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EVALUATION OF SHORT-TERM AND LONG-TERM STORAGE OF RED SNAPPER AND GRAY SNAPPER SPERM

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Short-term and long-term storage of sperm can offer benefits in the hatchery production of marine fish. Refrigerated and cryopreserved sperm can be used to improve artificial spawning by eliminating the need to hold male broodstock, allowing repeated use of sperm from high quality males, and enabling production of hybrids. As part of a larger study on the reproduction of lutjanids, we developed methods for the collection, handling, refrigerated storage, and cryopreservation of red snapper *Lutjanus campechanus* and gray snapper *Lutjanus griseus* spermatozoa.

Sperm were collected from red snapper ($n = 177$) and gray snapper ($n = 78$) males captured in the recreational fishery during the summers of 2000 and 2001. Sperm were diluted 1:4 with calcium-free Hanks' balanced salt solution (C-F HBSS), placed in 4-L Ziplock™ plastic bags, and transported to the laboratory on ice. Undiluted red snapper seminal plasma had an osmotic pressure of 428 ± 16 mOsmol/kg ($n = 19$) while gray snapper seminal plasma was 411 ± 5 mOsmol/kg ($n = 13$). Blood plasma osmolality was 440 ± 8 mOsmol/kg for red snapper and 419 ± 8 mOsmol/kg for gray snapper. Activation studies of red snapper and gray snapper sperm found that sperm motility was suppressed by decreasing the osmotic pressure of artificial seawater (Figure 1). In a series of refrigerated storage experiments, sperm samples suspended in 200 mOsmol/kg C-F HBSS retained motility for 12 d at 4 C. Refrigerated storage experiments with the addition of 0.1% and 1.0% solutions of an antibiotic-antimycotic mixture did not significantly extend refrigerated storage times. Dimethyl acetamide, dimethyl sulfoxide (DMSO), methanol, and glycerol were evaluated as cryoprotectants. Ten percent DMSO was chosen for further study because it was least toxic to sperm samples. Red snapper sperm used in cryopreservation trials had $90 \pm 6\%$ motility before freezing and $80 \pm 23\%$ after thawing. Gray snapper sperm used in cryopreservation trials had $89 \pm 6\%$ motility before freezing and $64 \pm 24\%$ after thawing. This study demonstrated that red snapper and gray snapper sperm can be stored for short-term and long-term use in the hatchery.

Figure 1. Percent motility of undiluted red snapper and gray snapper sperm activated with artificial seawater.

