Abstract: A spawning experiment was carried out to compare the egg size spawned between two groups reared under naturally fluctuating (20.2 to 27.3°C) and cooled water temperatures (20.2 to 22.2°C) in the red spotted grouper. The egg diameter was significantly larger in the natural temperature group than the cooled water temperature group in the beginning of the spawning season. However, the trend in egg diameter between the two groups was reversed from the middle of the spawning season and the diameter continued to be significantly different until the end of the spawning season. Rearing broodstock under a low temperature is concluded to be effective to delay the decrease of egg size in red spotted grouper.

Key words: Red spotted grouper; Egg size; Water temperature; Seed production.

The red spotted grouper, Epinephelus akerara, is a temperate rockfish with a high marketability for its tasty meat. Stocking experiments have been conducted in western Japan to enhance the decreased stock. Although seed production techniques for this species have been studied for more than 30 years, effective techniques have not been established because of high mortality which occurs during the early life stages. The early mortality of groupers generally occurs during the period from the endogenous to the exogenous change in feeding behavior owing to their smallness, namely poor nutritional reserves and their small mouth size. This phenomenon is observed in the red spotted grouper like other groupers. Kayano et al. suggested that the use of larger larvae hatched from larger eggs will be effective for preventing early mortality during larval rearing of red spotted grouper. They also noted that egg diameters were larger at the beginning of the spawning season and decreased in the later part of the season. The early mortality of groupers generally occurs during the period from the endogenous to the exogenous change in feeding behavior owing to their smallness, namely poor nutritional reserves and their small mouth size. This phenomenon is observed in the red spotted grouper like other groupers. Kayano et al. suggested that the use of larger larvae hatched from larger eggs will be effective for preventing early mortality during larval rearing of red spotted grouper. They also noted that egg diameters were larger at the beginning of the spawning season and decreased in the later part of the season. Rearing broodstock under a low temperature is concluded to be effective to delay the decrease of egg size in red spotted grouper.

The experiment was carried out at the Tamano Station of the Japan Sea-Farming Association from Jun. to Sep. 2001. Two groups of the red spotted grouper were used for the experiment. They were caught around Hakata Island or Ibuki Island in the middle region of the Seto Inland Sea in the summer season of 1999 and reared at the Tamano Station. The Hakata group (58 fish) was reared under naturally fluctuating water temperature (natural group) and the Ibuki group (89 fish) was reared under a cooled water condition (cooled group). The number of individuals by sex and body weight, mean±standard deviation (S.D.), of the two groups were female; 17; male, 41; 828±208 g in the natural group and female; 12; male, 74; unidentified, 3; 454±101 g in the cooled group. The two groups of fish were each reared in an indoor octagonal concrete tank 6 m wide and 2.7 m deep (1.7 m water depth). Sand filtered seawater was supplied to the natural temperature tank at the running water rate of 400 to 500% per day and the water temperature was allowed to fluctuate with the ambient temperature. The water exchange rate of the cooled tank was ca. 30% per day during the spawning season and water temperature was kept at ca. 22.0°C by using a filter and cooling system attached to the tank. Fish were fed with moist pellets to their satiation (1-3% of the body weight) once a day except on Sundays. The ingredients of the moist pellets were jack mackerel (Trachurus japonicus): 1 part, short-finned squid (Todarodes pacificus):1 part, shrimps (Metapenaeopsis spp, Trachypenaeus spp):1 part, mashed formula feed (for the Japanese flounder): 2.8 parts, and vitamin premix: 0.2 parts. The effluent from each tank was filtered through a pair of gauze nets set in attached tank for collecting eggs. The nets were inspected each morning and the collected eggs were transferred into bucket and filled up to 10 l with seawater. The eggs and seawater in the bucket were stirred gently and 2-5 ml was taken with a pipette to count the number of eggs. The eggs were counted as fertilized (normally developing) or unfertilized (not developing) eggs under a stereoscopic microscope. The total number of fertilized and unfertilized eggs for each tank were estimated from the respective number of each sampled eggs and the ratio of sampled water volume to the water volume in the bucket (10 l). The daily fertilization rate was calculated as the number of fertilized eggs divided by the total number of eggs. The egg diameter was used as an index of egg size, since eggs of this species are spherical. The egg diameters of 50 fertilized eggs from each of the two groups were measured using vernier calipers under a profile projector (Nikon V12, magnification of 50-power) to the nearest 2 μm at least once a week. A significance test was carried out with Student’s t-test on PC software (Excel 2000) between the mean egg diameters of the two groups collected on the same day.

Eggs were collected from Jun. 10 to Sep. 9 in the natural group and from Jun. 10 to Sep. 19 in the cooled group. The total number of collected eggs and fertilized rate were 79.1 million and 27.8% in the natural group, 52.3 million and 24.6% in the cooled group. Fig. 1 shows the changes of the water temperature and egg diameter of the two groups during the main spawning season (Jun. 15 to Sep. 10). The...
water temperature ranged from 20.2 to 27.3°C in the natural group, and 20.2 to 22.2°C in the cooled group except for Aug. 5 (25.0°C) when the cooling system broke down. The mean egg diameter values of the natural group were significantly larger than the cooled group in Jun. Significant differences of the mean egg diameter values were not found between the two groups from early to middle of Jul. The mean egg diameter of the cooled group became subsequently larger than the natural group from Jul. 23 to the end of the spawning season. The significant inversion occurred on Jul. 23 at the water temperature of 25.2°C in the natural group and 22.0°C in the cooled group. The egg diameter (mean ± S.D.) over the experimental period ranged 813 ± 15 to 812 ± 13 μm in the natural group and 727 ± 13 to 783 ± 19 μm in the cooled group.

The broodstock spawned normally in the two groups judged from previous data[9], although the fertilization rates approximated 25-28% in this study. The low fertilization rates are presumably derived from insufficient water depth of the spawning tank[15]. The water temperature suddenly rose to 25.0°C on early Aug. in the cooled group, however the temporarily high temperature scarcely affected the spawning since they continued spawning afterwards and the egg size did not change immediately.

The egg size of the natural group was significantly larger than the cooled group during the early part of spawning season when the two groups were reared at almost the same water temperatures. The difference in the egg size became smaller with the increase of the water temperature in the natural group. The comparative egg size of the two groups reversed after late Jul. Compared to the cooled group, the natural group showed a greater range in egg size which was inversely related to the ambient water temperature. These results indicate that a low water temperature is effective to delay the decrease of egg size with the seasonal increase of water temperature in red spotted grouper.

Komaki et al.[13] suggested that the egg diameter was not affected by the progression of the spawning season but by the water temperature in captive red seabream. In the present study, however, egg sizes decreased with the progression of the spawning season even under a constant water temperature. Moreover, the egg diameters of the two groups tended to fluctuate simultaneously through the spawning season. Not only water temperature but other undetermined factor(s) might affect the egg size of red spotted grouper.

The maximum egg diameter was 1.17 times larger than the minimum one in the natural group. This difference gives 1.60 times difference in egg volume. The difference of egg volume through the spawning season should be considered when the number of collected eggs is estimated from the total weight or total volume of the eggs. In this respect, the egg number estimation procedure employed in this study should be more precise and useful than the estimation method from total egg weight or total egg volume.

In conclusion, rearing broodstock under cooled conditions was effective to delay the decrease of egg diameter in red spotted grouper. The body size of the two spawning groups differed in this study. The difference of the body size might affect the changes of the egg size. Reexamination between the two temperature regimes using fish of the same body size will be necessary to verify the present results. Further, in order to confirm the superiority of larger eggs, comparison tests will be needed to examine larval quality, such as feeding ability and/or starvation tolerance, between the larvae hatched from larger and smaller eggs.

References


Fig. 1. Changes of the water temperature and egg diameter of the natural and cooled groups of the reared red spotted grouper. Dotted lines, natural group; Solid lines, cooled group. Solid squares, circles and vertical bars indicate mean ± S.D. (n = 50) in each egg batch. Horizontal arrows indicate that significant differences (Student's t-test, p<0.05) were observed between the groups during the respective periods.