

Innovative Approaches to Black Grouper (*Mycteroperca bonaci*) Aquaculture

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Abstract

The aquaculture potential of Black Grouper (*Mycteroperca bonaci*) within the southeastern United States has yet to be realized mainly due to challenges with their aggressive behavior with fellow tank inhabitants, limitations in space for adequate long term broodstock holding, and success in simulating natural spawning conditions. The College of the Florida Keys Southernmost Marine Aquaculture and Research Training (SMART) center located in the heart of the Florida Keys National Marine Sanctuary has worked with the *M. bonaci* species since awarded grant funding by the Gulf States Marine Fisheries Commission in 2020. Titled: 'From Guts to Glory', the project focuses on housing and maturing female brood (45 cm-75 cm) in an indoor recirculating aquaculture system equipped with natural spawning stimuli (photoperiod control, temperature regulation, and audio stimulus with male courtship calls) and using cryogenically preserved male *M. bonaci* gametes for in-vitro fertilization. Progress in advancing innovative hatchery procedure, husbandry, and best management practices continues with diet formulation and refining system technology in preparation for spawning. Alternating between diets for our inhouse female broodstock, we saw an average growth of 0.16 mm per day since their initial acclimation into our indoor recirculating aquaculture system in early April 2022. The broodstock maturation diet alternates between threadfin herring (*Opisthonema oglinum*), pink shrimp (*Penaeus Duorarum*), and "maturation sausages" developed as a prototype alternative feed using manual processing techniques.

Keywords: Black Grouper, *Mycteroperca Bonaci*, Grouper, In Vitro Fertilization.

Introduction

The aquaculture potential of black grouper, *Mycteroperca bonaci*, within the southeastern United States has yet to be realized, mainly due to challenges posed by their aggressive behavior toward tankmates, limited space for adequate long-term broodstock holding, and difficulty simulating natural spawning conditions. The College of the Florida Keys Southernmost Aquaculture Research and Training (SMART) center, located in the heart of the Florida Keys National Marine Sanctuary, has worked with the species since receiving grant funding from the Gulf States Marine Fisheries Commission in 2020. Titled: 'From Guts to Glory', the project focuses on domesticating and maturing female brood (45 cm-75 cm) in an indoor recirculating aquaculture system equipped with natural spawning stimuli (photoperiod control, temperature regulation, and audio stimulus with male courtship calls) and using cryogenically preserved male *M.*

bonaci gametes for attempts at in-vitro fertilization. Successfully spawning in captivity for this species would provide a critical species management strategy for one of the most overfished species in the Florida Keys and result in a one-of-a-kind Florida Keys stock enhancement plan for the coral reefs of the Florida Keys National Marine Sanctuary. Our ongoing work with this in-vitro fertilization process has the potential to spark a revolution in the marine finfish hatchery industry and the aquaculture community worldwide.

Ecology & Natural Spawning

As a prime target species for the fishing industry in the Florida Keys, pressure is put on the grouper family. The effects of overfishing fecund adults and the premature harvesting of developing juveniles ultimately contributed to a 94% population decline since 1990 (Figure 1).

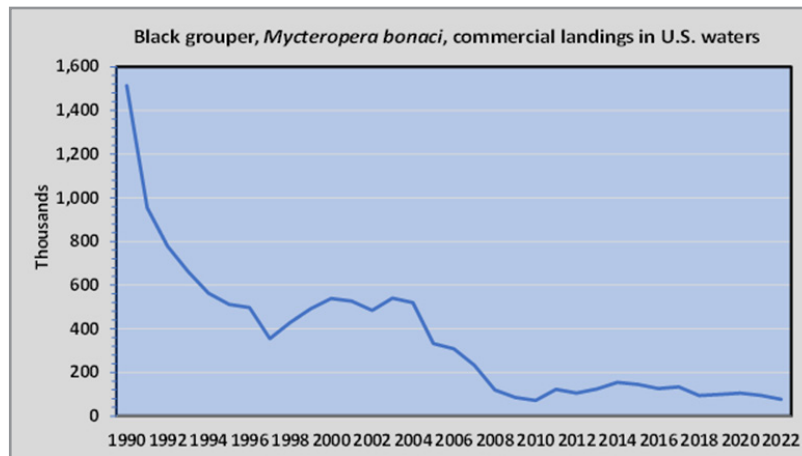


Figure 1: Commercial landings of black grouper, *Mycteroperca bonaci*, in U.S. waters. (Source: National Marine Fisheries Service, unpublished data)

Black grouper is one of the largest grouper species in the tropical and subtropical Western Atlantic, and though slow-growing, has a life expectancy of over 30 years [1-3]. Juveniles prefer relatively shallow reef patches or sand patches, ranging from 1 to 30m, while adults can be found at depths up to 100 meters [1, 4-6]. While juveniles feed primarily on crustaceans, adults feed on fish, crustaceans, and small squid, and are known to aggregate to feed in regions where their prey spawn.

As a protogynous species, black grouper transition from female to male, with length, age, and hierarchy contributing factors to sex transformation. Wild capture studies indicate that the transition from immature to mature female takes up to 5.2 years and occurs at a size of 82.6 cm. The most aggressive and dominant mature female will assume the role of alpha, after which she can transition to a male, usually at around 15.5 years of age or 121-121.4 cm total/fork length [1, 6].

Black grouper has seasonal reproduction, with peak gonad development during late winter and early spring. Spawning sites in Colombia showed temperatures of 20.5°C [7]. There are four color phases exhibited during natural courtship and spawning: blotched, white, dark, and white-headed, with the latter being more commonly seen in males during spawning [8].

Environmental cues of ecologically significant spawning grounds are critical triggers for spawning events [9]. Known to aggregate during the full moon, they would spawn after the last quarter moon. Natural spawning sites range in overall reef morphology but are almost always at greater depths. Courtship behavior is displayed in a harem by the male showing a white-head phase; he would follow a female to inspect her vent area at the posterior of the abdomen. The male's persistence in proximity would lead to success in courtship, as the pair would display a rapid vertical ascent in the water column to expel gametes for 3-5 minutes at dusk, then rejoin the harem below.

Spawning Simulation & Diet

Simulation of environmental conditions, such as lunar phases and depth, poses challenges for replication in recirculating aquaculture systems. Spawning tanks with the depth needed to replicate natural spawning events and the courtship ascension required to assist in releasing gametes into the water column are not achievable at the production scale currently in operation at the SMART center. Our preparations for winter spawning on a semi-commercial scale begin with temperature control. The simulation of the winter "season" begins with a 2-month simulated "summer" at 29°C to increase metabolism, followed by a steady decline towards a prolonged winter at 21°C. This period of controlled winter will help urge the brooding females into a state of egg production, and injectable hormone (GnRHa) treatments will be used to induce spawning in tandem with the natural spawning stimuli. Once gravid, females will be strip-spawned in-house, and in-vitro fertilization will be attempted for the first time with the *M. bonaci* species.

Alternating between diets for our in-house female broodstock, we observed an average growth rate of 0.16 mm/day since their initial acclimation to our system in early April 2022 (Figure 2). The broodstock maturation diet rotates among whole threadfin herring (*Opisthonema oglinum*), whole pink shrimp (*Penaeus duorarum*), and "maturation sausages" developed as a prototype alternative feed using manual processing techniques from a formulated dry powder (MadMac-MS Maturation Stimulator & Broodstock Conditioner 1) mixed with raw, ground seafoods. Batches of our maturation sausages include ground threadfin herring, pink shrimp, shortfin squid (*Illex illecebrosus*), and vitamin additives: New-Gar® Odorless Garlic Bulb Powder (*Allium sativum*) for appetite stimulation and Super B Complex (Vitamins: B1, B2, B6, B12, Niacinamide, Folate, & Dicalcium phosphate) for immune support. We have used garlic extract as an appetite stimulant since being awarded the project in 2020, with great success in increasing both feeding frequency and the average daily caloric intake per grouper.

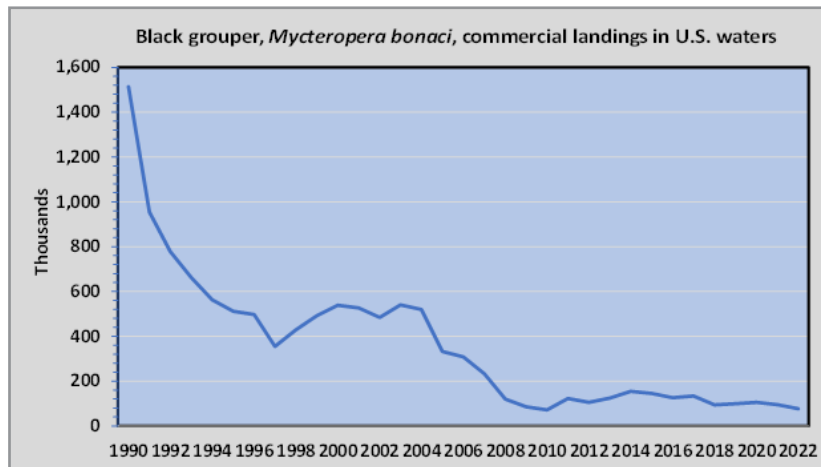


Figure 2. Seven female *M. bonaci* broodstock growth rates from delivery on 8 April, 2022, to 1 October, 2023. Broodstock identification is read as the first initial of their dorsal tag color, followed by their tank number (Example: Female with a pink tag in tank one is referred to as "P1")

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