast growth, productivity, delicious meat and high contributing to substantial employment for farmers who live in coastal areas (Vu et al., 2020b). The annual total production of C. angulata in Vietnam was around 50,000 tonnes in 2018 (Vu et al., 2020a). However, the source of seed supply has not met the domestic demand, and oyster have to be imported apart from China, leading to unstable production. Therefore, it is necessary to do research to increase the efficiency of oyster hatchery production and the enhancement of seed supply. To increase hatchery efficiency, one of the first steps is to optimise maturation protocols for C. angulata broodstock to have better spawning capacities in Vietnam.

MATERIALS AND METHODS

Experimental materials

C. angulata used in the experiment were 9 months old had an average weight of 50.2 ± 3.4 g, and originating from Quang Ninh province, Vietnam. Laboratory instruments included 9 composite tanks marked C1-C9 with the size of 4 m² per tank. UV light system for sterizing water, thermometer, oxygen meter, salinity meter, pH meter, chlorine kit, digital scales. The food used for

broodstock was live algae, Chlorella sp., Isochrysis galbana, Chaetoceros sp., meanwhile, Chaetoceros muelleri, Chaetoceros calcitrans, Isochrysis galbana, Tetraselmis sp. were used for oysters from D-larvae to spat stages. The algal density was supplied to satisfy the requirements of oyster and kept at least 40.000 cells/ml throughout the whole period of the experiment. The algal density was estimated by the Newbauer chamber.

Experimental design

The experiment was completely designed randomly based on 03 different treatments in the indoor tank system. The maturation densities of parents *C. angulata* were 50, 80 and 100 oysters/m³ for each treatment. The broodstock oyster completed spawning activities within one week. Each treatment was run with three replicates. The experiment was run for 30 days. Natural mating was applied for assessing spawning capacities during the experiment. The whole weights of oysters were recorded before carrying out the experiment.

Data collection and analysis

Environmental parameters observed according to the technical regulation on coastal water quality QCVN 10:2008/BTNMT of Vietnam including temperature (°C), pH, salinity (‰), DO (mg/L), NH₃-N (mg/L), NO₂-N (mg/L). Temperature, pH, DO were recorded daily from the start to the end of the experiment. Salinity was measured every two days. NH₃-N (mg/L), NO₂-N (mg/L) were measured every week.

Egg fertility rate (%) was the ratio of the total number of fertilized eggs/to the total number of eggs produced. Larval survival (%) was calculated as the total number of spat after 30 days of rearing/ the total number of initial larvae. Trochophore larval development rate (%) was the number of D-larvae per the number of trochophore larvae. The whole weights of oyster before and at the end of the experiment were measured for calculating the changes of oysters' weights during the experiment. Fertilization rate was measured

after 4-hour mating. The number of embyro and larvae used to calculate fertilization rate and trochophore larvae development rate were 100 embryos and larvae, respectively. Male and female ratios were calculated based on 50 oysters per treatment. Fertilized eggs were identified by microscopy.

All data were analyzed by SPSS software version 22.0 and comparisons between

treatments were made at a significant level (P < 0.05).

RESULTS AND DISCUSSION

The effect of rearing density on some environmental parameters

The results of monitoring of environmental factors during broodstock rearing are shown in Table 1.

Table 1. Environmental parameters in Crassostrea angulata broodstock rearing tanks

Environmental factors	Treatment 1	Treatment 2	Treatment 3
	(50 oysters/m ³)	(80 oysters/m ³)	$(100 \text{ oysters/m}^3)$
Temperature (°C)	24.1–26.1	24.2–26.2	24.1–26.2
pН	7.93 ± 0.42^{a}	7.97 ± 0.41^{a}	7.84 ± 0.38^{a}
Salinity (‰)	28.0-30.2	28.1-30.1	28.1-30.3
DO (mg/L)	4.74 ± 0.38^{a}	4.71 ± 0.34^{a}	4.83 ± 0.41^{a}
NH ₃ -N (mg/L)	0.03 ± 0.013^{a}	0.042 ± 0.012^{a}	0.035 ± 0.014^{a}
NO ₂ -N (mg/L)	0.042 ± 0.010^{a}	0.051 ± 0.013^{a}	0.043 ± 0.014^{a}

Note: The same letters in the same row show a significant difference in terms of statistics (P < 0.05).

Temperature and salinity are the two most important environmental factors affecting the gonadal development of oyster. The suitable salinity for broodstock rearing ranges from 28-30‰ (Giang et al., 2010). In the wild, oysters are distributed in waters with a salinity of 25% or more. Oyster grow well at the temperature range of 18–30 °C, but the suitable temperature range for oysters' gonadal development and spawning is 24–26 °C. Temperature is a factor that strongly influences the metamorphosis time of oyster larvae. Fertilized eggs develop into D-shaped larvae only after 12 hours when reared at 24-28 °C, while at 19 °C it takes 24 hours (Giang et al., 2010). The results of monitoring some environmental factors in the broodstock tanks showed that the monitored parameters were in the appropriate range for broodstock rearing (Table concentrations of NH3-N and NO2-N had no significant difference between the three experimental treatments and were within the allowable range (QCVN 10:2008/BTNMT).

Effects of maturation culture densities on whole weight before and after the experiment

The whole weights of oyster at the start and end of the experiment did not change significantly among the treatments (Fig. 1). The average whole weight of oyster at the start of the experiment was 50 grams/oyster, and these whole weights decreased after the completion of the experiment. However, the change was not statistically significant (P > 0.05). These decreases in whole weights of oyster can be due to the release of sperm and egg productions, resulting in the whole weight reductions.

The effect of the rearing density on fertility, sex ratio, trochophore development of the Portuguese oyster

The culture density has an effect on the maturation rate and fertility of mollusc species (O'Connor & Dove, 2009). The rate of egglaying maturity is an important indicator to evaluate the efficiency of the rearing process. After a 30-day rearing, the treatment 1 (50 oysters/m³) showed the highest maturation and survival rate (77.0 \pm 4.10%; 84.0 \pm 5.23%). In which, the treatment (100 oysters/m³) gave the lowest survival rate of broodstock (74.0 \pm 6.20%) with a statistically significant difference with the rest of treatment 2 (80 oysters/m³) with a survival rate of $83.0 \pm 6.23\%$ (P < 0.05) and treatment

1 (survival rate $84.0 \pm 5.23\%$; P < 0.05). Similar results were also found for the indicator of egg-laying maturity, treatment 3 gave the lowest result and had a statistically significant difference compared with the other treatment, treatment 1 and treatment 2 (Table 2). Other parameters such as fertility, fertilization rate, male-female ratio, and

Trochophore larvae development rate were not significantly different in all 3 experimental treatments (P > 0.05; Table 2). In addition, the number of brood stocks increased that may result in high genetic variability and promote higher fecundity and fertility rates in oyster (Bentsen & Olesen, 2002; Crawford, 2016).

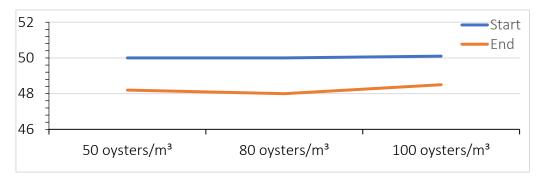


Figure 1. The whole weight of oyster at the start and end of the experiment

Treatment	Treatment 1	Treatment 2	Treatment 3
Indicators	(50 oysters/m ³)	(80 oysters/m ³)	$(100 \text{ oysters/m}^3)$
Survival rate after 30 rearing days (%)	84.0 ± 5.23^{a}	83.0 ± 6.23^{a}	74.0 ± 6.20^{b}
Rate of egg-laying maturity (%)	77.0 ± 4.10^{a}	75.0 ± 3.63^{a}	64.0 ± 3.60^{b}
Fertility rate (10 ⁶ eggs/mother oyster)	3.90 ± 0.75^{a}	3.74 ± 0.82^{a}	3.70 ± 0.79^{a}
Male-female ratio	40/60	40/60	40/60
Fertilization rate (%)	75.53 ± 8.49^{a}	77.2 ± 9.82^{a}	77.0 ± 9.81^{a}
Trochophore larvae development rate (%)	74.64 ± 8.66^{a}	75.42 ± 9.66^{a}	75.4 ± 9.60^{a}

Note: The same letters in the same row show significant differences in terms of statistics (P > 0.05).

CONCLUSION

The density of 50–80 broodstock oysters/m³ gave the highest results of the survival rate after 30-day rearing (> 84%), egg-laying maturation rate (75–77%). Fertility rate, fertilization rate, male-female ratio, and Trochophore larvae development rate were not significantly different for different rearing densities (50, 80 and 100 oysters/m³).

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