GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, REPRODUCTIVE BEHAVIOR, EARLY LIFE HISTORY, AND COMPETITIVE INTERACTIONS BETWEEN RED SNAPPER, *LUTJANUS CAMPECHANUS,* IN THE NORTHERN GULF OF MEXICO

Except where reference is made to the work of others, the work described in this dissertation is my own or was done in collaboration with my advisory committee. This dissertation does not include proprietary or classified information.

_____
Carrie MacKichan Simmons

Certificate of approval:

_____	_____
Stephen T. Szedlmayer, Chair Professor Fisheries and Allied Aquacultures	Jack W. Feminella Professor Biological Sciences
_____	_____
John M. Grizzle Professor Fisheries and Allied Aquacultures	Michael J. Maceina Professor Fisheries and Allied Aquacultures

_____
George T. Flowers
Interim Dean
Graduate School

GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, REPRODUCTIVE BEHAVIOR, EARLY LIFE HISTORY, AND COMPETITIVE INTERACTIONS BETWEEN RED SNAPPER, *LUTJANUS CAMPECHANUS,* IN THE NORTHERN GULF OF MEXICO

Carrie MacKichan Simmons

A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama
August 9, 2008

GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, REPRODUCTIVE BEHAVIOR, EARLY LIFE HISTORY, AND COMPETITIVE INTERACTIONS BETWEEN RED SNAPPER, *LUTJANUS CAMPECHANUS,* IN THE NORTHERN GULF OF MEXICO

Carrie MacKichan Simmons

_____
Signature of Author

_____
Date of Graduation

DISSERTATION ABSTRACT

GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, REPRODUCTIVE BEHAVIOR, EARLY LIFE HISTORY, AND COMPETITIVE INTERACTIONS BETWEEN RED SNAPPER, *LUTJANUS CAMPECHANUS,* IN THE NORTHERN GULF OF MEXICO

Carrie MacKichan Simmons

Doctor of Philosophy, August 9, 2008
(M.S. Georgia Southern University, 2003)
(B.A. University of North Carolina at Asheville, 2000)

121 Typed Pages

Directed by Stephen T. Szedlmayer

Gray triggerfish, *Balistes capriscus*, is a widely distributed species, important to both commercial and recreational fisheries. Other species of Balistidae display atypical behaviors compared to most other marine fishes such as demersal spawning and unusually long periods in the plankton as juveniles. However, for gray triggerfish there is limited documentation on spawning behavior, early life history, and interactions with other species. To examine these aspects of gray triggerfish, spawning behaviors were recorded in June and July 2004 to 2007 on artificial reefs in the northern Gulf of Mexico by SCUBA diver observation and unattended remote video. A single male gray

triggerfish established a territory around a reef, built 1 to 13 demersal nests in the sand, and attracted 1 to 5 female gray triggerfish to spawn. Eggs were collected from 13 of the 28 active nests and mean clutch size was 772,415 eggs. Based on male to female sex ratios, gray triggerfish displayed harem spawning behavior, with a single dominant male who attracted up to five spawning-condition females. Second, gray triggerfish larval development was described from laboratory-reared fish up to 6 d post hatching. Photographs of live larvae ($N = 10$) were taken daily to document development. Third, recruitment of age-0 gray triggerfish to benthic artificial reefs was documented by diver surveys from 2003 to 2007. Divers counted and estimated sizes of all gray triggerfish that recruited to three types of artificial reefs ranging in area from 1.2 to 4.0 m^2. Peak recruitment of age-0 gray triggerfish occurred from September through December 2003-2007. Fourth, competitive interactions between gray triggerfish and red snapper were studied in field removal and laboratory growth experiments. In the field, gray triggerfish removal experiments were completed to test for effects on red snapper. After removals, size frequency distributions of red snapper were significantly different between triggerfish removed and triggerfish not removed treatments. In the laboratory, growth rates were compared between control red snapper and red snapper mixed with gray triggerfish. Red snapper showed a significantly slower growth rate when mixed with gray triggerfish compared to red snapper control. Field and laboratory studies provided evidence of competitive interactions between these species. These studies documented the ecology of gray triggerfish reproductive behavior, described larval development, seasonality and size of benthic recruitment, and competitive interactions with red snapper.

ACKNOWLEDGMENTS

These projects were funded by the Mississippi-Alabama Sea Grant Consortium (MASGC R/SP-14) NOAA award number NA06OAR4170078 and Marine Resources Division, Alabama Department of Conservation and Natural Resources. This study is a contribution of the Alabama Agricultural Experiment Station and Department of Fisheries and Allied Aquacultures, Auburn University. I would like to thank my advisor for his guidance, support, and inspiration while pursuing my degree at Auburn. We share a common interest for marine science and he guided me towards the pursuit of limitless research projects. I thank my committee, for ideas, input, corrections and comments on these projects and continuing to challenge me in the classroom and in my research pursuits. I would also like to thank Adrienne Beck, Sabrina Beyer, Allison Chapin, David Maus, Dianna Miller, David Nadeau, Rebecca Redman, Melanie Rhodes, Darin Topping, and Rebecca Wingate for help with all field collections, larval rearing, and laboratory maintenance. I thank my parents, grandparents, and in-laws for always being supportive and a source of inspiration throughout my life. They have always put me first and taken a limitless interest in me. A special thanks to my husband Lane Simmons, for spending numerous weekends helping me feed fish, maintain the seawater laboratory system, and weighing and measuring gray triggerfish specimens.

Style manual or journal used: Transactions of the American Fisheries Society

Computer software used: Corel WordPerfect X4, Microsoft Excel 2007, Statistical Analysis Software (SAS) 9.1, Adobe Illustrator 10.0.3, Image Pro V4.5, Sigma Plot 10.0, Adobe Photoshop 9.0, and ArcView

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

CHAPTER 1

TERRITORIALITY, REPRODUCTIVE BEHAVIOR, AND PARENTAL CARE IN GRAY TRIGGERFISH, *BALISTES CAPRISCUS,* ON ARTIFICIAL REEFS IN THE NORTHERN GULF OF MEXICO

Abstract.—Gray triggerfish, *Balistes capriscus*, spawning behavior was documented by SCUBA diver observation and unattended remote video. Spawning behaviors were recorded in June and July 2004 to 2007 in the northern Gulf of Mexico, on artificial reefs ranging in size from 0.60 to 107.1 m^3. Male gray triggerfish were morphologically distinguishable from females by larger size and dark charcoal coloration. One male gray triggerfish established a territory around a reef, built 1 to 13 demersal nests in the sand, and attracted 1 to 5 female gray triggerfish to spawn. Nests were randomly distributed around the reef, with a mean diameter of 53 cm, mean depth of 24 cm, and mean distance from the reef of 8.7 m. Male gray triggerfish spent significantly more time building and maintaining nests, whereas female gray triggerfish spent significantly more time inspecting the nest during prefertilization and courtship than any other behavior. One spawning event was recorded where a male and female gray triggerfish circled tightly in the nest and rapidly changed shades of gray. Fertilized eggs were found shortly after this spawning event in a single cohesive mass at the bottom of the nest. Active nests with a

guarding female and male were observed 28 times on 18 different reef sites. Eggs were collected from 13 of the 28 active nests with a guarding female. The mean number of eggs per clutch was 772,415. The females stayed almost continuously in the nest, frequently blowing and fanning the eggs. Postspawning, the male gray triggerfish displayed territoriality and patrolled an approximately 15 m diameter area surrounding the nest. Males were observed guarding up to three active nests at any one time, and they continually attacked and chased away other fish from the nesting area. Guarding male triggerfish were significantly larger than other male triggerfish. Based on male to female sex ratios, gray triggerfish displayed harem spawning behavior, with a single dominant male and up to five spawning-condition females.

Introduction

Triggerfish (Balistidae) have atypical spawning behavior compared to most marine fishes, such as forming harem groups, building demersal nests, and showing parental care of eggs (Fricke 1980; Blumer 1982; Gladstone 1994; Ishihara and Kuwamura 1996; Kuwamura 1997). Previous studies on triggerfish reproductive behavior have found several variations on a given behavior, sometimes switching the roles between sexes. For example, the male yellowmargin triggerfish*, Pseudobalistes flavimarginatus,* migrated to spawning sites, established territories, built demersal nests and attracted several females to spawn (Gladstone 1994). In contrast, the female yellow-spotted triggerfish, *Pseudobalistes fuscus*, builds demersal nests and enticed the male into the nest to spawn,

while chasing away other courting females, resulting in a 1:1 male to female spawning ratio (Fricke 1980).

Reproductive behavior of several species of triggerfish in the Indo-Pacific and Red Sea have been described (Fricke 1980; Gladstone 1994; Ishihara and Kuwamura 1996; Kuwamura 1997). However, limited information is available on reproductive behavior of the gray triggerfish, *Balistes capriscus*. Gray triggerfish is a widely distributed species found in both temperate and tropical waters. This species is found throughout the western and eastern Atlantic Ocean, as far north as Nova Scotia and as far south as Argentina. Gray triggerfish distribution also extends into the Gulf of Mexico (Briggs 1958; Moore 1967). This species is associated with artificial reef structures (Frazer and Lindberg 1994; Vose and Nelson 1994; Wilson et al. 1995; Kurz 1995) and natural hard-bottom substrate (Johnson and Saloman 1984; Vose and Nelson 1994).

Studies have been completed on reproductive biology and seasonality of gray triggerfish spawning. For example, off the coast of Ghana, Africa, gray triggerfish showed peak spawning in the warmer months of November and December (Ofori-Danson 1990). In the Gulf of Mexico, gray triggerfish are multiple-batch spawners, peaking during June and July, and with a 1:2 male to female ratio. Males and females both reached sexual maturity at 250 mm fork length (FL), but males were age-1 and females were age-2 (Wilson et al. 1995; Ingram 2001). My study provides the first quantitative description of territoriality, reproductive behavior, and parental care in gray triggerfish.

Methods

The study sites were 26-50 km south to southeast of Dauphin Island, Alabama, in the northern Gulf of Mexico (Figure 1-1). Artificial reefs were surveyed because natural reefs are rare in the northern Gulf (Parker et al. 1983). These artificial reefs are similar in size and were located at depths of 20 to 30 m, which allowed SCUBA divers adequate time to quantify behaviors of gray triggerfish.

A wide variety of artificial reefs were surveyed from 2004 to 2007. In October 2003, 16 artificial reefs were deployed 50 m apart, and consisted of steel cages (2.5 x 1.2 x 1.5 m) in the Hugh Swingle reef building zone (Chapin et al., in press). An additional 44 artificial reefs were built 1 km apart in the same area in April and May 2004. During June and July 2004, 45 of these artificial reefs were surveyed for spawning gray triggerfish. The search for spawning gray triggerfish was expanded by completing 63 surveys on 18 submersed army tanks (M60, 9.3 x 3.6 x 3.2 m) in June, July, and August 2005, and 82 surveys on 28 army tanks in June and July 2006. In 2007, 62 surveys were completed on 49 reefs in June and July, and spawning gray triggerfish were collected by spearfishing for later identification of sex and maturity level in the laboratory.

Divers recorded the number and estimated lengths of all gray triggerfish and videotaped the fish on each reef. If divers observed demersal nests in the sand and a gray triggerfish displaying courtship or prefertilization behaviors, a video camera (Sony TR101 Hi-8 video camera) was placed 1-2 m from the nest (nest between camera and reef structure) to record gray triggerfish behaviors for 30 to 60 min in the absence of divers.

The same methods were used to remotely videotape postfertilization behavior of females while on their nest and the behavior of the dominant male. When possible, divers measured all nests around a reef, recording the largest diameter, depth, distance from the reef, and compass bearing from the reef to the nest. Eggs and sediment samples were collected in plastic jars (8x5 cm) from active nests with a guarding female. Sediment samples were taken from the surface of the nest where the eggs were attached.

Egg clutches were removed from the nest and stored on ice for later laboratory analysis. Prior to placing the eggs in preservative (NOTOXhisto fixative, Scientific Device Laboratory, Glenview, Illinois), 10 eggs from each clutch (N = 9) were measured to the nearest 0.01 mm with an Olympus BH-2 compound microscope, Sony AVC-D7 video camera, and FlashPoint 128-4M digitizing board (Integral Technologies Inc. Indianapolis, Indiana), using Image Pro V4.5 software. Different egg clutches (N = 13) were weighed to the nearest 0.01 g with a Scout Pro balance (Ohaus, Pine Brook, New Jersey). For egg counts, five 0.054-g samples were taken from each clutch with a Ohaus portable advanced balance. Each egg sample was placed into a Ward Counting Wheel (Wildco, Buffalo, New York) and counted under a Leica MZ6 (Leica Microscopy Systems Ltd, Heerbrugg, Switzerland) dissecting scope at 10x magnification. Means of the five egg count samples were used to estimate number of eggs per clutch.

Depth, temperature, salinity, and dissolved oxygen were measured with a YSI 6920 meter (Yellow Springs Instruments, Yellow Springs, Ohio) at all sites where spawning was observed. Mean values of temperature, salinity, and dissolved oxygen were calculated from recordings taken from the bottom 3 m.

Behaviors were divided into prefertilization and postfertilization for both male and female gray triggerfish. The number of observations for each behavior was divided by total minutes of recorded video and then multiplied by 60 minutes to calculate number per hour.

Gray triggerfish were collected by SCUBA diver spearfishing for sex ratio estimation from 19 army tanks in August, October, and November 2005, and June to September 2006. In June and July 2007, gray triggerfish were collected from 26 artificial reefs for sex ratio estimation. These reef sites included: steel cages, concrete pyramids, a barge, concrete block reefs, and a gas pipeline covered with a concrete mat. Ten gray triggerfish were collected in the nest guarding eggs from 2004-2007 for sex identification; weight (kg), standard length (SL), fork length (FL), total length (TL), sex, and gonad weight (nearest 0.01 g) were recorded.

Substrate samples from active gray triggerfish nests were oven dried at 100-150°C for 24-72 h. Samples were then partitioned by sorting substrate through 2000, 1000, 500, 250, 106, and 63 μm sieves. Sieve sizes were based on the Wentworth grade limits (Buchanan and Kain 1971). After sieving the dried substrate, each portion was weighed to the nearest 0.01 g with a Scout Pro Ohaus balance (Ohaus, Pine Brook, New Jersey).

Prefertilization behaviors for males, i.e., nest maintenance, fish chasing, camera attacks, and circling in nest with female were analyzed separately with an analysis of variance (ANOVA). Female prefertilization behaviors, i.e., nest inspection, foraging, camera attacks, and female nest competition were also analyzed separately with an ANOVA. Postfertilization behaviors for males i.e., nest visits, fish chasing, and camera

attacks were analyzed separately with ANOVA. Female postfertilization behaviors i.e., blow and fan eggs, fish chasing, camera attacks, and foraging were analyzed separately with ANOVA. All tests in this study were considered significant at $\alpha = 0.05$. If significant differences were detected, a Student-Newman-Kuels test was used to show specific differences (Zar 1999). Male and female fork lengths (FL, mm) and weights (kg) were compared each year with a two-way ANOVA. Fork length (mm) and weight (kg) of dominant males guarding active nests were compared to other non-guarding males with an ANOVA. The length and weight of females on an active nest were compared to other females with a separate ANOVA. Sediment grades were analyzed with an ANOVA.

Results

Two hundred and fifty-two SCUBA diver surveys were completed on 153 different artificial reefs. Spawning behaviors were observed in June and July 2004 to 2007. Active nests with a guarding female and male were observed 28 times, on 18 different reef sites. Gray triggerfish were sexually dimorphic during the spawning season, males being identified by their large size, darker coloration, black fins, and aggressive behavior towards other fish and SCUBA divers. Other female gray triggerfish were distinguished from one another by size, external parasites and scars.

Prefertilization behaviors of gray triggerfish were the most difficult to document on the reef because I could not predict if actual fertilization followed. Prefertilization behaviors were observed once in 2004 and courtship was documented four times from 2004 through

2006. The following behaviors were defined for male gray triggerfish prefertilization: nest maintenance, fish chasing, camera attacks, and circling in the nest with a female. Nest maintenance was observed significantly more times than other male prefertilization behaviors (mean = 33.3/h, ANOVA: df = 3,8, F = 6.0, $P<0.05$; Figure 1-2), which included nest building by removing sediment with the mouth, blowing sand out of the nest, and lying laterally while in the nest and fanning with the dorsal and anal fins to excavate sand. The circling of a male and female in a nest was only observed one time (Figure 1-2). Gamete release and fertilization occurred shortly thereafter based on subsequent SCUBA divers observations of a female on a nest with eggs. This guarding female on a nest was not present prior to the circling behaviors. The dominant male was also observed chasing away other gray triggerfish.

Four prefertilization behaviors were documented for female gray triggerfish; these included nest inspection, foraging, camera attacks, and female nest competition. Female gray triggerfish spent significantly more time on nest inspection than other prefertilization behaviors (mean = 11.6/h, ANOVA: df = 3,12. F = 15.8, $P<0.001$; Figure 1-3). Females also were observed foraging near the nest and attacking the remote camera. Another behavior observed was two females circling in a nest, which was followed by one female chasing the other female from the nest and this was defined as nest competition.

During the 2006 spawning season, I returned to three artificial reefs over four separate surveys and found at least one active nest with the same dominant male. Postspawning, the male gray triggerfish displayed territoriality and patrolled a 15-m diameter area surrounding the nest. Males were observed guarding up to three active nests at a time.

Three behaviors were documented for male gray triggerfish postfertilization including nest visits, chasing fish, and camera attacks. Guarding male gray triggerfish displayed significantly more nest visit behavior than camera attacking behavior (mean = 16.1/h, ANOVA: df = 2,39, F = 3.8, $P<0.05$; Figure 1-4).

Similar to males, postfertilization females also were observed chasing other fish and attacking the camera. Postfertilization behavior restricted to females included blowing and fanning the eggs and foraging. Also, females were the only fish that stayed on the nest. Females spent significantly more time blowing and fanning the eggs than other recorded behaviors (mean = 113.9/h, ANOVA: df = 4,15, F = 17.6, P<0.001; Figure 1-5). Chasing patterns of females differed from males as they only made short, quick attacks (< 2 m) on other fish before returning to the nest. When females were observed searching for food by blowing in the sand, they were never observed greater than 1 m from the nest. In addition, no consumption of food was recorded in the prefertilization or postfertilization behaviors (Figure 1-5). Females displayed a distinct coloration while guarding the nest, i.e., a sharply contrasting white and black vertical bar pattern across the whole body.

Nests were distributed around the reef without any pattern. The maximum number of nests counted around a reef was 13, with a mean (± SE) of 4 ± 0.02 per reef during the spawning season. Mean (± SE) nest diameter was 53.1 ± 1.3 cm, mean (± SE) depth was 23.6 ± 0.6 cm, and mean (± SE) distance from the reef was 8.7 ± 0.4 m (N = 154). Eggs were deposited in a single cohesive mass at the bottom of the nest. Eggs were negatively buoyant, even when separated from the clutch. Large pieces of shell and sediment were

attached to the bottom of the egg mass. Mean (± SE) egg diameter was 0.62 ± 0.003 mm (N = 90) based on samples from nine different nests. Mean (± SE) clutch size was 772,415 ± 20,545 eggs (range: 420,486 to 1,371,409, N = 13). Eggs were observed hatching between 24 to 48 h after fertilization based on samples returned to the laboratory.

Gray triggerfish spawning was observed at a mean (± SE) depth of 23.7 ± 0.2 m (range:14.6 to 31.1 m, N = 21), a mean (± SE) temperature of 23.4 ± 0.1°C (range: 21.9-29.9°C, N = 21), a mean (± SE) salinity of 34.6 ± 0.1‰ (range: 29.8-36.8‰, N = 21) and a mean (± SE) dissolved oxygen concentration of 5.7 ± 0.7 mg/L (range: 4.8-6.8 mg/L, N = 16).

Nest sediments were 66% fine sand (250 μm), significantly higher than other size fractions (ANOVA: df = 5,162, F = 428, P<0.001; Figure 1-6). Other sediment types found in the nests were 9.3% coarse shell (2000 μm), 10.3% coarse sand (1000 μm), 9.6% medium sand (500 μm), and 4% very fine sand and silt (106 and 63 μm). When eggs were removed from the nest, several large pieces of shell were attached, but not included in the sediment grain size analysis.

Males were identifiable by their large size and dark charcoal coloration during the spawning season. When collecting gray triggerfish, divers identified what they assumed to be a male based on this color pattern, and collected these fish first. This color pattern identification of males was 100% accurate (N = 9). I failed to collect all gray triggerfish on three of the artificial reefs where an active nest with a guarding male was observed. On reef 10, based on the large size and dark coloration, the missed fish was considered

the dominant male (Table 1-1). Males were significantly heavier (ANOVA: df = 1, 288, F = 3.8, $P<0.001$) and longer (ANOVA: df =1, 288, F=4.8, $P<0.001$) than females. Males had a mean (± SE) weight of 0.60 ± 0.03 kg and mean (± SE) fork length of 282 ± 4.3 mm (N = 139). In contrast females had a mean (± SE) weight of 0.46 ± 0.02 kg and mean fork length (± SE) of 262 ± 3.2 mm (N = 156). A two-way ANOVA for gray triggerfish size differences by sex was significant ($P<0.001$), but there was no significant time ($P>0.1$), or time-sex interaction (ANOVA: df = 3, 284, F = 2.5, $P>0.05$). Mean male:female sex ratios at sites without spawning (lack of active nests) was 1:1.3 (N = 49 reefs). Harems (one male and several females) were found 50% of the time at sites with active nests (Table 1-1). All fish removed from active nests were females. Females on nests (N = 10) were not significantly different in length or weight than other females ($P>0.8$, N = 146). Females on active nests had a mean (± SE) weight of 0.44 ± 0.1 kg (range: 0.23 to 0.8 kg) and mean (± SE) fork length of 261 ± 13 mm (range: 215 to 320 mm). Territorial dominant guarding males were significantly larger with a mean (± SE) weight of 1.0 ± 0.2 kg and a mean (± SE) fork length of 337 ± 23.0 mm than other males on the reef with a mean (± SE) weight of 0.54 ± 0.03 kg and mean (± SE) fork length of 278 ± 4.2 mm (ANOVA: df = 1,137, F = 10.7, $P<0.01$).

Discussion

Past studies of gonadal somatic indexes (GSI) for male and female gray triggerfish supported our findings of spawning time when GSI peaked during June and July in the South Atlantic Bight and northern Gulf of Mexico (Wilson et al. 1995; Ingram 2001; Moore 2001). Gray triggerfish collected along coastal Alabama were sexually dimorphic, with males being significantly larger than females. Similar results were shown by Ingram (2001) for gray triggerfish in the Gulf of Mexico and by Moore (2001) in the Atlantic; however, in both previous studies, fish showed larger fork length than in my study. The larger sizes collected by Ingram (2001) and Moore (2001) were probably due to samples taken from fishers that tend to keep only the largest fish. Other triggerfish species have also shown size differences by gender including: redtoothed triggerfish, *Odonus niger*; blackbar triggerfish, *Rhinecanthus aculeatus*; halfmoon triggerfish, *Sufflamen chrysopterum*; and yellow-spotted triggerfish (Fricke 1980; Ishihara and Kuwamura 1996; Kuwamura 1997). Gray triggerfish built smaller nests, both in diameter and depth than other species of Balistidae, including the yellowmargin triggerfish. This pattern was expected because male gray triggerfish build nests primarily with their body and fins and are smaller than most other triggerfish (Lobel and Johannes 1980; Gladstone 1994).

Dominant male gray triggerfish also were identified by coloration and behavior. Typically, one dominant male established a territory around an artificial reef during the spawning season. After establishing a territory, the dominant male was observed building and maintaining several nests. This behavior also was shown in male yellowmargin

triggerfish maintaining between one to three nest sites (Gladstone 1994). Similar to gray triggerfish, prefertilization behaviors of the yellowmargin triggerfish included fish chasing and camera attacks (Gladstone 1994). The male halfmoon triggerfish also was observed chasing fish from his territory and the nest prior to spawning (Kawase and Nakazono 1992). Finally, a behavior rarely observed was the male and female gray triggerfish circling in the nest and rapidly changing colors. This behavior has not been described for any other species of Balistidae.

During courtship and prefertilization female gray triggerfish spent significantly more time inspecting nests, compared to other behaviors. Nest inspection behavior also has been observed in female yellowmargin triggerfish (Gladstone 1994). In my study, female gray triggerfish rarely showed foraging behavior. In contrast, the halfmoon triggerfish commonly showed feeding within the males territory or their own territory prior to spawning (Ishihara and Kuwamura 1996). The circling behavior in the nest shown by two female gray triggerfish has not been previously reported. This display may have been a mechanism to identify the sex of the other fish or perhaps establish dominance. During courtship, female yellow-spotted triggerfish also have been observed competing with other females for spawning access to a territorial male (Fricke 1980). Most other species of Balistidae did not show aggressive behavior by female fish; however, the studies were only based on diver observations. In my study, a remote video camera was used to record female competitive interactions, suggesting that these female interactions were inhibited by diver presence.

There were few reported estimates for the number of eggs per clutch for any species of triggerfish. Lobel and Johnannes (1980) estimated 430,000 eggs per clutch (N = 1) for yellowmargin triggerfish, and Kawase and Nakazono (1992) estimated that halfmoon triggerfish produced 132,800 eggs per clutch (N = 2). Both of these previous estimates mentioned above were lower than clutch size estimated for gray triggerfish in my study (mean number = 772,415 for mean fish size = 261 mm FL). Ingram (2001) also showed high fecundity for gray triggerfish ranging from 96,379 to 2,649,027 oocytes for mean fish size of 326 mm FL.

Parental care of the eggs was displayed by both male and female gray triggerfish. The dominant male continually patrolled around the reef, visited the active nests, chased away other fish, and attacked the camera. These males also were observed building more nests and courting other females. Similar behaviors were shown for the male redtoothed and yellowmargin triggerfish (Fricke 1980; Gladstone 1994).

All gray triggerfish collected on nests were females. In all other species of triggerfish, females also have been the primary fish on the nest (Fricke 1980; Kawase and Nakazono 1992; Gladstone 1994; Ishihara and Kuwamura 1996; Kuwamura 1997). Female gray triggerfish spent most of their time fanning and blowing the eggs, rarely moving off the nest. Most of the time females only moved off the nest to chase other fish or attack the camera but always quickly returned to the nest. Female gray triggerfish were recorded chasing potential egg predators from the following families: Lutjanidae, Labridae, Haemulidae, and Serranidae. On a few occasions, females were observed swimming rapidly off the nest up to the guarding male, but quickly retreated back to the nest after

recognition of the dominant male. This behavior was different than the female yellow-spotted triggerfish, which was observed specifically chasing the dominant male triggerfish from the nest (Fricke 1980).

Female gray triggerfish were rarely observed leaving the nest because of SCUBA divers, competition, or potential predators, except on one occasion recorded on the remote video. The female quickly left the nest for shelter in the artificial reef (army tank) when a 2-m sandbar shark, *Carcharhinus plumbeus,* passed directly over the nest. After the shark passed she quickly returned to the nest; 3 min was the longest time period that the female was observed off an active nest. The redtoothed triggerfish was not as diligent about attending the nest, often leaving eggs and swimming up in the water column for short periods, and also visiting her sleeping site during the day (Fricke 1980). Female gray triggerfish were rarely observed foraging while guarding eggs, unless foraging was less than 1 m from the nest. The postfertilization behaviors of the female halfmoon triggerfish were similar to the female gray triggerfish except that they foraged more often and further from the nest (Kawase and Nakazono 1992). On two separate diver surveys the female gray triggerfish was observed repeatedly blowing on the eggs; subsequent collection of the eggs showed that they were actively hatching. This behavior also has been recorded by the halfmoon triggerfish for dispersion of the larvae (Kawase and Nakazono 1992). In fact, the female yellow-spotted triggerfish blows on the nest site so vigorously to disperse the hatching larvae that the nest pit is nearly flattened (Fricke 1980).

Gray triggerfish were observed spawning at temperatures ranging from 21.9 to 29.9°C, which suggested a broader spawning temperatures than other species. The masked triggerfish, *Sufflamen fraenatum,* and the yellow-spotted triggerfish were observed spawning at temperature ranges of 24 to 27.8°C (Fricke 1980; Kawabe 1984). The halfmoon triggerfish was observed spawning in warmer water temperatures from 26.6 to 28.8°C (Ishihara and Kuwamura 1996). The mean temperature observed for gray triggerfish spawning was comparable to other species. However, I believe that the maximum temperature of 29.9°C observed during spawning was above the suitable range. This maximum temperature was observed in late July towards the end of the spawning season. The eggs found at this temperature never hatched and microscopically they appeared deformed.

Gray triggerfish spawned at greater depths compared to most other triggerfish. Almost all other triggerfish were observed spawning in depths ranging from 1 to 8 m (Fricke 1980; Lobel and Johannes 1980; Ishihara and Kuwamura 1996; Kumamura 1997; Gladstone 1994). Kawase and Nakazono (1992) observed deeper spawning for the halfmoon triggerfish in 10 to 13 m of water. Gray triggerfish in my study spawned in a broad range of depths from 14.6 to 31.1 m. Gray triggerfish could potentially spawn in deeper depths, but SCUBA diving limits prevented us from exploring any deeper. These fish could also spawn shallower, but artificial reefs shallower than 14.6 m were not examined.

Significantly more fine sand (250 μm) than the other sediment size fractions was taken from active nests and was consistent with the Mississippi-Alabama sand sediment

information documented by Ludwick (1964). Other triggerfish species, i.e., halfmoon, orange-lined (*Balistapus undulatus*), and redtoothed also showed a preference for attaching their eggs to sand and some even mixed the eggs with sand (Kawase and Nakazono 1992; Ishihara and Kuwamura 1996). Yellowmargin triggerfish also laid their eggs in sandy nests, with the slight variation of lining the nests with pieces of coral rubble (Gladstone 1994). They also appeared to weigh down egg masses with small pieces of coral rubble (Lobel and Johannes 1980). In my study, gray triggerfish were never observed using coral rubble or stones in nests. However, coarse shell was attached and mixed with the egg mass.

When active nests were found, harems were observed 50% of the time. There were several possibilities why harems were not observed on all spawning sites. One reason may be that guarding males were unable to exclude all other males from the reef. On one survey the dominant male was observed chasing another smaller gray triggerfish away from the reef. However, this smaller fish would soon return only to be chased again. This smaller fish was probably a male, because several other gray triggerfish present on the same reef, probably females, were not chased.

Among species of triggerfish where reproductive behavior has been recorded, the yellow-spotted triggerfish is the only species that did not display a harem mating system; instead, males were defined as successively polygynous (Fricke 1980). This mating system was defined as the male mating with one female at a time sequentially (Fricke 1980).

Fricke (1980) suggested that environmental factors probably control whether a fish is monogamous (one male mates with one female in a breeding season) or polygynous (a male fertilizes the eggs of several females in a spawning season) based on finding and establishing a territory and attracting enough mates (Alcock 1998). Ishihara and Kuwamura (1996) found that halfmoon triggerfish can be monogamous or polygynous based on size of the male and location of their territory. These variations in spawning behavior also may occur for gray triggerfish. For example, reef number 6 had an active nest with only one male and one female, potentially the male was unable to attract another female (Table 1-1).

An important aspect of gray triggerfish spawning behavior that remains unresolved is whether spawning sites on particular artificial reefs are specific. Other species of triggerfish including the redtoothed and yellowmargin triggerfish move to spawning grounds (Fricke 1980; Gladstone 1994). In my study, during observation of postfertilization behaviors of gray triggerfish in 2006, several surveys were completed on the same reef and the same dominant male (with recognizable scars and damaged fins) was observed spawning up to four times. The continued presence of this particular male and appearance of new females suggests male territoriality, attraction of new females, and harem spawning as observed in other triggerfish (Fricke 1980; Gladstone 1994).

Egg size of gray triggerfish was small (mean = 0.62 mm) and similar to other species of triggerfish including the masked triggerfish, halfmoon triggerfish, yellowmargin triggerfish, and orange-lined triggerfish (Lobel and Johannes 1980; Kawabe 1984; Kawase and Nakazono 1992; Gladstone 1994). Parental care is not typically observed in

species with small egg size (Shine 1978; Gross and Sargent 1985; Sargent et al. 1987; Nassbaum and Schultz 1989). An explanation for smaller eggs produced by gray triggerfish and increased parental care may be from environmental factors (Shine 1978; Nassbaum and Shultz 1989). One of these limitations might be oxygen, as small eggs with a higher surface area to volume ratio would be more suited to a low oxygen environment that might result from excavating nest sites in deeper continental shelf habitats. In support of this contention, the female gray triggerfish was observed fanning and blowing eggs postfertilization more than any other behavior. In other species such as the green bubble goby, *Eviota prasina,* the male fish also provides parental care by fanning the eggs (Karino and Arai 2006). Studies of female mate preference and hatching success found that the time male gobies spent fanning eggs was the most important factor affecting survival of eggs, and fanning occurred at a higher frequency for larger clutches (Karino and Arai 2006).

One male gray triggerfish established dominance on an artificial reef and built 1 to 13 demersal nests in the sand to attract females to spawn. Females inspected the nests and continuously chased fish from the spawning site prior to fertilization. Gray triggerfish displayed parental care of the eggs with the female aerating the eggs by fanning and blowing, only swimming off the nest to chase egg predators away. The dominant male defended his territory and guarded 1 to 3 nests from potential egg predators. Gray triggerfish were observed forming harems. This mating system and display of parental care is consistent with other species in the family Balistidae. These observations of elaborate courtship, parental care, and territory defense are typically shown in coral reef

fishes (Mumby and Wabnitz 2002), but are unusual for marine fish species found on artificial reefs in the northern Gulf of Mexico, and provide new information on the ecology of marine fishes from this system.

References

Alcock, J. 1998. Animal Behavior. 6th edition. Sinauer Associates, Sunderland, Massachusetts.

Blumer, L.S. 1982. A bibliography and categorization of bony fishes exhibiting parental care. Zoological Journal of the Linnean Society 76: 1-22.

Briggs, J.C. 1958. A list of Florida fishes and their distribution. Bulletin of Florida State Museum, Biological Sciences 2: 222-319.

Buchanan, J.B., and J.M. Kain. 1971. Measurement of the physical and chemical environment. In: Holme NA, McIntyre AD (eds) Methods for the study of marine benthos, 1st ed. Blackwell, Oxford and Edinburgh.

Chapin, A.M., S.T. Szedlmayer, and R.P. Phelps. (in press) Survival, movement, and residency of hatchery-reared red snapper on artificial reefs in the northern Gulf of Mexico. Fisheries Management and Ecology.

Frazer, T.K., and W.J., Lindberg. 1994. Refuge spacing similarly affects reef-associated species from three phyla. Bulletin of Marine Science 55: 388-400.

Fricke, H.W. 1980. Mating systems, maternal and biparental care in triggerfish (Balistidae). Zeitschrift fur Tierpsychologie 53: 105-122.

Gladstone, W. 1994. Lek-like spawning, parental care and mating periodicity of the triggerfish *Pseudobalistes flavimarginatus* (Balistidae). Environmental Biology of Fishes 39: 249-257.

Gross, M.R., and R.C. Sargent. 1985. The evolution of male and female parental care in fishes. American Zoologist 25: 807-822.

Ingram, G.W. Jr. 2001. Stock structure of gray triggerfish, *Balistes capriscus*, on multiple spatial scales in the Gulf of Mexico. PhD Dissertation, University of South Alabama, Mobile, Alabama.

Ishihara M., and T. Kuwamura. 1996. Bigamy or monogamy with maternal egg care in the triggerfish, *Sufflamen chrysopterus*. Ichthyological Research 43: 307-313.

Johnson, A.G., and C.H. Saloman. 1984. Age, growth, and mortality of gray triggerfish, *Balistes capriscus*, from the northeastern Gulf of Mexico. Fishery Bulletin 82: 485-492.

Karino K., and R. Arai. 2006. Effect of clutch size on male egg-fanning behavior and hatching success in the goby, *Eviota prasina* (Klunzinger). Journal of Experimental Marine Biology and Ecology 334: 43-50.

Kawabe, R. 1984. Spawning behavior of the bridled triggerfish, *Sufflamen fraenatus*, in the aquarium. Japanese Journal of Ichthyology 31:193-197.

Kawase, H., and A. Nakazono. 1992. Reproductive Behavior of the flagtail triggerfish, *Sufflamen chrysopterus*. Proceedings of the Seventh International Coral Reef Symposium 2: 905-907.

Kurz, R.C. 1995. Predator-prey interactions between gray triggerfish (*Balistes capriscus* Gmelin) and a guild of sand dollars around artificial reefs in the northeastern Gulf of Mexico. Bulletin of Marine Science 56: 150-160.

Kuwamura, T. 1997. Evolution of female egg care in haremic triggerfish, *Rhinecanthus aculeatus.* Ethology 103:1015-1023.

Lobel, P.S., and R.E. Johannes. 1980. Nesting, eggs and larvae of triggerfishes (Balistidae). Environmental Biology of Fishes 5: 251-252.

Ludwick, J.C. 1964. Sediments in Northeastern Gulf of Mexico. In: Miller RL (ed) Papers in Marine Geology: Shepard Commemorative volume, 1920-edition. Macmillan, New York.

Moore, D. 1967. Triggerfishes (Balistidae) of the Western Atlantic. Bulletin of Marine Science 17: 689-722.

Moore, J.L. 2001. Age, growth and reproductive biology of the gray triggerfish (*Balistes capriscus*) from the southeastern United States, 1992-1997. MS Thesis, University of Charleston, Charleston, South Carolina.

Mumby, P.J., and C.C.C. Wabnitz. 2002. Spatial patterns of aggression, territory size, and harem size in five sympatric Caribbean parrotfish species. Environmental Biology of Fishes 63: 265-279.

Nassbaum, R.A., and D.L. Schultz. 1989. Coevolution of parental care and egg size. American Naturalist 133: 591-603.

Ofori-Danson, P. K. 1990. Reproductive ecology of the triggerfish, *Balistes capriscus* from the Ghanaian coastal waters. Tropical Ecology 31: 1-11.

Parker, R.O., D.R. Colby, and T.D. Willis. 1983. Estimated amount of reef habitat on a portion of the U.S. South Atlantic and Gulf of Mexico continental shelf. Bulletin of Marine Science 33: 935-940.

Sargent, R.C., P.D. Taylor, and M.R. Gross. 1987. Parental care and the evolution of egg size in fishes. American Naturalist 129: 32-46.

Shine, R. 1978. Propagule size and parental care the "safe harbor" hypothesis. Journal of Theoretical Biology 75: 417-424.

Vose, F.E., and W.G. Nelson. 1994. Gray triggerfish (*Balistes capriscus* Gmelin) feeding from artificial and natural substrate in shallow Atlantic waters of Florida. Bulletin of Marine Science 55: 1316-1323.

Wilson, C.A., D.L. Nieland, and A.L. Stanley. 1995. Age, growth, and reproductive biology of gray triggerfish, *Balistes capriscus*, from the Northern Gulf of Mexico commercial harvest. MARFIN Final Report. Louisiana State University, Baton Rouge, Louisiana.

Zar, J. H.1999. Biostatistical Analysis. 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

TABLE 1-1.—Sex ratios of all gray triggerfish on a reef when an active nest and guarding male were observed. The asterisk indicates that not all gray triggerfish were collected from that reef. Two fish were missed from a concrete pyramid (8) and one fish was missed from each concrete block reef (9 and 10).

Reef number	Reef type	Reef size (m)	Males	Females
1	army tank	9.3 x 3.6 x 3.2	1	4
2	concrete pyramid	3.7 x 3.7 x 3.1	1	4
3	steel cage	2.5 x 1.2 x 1.5	1	3
4	steel cage	2.5 x 1.2 x 1.5	1	5
5	concrete pyramid	3.7 x 3.7 x 3.1	1	5
6	army tank	9.3 x 3.6 x 3.2	1	1
7	army tank	9.3 x 3.6 x 3.2	2	4
8	concrete pyramid	3.7 x 3.7 x 3.1	10	8 *
9	concrete block reef	1.2 x 1.2 x 0.4	3	2 *
10	concrete block reef	1.2 x 1.2 x 0.4	0	2 *

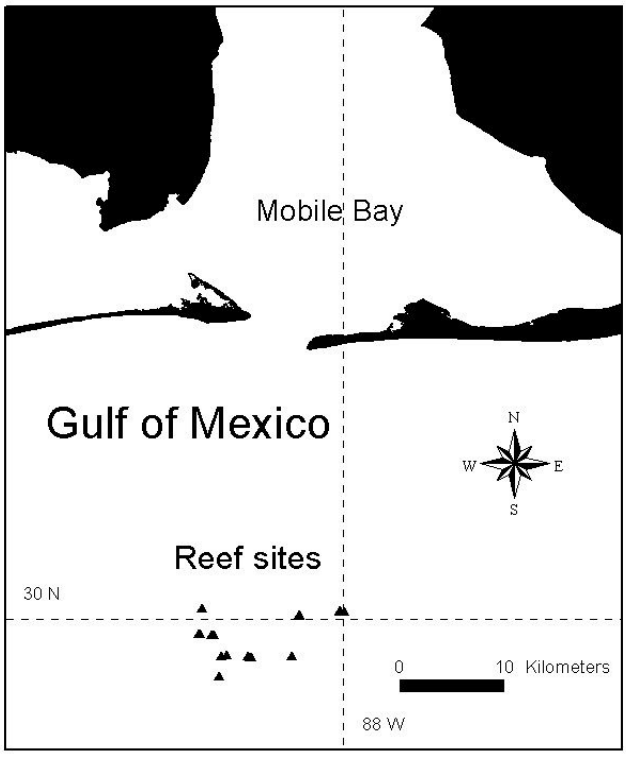


FIGURE 1-1.—Study location in the northern Gulf of Mexico. Black triangles are reef sites.

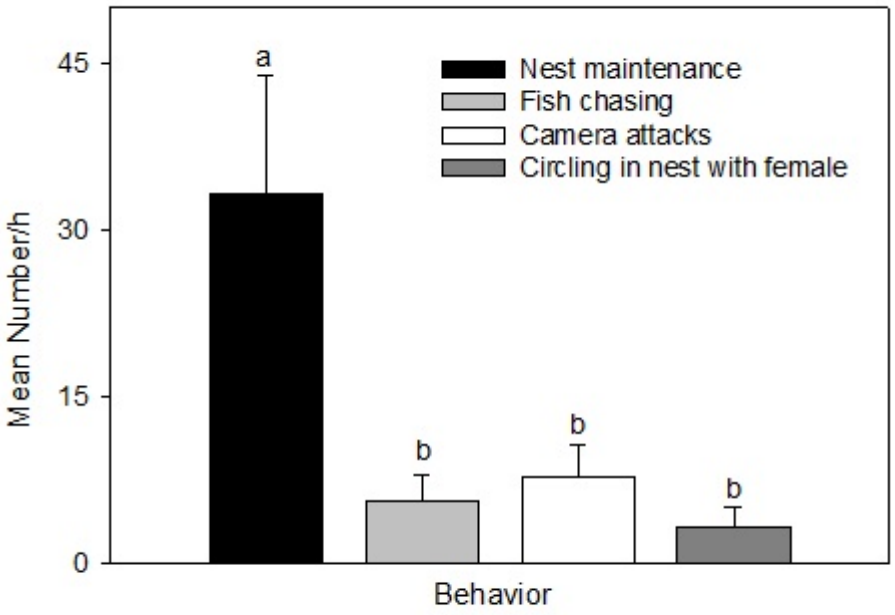


FIGURE 1-2.—Courtship and prefertilization behaviors of male gray triggerfish (N = 4). Error bars represent standard error. Significant differences ($\alpha = 0.05$) are shown by different letters.

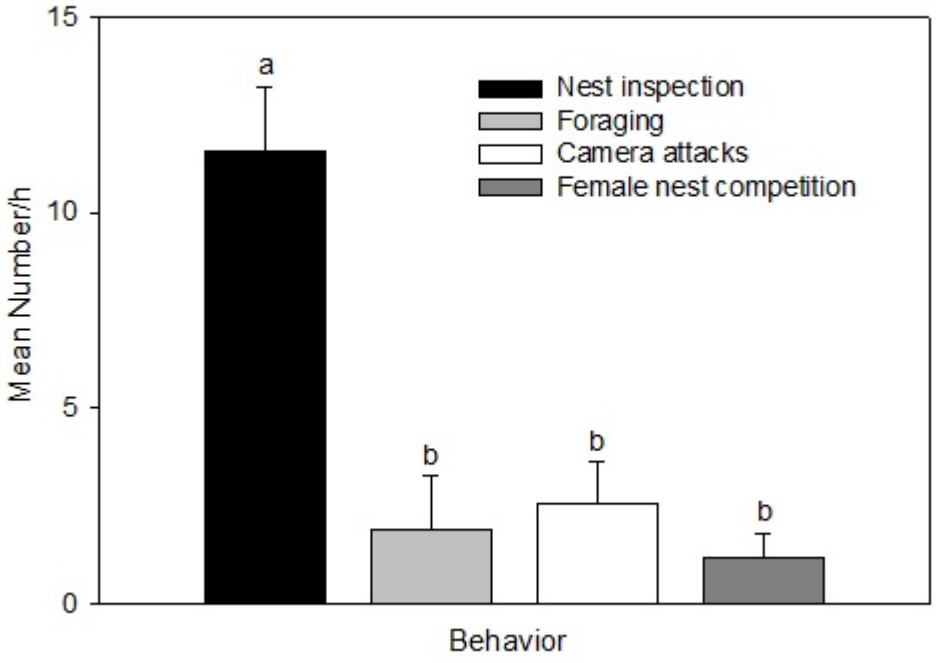


FIGURE 1-3.—Courtship and prefertilization behaviors of female gray triggerfish (*N* = 4). Error bars represent standard error. Significant differences (α = 0.05) are shown by different letters.

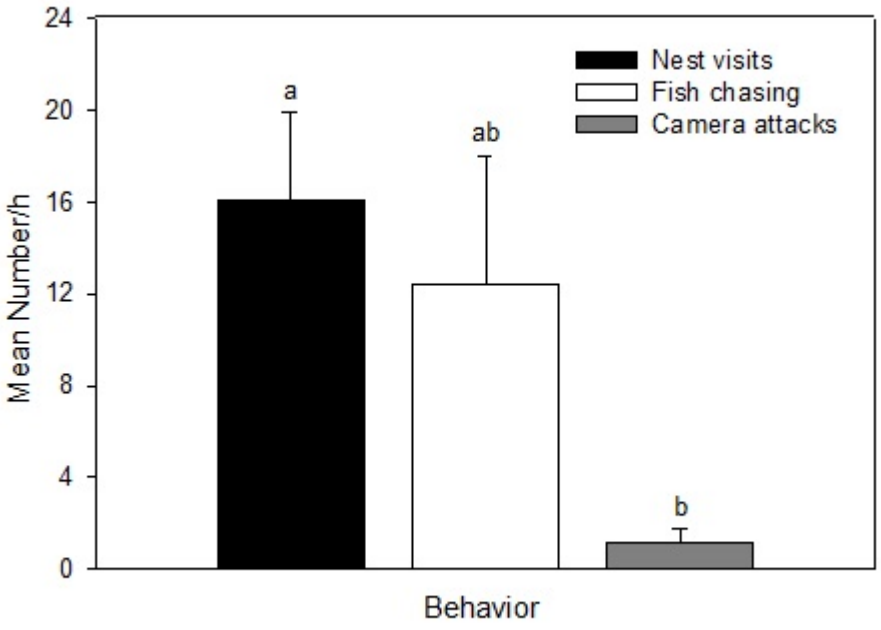


FIGURE 1-4.—Postfertilization behaviors of guarding male gray triggerfish (N = 14). Error bars represent standard error. Significant differences ($\alpha = 0.05$) are shown by different letters.

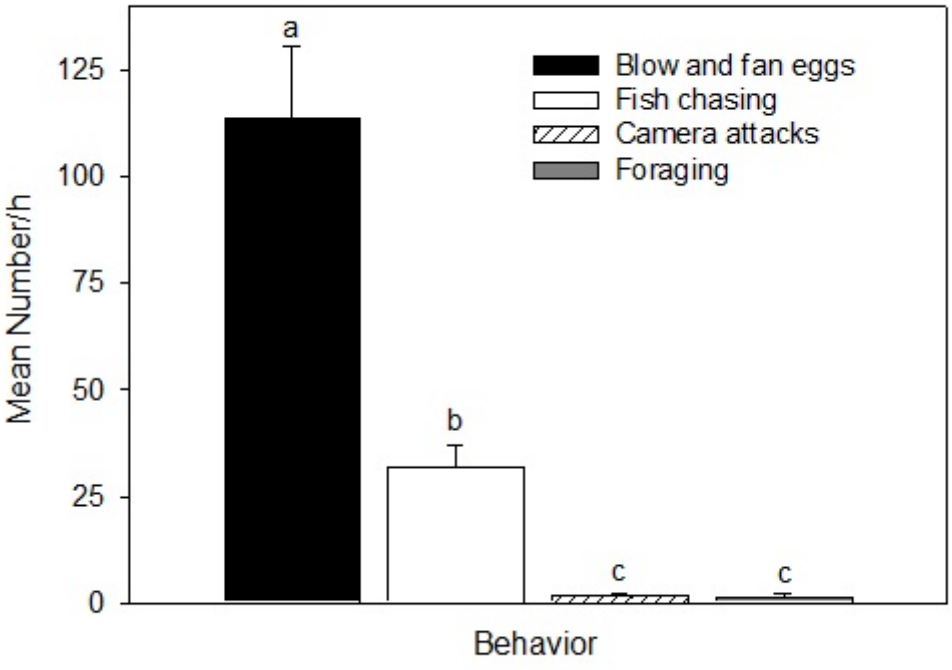


FIGURE 1-5.—Postfertilization behaviors of female gray triggerfish (N = 16) on active nests. Error bars represent standard error. Significant differences ($\alpha = 0.05$) are shown by different letters.

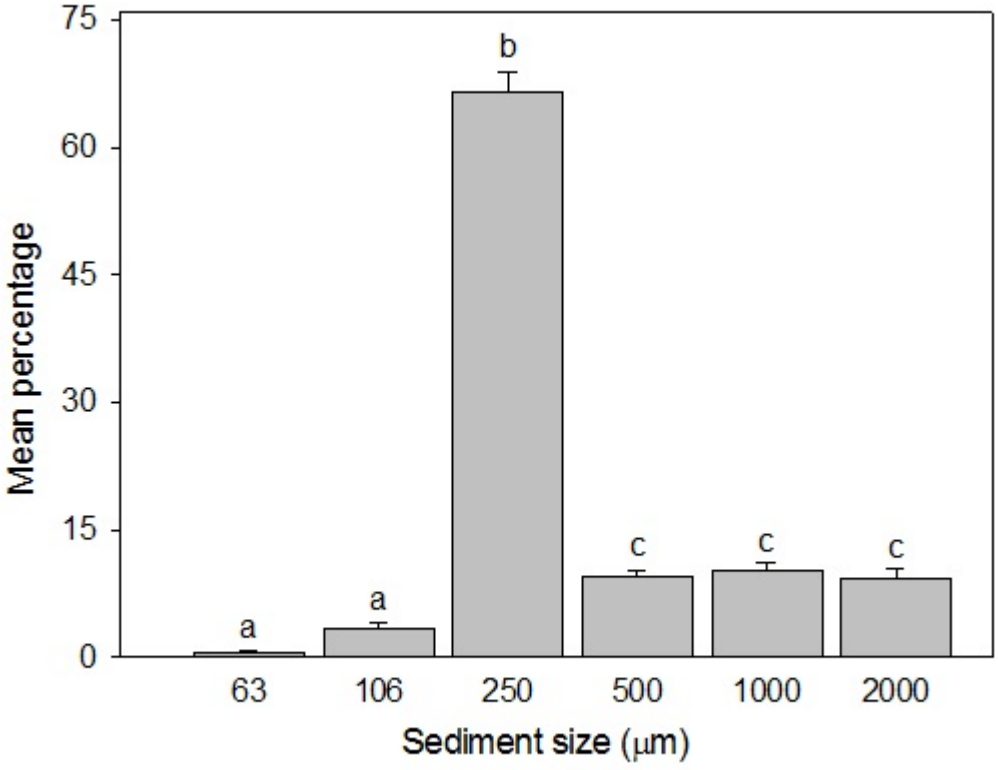


FIGURE 1-6.—Sediment size from active gray triggerfish nests (N = 28). Error bars represent standard error. Significant differences ($\alpha = 0.05$) are shown by different letters.

CHAPTER 2

DESCRIPTION OF LARVAL GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, FROM THE NORTHERN GULF OF MEXICO

Abstract. —Little descriptive information is available on larval gray triggerfish, *Balistes capriscus*, particularly those less than 3 mm notochord length (NL). Gray triggerfish eggs were collected from an active nest with a guarding female and were reared until 6 d old in the laboratory. Photographs of live larvae (N =10) were taken each day and morphometric measures were taken from digitized images. Illustrations of larvae were completed using photographs of the live larvae as well as preserved specimens for placement of melanophores. Gray triggerfish eggs had a mean (± SD) diameter of 0.62 ± 0.03 mm. Larvae hatched (day 0) at a mean size of 2.2 mm NL, without pigmented eyes, no functional mouth, and 20 myomeres. On day 1, larvae had the same number of myomeres. Melanophores were visible on the anterior and dorsal margin of the eye. On day 2, eyes became completely pigmented and a coiled gut was visible. Melanophores were visible on the ventral portion of the myomeres starting on the third myomere posterior to the anus. On day 3, melanophores on ventral portion of the myomeres were visible on the first myomere posterior to the anus. On day 4, the yolk sac was greatly reduced. Melanophores were numerous on the dorsal and anterior margin of the

peritoneal cavity and formed a continuous line. On day 5, one large melanophore was visible on the dorsal side of the posterior end of the notochord. Four distinct melanophores were visible on the hindbrain, but only two from the lateral view. The yolk sac and oil globule were no longer visible. On day 6, the melanophores on the hindbrain were not visible as separate melanophores as they were on day 5, instead one large, pigmented area was visible in 67% of the larvae examined. This early description of laboratory-reared gray triggerfish will aid in the identification of wild-caught larvae of this species.

Introduction

Gray triggerfish, *Balistes capriscus*, are found throughout the Gulf of Mexico and the Eastern and Western Atlantic (Briggs 1958; Moore 1967). Gray triggerfish can be found on both artificial and natural reefs (Johnson and Saloman 1984; Frazer and Lindberg 1994; Vose and Nelson 1994; Wilson et al. 1995; Kurz 1995). Spawning occurs from May through July in the Gulf of Mexico and South Atlantic Bight, and males mature at age-1 and females age-2, when they are 250 mm fork length (FL) (Wilson et al. 1995; Ingram 2001; Moore 2001). Gray triggerfish have an atypical spawning behavior compared to most other marine fishes because they form harem groups and provide parental care of the eggs for 24-48 h after spawning (MacKichan and Szedlmayer 2007).

Gray triggerfish larvae have been collected by plankton tows and described in several studies (Matsuura and Katsuragawa 1981, 1985; Lyczkowski-Shultz and Ingram 2005).

However, past descriptions were of preserved larvae and most larvae were greater than 3 mm notochord length (NL). Lyczkowski-Shultz and Ingram (2005) found few larval gray triggerfish less than 3 mm NL in the water column. Similarly, a study completed off the coast of Brazil did not find any gray triggerfish that were smaller than 3 mm NL that could be positively identified (Matsuura and Katsuragawa 1981, 1985). Therefore, little information is available on larval gray triggerfish after hatching. In my study, gray triggerfish were reared for 6 d after hatching and used to describe larval development.

Methods

Eggs were collected from gray triggerfish nests by SCUBA divers and placed in 3.8-L plastic bags underwater. At the surface eggs were placed into aerated coolers at 20 to 21°C, slightly cooler than the bottom water temperature, and transported to the laboratory. Larval rearing was attempted six times from 2004 through 2006 spawning season. Several different methods were attempted to rear gray triggerfish larvae in circular polyvinyl chloride (PVC) 200-L containers. All larval rearing was completed in a closed system, with gentle aeration. Larvae from three different gray triggerfish egg masses were reared in a controlled system, with temperature maintained between 21 to 22.5°C and salinity constant at 33‰. In 2006, one rearing attempt was completed outdoors in 200-L containers, where temperature ranged from 27 to 29°C and salinity was 33 to 34‰. Stocking density for all rearing trials ranged from 1,000 to 1,500 larvae/L. Live wild zooplankton were collected with two 63-µm plankton nets every 2 d. Larvae

were fed all zooplankton within the 63- to 150- µm size fraction. Two attempts were made to rear gray triggerfish larvae in 4,490-L tanks. Fish were stocked at 1,133 larvae/L and 12.5 L of *Isochrysis* algae was added. Salinity was maintained at 29‰ and temperatures ranged from 26 to 27.8°C. Cyclopoid copepod naupli were stocked at 2000 naupli/L as supplemental food.

Photographs were taken of live eggs and larvae each day. Digital photographs of larvae and a micrometer were taken with an Olympus BH-2 compound microscope with 4 and 10x objective lens, Sony AVC-D7 video camera, and FlashPoint 128-4M digitizing board (Integral Technologies Inc., Indianapolis, Indiana). Digitized egg and larval images were measured (nearest 0.01 mm) using Image Pro V4.5 software. Measurements of 10 larvae were taken each day and included: (1) total length (TL) = tip of snout to end of tail; (2) snout length (SL) = tip of snout to anterior margin of the eye; (3) notochord length (NL) = tip of the snout to end of the notochord; (4) oil globule in the yolk sac (Oil) = largest horizontal measure from the anterior to the posterior margin of the oil globule; (5) yolk sac (YS) = largest horizontal measure from the anterior to the posterior margin of the yolk sac; (6) eye diameter (ED) = largest horizontal measure from the anterior to the posterior margin of the eye; (7) lower jaw length (JL) = tip of the lower jaw to posterior margin of the jaw. Identification of the measured parts listed above and description followed Matsuura and Katsuragawa (1981), Kimmel (1993), Moser et al. (1984), and Moser (1996). Illustrations of the larvae were made each day using the digitized images. For pigment location, preserved larvae were examined using a Leica MZ6 (Leica

Microscopy Systems Ltd, Heerbrugg, Switzerland) dissecting scope and compared with several (3-5) digitized images of live larvae.

Results

Mean (± SD) egg diameter was 0.62 ± 0.03 mm (N = 90) based on samples from nine different nests (Figure 2-1). Gray triggerfish eggs hatched from 24 to 48 h after fertilization based on samples brought back to the laboratory. Mean morphometric characters of larvae from hatching through day 6 are shown in Table 2-1.

Day 0 (Figure 2-2a)

The mean TL (± SD) of gray triggerfish larvae at hatching was 2.27 ± 0.04 mm (Table 2-1). Larvae hatched without pigmented eyes or functional mouth. At day 0, there were 20 myomeres, with four to five pre-anus, and 15 to 16 post-anus, and the otoliths were visible. Nine to twelve melanophores were visible on the anterior portion of the oil globule and yolk sac, with 4-5 melanophores on the gut ventral to the myomeres. One to two melanophores were visible on the dorsal portion of the yolk sac. The forebrain, midbrain, and hindbrain were visible, with one melanophore on the forebrain and one to two melanophores on the midbrain (Figure 2-2a).

Day 1 (Figure 2-2b)

At day 1, mean TL (± SD) was 2.32 ± 0.02 mm. There were 20 myomeres, four to five pre-anus and 15 to 16 post-anus. The pectoral fins and otoliths were visible, but pectoral fins were not included in illustrations. Eyes were partially pigmented, with melanophores spreading from the anterior and dorsal portion of eye towards the ventral margin of the eye. Heart and gills were visible but not included in illustrations. Many overlapping melanophores were visible on the anterior margin of the yolk sac and oil globule. The gut was more visible as the yolk sac decreased in size. Two melanophores are present on the ventral side of the gut, adjacent to the yolk sac. Two large melanophores were visible on the forebrain and the midbrain had one large melanophore (Figure 2-2b).

Day 2 (Figure 2-3a)

At day 2, mean TL (± SD) was 2.34 ± 0.08 mm. The number of myomeres did not change from day 1. The heart and gills were visible but not included in illustrations. The first one or two myomeres posterior to the anus did not have melanophores, but the more posterior myomeres each had one ventral melanophore. Eyes were completely pigmented. The gut was coiled. The dorsal margin of the peritoneal cavity, just ventral to the myomeres had numerous melanophores that formed a continuous line. Posterior to the eye, across the otoliths and into the hindbrain were numerous melanophores that formed a short line. The lower jaw was visible. Pectoral fins were visible as they continue to grow longer but were not included in the illustration. The midbrain had two single distinct melanophores. The yolk sac and oil globule had larger, more numerous

melanophores on the anterior margin. In addition, on the posterior margin of the yolk sac there were two to three melanophores, anterior to the coil in the gut (Figure 2-3a).

Day 3 (Figure 2-3b)

At day 3, mean TL (± SD) was 2.34 ± 0.02 mm. The number of myomeres did not change. The heart, gills, and otoliths were visible, but the heart and gills were not included in the illustration. Eye pigment did not change. The melanophores on the ventral portion of the myomeres were visible on the first myomere posterior to the anus. Large single melanophores were present on the five myomeres posterior to the anus, followed by two melanophores on the next seven myomeres, followed by single melanophore on the next three myomeres. Three to four melanophores were also located on the ventral side of the notochord at the posterior end. The forebrain lobes became more distinct and larger just anterior to the eye. The yolk sac decreased in size and melanophores on the anterior portion of the yolk sac were so numerous that they were not visible as separate melanophores. The pectoral fins were longer, but not included in the illustration. The gut was larger and melanophores formed a continuous line on the dorsal portion of the peritoneal cavity just ventral to the myomeres. The lower jaw became more prominent and projected anteriorly. The midbrain had one and the hindbrain had two distinct melanophores (Figure 2-3b).

Day 4 (Figure 2-4a)

At day 4, mean TL (± SD) was 2.27 ± 0.06 mm. The number of myomeres did not change and the otoliths were visible. The heart and gills were visible, but not included in the illustration. Eye pigment did not change. The melanophores on the ventral portion of the myomeres formed a continuous line to the end of the notochord. The hindbrain was more developed. The yolk sac was smaller and more difficult to distinguish from the oil globule. The yolk sac was not included in the illustration. Melanophores formed a continuous line on the dorsal and anterior portion of the peritoneal cavity, and also posterior to the eye across the otoliths into the hindbrain. The lower jaw was pronounced and extended anteriorly beyond the adjacent margin of the head. Two large melanophores were located on the indented portion of the hindbrain, but only one was visible from a lateral view (Figure 2-4a).

Day 5 (Figure 2-4b)

At day 5, mean TL (± SD) was 2.29 ± 0.05 mm. The number of myomeres did not change. Eye pigment did not change. The heart and gills were visible, but not included in the illustration. The otoliths were no longer visible. Melanophores on the ventral portion of the myomeres have increased in thickness and still form a continuous line. One large melanophore is visible on the dorsal side of the posterior end of the notochord. The lower jaw was more prominent and extended anteriorly beyond the adjacent margin of the head. The yolk sac and oil globule were no longer visible. On the anterior and dorsal margin of the peritoneal cavity, melanophores still formed a continuous line.

Melanophores also formed a line on the anterior portion of the gut. Melanophores increased in thickness and formed a complete line from the posterior margin of the eye along the ventral portion of the myomeres. Melanophores also formed a thicker line from the posterior margin of the eye to the hindbrain. The forebrain and hindbrain continued to show more distinctive lobes. Four large melanophores were located on the indented portion of the hindbrain, but only two are visible from the lateral view (Figure 2-4b).

Day 6 (Figure 2-5)

At day 6, mean TL (± SD) was 2.22 ± 0.05 mm, myomere count did not change, eye pigment did not change, and otoliths were not visible. The heart and the gills were visible, but were not included in the illustration. Similar to day 5, melanophores on the ventral portion of the myomeres have increased in thickness and formed a line. The melanophore present on the dorsal portion of the posterior end of the notochord on day 5 was no longer visible. The lower jaw was similar to day 5, prominent and extended anteriorly beyond the adjacent margin of the head. The peritoneal cavity was similar to day 5, except the gut had larger more numerous coils. Melanophores have increased in thickness and formed a line from the posterior margin of the eye along the ventral portion of the myomeres. Similar to day 5, melanophores also formed a line from the posterior margin of the eye to the hindbrain. The yolk sac and oil globule were no longer visible. Melanophores still formed a line on the anterior and dorsal portion of the peritoneal cavity. The coiled gut had increased in size and the line of melanophores on the anterior side of the gut was thinner and less visible. The four melanophores on the hindbrain were

not visible as separate melanophores as they were in day 5; instead they formed one large area of pigmentation on 67% of the photographs and preserved specimens (Figure 2-5).

Discussion

Gray triggerfish larvae were collected from active nests and reared in the laboratory. All rearing attempts resulted in larvae living through 6 d. Gray triggerfish larvae hatched at a mean size of 2.16 mm NL, larger than the size of (1.7 mm NL) suggested by Lyczkowski-Shultz and Ingram (2005). Another difference documented from this study was that gray triggerfish had 20 myomeres from hatching until at least 6 d posthatch. However, Lyczkowski-Shultz and Ingram (2005) documented gray triggerfish at 1.7 mm NL with 18 myomeres. These differences may result from preservation effects on larvae, or misidentification of gray triggerfish larvae.

The maximum mean total length and notochord length measured for larval gray triggerfish was at 3 d. After that age, larvae continued to develop but decreased in length. The mean decrease in gray triggerfish larvae length after 3 d could be an artifact of rearing conditions; however, it has been documented in other species of larvae such as the Indo-Pacific tarpon, *Megalops cyprinoides*. Rearing experiments of the Indo-Pacific tarpon documented shrinkage during metamorphosis before resuming normal growth one month later (Tsukamoto and Okiyama 1993).

The lower jaw was present at day 3 and continued to grow in length through day 6. The yolk sac and oil globule were not visible after day 4, when fish apparently feed

exogenously. Food was visible in the gut of a few larvae at this time, but difficult to discern in many larvae because of the coiled gut. Lyczkowski-Shultz and Ingram (2005) suggested that gray triggerfish had a coiled gut by 3 mm NL. In my study, larval gray triggerfish had a coiled gut by day 2, when mean size was 2.2 mm NL. These size differences might be due to the fact that larval gray triggerfish less than 3 mm NL were not collected and identified in previous studies (Matsuura and Katsuragawa 1981; Lyczkowski-Shultz and Ingram 2005). Eye diameter was largest at day 2, and remained consistent in size through day 6. Lyczkowski-Shultz and Ingram (2005) also suggested that a dorsal spine is present when larval gray triggerfish are greater than 2 mm NL; but the reared fish in my study showed no development of the dorsal spine during the first 6 d posthatch.

Benthic habitat use by larval gray triggerfish has been suggested in other studies because of their absence in plankton tows (Matsuura and Katsuragawa 1981; Lyczkowski-Shultz and Ingram 2005). However, in my study, larvae were observed in the water column of all rearing tanks, which suggests use of pelagic habitat. It is possible that preserved gray triggerfish larvae are difficult to distinguish from other larval fish found in the plankton before formation of dorsal spines, which is one of the main distinguishing characteristics (Matsuura and Katsuragawa 1981; Lyczkowski-Shultz and Ingram 2005). Identification of larval fish less than 3 mm NL caught in plankton tows is difficult for many species (Moser et al. 1984; Lyczkowski-Shultz and Ingram 2005; Moser 1996). However, my study provides new information based on larvae known to be gray triggerfish.

There are several possibilities why larval gray triggerfish did not survive past 6 d. In this study wild-caught plankton were fed to larvae, and prey concentrations may have been too low or the plankton provided not the correct food type. For example, red snapper larval rearing studies found higher rates of survival in captivity when a ciliate, *Fabrea salina,* was fed in addition to copepod naupli (Rhodes and Phelps in press). Field studies also suggest that ciliates play an important role in early feeding of larval fish; however, most ciliates do not have hard parts so they are difficult to detect in the gut of larvae (Stoecker and Govoni 1984; Fukami et al. 1999).

My study describes the early development of gray triggerfish that has not been previously reported. Myomere counts, illustrations for each day, and description of melanophores could be used for identification of the smallest larval gray triggerfish (< 3 mm NL) from other similar species that are often captured in the same plankton tows. However, presently there are few descriptions for comparison of other larvae less than 3mm NL that might be found in the same plankton tows, such as species from the family Monacanthidae (Lyczkowski-Shultz and Ingram 2005).

References

Briggs, J.C. 1958. A list of Florida fishes and their distribution. Bulletin Florida State Museum Biological Science 2: 222-319.

Frazer, T.K., and Lindberg W.J. 1994. Refuge spacing similarly affects reef-associated species from three phyla. Bulletin Marine Science 55: 388-400.

Fukami, K., A. Watanbe, S. Fujita, K. Yamaoka, T. Nishijima. 1999. Predation on naked protozoan microzooplankton by fish larvae. Marine Ecology Progress Series 185: 285-291.

Ingram, G.W. Jr. 2001. Stock structure of gray triggerfish, *Balistes capriscus*, on multiple spatial scales in the Gulf of Mexico. PhD Dissertation, University of South Alabama, Mobile, Alabama.

Johnson, A.G., and Saloman C. H. 1984. Age, growth, and mortality of gray triggerfish, *Balistes capriscus*, from the northeastern Gulf of Mexico. Fishery Bulletin 82: 485-492.

Kimmel, C.B. 1993. Patterning the brain of the zebrafish embryo. Annual Reviews in Neuroscience. 16:707-732.

Kurz, R.C. 1995. Predator-prey interactions between gray triggerfish (*Balistes capriscus* Gmelin) and a guild of sand dollars around artificial reefs in the northeastern Gulf of Mexico. Bulletin of Marine Science 56: 150-160.

Lyczkowski-Shultz, J., and G. W. Ingram, Jr. 2005. Balistidae: Triggerfishes. Pages 2,387-2,398 *in* W.F. Richards, editor. Early stages of Atlantic fishes: An identification guide for the western central North Atlantic. CRC Press, Boca Raton, Florida.

MacKichan, C.A., and Szedlmayer, S.T. 2007. Reproductive behavior of gray triggerfish, *Balistes capriscus*, in the Northeastern Gulf of Mexico. Proceedings of the 59th Gulf and Caribbean Fisheries Institute 59: 231-235.

Matsuura, Y., and M. Katsuragawa. 1981. Larvae and juveniles of grey triggerfish, *Balistes capriscus*, from southern Brazil. Japanese Journal of Ichthyology 28: 267-275.

Matsuura, Y., and M. Katsuragawa. 1985. Osteological development of fins and their supports of larval grey triggerfish, *Balistes capriscus*. Japanese Journal of Ichthyology 31: 411-420.

Moore, D. 1967. Triggerfishes (Balistidae) of the Western Atlantic. Bulletin Marine Science 17: 689-722.

Moore, J.L. 2001. Age, growth and reproductive biology of the gray triggerfish (*Balistes capriscus*) from the southeastern United States, 1992-1997. Thesis, University of Charleston, Charleston, South Carolina.

Moser, H.G., W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall, Jr. and S.L. Richardson (eds). 1984. Ontogeny and systematics of fishes. American Society of Ichthyology and Herpetology. Special Publication. Allen Press, Kansas.

Moser, H. G. 1996. The early stages of fishes in the California current region. H.G. Moser (ed). CalCOFI Atlas 33. Allen Press, Kansas.

Rhodes, M., and R. P. Phelps. In press. Evaluation of the ciliated protozoan *Fabrea salina* as the first food for larval red snapper, *Lutjanus campechanus*, in a large scale rearing experiment. Journal of Applied Aquaculture.

Stoecker, D.K., and J.J. Govoni. 1984. Food selection by young larval Gulf menhaden (*Brevoortia patronus*). Marine Biology 80:299-306.

Tsukamoto Y., and M. Okiyama. 1993. Growth during the early life history of the Pacific tarpon, *Megalops cyprinoides*. Japanese Journal of Ichthyology 39:379-386.

Vose F.E., and W. G. Nelson. 1994. Gray triggerfish (*Balistes capriscus* Gmelin) feeding from artificial and natural substrate in shallow Atlantic waters of Florida. Bulletin Marine Science 55: 1316-1323.

Wilson, C.A., D.L. Nieland, and A.L. Stanley. 1995. Age, growth, and reproductive biology of gray triggerfish, *Balistes capriscus*, from the Northern Gulf of Mexico commercial harvest. MARFIN Final Report. Louisiana State University, Baton Rouge, Louisiana.

TABLE 2-1.—Mean (± SD) morphometric measures of larval gray triggerfish by day ($N = 10$).

Day	Total length	Snout length	Notochord length	Eye diameter	Oil globule	Yolk sac	Jaw length
0	2.27 ± 0.04	0.10 ± 0.01	2.16 ± 0.04	0.16 ± 0.01	0.18 ± 0.01	0.46 ± 0.02	
1	2.32 ± 0.02	0.11 ± 0.02	2.19 ± 0.05	0.18 ± 0.02	0.18 ± 0.01	0.35 ± 0.02	
2	2.34 ± 0.08	0.12 ± 0.02	2.20 ± 0.06	0.22 ± 0.01	0.15 ± 0.01	0.22 ± 0.02	
3	2.34 ± 0.02	0.13 ± 0.01	2.20 ± 0.03	0.20 ± 0.02	0.11 ± 0.01	0.20 ± 0.02	0.17 ± 0.03
4	2.27 ± 0.06	0.10 ± 0.01	2.12 ± 0.05	0.20 ± 0.01	0.08 ± 0.01	0.12 ± 0.03	0.22 ± 0.03
5	2.29 ± 0.05	0.11 ± 0.01	2.13 ± 0.04	0.20 ± 0.01			0.21 ± 0.03
6	2.22 ± 0.05	0.06 ± 0.01	2.08 ± 0.05	0.20 ± 0.01			0.25 ± 0.02

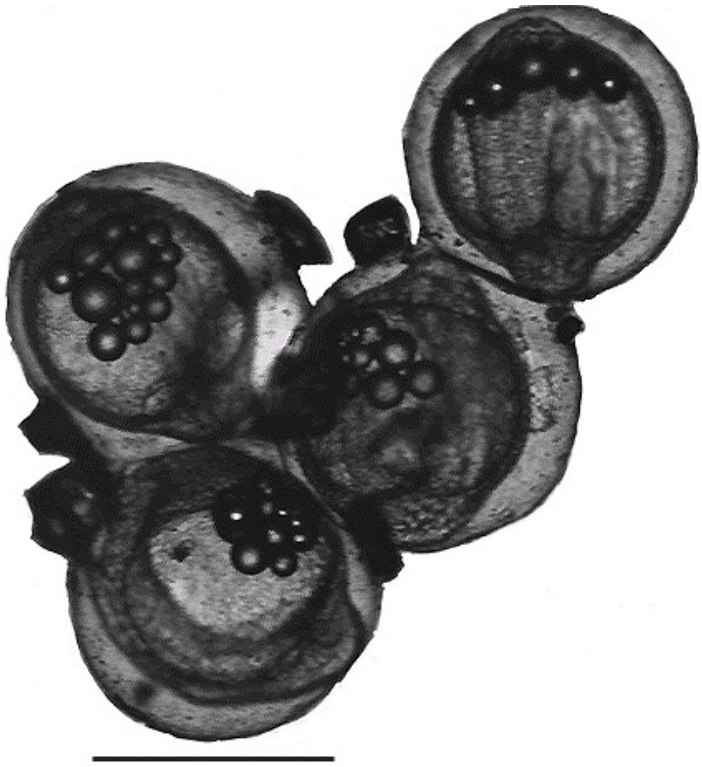
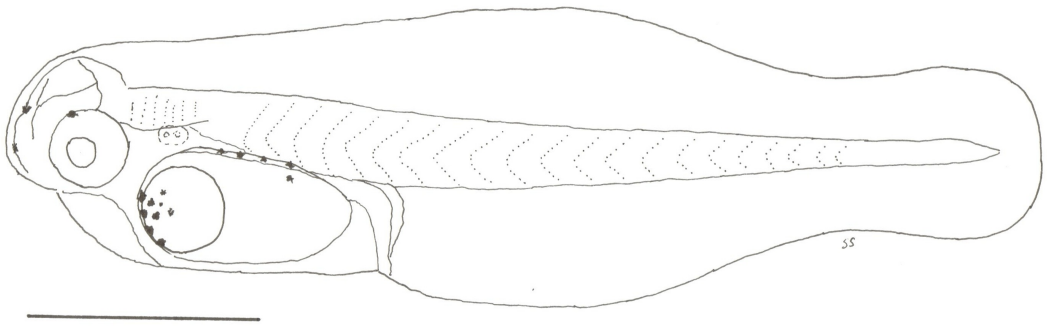


FIGURE 2-1.— Gray triggerfish eggs. Mean (± SD) egg diameter was 0.62 ± 0.03 mm. Scale bar = 0.5 mm.

(a)



(b)

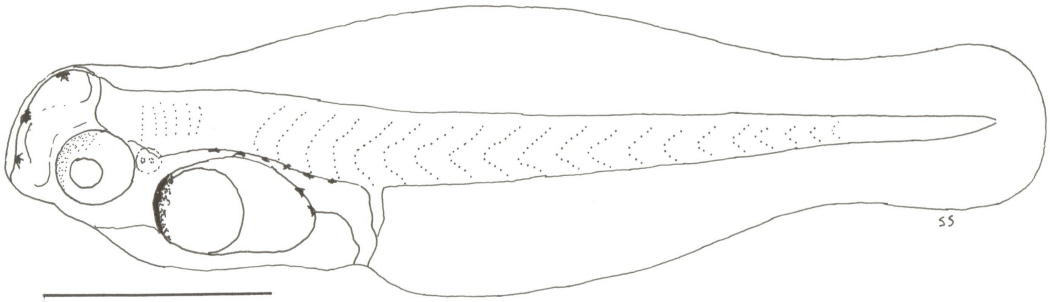
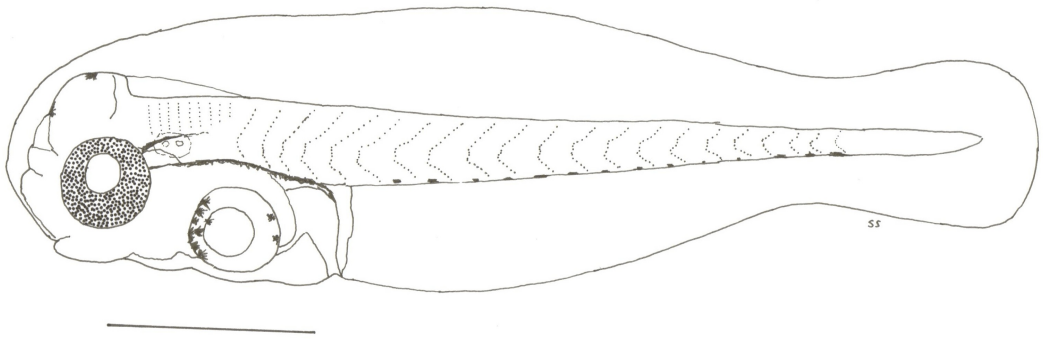


FIGURE 2-2.— Gray triggerfish larvae: **(a)** 0 d old, hatch without pigmented eyes or a functional mouth; **(b)** 1 d old, eyes are partially pigmented, and many overlapping melanophores are visible on the anterior margin of the yolk sac. Scale bar = 0.5 mm.

(a)



(b)

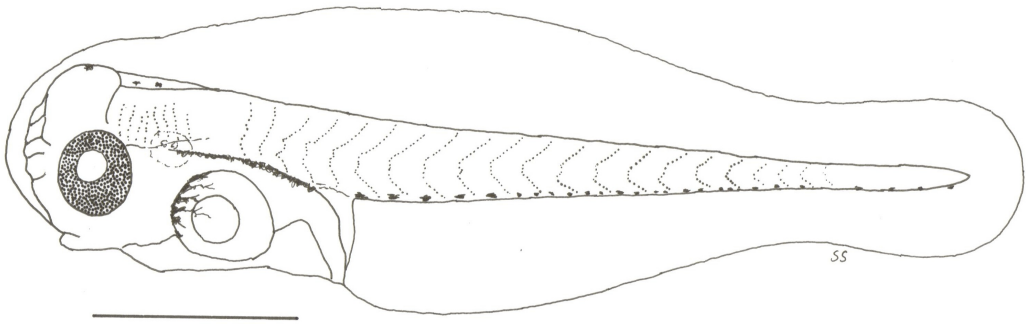
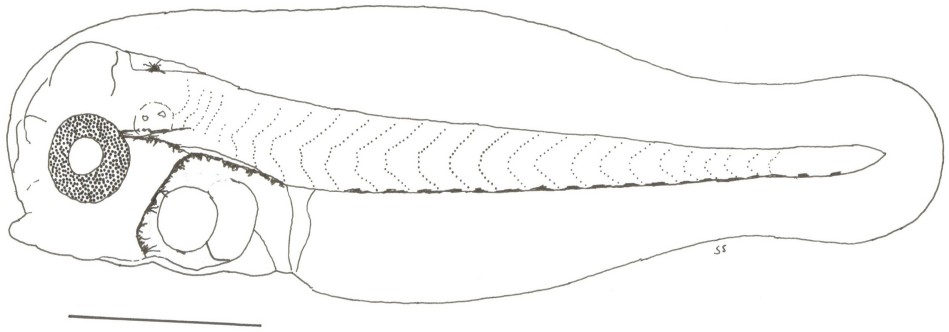


FIGURE 2-3.— Gray triggerfish larvae: **(a)** 2 d old, eyes are completely pigmented and gut is coiled; **(b)** 3 d old, melanophores are visible on the ventral portion of the myomeres posterior to the anus. Scale bar = 0.5 mm.

(a)



(b)

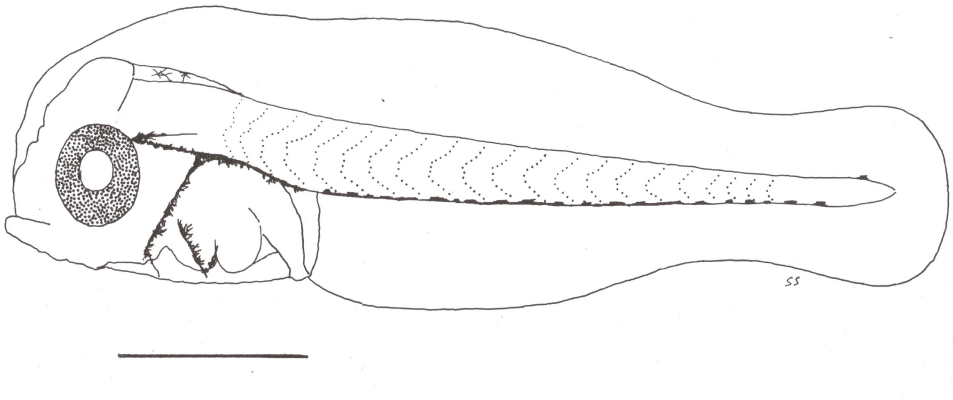


FIGURE 2-4.— Gray triggerfish larvae: **(a)** 4 d old, melanophores on the ventral portion of the myomeres form a continuous line to the end of the notochord; **(b)** 5 d old, the yolk sac and oil globule were no longer visible. Scale bar = 0.5 mm.

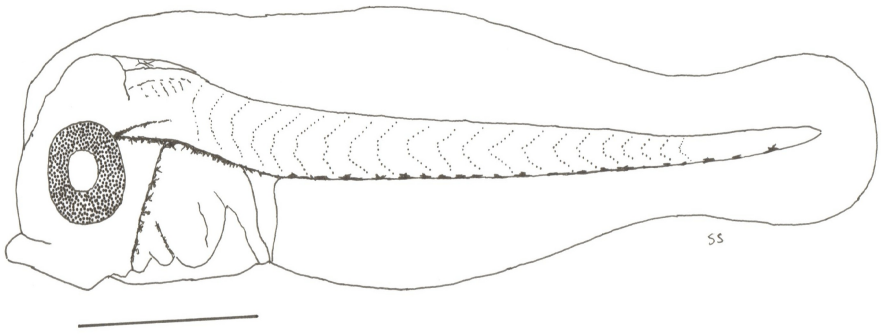


FIGURE 2-5.— Gray triggerfish larvae 6 d old, the lower jaw is prominent and extends anteriorly beyond the adjacent margin of the head. Scale bar = 0.5 mm.

CHAPTER 3

RECRUITMENT OF AGE-0 GRAY TRIGGERFISH, *BALISTES CAPRISCUS,* TO ARTIFICIAL REEFS IN THE NORTHERN GULF OF MEXICO

Abstract.—Recruitment of age-0 gray triggerfish, *Balistes capriscus*, to benthic artificial reefs was documented by diver surveys from 2003-2007. Divers counted and estimated sizes of all gray triggerfish that recruited to three types of artificial reefs ranging in area from 1.2 to 4.0 m^2 in 20 m of water, 28 km south of Dauphin Island, Alabama. Forty artificial reefs were built in June 2003, surveyed in October, November-December 2003, and May 2004; 20 artificial reefs were built in October 2005 and surveyed in October and December 2005, May, August, and December 2006; 40 artificial reefs were built July 2006 and surveyed June 2007; and 30 artificial reefs were built in August 2007 and surveyed in September, October, and December 2007. Recruitment patterns were similar in the fall and winter of 2003 and 2007. In 2005 significantly lower numbers of recruits were detected compared to other years, which may have been caused by a major hurricane. Peak recruitment of age-0 gray triggerfish occurred from September to December. Based on known spawning seasonality and the first appearance of recruits in September in this study, gray triggerfish spend 4 to 7 months in the pelagic environment before becoming benthic.

Introduction

Limited information is available on the early life history of gray triggerfish, *Balistes capriscus*. Previous studies have documented spawning seasonality and showed spawning as early as May with a peak in June and July in the Gulf of Mexico and South Atlantic Bight (Wilson et al. 1995; Ingram 2001; Moore 2001; Ismen et al. 2004; MacKichan and Szedlmayer 2007). Other studies have also shown a pelagic stage after hatching, which is closely associated with floating *Sargassum* (Dooley 1972; Fahay 1975; Bortone et al. 1977; Wells and Rooker 2004). Seasonal patterns of gray triggerfish found in the pelagic stage were similar among studies. Fahay (1975) showed peaks in July through August, Dooley (1972) from August through October, and Wells and Rooker (2004) from May through August. Pelagic gray triggerfish showed relatively larger sizes in the plankton compared to other northern Gulf of Mexico fishes (Dooley 1972; Fahay 1975; Bortone et al. 1977; Szedlmayer and Conti 1999; Wells and Rooker 2004). For example, gray triggerfish were 11 to 39 mm SL from July to August, but still collected in the plankton in October at 21 to 83 mm SL (Fahay 1975).

Adult gray triggerfish are closely associated with both natural and artificial reefs (Johnson and Saloman 1984; Frazer and Lindberg 1994; Vose and Nelson 1994; Kurz 1995; Blitch 2000; Ingram 2001; Lingo and Szedlmayer 2006). However, no information is available on the size and season juvenile gray triggerfish drop out of the plankton and recruit to benthic artificial reefs. Past studies have suggested that multiple bottlenecks occur in the early juvenile stages, and that the shift from pelagic to benthic habitat may

represent a critical period (Doherty 1987; Richards and Lindeman 1987; Sogard 1997). For example, during habitat shifts gray triggerfish may be more susceptible to predation as they encounter new predators on benthic reefs (Sogard 1997).

In this experiment, the size and season that gray triggerfish leave the pelagic zone and settle on benthic artificial reefs in the northern Gulf of Mexico was examined. In this study recruitment was defined as "the addition to the population from reproduction" as opposed to movement of fish from other benthic reefs (Helfman et al. 1997).

Methods

Three types of artificial reefs were built from 2003 to 2007 to study gray triggerfish recruitment to benthic reefs. All artificial reefs were placed 28 km south of Dauphin Island, Alabama, in 20 m of water. All gray triggerfish were counted by SCUBA divers and placed into 25-mm increment size classes.

Forty artificial reefs (2 x 2 x 0.2 m) were built 15 to 25 June 2003. Each of the 2003 artificial reefs consisted of 10 concrete blocks (41 x 20 x 20 cm) randomly placed on a polyethylene mat (4 m^2, mesh size 0.64 cm) by divers using two 91-cm cable ties to secure each block to the mat (Figure 3-1a). Ten artificial reefs were placed 30 m apart in four transects. Artificial reefs were visually surveyed by SCUBA divers and videotaped for gray triggerfish in the fall (4-5 October 2003), winter (21 November-8 December 2003), and spring (5-20 May 2004).

Forty new artificial reefs (1.2 x 1.2 x 0.41 m) were built from 11 to 24 August 2005; however, before any surveys were completed, all were destroyed by Hurricane Katrina. After the storm, an additional 20 artificial reefs were built 10 October 2005. Each of the 2005 artificial reefs consisted of 12 blocks (41 x 20 x 20 cm) attached with two 91-cm cable ties to plywood (1.22 x 1.22 x 0.006 m). Two sets of four concrete blocks each were stacked in the center of the board, and four blocks were secured on the outside edge (Figure 3-1b). Each reef also had four small concrete bricks (9 x 6 x 20 cm) placed on the top of the outside larger blocks and secured with 30-cm cable ties. Each reef was tied to a 1.8-m ground anchor with a 1.3-cm diameter nylon rope, to reduce movement and destruction from storm. Ten artificial reefs were placed 30-m apart in two transects. All the 2005 artificial reefs were visually surveyed by SCUBA divers and videotaped for gray triggerfish in the fall (20 October 2005), winter (1 December 2005), spring (24 May 2006), summer (2 August 2006), and the following winter (13 December 2006).

An additional set of 40 artificial reefs were built 18-26 July 2006 using the same reef design as in 2005 (Figure 3-1b). All of the 2006 artificial reefs were visually surveyed by SCUBA divers and videotaped for gray triggerfish from 6 to 11 June 2007.

A third reef design (1.22 x 1.02 x 0.42 m) was built ($N = 30$) from 1 to 9 August 2007 (Figure 3-1c). Each of the 2007 artificial reefs comprised 10 concrete blocks (41 x 10 x 20 cm), and each block was attached to a plastic pallet (1.22 x 1.02 x 0.14 m) secured with two cable ties. Five blocks were arranged in rows on each side of the pallet and a plastic crate (61 x 30.5 x 28 cm) was placed in the middle to increase reef complexity. The plastic crate had various sized holes (12.1 x 3.2, 14 x 3.8, 7.6 x 3.2 cm) on the top

and sides. One float (12.7 x 5.1 cm) was attached with 0.64-cm diameter nylon rope to each corner block of the pallet ($N = 4$), and floated 1 m above the reef. A large circular float (15.2 cm diameter) also was tied with 0.64-cm diameter nylon rope in the center of the plastic pallet 1 m above the blocks (Figure 3-1c). After the reef was deployed, it was tied to a 1.8-m ground anchor with a 1.3-cm diameter nylon rope. Artificial reefs were placed in six rows with five reefs per row, with each reef 500 m from the other reefs. Ten different artificial reefs were video and visually surveyed by two SCUBA divers for gray triggerfish on 27 September, 26 October, and 7 to 10 December 2007.

Temperature, salinity, and dissolved oxygen were measured with a YSI 6920 meter (Yellow Springs Instruments, Yellow Springs, Ohio). Values reported were measured at 1 m above the bottom. Temperature data also were obtained from a National Oceanic Atmospheric Administration (NOAA) data buoy 41 km south of Biloxi, Mississippi, to calculate mean surface water temperatures for 2003 through 2007.

From monthly visual surveys, age-0 and age-1 recruits were separated based on comparisons to previously reported age-length relations (Johnson and Saloman 1984; Ingram 2001; Moore 2001). A June 1 birth date was applied based on observations of gray triggerfish spawning in June and July in the northern Gulf of Mexico (MacKichan and Szedlmayer 2007). Previous studies have also shown that gray triggerfish reach 200 mm FL by age 1 (January), with an estimated growth rate of 0.95 mm/d (Ingram 2001; Moore 2001). All juvenile gray triggerfish observed in May were age 1, based on the above studies that showed spawning was first initiated at the end of May, and peaks in June and July (Ingram 2001; Moore 2001; Ismen et al. 2004). In June, most gray

triggerfish on benthic artificial reefs were considered age 1, except those less than 30 mm FL based on 0.95 mm/d growth rate. In August, gray triggerfish less than 100 mm FL were considered age 0. In September, gray triggerfish less than 125 mm FL were age 0. In October, gray triggerfish less than 150 mm FL were age 0. In November, gray triggerfish less than 175 mm FL were age 0. In December, all gray triggerfish less than 200 mm FL were age 0. In January, all juvenile gray triggerfish on benthic artificial reefs were considered age 1.

Artificial reefs built in 2003, 2005, 2006, and 2007 were compared separately for number and size of gray triggerfish across survey periods (months). Abundance estimates were standardized to mean number of gray triggerfish per square meter. For example, artificial reefs built in 2003 were 4 m^2, therefore the number of gray triggerfish counted on each reef was divided by four. This was repeated for each set of reefs based on reef area and number of reefs surveyed. The 2003 and 2005 artificial reefs were analyzed with a repeated-measures analysis of variance (rmANOVA), whereas the 2007 artificial reefs were analyzed with a one-way ANOVA because 10 different reefs were sampled each survey only once (not repeated). Mean abundance of age-0 and age-1 fish were compared separately for each survey with ANOVA. Length-frequency distributions of age-0 gray triggerfish were compared by month using a chi-square. Mean number of fish were compared across years for October and December surveys with a one-way ANOVA. Surface water temperatures were compared to temperatures taken within 1-m from the bottom by month and year with a paired t-test. All tests were considered significant at

$\alpha = 0.05$. If significant differences were detected a Student-Newman-Kuels test was used to show specific differences (Zar 1999).

Results

Peak abundances of age-0 gray triggerfish were found on artificial reefs from September to December 2003 through 2007, with the first recruits in September (Figures 3-2, 3-3, and 3-4). In 2003, significantly more age-0 gray triggerfish were observed in October (mean ± SE = 1.8 ± 0.3 fish/m^2) than in November-December (mean ± SE = 0.2 ± 0.05 fish/m^2, rmANOVA: df = 1,39, F = 35.0, P <0.001; Figure 3-2). In May 2004, age-0 gray triggerfish were not observed on artificial reefs. In 2005, significantly more age-0 fish were found on artificial reefs in December (mean ± SE = 0.32 ± 0.1 fish/m^2) than in October (mean ± SE = 0.04 ± 0.03 fish/m^2, rmANOVA: df = 1,19, F = 4.8, P < 0.05). In May and August 2006, age-0 gray triggerfish were not observed on artificial reefs, but did recruit to benthic artificial reefs by December 2006 (mean ± SE = 2.5 ± 0.3 fish/m^2; Figure 3-3). In 2007, significantly more age-0 gray triggerfish were found in December (mean ± SE = 7.1 ± 1.1 fish/m^2) than in September (mean ± SE = 3.1 ± 0.7 fish/m^2) or October (mean ± SE = 4.2 ± 0.6 fish/m^2, ANOVA: df = 2, 27, F = 6.0, P<0.01; Figure 3-4).

During October and November-December 2003 there were significantly more age-0 than age-1 gray triggerfish (October ANOVA: df = 1,78, F = 43.8, P<0.001; November-December ANOVA: df = 1,78, F = 8.7, P< 0.01; Figure 3-2). In October 2005 only two

gray triggerfish were observed. In December 2005, no significant differences were detected between age-0 and age-1 gray triggerfish (ANOVA: df = 1, 38, F = 1.0, P>0.1; Figure 3-3). In December 2006, significantly more age-0 gray triggerfish were observed than age-1 (ANOVA: df = 1,38, F = 23.3, P<0.001; Figure 3-3). In 2007, no significant differences were detected in the number of age-0 versus age-1 gray triggerfish in September (ANOVA: df = 1,18, F = 1.4, P > 0.1). However, in October and December 2007, significantly more age-0 gray triggerfish were observed than age-1 (October ANOVA: df = 1,18, F = 13.3, P <0.01; December ANOVA: df = 1,18, F = 30.3, P < 0.001; Figure 3-4). Only age-1 gray triggerfish were observed in May 2004 (mean ± SE = 0.24 ± 0.04 fish/m^2, Figure 3-2), May 2006 (mean ± SE = 1.1 ± 0.2 fish/m^2, Figure 3-3), June 2007 (mean ± SE = 1.4 ± 0.2 fish/m^2, Figure 3-5), and August 2006 (mean ± SE = 1.3 ± 0.2 fish/m^2, Figure 3-3).

Age-0 gray triggerfish length increased from initial recruits in fall to older recruits in early winter. In 2003, age-0 gray triggerfish were significantly smaller in October (range 25-150 mm FL) than in November-December (range 51-175 mm, df = 5, X^2 = 26.0, P<0.001; Figure 3-2). In 2005, the number of age-0 gray triggerfish was too low to make length-frequency comparisons. There was only one age-0 gray triggerfish surveyed in October 2005 (138 mm FL, N = 1). In December 2005 the age-0 length-frequency ranged from 101-200 mm FL (Figure 3-3). Artificial reefs surveyed in December 2006 had similar length-frequencies distributions (range 101-200 mm FL) of age-0 gray triggerfish compared to December 2005 (Figure 3-3). In 2007, length-frequency distributions of age-0 gray triggerfish in September (range 25-125 mm FL), were significantly smaller than

October (range 51-150 mm FL, df = 6, $X^2 = 27$, $P < 0.001$) and December (25-200 mm FL, df = 6, $X^2 = 14.5$, $P < 0.02$); and distributions in October (range 51-150 mm FL) were significantly smaller than December (range 25-200 mm FL, df = 6, F= 19.4, $P < 0.01$; Figure 3-4).

Age-0 gray triggerfish recruits in 2003 had similar size ranges (51-175 mm FL) compared to recruits in 2007 ranges (25-200 mm FL, Figures 3-2 and 3-4). In December 2005 and 2006, gray triggerfish were larger than other years, i.e., no fish less than 100 mm FL were observed (Figure 3-3). Comparisons of recruitment across years showed significantly more age-0 gray triggerfish in October 2007, than in October 2003 and 2005 (ANOVA: df = 2, 67, F = 27.4, $P < 0.001$; Figure 3-6 (a)) and more in December 2007 than December 2003, 2005 and 2006 (ANOVA: df = 3, 86, F = 73.5, $P < 0.001$; Figure 3-6 (b)).

Seasonal surface water temperatures were similar from among years (2003-2007) and were not significantly different from bottom water temperatures taken during reef surveys (paired t-test: df = 8, t = 0.16, $P > 0.05$; Figure 3-7). Salinity and dissolved oxygen measures taken 1-m above the bottom showed little variation over the study period. Salinity ranged from 32.5 to 36.2‰, (mean ± SE = 34.4 ± 0.1‰, $N = 9$) and dissolved oxygen ranged from 5.6-8.3 mg/L, (mean ± SE = 7.2 ± 0.2 mg/L, $N = 7$).

Discussion

This study showed peak recruitment of age-0 gray triggerfish from September to December in the northern Gulf of Mexico. Fish were age-0 when they recruited to the benthic artificial reefs, based on my observations and previous seasonal information on spawning and pelagic stages. Gray triggerfish spawn as early as May, with a peak in June and July in the Gulf of Mexico and South Atlantic Bight (Wilson et al. 1995; Ingram 2001; Moore 2001; Ismen et al. 2004; MacKichan and Szedlmayer 2007). Studies revealed high numbers in the plankton in late summer and fall (August-October), with sizes ranging from 13-132 mm SL (Dooley 1972). Bortone et al. (1977) also found gray triggerfish in the plankton in the eastern Gulf of Mexico from May through October, with sizes ranging from 9 to 78 mm SL. A more recent study of *Sargassum* mats in the northwestern Gulf of Mexico also found high abundance of gray triggerfish in the pelagic zone from May through August, ranging in size from 13 to 106 mm SL (Wells and Rooker 2004). Data from my study combined with these earlier studies indicate that most juvenile gray triggerfish have dropped out of the plankton by December, and juvenile gray triggerfish spend 4 to 7 months in the pelagic zone before recruiting to benthic artificial reefs.

Similar to gray triggerfish, queen triggerfish, *Balistes vetula*, also recruited to reef structures as age-0 fish, but recruited in the spring rather than in fall (Robsertson 1988). Queen triggerfish recruits ranged in size from 49 to 70 mm TL and were 66-86 days old based on otolith growth increments (Robsertson 1988). Ingram (2001) used back-

calculations of gray triggerfish collected in October and November 1998, and estimated mean size of 81 mm FL when fish recruited to benthic reefs. This mean estimate was smaller than my estimates of 95 mm FL (October 2003), 138 mm FL (October 2005) and 135 mm FL (October 2007) for mean size on benthic artificial reefs. However, in the present study, sizes of fish were estimated on a reef after recruitment had already occurred, and these fish probably recruited at smaller sizes sometime before the visual census. In addition, the smallest recruits in the present study were 25 to 50 mm FL.

In contrast to my study and Ingram (2001), gray triggerfish off the Brazilian coast recruited later than age-0. Bernardes (2002) completed ageing studies on gray triggerfish off the Brazilian coast and concluded that they recruited to benthic habitat at age-1. However, these age-1 fish were similar in size (90-120 mm FL) to recruitment sizes of age-0 gray triggerfish I observed in the Gulf of Mexico and recruitment sizes reported by Moore (2001) in the South Atlantic Bight. Bernardes (2002) age-length relation for gray triggerfish off the Brazilian coast was not used in the present study for several reasons: (1) only fish up to 4 years were aged, (2) reported two complete annular rings on the spine per year, (3) few fish less than 200 mm FL were aged, and (4) the smallest fish aged was 139 mm FL. Also, Bernardes (2002) studied gray triggerfish in the Southern Hemisphere, where numerous differences in environmental factors could affect growth rate and increment formation. The present study applied the age-length relation from Ingram (2001) on gray triggerfish in the Gulf of Mexico. Ingram's collections included many ($N = 80$) small gray triggerfish (< 200 mm FL) and ages were not based on back

calculations. In fact, 200 mm FL was a conservative estimate for age-0 gray triggerfish, because 12% of the fish aged from 200 to 250 mm FL were aged 0 (Ingram 2001).

Gray triggerfish showed similar recruitment patterns in fall and winter 2003, 2006, and 2007; however, in 2005 recruitment was lower. Clearly destruction of artificial reefs due to Hurricane Katrina affected our ability to quantify recruitment. After the first set of 2005 artificial reefs were destroyed, artificial reefs were rebuilt in October 2005 and surveys were taken at the end of the same month. These rebuilt reefs probably showed lower recruitment due to mortality or movement of juvenile gray triggerfish caused by Hurricane Katrina or artificial reefs were not in place for long enough time period before surveys were completed.

Salinity and dissolved oxygen showed little variation across surveys. Szedlmayer and Conti (1999) showed similar salinity and dissolved oxygen readings in an earlier study from the same area. Bottom water temperatures taken during my study were similar to surface water temperatures from the NOAA data buoys and declined in the fall. These decreasing water temperatures in the fall (October and November) and winter (December) may be a cue for gray triggerfish to drop out of the plankton to benthic reefs.

Further studies are needed, but reef type and complexity may affect annual recruitment of gray triggerfish (Shulman 1984; Lingo and Szedlmayer 2006; Piko and Szedlmayer 2007). All three types of artificial reefs had concrete blocks with some variation in area and type of material used as the base. Two differences in the 2007 artificial reefs compared to other years were the vertical floats 1 m above the reef, and the plastic crate in the center of the reef between the concrete blocks. Thus, the 2007 artificial reefs were

more complex than 2003, 2005 and 2006 reefs and this increased complexity may account for some of the higher recruitment observed in 2007. However, different artificial reef types in the present study were constructed different years and it was difficult to separate complexity aspects from interannual recruitment differences. The 2007 design is recommended over the 2003, 2005, and 2006 reef designs because it attracted more gray triggerfish, was easier to build, and better able to withstand winter storms. Diver visual surveys completed in May and June 2008 found 27 out of 30 artificial reefs completely intact.

This study found recruitment of age-0 gray triggerfish from September to December in the northern Gulf of Mexico. Gray triggerfish recruited at age-0, which was younger than previously inferred (Bernardes 2002) and provides new information for size and season of recruitment. Recruitment was consistent in the months of September to December 2003, 2006, and 2007, but significantly lower in 2005 following a major hurricane. Patterns of recruitment in this study concur with previous literature on the seasonality of reproductive biology and pelagic stage duration of gray triggerfish.

References

Bortone, S.A., P.A. Hastings, and S.B. Collard. 1977. The pelagic-*Sargassum* ichthyofauna of the eastern Gulf of Mexico. Northeast Gulf Science 1: 60-67.

Bernardes, R.A. 2002. Age, growth, and longevity of the gray triggerfish, *Balistes capriscus* (Tetraodonitformes: Balistidae), from the Southeastern Brazilian Coast. Scientia Marina 66: 167-173.

Blitch, K.M. 2000. The feeding habits of gray triggerfish, *Balistes capriscus* (Gmelin) from the northeast Gulf of Mexico. Master's thesis, Auburn University, Auburn.

Doherty, P.J. 1987. The replenishment of populations of coral reef fishes, recruitment surveys and the problems of variability manifest on multiple scales. Bulletin of Marine Science 41: 411-422.

Dooley, J.K. 1972. Fishes associated with the pelagic sargassum complex, with a discussion of the sargassum community. Contributions in Marine Science 16: 1-32.

Fahay, M.P. 1975. An annotated list of larval and juvenile fishes captured with surface-towed meter net in the South Atlantic Bight during four RV *Dolphin* cruises between May 1967 and February 1968. NOAA Technical Report NMFS SSRF-685:1-39.

Helfman, G.S., B.B. Collette, and D.E. Facey. 1997. Individuals, populations, and assemblages. Pages 406-425 *in*: The Diversity of Fishes. Blackwell Science, Malden, Massachusetts.

Ingram, G.W. Jr. 2001. Stock structure of gray triggerfish, *Balistes capriscus*, on multiple spatial scales in the Gulf of Mexico. PhD dissertation, University of South Alabama, Mobile.

Ismen, A., M., Turkoglu, and C.C. Yigin. 2004. The age, growth, and reproduction of gray triggerfish (*Balistes capriscus*, Gmelin, 1979) in Iskenderun Bay. Pakistan Journal of Biological Sciences 7: 2135-2138.

Johnson, A.G., and C.H. Saloman. 1984. Age, growth and mortality of gray triggerfish, *Balistes capriscus*, from the Northeastern Gulf of Mexico. Fishery Bulletin 82: 485-492.

Kurz, R.C. 1995. Predator-prey interactions between gray triggerfish, *Balistes capriscus* (Gmelin), and a guild of sand dollars around artificial reefs in the northeastern Gulf of Mexico. Bulletin of Marine Science 56: 150-160.

Lingo, M.E., and S.T. Szedlmayer. 2006. The influence of habitat complexity on reef fish communities in the northeastern Gulf of Mexico. Environmental Biology of Fishes 76: 71-80.

MacKichan, C.A., and S.T. Szedlmayer. 2007. Reproductive behavior of gray triggerfish, *Balistes capriscus*, in the Northeastern Gulf of Mexico. Proceedings of the 59th Gulf and Caribbean Fisheries Institute 59: 231-235.

Moore, J.L. 2001. Age, growth and reproductive biology of the gray triggerfish (*Balistes capriscus*) from the southeastern United States, 1992-1997. Master's thesis, University of Charleston, Charleston.

Piko, A.A., and S.T. Szedlmayer. 2007. Effects of habitat complexity and predator exclusion on the abundance of juvenile red snapper. Journal of Fish Biology 70: 758-769.

Richards, W.J., and K.C. Lindeman. 1987. Recruitment dynamics of reef fishes: planktonic processes, settlement and demersal ecologies and fishery analysis. Bulletin of Marine Science 41: 392-410.

Robertson, D.R. 1988. Extreme variation in settlement of the Caribbean triggerfish *Balistes vetula* in Panama. Copeia 1988: 698-703.

Shulman, M.J. 1984. Resource limitation and recruitment patterns in coral reef fish assemblage. Journal of Experimental Marine Biology and Ecology 74: 85-109.

Sogard, S.M. 1997. Size-selection mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science 60: 1129-1157.

Szedlmayer, S.T., and J. Conti. 1998. Nursery habitats, growth rates, and seasonality of age-0 red snapper, *Lutjanus campechanus*, in the northeast Gulf of Mexico. Fishery Bulletin 97: 626-635.

Vose, F.E., and W.G. Nelson. 1994. Gray triggerfish (*Balistes capriscus* Gmelin) feeding from artificial and natural substrate in shallow Atlantic waters of Florida. Bulletin of Marine Science 55: 1316-1323.

Wells, R. J. D., and J.R. Rooker. 2004. Spatial and temporal patterns of habitat use by fishes associated with *Sargassum* mats in the northwestern Gulf of Mexico. Bulletin of Marine Science 74: 81-99.

Wilson, C.A., D.L. Nieland, and A.L. Stanley. 1995. Age, growth, and reproductive biology of gray triggerfish, *Balistes capriscus*, from the Northern Gulf of Mexico commercial harvest. MARFIN Final Report. Louisiana State University, Baton Rouge, Louisiana.

Zar, J. H.1999. Biostatistical Analysis. 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

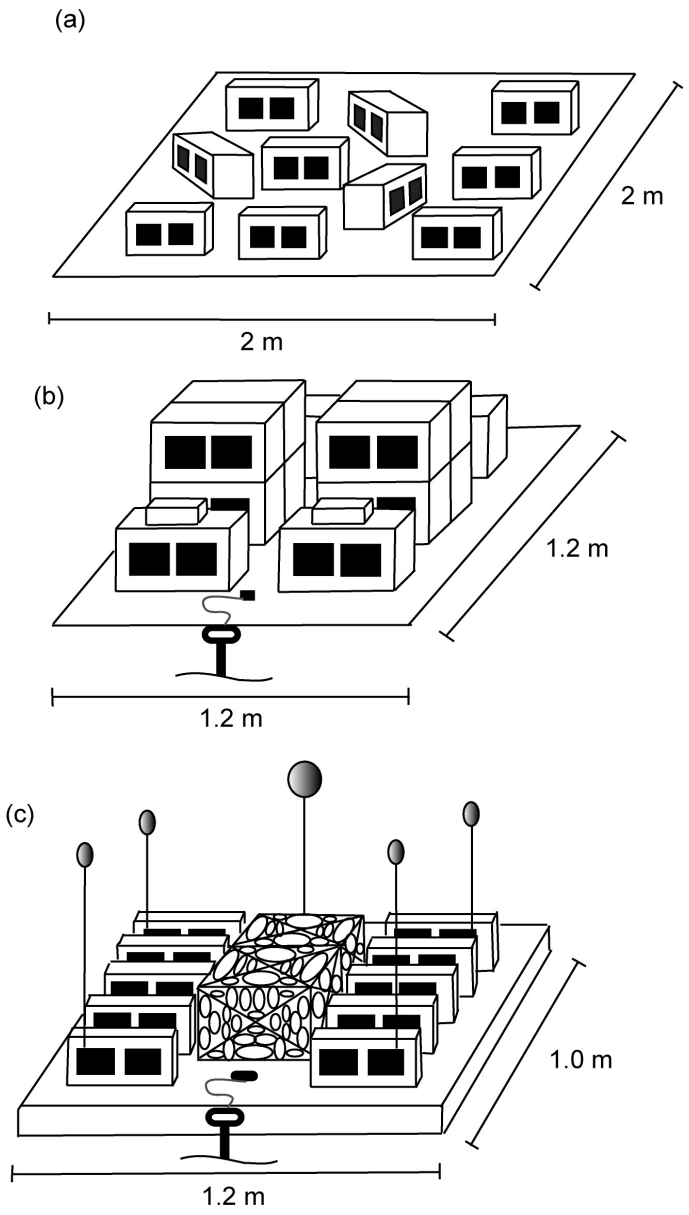


FIGURE 3-1.— Artificial reef design of **(a)** 2003, **(b)** 2005 and 2006, and **(c)** 2007.

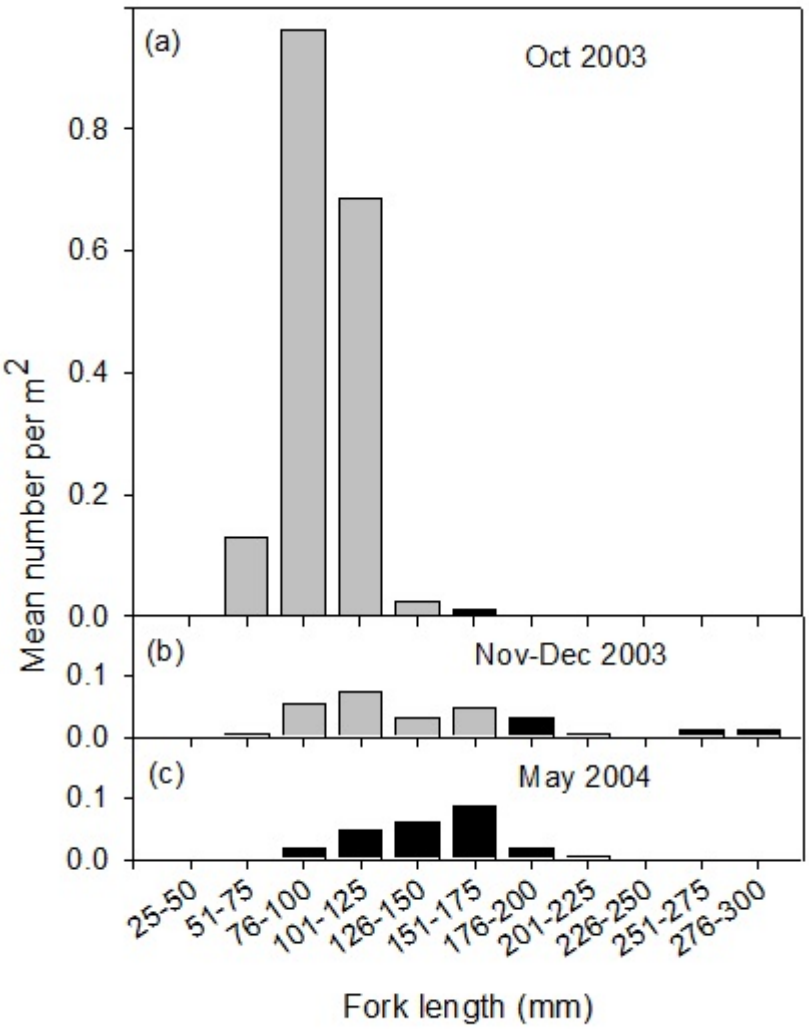


FIGURE 3-2.—Length-frequency distributions of gray triggerfish in **(a)** October 2003, **(b)** November-December 2003, and **(c)** May 2004 from the northern Gulf of Mexico. Age 0 = gray bars and age 1 = black bars.

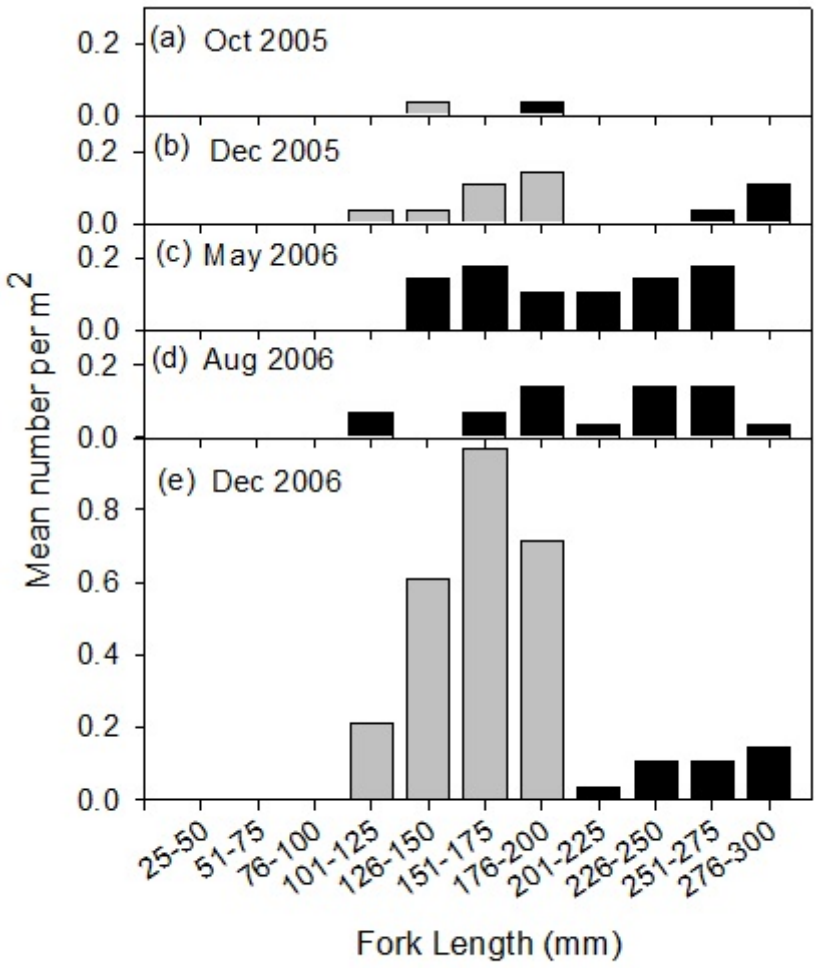


FIGURE 3-3.—Length-frequency distributions of gray triggerfish in **(a)** October 2005**, (b)** December 2005, **(c)** May 2006, **(d)** August 2006, and **(e)** December 2006 from the northern Gulf of Mexico. Age 0 = gray bars and age 1 = black bars.

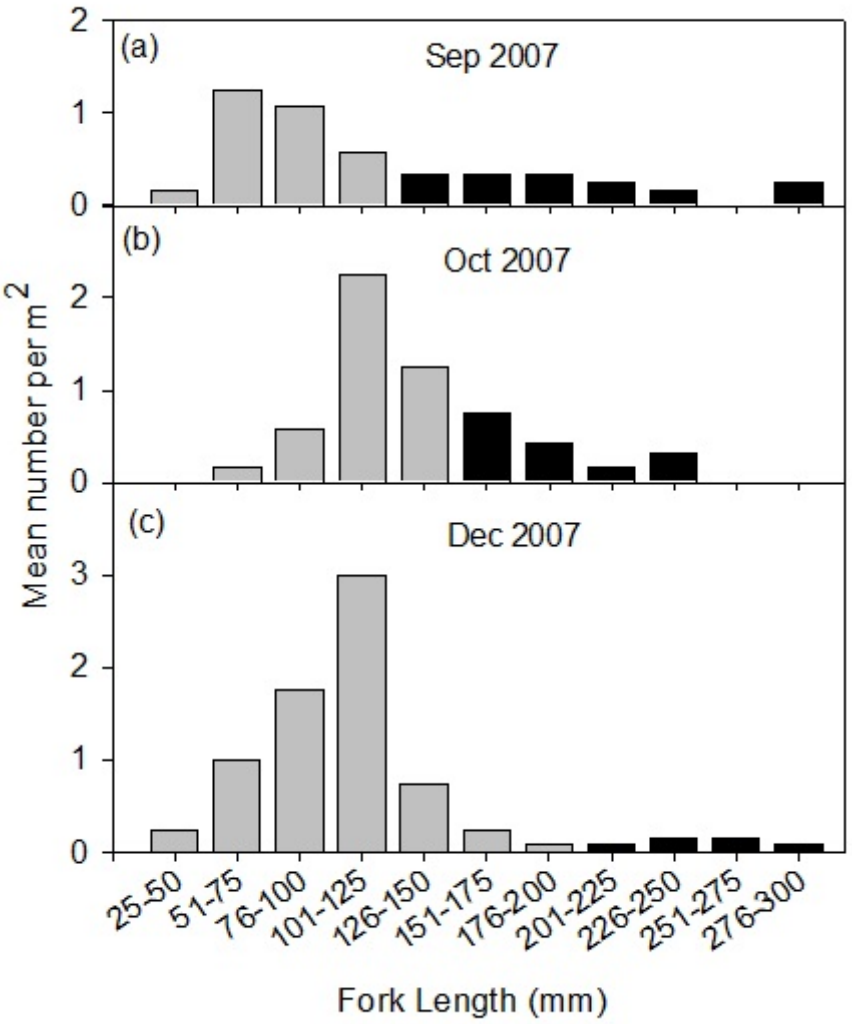


FIGURE 3-4.—Length-frequency distributions of gray triggerfish in **(a)** September 2007, **(b)** October 2007, and **(c)** December 2007 from the northern Gulf of Mexico. Age 0 = gray bars and age 1 = black bars.

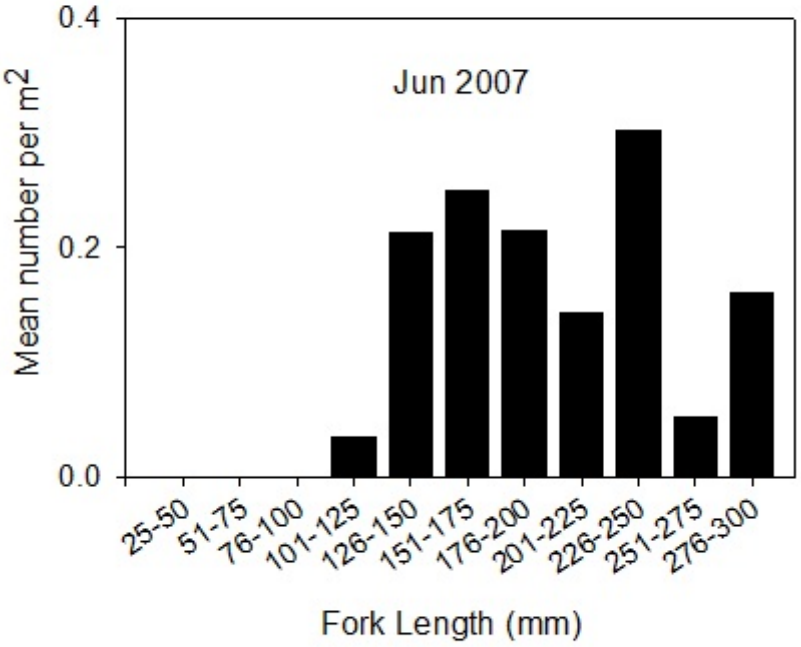


FIGURE 3-5.—Length-frequency distributions of age-1 gray triggerfish from 2006 artificial reefs surveyed June 2007 from the northern Gulf of Mexico. No age-0 gray triggerfish were observed.

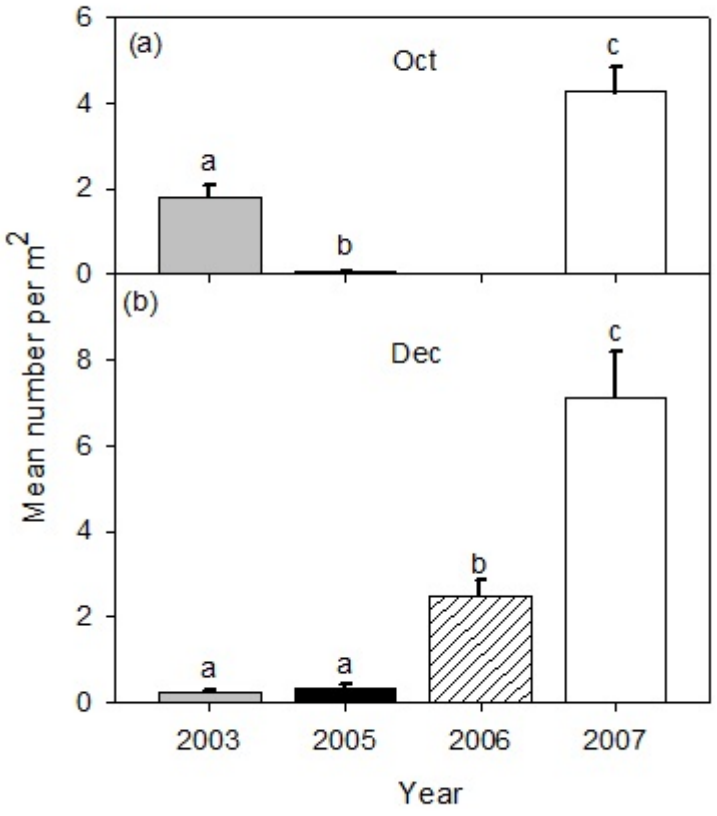


FIGURE 3-6.—Comparison across years for mean (± SE) number of gray triggerfish in **(a)** October and **(b)** December from the northern Gulf of Mexico. Significant differences ($\alpha = 0.05$) are represented by different letters in each panel.

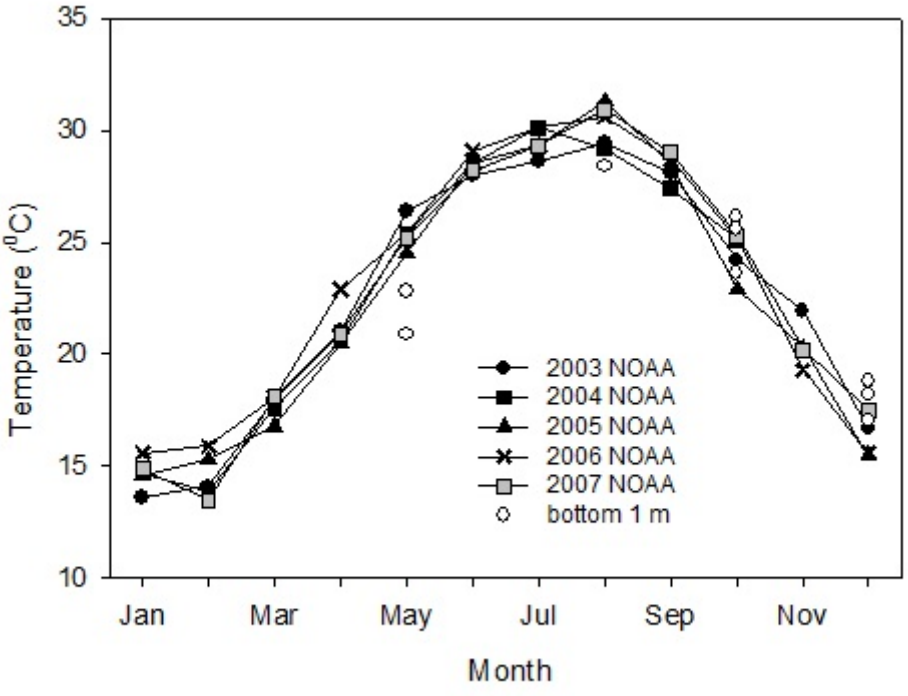


FIGURE 3-7.—Mean surface water temperatures calculated from the NOAA data buoy 41 km south of Biloxi, Mississippi, from 2003 through 2007. NOAA surface water data 2003 = black circles, 2004 = black squares, 2005 = black triangles, 2006 = black x's, 2007 = gray squares, and data collected from 2003 to 2007 during this study 1-m above the bottom = open circles.

CHAPTER 4

COMPETITIVE INTERACTIONS BETWEEN GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, AND RED SNAPPER, *LUTJANUS CAMPECHANUS*, IN FIELD AND LABORATORY EXPERIMENTS

Abstract.—Competitive interactions between gray triggerfish, *Balistes capriscus*, and red snapper, *Lutjanus campechanus*, were studied in field removal and laboratory growth experiments. In the field, all gray triggerfish and red snapper were counted and sizes estimated on 24 artificial reefs (army tanks) by SCUBA divers. Gray triggerfish were removed from half of the artificial reefs ($N = 12$). After 7 months all reefs were re-surveyed. At the end of the experiment, red snapper numbers increased on both removal and non-removal artificial reefs. No significant differences were detected between red snapper numbers on the removal versus non-removal artificial reefs after 7 months. Length-frequency distributions for red snapper at the start of the experiment were not significantly different across treatments. After 7 months the length-frequency distribution from removal artificial reefs was significantly different from non-removal artificial reefs. There were higher numbers of red snapper for all size classes, except the 400-mm TL size class, which showed lower numbers on the removal than non-removal artificial reefs. In the laboratory, competitive interactions were quantified by measuring change in growth

rate of gray triggerfish and red snapper randomly assigned to the following three treatments: (1) control treatment of 6 red snapper, (2) control treatment of 6 gray triggerfish, and (3) mixed treatments with 3 red snapper and 3 gray triggerfish. Each treatment was replicated 2-3 times for seven 30-35 d trial periods. Temperature, salinity, and dissolved oxygen were maintained within a narrow range throughout the seven trials. Red snapper showed a significantly slower growth rate when mixed with gray triggerfish compared to red snapper control. Red snapper and gray triggerfish showed competitive interactions based on significant differences in red snapper length-frequency distributions in field experiments and significant differences in laboratory growth rates. These competitive interactions could lead to negative effects on red snapper on artificial reefs with gray triggerfish in the northern Gulf of Mexico.

Introduction

Competitive interactions between species can be significant when resources are limited (Begon et al. 1996;Wootton 1998). Several studies in the coral reef community have shown that shelter type and food were limiting resources for reef fish (Shulman et al. 1983; Hixon and Beets 1989; Jones 1986; Jones 1991). Field studies such as species-removal experiments have provided examples of competition for a limited resource such as habitat or food, by removing one species and assessing change in abundance or growth of the other (Connell 1983; Schoener 1983). Hixon (1980) and Schmitt and Holbrook (1986) found that black seapearch, *Embiotoca jacksoni,* avoided foraging in areas with

striped seaperch *E. lateralis.* Sano (1990) examined two species of territorial male sandperches, (speckled sandperch *Parapercis hexophtalma* and latticed sandperch *P. clathrata*), that have similar habitat and diet, and found that new territories were established and filled by either species after removal of the other species. Fish removal experiments on unrelated species in freshwater lakes found similar results. Persson (1986) found when roach, *Rutilus rutilus,* abundance and biomass were reduced the abundance and growth of the European perch, *Perca fluviatilis,* increased.

Growth experiments also have examined interspecific competition between species in confined cages or controlled laboratory conditions. Persson (1987) completed a growth experiment between roach and European perch in cage enclosures built in a freshwater lake and showed lower growth rates of age-0 perch when roach were present. Buckel and McKown (2002) investigated interspecific competition in the laboratory between juvenile striped bass, *Morone saxatilis*, and bluefish, *Pomatomus saltatrix*. After 60 days, they did not detect a significant difference in growth rate when the species were sympatric (held together) versus allopatric (separate) (Buckel and McKown 2002).

Fish species with high niche overlap often compete for habitat or food (Begon et al. 1996; Wootton 1998). Thus, competition might be expected between gray triggerfish, *Balistes capriscus*, and red snapper, *Lutjanus campechanus*, because they are the most abundant co-occurring species on artificial reefs in the northern Gulf of Mexico (Lingo and Szedlmayer 2006) with overlapping diets (Blitch 2000; Ouzts and Szedlmayer 2003; Szedlmayer and Lee 2004), and natural reefs are limited (Parker et al. 1983; Gallaway et al., in press). I used both field manipulations and controlled laboratory experiments to

test for possible interactions between gray triggerfish and red snapper. For this study, exploitative competition was examined in field removal experiments and defined as adverse affects on an organism resulting from reductions in resources caused by competing organisms (Begon et al. 1996; Wootton 1998). Inference competition was examined in laboratory growth rate experiments and occurs when resources are not limited, but competitors exclude the other species from the resource (Begon et al. 1996; Wootton 1998).

Methods

Field Experiment Design

The study site was located 28-50 km south to southeast of Dauphin Island, Alabama, in the northern Gulf of Mexico. Twenty-four M60 army tanks (9.3 x 3.6 x 3.2 m, 51.3 metric tons (t)) were visually and video (Sony TR101 Hi-8 video camera) surveyed by two SCUBA divers. Gray triggerfish and red snapper were identified, counted, and size classes estimated. All artificial reefs used for the experiment contained both species at the start of the experiment. Gray triggerfish sizes were estimated at 25-mm increments, from 125 to 450 mm fork length (FL), and red snapper were estimated at 100-mm increments, from 200 to 1,000 mm total length (TL). Fork length (FL) was used for gray triggerfish because of the long extensions on gray triggerfish caudal fins, which are difficult to estimate underwater. On 50% of the army tanks ($N = 12$) gray triggerfish were removed by SCUBA divers spearfishing. On occasions multiple SCUBA dives on

consecutive 2 days were needed to remove all the gray triggerfish from particular tanks. The removal experiment began on 13 October 2005 and all artificial reefs ($N = 24$) were surveyed by 7 November 2005. After seven months, the artificial reefs were surveyed from 1 to 23 June 2006, and all gray triggerfish and red snapper were counted and size classes estimated.

Temperature (°C), salinity (‰), and dissolved oxygen (mg/L) were measured at each reef site with a remote recording YSI-6920 meter (Yellow Springs Instruments, Yellow Springs, Ohio). Mean values of these measures were calculated from continuous vertical recordings from the bottom to 9.1 m from the surface.

Data Analysis

Temperature, salinity, and dissolved oxygen were compared for each survey period (i.e., start, end) of the experiment using an analysis of variance (ANOVA). The total number of red snapper and gray triggerfish were calculated for each reef, and compared across treatments within each survey period with (ANOVA). The increase in number of red snapper over survey periods between treatments was also compared with ANOVA. Size of red snapper (TL) and gray triggerfish (FL) were also compared separately for each survey period across treatments with an ANOVA. A two-way ANOVA was used to detect interactions in size of red snapper and gray triggerfish over survey period and treatment. Length-frequency distributions for red snapper were compared across treatments within each survey period with chi-square analyses.

Laboratory Experiment

To test for competitive interactions in the laboratory, gray triggerfish and red snapper were caught by hook and line and returned to the laboratory in circular containers (85 x 52 cm, 250 L). Each container was aerated and held 10-15 fish. To limit barotrauma, fish were collected from artificial reefs at depths of less than 22 m. Each fish was vented (relieve expanded gases in swim bladder) in the field using a 12-gauge needle inserted into the swim bladder. Each fish was tagged with a passive integrated transponder (PIT) tag placed in the peritoneal cavity, for individual identification. All fish were held at a narrow range of temperature and salinity in a 14,800-L closed recirculating seawater system. All fish were acclimated to captivity for 3-4 weeks before starting experiments.

At the start of a trial all fish were anesthetized with 150 mg/L of tricaine methanesulfonate (MS-222), scanned for PIT tags, weighed to 0.1 g and length measured to 0.1 mm. Fish were randomly assigned to one of the following three treatments: (1) control treatment of 6 red snapper, (2) control treatment of 6 gray triggerfish, or (3) mixed treatment of 3 red snapper and 3 gray triggerfish. Each trial had 2 replicate tanks of control red snapper ($N = 12$) and control gray triggerfish ($N = 12$), and 3 replicate tanks of mixed red snapper ($N = 9$) and gray triggerfish ($N = 9$). Each replicate was carried out in a circular 1,230-L tank (1.5 m diameter x 0.7 m deep) that contained two PVC refuge boxes (28.5 x 18.5 x 39.5 cm) with holes (14 x 14 cm) cut into each side. All replicate tanks were part of the same seawater system and had the same lighting. Every 2 d, temperature, salinity, and dissolved oxygen were recorded with a YSI-85 meter (Yellow Springs Instruments, Yellow Springs, Ohio) and ammonia, nitrite, and nitrate levels were

measured with LaMotte test kits (LaMotte Company, Chestertown, Maryland). Fish were fed weighed rations of squid and shrimp typically ranging from 100 to 250 g per tank every 2 d until satiation. When feeding slowed, the remaining portion of food was weighed, to estimate ration consumed for each replicate tank. Seven trials were completed using different fish every trial, with each trial lasting 30 to 35 d.

Data Analysis

Change in specific growth rate per day was calculated for each fish as the change in weight (g)/start weight (kg)/number of experiment days (d). These changes in specific growth rate were then compared across treatments with ANOVA. Specific ration consumed was defined as the weigh of food consumed in each tank (g)/ total weight of 6 fish in the tank (kg)/ number of experiment days (d). These specific rations were then compared across treatments with ANOVA. All tests were considered significant at $\alpha = 0.05$. If significant differences were detected, a Student-Newman-Kuels test was used to show specific differences (Zar 1999).

Results

Field Experiment

Experimental artificial reefs were at similar depths, non-removal treatments ranged from 23.4 to 29.7 m and removal treatments ranged from 20.7 to 29.4 m. At the start of the experiment significant differences were not detected between temperature (ANOVA:

df = 1,17, F = 1.21, *P* >0.1), salinity (ANOVA: df = 1,17, F = 0.36, *P*>0.5), and dissolved oxygen (ANOVA: df = 1,17, F = 1.7, *P*>0.2) across treatments (Table 4-1). Similarly, at the end of the experiment significant differences were not detected between temperature (ANOVA: df = 1,22, F = 0.01, *P*>0.5), salinity (ANOVA: df = 1,22, F = 0.86, *P*>0.1), and dissolved oxygen (ANOVA: df = 1,22, F = 1.5, *P*>0.1) across treatments (Table 4-1). At the start of the experiment no significant differences were detected in the number of gray triggerfish across treatments (ANOVA: df = 1, 22, F = 2.5, *P*>0.1; Figure 4-1). Gray triggerfish numbers decreased for both removal and non-removal reefs between the start and the end of the experiment. Removal reefs showed significantly fewer gray triggerfish compared to non-removal reefs at the end of the experiment (ANOVA: df = 1, 22, F = 5.2, *P*<0.05; Figure 4-1). At the start of the experiment, no significant differences were detected in the number of red snapper across treatments (ANOVA: df = 1,22, F = 0.05, *P*>0.5; Figure 4-2). At the end of the experiment, no significant differences were detected in the number of red snapper across treatments (ANOVA: df = 1,22, F = 0.04, *P*>0.5; Figure 4-2). Red snapper abundance increased between the start and the end of the experiment, but significant differences were not detected across treatments (ANOVA: df = 1,22, F = 0.1, *P*>0.5; Figure 4-3).

Size comparisons

Red snapper showed similar mean lengths (± SE) across treatments at the start of the experiment (removal = 392 ± 5.6 mm TL; non-removal = 393 ± 5.7 mm TL; ANOVA: df = 1, 926, F = 0.05, *P*>0.5). At the end of the field experiment, significant differences in

red snapper length were not detected between removal (388 ± 4.8 mm TL) and non-removal (396 ± 4.4 mm TL; df = 1,165, F = 1.2, P>0.1). However, at the start of the experiment significant differences were detected in gray triggerfish length between removal (316 ± 5.3 mm FL) and non-removal (271 ± 6.7 mm FL; ANOVA: df = 1,190, F = 23.8, P<0.001). At the end of the experiment, significant differences in gray triggerfish length were again detected between removal (298 ± 15.2 mm FL) and non-removal (261 ± 5.8 mm FL; ANOVA: df =1,71, F=7.8, P<0.01). Two-way ANOVA for gray triggerfish size comparisons showed a significant treatment (P< 0.001), but no time (P>0.1), or time-treatment interaction (ANOVA: df =1,257, F=0.21, P>0.5).

Length-frequency distributions

At the start of the experiment, red snapper length-frequency distributions were not significantly different between removal versus non-removal reefs (df = 8, X^2 = 12.9, P>0.1; Figure 4-4a). After seven months the length-frequency distribution from removal reefs was significantly different from non-removal reefs. There were higher numbers for all size classes, except the 400-mm TL size class that showed lower numbers on the removal than non-removal reefs (df = 8, X^2 = 20.1, P<0.01; Figure 4-4b).

Laboratory experiment

Temperature ranged from 18.8 to 24.8°C, salinity ranged from 32 to 38‰, and dissolved oxygen ranged from 5.3 to 7.4 mg/L in the recirculating seawater system throughout the seven trials (Table 4-2). In the laboratory experiment, red snapper had a

mean (± SE) length of 299 ± 2.6 mm FL and mean weight of 0.49 ± 0.01 kg (N = 161), and gray triggerfish had a mean (± SE) length of 292 ± 2.8 mm FL and mean weight of 0.58 ± 0.02 kg (N = 156). Significant differences were not detected in specific ration consumed ($g \cdot kg^{-1} \cdot d^{-1}$) across treatments (ANOVA: df = 2,764, F = 1.6, P>0.1; Figure 4-5). Red snapper in the mixed treatments showed significantly slower growth rate ($g \cdot kg^{-1} \cdot d^{-1}$) than control red snapper, control gray triggerfish, and gray triggerfish in the mixed treatments (ANOVA: df = 9, 291, F = 14.1, P< 0.001; Figure 4-6).

Discussion

Although difficult to document in field experiments, interspecific competition between gray triggerfish and red snapper was clearly observed in laboratory experiments. Gray triggerfish negatively affected the growth rate of red snapper in mixed treatments compared to red snapper growth rates in single species treatments. Similar results were shown when age-0 European perch were mixed with roach in enclosed cages, growth rates of perch were lower, and sometimes negative (Persson 1987). Olson et al. (1995) also used enclosures and observed slower growth rate in age-0 largemouth bass, *Micropterus salmoides*, at high densities of bluegill, *Lepomis macrochirus*.

Interference competition was documented in the laboratory. Further evidence of competitive interactions was bite marks on red snapper in mixed treatments but not in single species treatments. Aggression by gray triggerfish may be related to spawning as

they are known to be highly aggressive during spawning (MacKichan and Szedlmayer 2007).

Significant differences in red snapper length-frequency distributions at the end of the experiment between removal and non-removal reefs indicated the potential for competition between red snapper and gray triggerfish. Other field studies have removed the more dominant species and found an increase in the abundance of another species. Hixon (1980) and Schmitt and Holbrook (1986) found a difference in abundance of the black seaperch, when the striped seaperch, was removed in both shallow water and deep reefs. In bicolor damselfish, *Stegastes partitus*, both juveniles and adults increased, after removal of the more dominant three-spot damselfish, *S. planifrons* (Robertson 1996). Similarly, the gopher rockfish, *Sebastes carnatus*, increased in abundance after the removal of the black-and-yellow rockfish, *S. chrysomelas*, and in a reciprocal removal the black and yellow rockfish increased in the absence of the gopher rockfish (Larson 1980). Fausch and White (1981) found that after removing dominant brown trout, *Salmo trutta,* brook trout, *Salvelinus fontinalis*, occupied the brown trout's more advantageous positions for feeding and resting which were areas with greater water velocity, depth, and shade. Dominant territorial species can also affect recruitment. Shulman et al. (1983) showed when more dominant or territorial fishes settled on artificial reefs first, such as yellowtail snapper, *Ocyurus chrysurus*, blackfin snapper, *Lutjanus buccanella*, or mahogany snapper, *Lutjanus mahogoni*, the number of other fishes such as grunts *Haemulon* spp. and high-hats, *Pareques acuminatus* were reduced.

Although patterns of increased numbers of red snapper on removal reefs were consistent with a competitive interaction effect, significant differences in field experiments were not detected. Perhaps sample sizes were low ($N = 12$) for both removal and non-removal reefs and further field experiments should attempt to increase sample size. Another possible difficulty may have been that too much time had elapsed (7 months) before surveys were repeated. For example, Hixon's (1980) removal experiment of surfperches found competitive interactions after 2 weeks and that the population density of each species had returned to the baseline levels after 6 months. In addition, Sano (1990) found that after removal of the dominant speckled sandperch, within two weeks the latticed sandperch, moved into the speckled sandperch territory. A third difficulty may have been that experimental artificial reefs used during this study were open to public fishing. Fishing may have caused unexpected gray triggerfish decreases in non-removal reefs as well as the removal reefs. Future removal studies should attempt to limit public access to manipulated experimental reef sites.

Questions remain as to the importance of food or habitat limitation, but gray triggerfish and red snapper showed competitive interactions, based on significant differences in red snapper length-frequencies in removal field experiments, and significant growth rate differences in laboratory experiments. These competitive interactions could lead to negative effects for red snapper on artificial reefs with aggressive gray triggerfish. Red snapper is probably the most important commercial and recreational fish species in the northern Gulf of Mexico (Gallaway et al., in press). It is also overfished, and

management strategies that involve artificial reef construction may need to consider side effects that could be reducing red snapper numbers.

References

Begon, M., J.L. Harper, and C.R. Townsend. 1996. Ecology, individuals, populations, and communities. 3rd edition. Blackwell Science. Cambridge, Massachusetts.

Blitch, K.M. 2000. The feeding habits of gray triggerfish, *Balistes capriscus* (Gmelin) from the northeast Gulf of Mexico. Master's thesis, Auburn University, Auburn.

Buckel, J.A., and K.A. McKown. 2002. Competition between juvenile striped bass and bluefish: resource partitioning and growth rate. Marine Ecology Progress Series 234:191-204.

Connell, J.H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist 122:661-689.

Fausch, K.D., and R.J. White 1981. Competition between brook trout *(Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. Canadian Journal of Fisheries and Aquatic Science 38: 1220-1227.

Gallaway, B., S.T. Szedlmayer, and W.J. Gazy (in press). A life history review for red snapper in the Gulf of Mexico with an evaluation of the importance of offshore petroleum platforms and other artificial reefs. Fisheries Review.

Hixon, M.A. 1980. Competitive interactions between California reef fishes of the genus *Embiotoca*. Ecology 61: 918-931.

Hixon, M.A., and J.P. Beets. 1989. Shelter characteristics and Caribbean fish assemblages: experiments with artificial reefs. Bulletin of Marine Science 44: 666-680.

Jones, G.P. 1986. Food availability affects growth in a coral reef fish. Oecologia (Berlin) 70:136-139.

Jones, G.P. 1991. Postrecruitment processes in the ecology of coral reef fish populations: a multifactorial perspective. Pages 294-328 *in* P. F. Sale, editor. Ecology of fishes on coral reefs. Academic Press, San Diego, California.

Larson, R.J. 1980. Competition, habitat selection, and the bathymetric segregation of two rockfish (*Sebastes*) species. Ecological Monographs 50:221-239.

Lingo, M.E., and S.T. Szedlmayer. 2006. The influence of habitat complexity on reef fish communities in the northeastern Gulf of Mexico. Environmental Biology of Fishes 76: 71-80.

MacKichan, C.A., and S.T. Szedlmayer 2007. Reproductive behavior of gray triggerfish, *Balistes capriscus*, in the Northeastern Gulf of Mexico. Proceedings of the 59th Gulf and Caribbean Fisheries Institute 59:231-235.

Olson, M.H., G.G. Mittelbach, and C.W. Osenberg. 1995. Competition between predator and prey resource-based mechanism and implications for stage-structured dynamics. Ecology 76:1758-1771.

Outzs, A.C., and S.T. Szedlmayer. 2003. Diel feeding patterns of red snapper on artificial reefs in the north-central Gulf of Mexico. Transactions of the American Fisheries Society 132:1186-1193.

Parker, R.O., D.R. Colby, and T.D. Willis. 1983. Estimated amount of reef habitat on a portion of the U.S. South Atlantic and Gulf of Mexico continental shelf. Bulletin of Marine Science 33:935-940.

Persson, L. 1986. Effects of reduced interspecific competition on resource utilization in perch (*Perca fluviatilis*). Ecology 67:355-364.

Persson, L. 1987. Competition-induced switch in young of the year perch, *Perca fluviatilis*: an experimental test of resource limitation. Environmental Biology of Fishes. 19:235-239.

Robertson, D.R. 1996. Interspecific competition controls abundance and habitat use of territorial Caribbean damselfishes. Ecology 77:885-899.

Sano, M. 1990. Patterns of habitat and food utilization in two coral-reef sandperches (Mugiloididae): competitive or noncompetitive coexistence? Journal of Experimental Marine Biology and Ecology 140: 209-223.

Schmitt, R.J., and S.J. Holbrook. 1986. Seasonally fluctuating resources and temporal variability of interspecific competition. Oecologia 69:1-11.

Schoener, T W. 1983. Field experiments on interspecific competition. American Naturalist 22: 240-285.

Shulman, M.J., J.C. Ogden, J.P. Ebsersole, W.N. McFarland, S.L. Miller, and N.G. Wolf. 1983. Priority effects in the recruitment of juvenile coral reef fishes. Ecology 64:1508-1513.

Szedlmayer, S.T., and J.D. Lee. 2004. Diet shifts of juvenile red snapper (*Lutjanus campechanus*) with changes in habitat and fish size. Fishery Bulletin 102: 366-375.

Wootton, R.J. 1998. Ecology of teleost fishes, 2nd edition. Kluwer Academic, Dordrecht, The Netherlands.

Zar, J. H.1999. Biostatistical Analysis. 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

TABLE 4-1.—Mean (± SE) depth, temperature, salinity, and dissolved oxygen (DO) measured at the start (13 Oct -7 Nov 05) and end (1-23 Jun 06) of the field experiment. Significant differences were not detected for characteristics between treatments (*N* = sample size).

	Start		End	
Characteristic	Triggerfish removed	Triggerfish not removed	Triggerfish removed	Triggerfish not removed
Depth (m)	25.2 ± 0.8	27.9 ± 0.6	25.2 ± 0.8	27.9 ± 0.6
Temperature (˚C)	24.9 ± 0.5	25.6 ± 0.5	24.2 ± 0.5	24.1 ± 0.4
Salinity (‰)	34.1 ± 0.2	33.9 ± 0.2	34.2 ± 0.2	34.4 ± 0.2
DO (mg/L)	5.6 ± 0.2	7.0 ± 0.9	6.8 ± 0.2	6.5 ± 0.1
N	9	10	12	12

TABLE 4-2.—Mean (± SE) and range of temperature, salinity, and dissolved oxygen measured every 2 days throughout the 7 laboratory trials (*N* = sample size).

Characteristic	Mean ± SE	range	*N*
Temperature (°C)	21.2 ± 0.1	18.8 to 24.8	100
Salinity (‰)	34.9 ± 0.1	32 to 38	100
Dissolved oxygen (mg/L)	6.1 ± 0.1	5.3 to 7.4	80

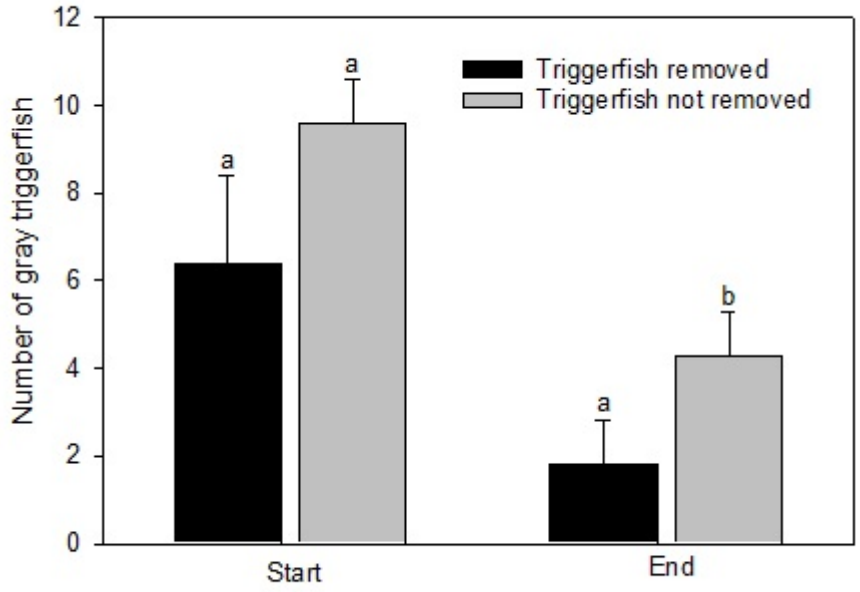


FIGURE 4-1.—Mean (± SE) number of gray triggerfish surveyed on triggerfish removal and non-removal artificial reefs at the start (13 Oct -7 Nov 05) and the end (1-23 Jun 06) of the experiment. Significant differences ($\alpha = 0.05$) are shown by different letters between treatments.

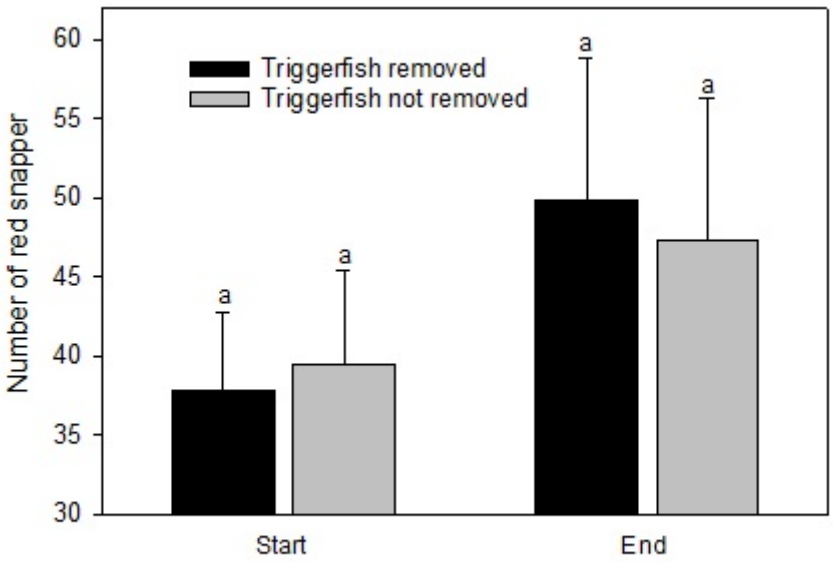


FIGURE 4-2.—Mean (± SE) number of red snapper surveyed on triggerfish removal and non-removal artificial reefs at the start (13 Oct -7 Nov 05) and the end (1-23 Jun 06) of the experiment. Significant differences ($\alpha = 0.05$) are shown by different letters between treatments.

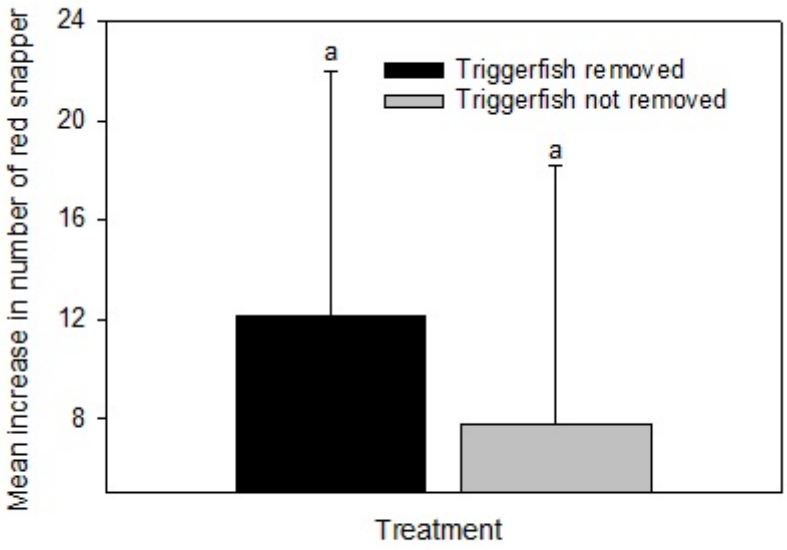


FIGURE 4-3.— Mean (± SE) increase in the number of the red snapper surveyed from the start (13 Oct -7 Nov 05) and the end (1-23 Jun 06) on triggerfish removal and non-removal artificial reefs. Significant differences ($\alpha = 0.05$) are shown by different letters.

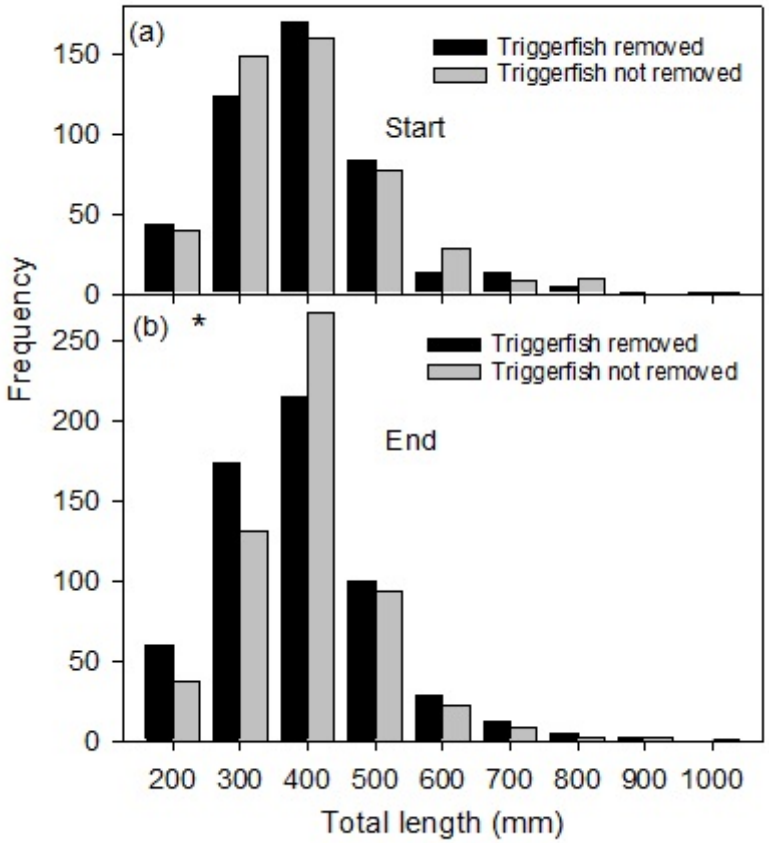


FIGURE 4-4.—Red snapper length-frequency distributions from triggerfish removal and non-removal artificial reefs at the **(a)** start (13 Oct -7 Nov 05) and **(b)** end (1-23 Jun 06) of the experiment. An asterisk indicates significant differences ($\alpha = 0.05$) in length-frequency distributions across treatments.

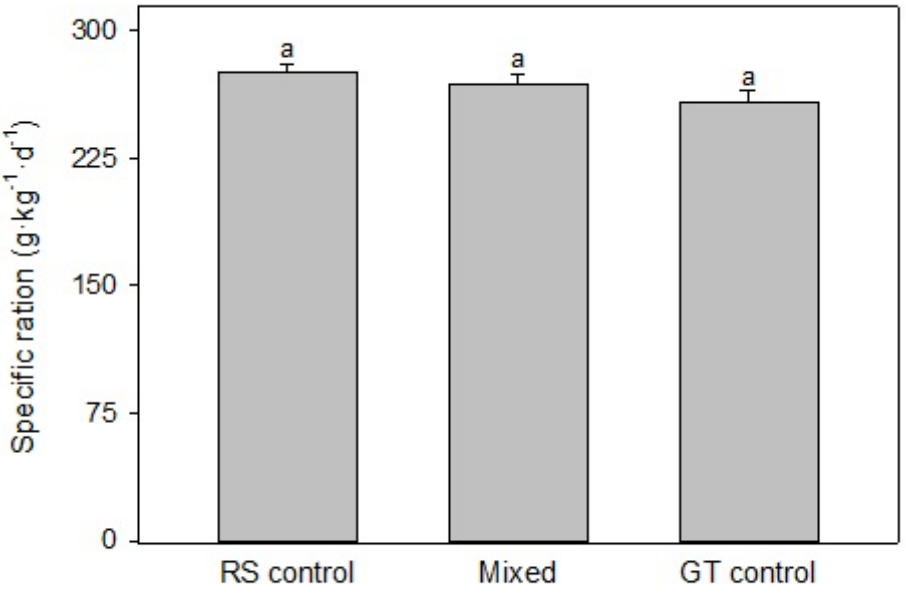


FIGURE 4-5.—Mean (± SE) specific ration ($g \cdot kg^{-1} \cdot d^{-1}$) consumed across the following three treatments: red snapper control (RS control), red snapper and gray triggerfish (mixed), and gray triggerfish control (GT control). Significant differences ($\alpha = 0.05$) are shown by different letters.

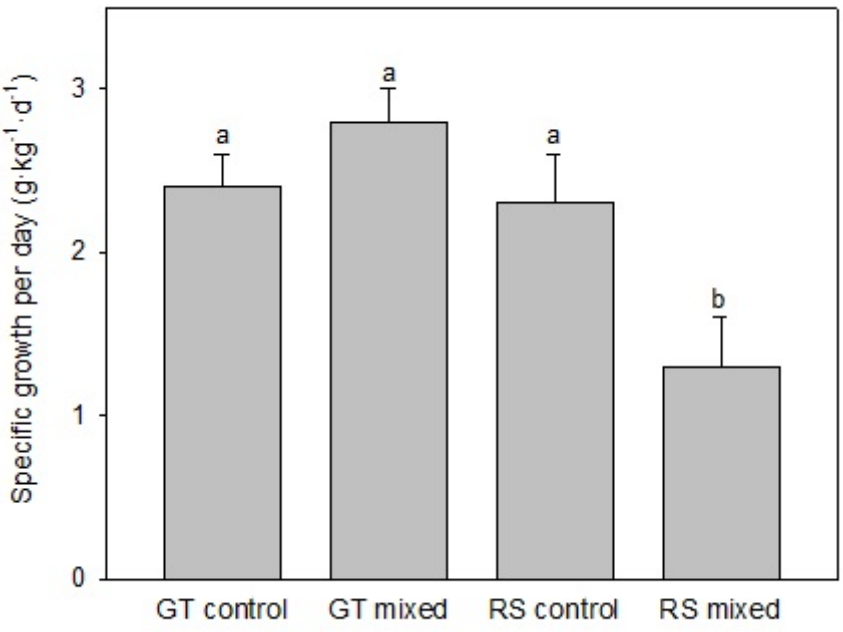


FIGURE 4-6.—Mean (± SE) specific growth rate per day ($g \cdot kg^{-1} \cdot d^{-1}$) across the following four treatments: gray triggerfish control (GT control), gray triggerfish mixed (GT mixed), red snapper control (RS control), and red snapper mixed (RS mixed). Significant differences ($\alpha = 0.05$) are shown by different letters.