

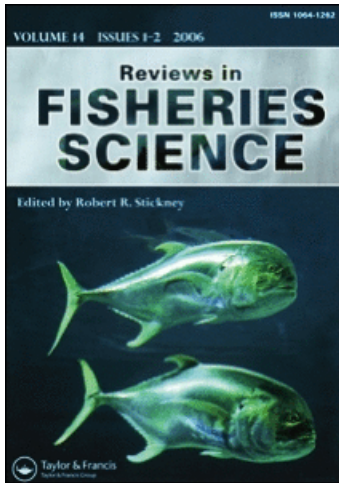
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Spawning of Red Snapper (*Lutjanus campechanus*) in Response to Hormonal Induction or Environmental Control in a Hatchery Setting

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Red snapper (*Lutjanus campechanus*) is a popular marine sportfish and is a significant part of the commercial fisheries in the Gulf of Mexico. It can be reproduced under hatchery conditions by induced spawning of wild-caught adults or by conditioning wild-caught adults to spawn naturally under controlled conditions. Mature females (Gondosomatic Index ≥ 1) occur in the northern Gulf of Mexico from May to September. When such females were collected off the Alabama coast and induced to spawn with HCG, $56 \pm 17.3\%$ of the females ovulated. Fecundity (ovulated floating eggs) varies, for 60 spawns averaged $197,212 \pm 173,349$ eggs/kg female. Percent hatch averaged $42.1 \pm 3.44\%$. Wild-caught adult red snapper can be held in confinement and natural spawning obtained when temperature and photoperiod are controlled. Adult snapper held in 13.2-m^3 tanks (8–10 fish/tank), where photoperiod and temperatures were adjusted, spawned naturally 63 times over a 105-day period, with a mean egg production/tank of 4.2 million eggs. Mean fertilization rate, hatch rate, and survival at 36–40 hph were 90.5%, 83%, and 49%, respectively. Natural spawns produce better quality seed, but the occurrence of fertilized spawns is unpredictable. Hormone-induced spawns are easier to schedule but egg quality can be variable.

Keywords red snapper, reproduction, fecundity, spawning

I INTRODUCTION

Red snapper *Lutjanus campechanus*, a popular restaurant fish highly prized by anglers, is considered overfished, and is highly regulated through controversial regulations that affect both the recreational/charter boat industry as well as the commercial shrimp fishery. Annual commercial landing of red snapper have been as high as 6,363 mt in 1965 (NMFS, 2007) but have since declined. In 2008 the commercial fisheries were restricted to a total harvest of 1,157 mt (NMFS, 2008). Hatcheries can make significant contributions to the understanding of the life history of a fish, re-establishment of populations, as well as being a source of seed for aquaculture (Stickney, 2001). There is interest in reproducing red snapper under controlled conditions to gain a better understanding of its reproductive characteristics,

issues associated with larval development and survival, as well as providing fingerlings for mark and recapture studies, stock enhancement, and aquaculture.

Red snapper can be reproduced in captivity using wild-caught fish that are hormone-induced to spawn soon after capture (Minton et al., 1983). Red snapper can also be domesticated under controlled conditions and stimulated to spawn by controlling the temperature and photoperiod (Arnold et al., 1978) or through hormone administration (Laidley and Ostrowski, 2001). Snapper larvae are difficult to culture through the first 30 days post-hatch because of their small size and limited endogenous nutrient reserves. Part of the difficulty associated with the quality of larvae results from the quality of the brood stock and how they were reproduced. Auburn University and the Marine Resources Division of the Alabama Department of Conservation and Natural Resources have been collaborating at the Claude Peete Mariculture Center (CPMC) in Gulf Shores, Alabama, on various aspects of red snapper biology and its reproduction. The following is a review of those efforts as well as work done by

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others regarding snapper biology and reproduction. Emphasis is given to the natural reproductive cycle, induced spawning of wild-caught fish, and natural spawning under controlled hatchery conditions.

II NATURAL REPRODUCTIVE CYCLE

One of the first steps in the successful reproduction of any fish is to understand its natural life history, when mature animals occur in the wild and under what conditions. Red snapper is one of some 103 species of the tropical and subtropical family Lutjanidae (Froese and Pauly, 2006). It has a natural distribution in the Atlantic on the coastal shelf from Massachusetts to Florida and around the Gulf of Mexico (Hoese and Moore, 1977). Red snapper reach sexual maturity at approximately two years of age when they are about 26–27 cm in length (Moran, 1988). They are asynchronous spawners with an extended spawning season. In the Northern Gulf of Mexico, mature fish can be found from May through September (Collins et al., 1996; Bueno, 2002; Woods et al., 2003), and also in the Atlantic during the same timeframe (White and Palmer, 2004). The frequency of mature fish varies over this period, influenced by water temperature and the lunar cycle. In 1999, the mean gonadosomatic index (GSI) of female red snapper caught off the Alabama coast was 0.68 ± 0.31 on April 1, where the surface water was 19.5°C . The temperature had increased to 24.8°C on May 8, and the mean female GSI was 1.28 ± 1.59 . Mean GSIs remained above 1 through the last sampling date of September 9, 1999, where the water temperature was 29.8°C (Bueno, 2002). Mature eggs were found in the ovaries when the GSI was 1 or more. Fish with high GSIs were more common from mid May to mid July. On May 18 and 19, 1999, 8 of the 16 females sampled had a $\text{GSI} > 4$ (Bueno, 2002). White and Palmer (2004) found that, in the Atlantic, the monthly mean GSI for female red snapper was greater than 1.0 from May to September, with a peak in June of 2.7.

The reproductive cycle of many asynchronous spawning temperate and tropical reef fishes is influenced by the lunar

cycle. Wild gray snapper *Lutjanus griseus* and cubera snapper *L. cyanopterus* spawn primarily around the dark of the moon (Domeir et al., 1996; Heyman et al., 2005). Captive mangrove red snapper *L. argentimaculatus* were reported by Emata (2003) to spawn more frequently three days before or after the last quarter moon (43.4% of the monthly spawns) and near a new moon (37.1%). Davis and West (1993) concluded that wild *L. vittus* has two peak periods of spawning during a lunar cycle, the largest peak 6 days after a full moon and a smaller peak 3 days after a new moon. Bueno (2002) found that the GSI of red snapper caught off the Alabama coast during May to August 1999 varied considerably, with distinct highs and lows (Figure 1). On several occasions, the mean female GSI would increase soon after a new moon and then drop a percentage point and increase again until the next new moon period, when it would drop again. However, the lack of samples on a few key dates did not allow a complete view of the spawning period. Even though there were peaks and valleys in the mean GSI, at any one point in the season females with high GSIs could be found, but the frequency of such fish was greater soon after a new moon. Buenos (2002) found that hydrated eggs occurred in four consecutive weekly samples of ovaries taken in June. However, he found no correlation between GSI and relative abundance of hydrated eggs where weekly mean GSIs ranged from 1.0 ± 1.18 to 3.0 ± 1.72 and relative abundance of hydrated eggs ranged from 20.4 ± 9.3 to $35.1 \pm 12.8\%$ of the vitellogenic eggs. Jackson et al. (2006) concluded that red snapper showed no lunar-related spawning cycle as females with hydrated eggs were found every day throughout the lunar cycle during the spawning season. Any periodicity in GSI or abundance of hydrated eggs may influence when quality brooders can be collected from the wild for induced spawning or when domesticated fish might be expected to spawn naturally under controlled conditions.

Red snapper are typical of many asynchronous spawners in that the GSI of a mature female is not very high, but the ovary contains eggs in various states of maturity. Bueno (2002) collected ovaries from late April to early September 1999 from red snapper caught by hook and line off the Alabama coast and dissolved the ovary matrix in Gilson's to determine the number of non-vitellogenic (<0.2 mm diameter), vitellogenic (>0.2 mm

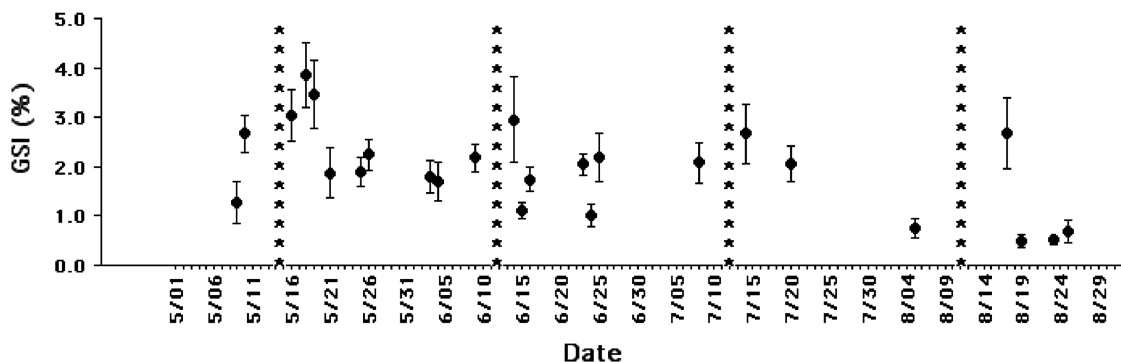


Figure 1 Gonadosomatic index (GSI) (mean \pm SD) for female red snapper (≥ 1 kg) collected off the Alabama coast in 1999 as related to moon phase, where vertical bars indicate a new moon.

diameter), and hydrated vitellogenic (large clear eggs) eggs/kg female. The total number of oocytes/kg female was $5,282,147 \pm 3,426,815$ and $459,096 \pm 375,699$ vitellogenic eggs/kg. From weekly samples taken in June, $20.36 \pm 9.34\%$ to $35.07 \pm 12.83\%$ of the vitellogenic eggs were hydrated. This would correspond to approximately 114,000 to 293,000 eggs/kg female/spawn. This level of fecundity is within the range obtained when red snapper are hormone induced to spawn as discussed later in this article.

III INDUCED SPAWNING

Mature red snapper captured by hook and line can be successfully induced to spawn if given human chorionic gonadotropin (HCG) or luteinizing releasing hormone (LH-RHa) within 24 hr after capture. Fish are ideally captured in relatively shallow water (15–25 m) and the swim bladder deflated with a 3-mm diameter cannula when fish are landed (Collins et al., 1999). Brood fish of approximately 1.5–2 kg are preferred for their ease of handling and spawning characteristics. Females can be identified when captured by the opening of the oviduct on the genital papilla, appearing as a slit across the papilla; males should be running sperm when captured. Fish are held in oxygenated transport tanks at 75–100 g of fish/L of water at temperatures similar (22–28°C) to that at which they were captured. At higher temperatures, successful brood transport becomes more difficult. Fish are often held for 6–8 hr from the time of capture to their arrival at the hatchery.

HCG is the most commonly used hormone to induce spawn red snapper following the procedures developed by Minton et al. (1983). Red snapper will ovulate when given LH-RHa as a series of injections or an implant, but the response time is longer relative to that with HCG (Phelps et al., 1996). Upon arrival at the hatchery, females are given a single injection of HCG at 1,100 IU/kg body weight, and the males an injection at 550 IU/kg. Females can be selected for injection based on the abundance of mature eggs found in a sample taken from the ovary with a catheter (Minton et al., 1983). However, if brooders are collected during the peak of the spawning season, this step is unnecessary and avoids the risk of damaging the oviduct. Sexes are held separately at 24–28°C in aquaria or tanks where the fish can be observed easily. Over a 4-year period for fish collected during May to July, the average frequency of fish that ovulated in response to HCG was $55.8 \pm 17.33\%$. Ovulation can occur within <12 to 36+ hr post-injection, most frequently between 24 and 32 hr. Response time varies as a function of water temperature, maturity of females, and how they are handled. It is not uncommon to have a 12-hr difference in response time among females injected within minutes of each other.

Ovulation can be anticipated by observing the degree of extension of the abdomen and by palpation of the abdomen. As ovulation approaches, the abdomen will become more distended

near the vent and soft to touch, and once ovulation has begun, eggs can be squeezed from the vent. Beginning at approximately 24 hr post injection, females are palpated to determine how soft the abdomen is and if there is egg flow. As snapper eggs complete final maturation, oil globules merge, and the eggs swell in volume and become clear. Egg samples can be taken by inserting a catheter in the oviduct into the ovary and degree of maturity observed. Eggs will become larger, clearer, and with a single oil vacuole as the time of ovulation approaches. Ovulated eggs in the ovary or oviduct are clear with one or two oil globules. As more eggs are ovulated into the lumen of the ovary, the eggs press against each other, filling the voids between eggs, and appear hexagonal in shape. Females that are thought to have ovulated are anesthetized by adding MS-222 to the holding water. Eggs should flow freely from the vent and appear clear and hexagonal when observed under a microscope (Figure 2). This final maturation and ovulation process is very similar to that of striped bass (Kerby, 1986) or snook (Neidig et al., 2000).

Eggs are stripped from the female by hand into a pan of seawater and at the same time sperm is stripped from the males into the pan and mixed with the eggs. Two to five minutes are allowed for fertilization. Egg quality depends on how soon eggs are collected after ovulation. Within an hour after ovulation, if eggs remain in the lumen of the ovary, egg quality drops. Egg quality is apparent after attempting to fertilize the eggs. After eggs and sperm are mixed in seawater and held for a few minutes, the eggs are poured into a graduated cylinder or MacDonald jar and allowed to separate. Good quality eggs float and form a pale orange-colored band at the surface, while poor quality eggs sink (Watanabe et al., 2005). The average number of eggs/mL of floating eggs within 5 min post-fertilization was $2,217 \pm 94.2$ /mL for 14 induced spawns (Papanikos, 2004). A quality spawn is >90% floating eggs. Fertilization rates of floating eggs is variable and for 54 spawns averaged $63.1 \pm 25.87\%$. Egg diameter averages 0.78 mm (0.73–0.93 mm). Eggs that

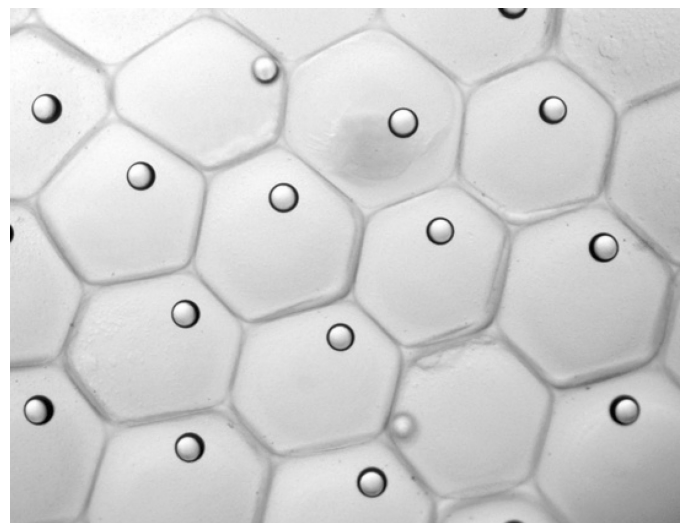


Figure 2 Quality red snapper eggs at the time of stripping, appearing clear and hexagonal, with a single oil globule when observed under a microscope.

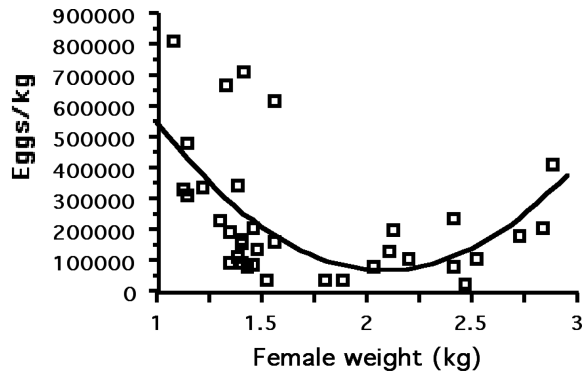


Figure 3 Fecundity (mean \pm SD of floating eggs/kg female) as a function of brood weight for wild females captured over a 4-year period during the natural spawning season and induced to spawn with human chorionic gonadotropin ($R^2 = 0.35$, $p = 0.001$).

sink are poorly fertilized and are discarded. Papanikos (2004) found lower rates of fertilization the greater the variation in egg diameter within a spawn ($R^2 = 0.27$, $p = 0.05$). A possible issue with induced spawning in hormone induction may result in the release of eggs that are not truly mature; thus, with the size variation and the lower fertilization rate, the more variation in egg size.

The mean fecundity for 60 induced spawns obtained over a 4-year period was $197,212 \pm 173,349$ eggs/kg. The high variability may be explained by a number of factors. Females are, on occasions, releasing eggs when captured or upon arrival at the hatchery before the fish are hormone induced to spawn. Such eggs are lost and not included in the fecundity estimates. Fecundity also varies as a function of brood size and season. Smaller females (1.0–1.5 kg) have higher average fecundities/kg than larger fish ($R^2 = 0.41$, $p = 0.002$; Figure 3). There are seasonal differences in fecundity with fish spawned in May giving fewer eggs/kg ($120,289 \pm 73,895$) than fish spawned in June ($234,586 \pm 185,2700$) or July ($416,448 \pm 329,719$) ($p = 0.002$).

IV NATURAL SPAWNING

Wild-caught red snapper can be domesticated and stimulated to spawn by controlling the water temperature and photoperiod. In the wild, snapper form spawning aggregations, where a female would rise toward the surface followed by several males, and eggs and sperm are released near the surface (Domeir et al., 1996; Heyman et al., 2005). Hamamoto et al. (1992) observed a similar spawning pattern with captive *L. stellatus*, when held in a 242-m³ tank. Arnold et al. (1978) obtained natural spawns of red snapper *L. campechanus* in May and June from wild-caught brooders that had been held approximately 20 months in a 29.9-m³ temperature/photoperiod controlled system. Eight fish were held in the tank during the spawning period. Seven egg releases, each giving a few thousand eggs, were documented. Fertilization rates were greater than 90%. Environmental conditions in the tank during the May–June period were 15L–9D photoperiod,

23–25°C water temperature, and 31–34 ppt salinity. Other snapper also have been held under controlled conditions and natural spawns obtained: mangrove red snapper, *L. argentimaculatus* (Emata, 2003; Leu et al., 2003); yellowtail snapper, *Ocyurus chrysurus* (Soletchnik et al., 1989; Turano et al., 2000); *L. kasmira* (Suzuki and Hioki., 1979); and *L. stellatus* (Hamamoto et al., 1992).

At the Claude Petet Mariculture Center, six closed 13.2-m³ tanks were constructed, where photoperiod was adjusted daily and temperatures adjusted weekly, following the natural temperature and photoperiod cycle of the northern Gulf of Mexico off the Alabama coast. Each tank has its own particulate and biofiltration system. Temperature, controlled with a heat pump, ranged from 15–28°C. A dim-up/dim-down fluorescent lighting system provided an average daily maximum of 500 lux at the water surface. Lunar cycle was also regulated to match the calendar date with a dim-up/dim-down incandescent lighting system. Photoperiod ranged from 10 hr 11 min to 14 hr 6 min light/day.

A difficulty with natural spawning of red snapper is the unpredictability of when a spawn will occur. However, brood behavior can give some indication of anticipated spawning. A few hours before sunset, males have been observed to follow a female with a swollen abdomen, swimming close to her vent area. Spawning behavior included fish schooling and slow swimming interrupted by sudden group fast swimming and rushing toward the water surface. This pattern was repeated several times. Fertilized eggs were found in the egg collector 30 min to 2 hr after the spawning behavior had been observed. Spawning occurs mainly during the late afternoon/evening hours. At CPMC, several fertilized spawns with egg development at the blastula stage were collected in the late afternoon to early evening hours, indicating that spawning occurred between 1700–1900 hours, before lights start dimming off (at approximately 1900 hours). Jackson et al. (2006) examined the abundance of hydrated eggs in ovaries of wild-caught red snapper along the northern Gulf of Mexico and concluded that spawning peaks around 1600 hours. Other snappers are reported to spawn later, including at *L. synagris* (Wicklund, 1969), *L. cyanopterus* and *L. jocu* (Heyman et al., 2001) at dusk, *Ocyurus chrysurus* (Watanabe et al., 2005) near dusk and at night, and *L. kasmira* (Suzuki and Hioki, 1979), *L. argentimaculatus* (Emata, 2003; Leu et al., 2003) at night, while *L. vittus* is reported to spawn during the day, 1100 to 1500 hours (Davis and West, 1993).

At CPMC, floating eggs are carried by the water current from the spawning tank into an egg collector. The egg collector is a 200-L trough attached to the side of the brood tank. The inlet is set at surface water level of the brood tank, and the drain, set on the opposite end of the tank, is covered by a 125- μ m mesh screen. An air lift is used to control water flow through the egg-collecting tank. The brood tank is adequately aerated with enough current that both fertilized and unfertilized eggs are suspended in the water column. Eggs flow into the egg collector and accumulate during the night. The egg collector is checked nightly or the first thing each morning once the temperature is 24°C or above. Eggs are collected with a fine-mesh dip net held

in seawater, then poured into a graduated cylinder to separate floating and sinking eggs. Within 5 min, floating eggs separate from sinking eggs. Floating eggs are examined to determine the degree of fertility. On occasion, unfertilized eggs will float for up to an hour after being collected. Fertile eggs collected from the egg collector are distinct in that snapper eggs are relatively clear and have already gone through numerous cell divisions. When eggs are examined under a microscope, the cell division is evident. Eggs are enumerated by volume based on an average count of eggs/mL.

Natural spawns of red snapper were first obtained at CPMC the spring of 2001. Wild-caught adult snapper were stocked at approximately 5 males and 7 females per tank in mid October 2000. Average weights were 1.66 kg and 1.49 kg, respectively. Fish were fed to satiation a diet of fresh shrimp, squid, and fish, as well as gelatin cubes containing the above ingredients supplemented with vitamins, minerals, and fish oil. The first egg release was obtained on May 12, 2001 at a temperature of 24.5°C and a 13-hr 35-min light cycle. Fish in four of the six tanks released eggs, with two tanks more consistently. One tank gave eggs on 23 days over an 85-day period; however, most spawns were not fertilized. As many as 120,000 eggs were collected/tank/day. No pattern to the egg releases was observed. Typically, one egg release would be obtained followed by a 1- to 2-day lull before another release. On several occasions, eggs would be released for two consecutive days. No evidence of more spawning occurring on a given lunar phase was seen. Leu et al. (2003) obtained 71 natural spawns of mangrove red snapper from May 21 to September 15, 1999, with eggs collected for 25 consecutive days during the first spawning period. This was followed by intervals of 2 to 22 days with no spawning, then periods of spawning for 5 to 14 days. Emata (2003), also working with mangrove red snapper, obtained 83 spawns over a 773-day period and observed a pattern of spawns occurring 3 days prior and after the last quarter moon and new moon phases.

In August 2002, brooders were restocking at 4 females and 5 males per tank. Average weights were 2.92 ± 0.89 and 2.70 ± 0.76 kg, respectively. Fish in three tanks were fed a basal diet,

while fish in three other tanks received the basal diet enriched with omega-3 fatty acids (Papanikos et al., 2008). Fish given the non-enriched diet gave a total of 70 spawns, while those given the enriched diet gave 23 spawns. Egg release was first observed when the temperature rose to 26°C at a photoperiod equivalent to May 8. Five of the six tanks gave spawns, 15.1% of the spawns occurred during the May photoperiod, 24.4%, 33.7%, and 26.7% during the June, July, and August photoperiods, respectively. The temperature averaged 26.5°C during that time. No correlations to change in water quality and the occurrence of spawning or fertilization success were observed. As with the 2001 season, no evidence of more spawning occurring on a given lunar phase was seen.

Mean egg production per tank was 4.2 and 0.4 million eggs for the non-enriched and enriched diet groups, respectively. When spawns occurred, the number of eggs collected per tank per day ranged from <2,000 to 441,000. There was no pattern to frequency of egg release (Figure 4). The maximum number of days of consecutive spawns was six, with each spawn containing over 100,000 eggs. One tank gave 28 spawns, with an average fertilization rate of 87%. Another tank gave a total of 28 spawns and none were fertilized. Fertilized spawns of more than 50,000 eggs per spawn were obtained from both treatments, 22 from the non-enriched and just one from the enriched treatment. The mean fertilization rate, the hatch rate, and the survival at 36–40 hph were 90.5%, 83.0%, and 49.0%, respectively for the non-enriched treatment. The results for the above parameters for the one fertilized spawn of the enriched diet group were 99.0%, 87.0%, and 70.0%. Egg diameters were $796.4 \pm 7.9 \mu\text{m}$ and $810.9 \pm 3.6 \mu\text{m}$ for the un-enriched and enriched treatments, with average egg globule diameters of 139.1 ± 8.0 and $141.2 \pm 3.9 \mu\text{m}$, respectively (Papanikos et al., 2008).

Average brood weight gain over the 356-day cycle was 50.1% for males and 42.7% for females. At the end of the study period, at 26.5°C, the brood fish consumed approximately 2.9% (wet to wet) of their body weight/day. Brood mortality per tank ranged from 0% to 44%, losing 6.7% of the males and 25% of the females.

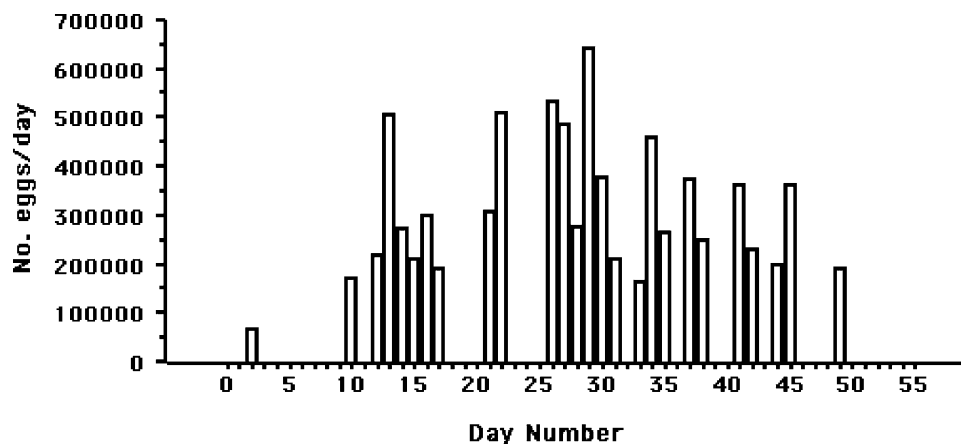


Figure 4 Natural spawns of red snapper in one tank over a 55-day period given as the total number of eggs collected.

Table 1 A comparison of natural ($n = 23$) and induced spawns ($n = 29$) of red snapper obtained during the 2002 season and the egg and larval characteristics

	Natural	Induced
Floating eggs/spawn ($\times 10^3$)	351.2 \pm 33.0	328.8 \pm 43.6
Fertilization rate (%)	91.6 \pm 3.2	79.3 \pm 4.0
Hatch rate (%)	87.7 \pm 2.5	63.6 \pm 7.7
Larval survival, 36 hr post-hatch (%)	60.3 \pm 3.7	48.8 \pm 4.8
Egg diameter (μm)	804.1 \pm 2.4	793.2 \pm 3.2
Oil globule diameter (μm)	139.2 \pm 1.2	125.0 \pm 1.3
Larval length (SL mm) at hatch	2.2 \pm 0.04	2.1 \pm 0.03
Oil globule diameter at hatch (μm)	141.8 \pm 1.19	123.8 \pm 2.05

Source: Data from Papanikos (2004).

The challenge with obtaining natural spawns by red snapper under controlled conditions is the unpredictability of when quality spawns will be available. Egg incubators and larval rearing tanks must be held in reserve, and live foods must be available on demand as a first food within two days after a spawn is obtained. The high frequency of unfertilized spawns is also a major limitation. Attempts to stimulate and synchronize natural spawning of red snapper in tanks using LH-RHa and Gn-RH with and without dopamine blockers have resulted in egg releases, but few fertilized eggs have been obtained. As much as 579,700 eggs were released by four females (1.1 kg av. wt.) in a common tank over a 4-day period when given a LH-RHa implant (Bourque, 2001), but none were fertile. Laidley and Ostrowski (2001) tried various hormone-injection protocols with red snapper to obtain a natural egg release and successful fertilization, and did get some limited fertilization when LH-RHa and the dopamine inhibitor pimozide were given.

V COMPARISON OF SPAWNING TECHNIQUES

When fertilized natural ($n = 23$) and induced spawns ($n = 29$) obtained in the same season are compared (Table 1), better fertilization rates (91.6 \pm 3.2%) and hatching rates (83.6 \pm 2.3%) were obtained from natural spawns. Egg diameter and oil globule diameter were larger for natural spawned eggs relative to those from induced spawns. The difference in oil globule diameter remained with recently hatched larvae, where larvae from natural spawns had a mean oil globule diameter of 141 \pm 1.19 μm , while those from induced spawns averaged 123.8 \pm 2.05 μm . Overall, the quality of seed obtained through natural spawns is considered to be better relative to induced spawns. More consistent survival during the first 30 dph has been obtained using larvae from natural spawns.

There are tradeoffs between natural spawning of red snapper in a hatchery setting and the induced spawning of wild-caught fish. Natural spawns produce better quality seed, but the occurrence of fertilized spawns is unpredictable. A significant investment in infrastructure is required, and daily attention must be given to the fish and facilities for an extended period of time.

Along the Alabama coast, red snapper are abundant, and mature fish can be captured during the peak of the natural spawning season and induced to spawn. Greater genetic diversity is possible with induced spawning, as females are used only once for spawning. However, induced spawning gives variable results due to differences in fish-to-fish quality, how fish are handled during capture and transport, and how successful the biologist is in recognizing when ovulation occurs and collecting the eggs and fertilizing them in a timely fashion. Continued improvement in larval rearing as well as in brood spawning will make hatchery-based red snapper production more practical.

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