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Intensive (Non-pond) Culture of Gulf Killifish

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The Gulf killifish *(Fundulus grandis)* inhabits the estuarine waters of the Gulf of Mexico and Atlantic coast of Florida where it is commonly used as a baitfish by anglers for speckled trout, flounder, and red drum (Fig. 1). From Texas to Florida it is referred to by many regional names including but not limited to: mudminnow, mudfish, cocahoe minnow, and bull minnow. The Gulf killifish is closely related to the mummichog *(F. heteroclitus),* which inhabits the Atlantic coast and is similarly utilized by anglers as a live bait. Both species are characterized as "hardy" bait tolerant of wide swings in salinity, temperature, and other conditions encountered by live marine bait.

The majority of bait sales for Gulf killifish currently originate from wild harvest. A survey on Gulf killifish sales conducted at marinas and bait shops within Louisiana indicated that approximately half (53 percent) of bait sellers were unable to meet the demands of anglers, even when using multiple suppliers (2 to 3). Seasonal availability and inconsistency of harvest throughout the range of this species has been apparent since the 1970s when research on the culture of Gulf killifish began. Initial research on pond growth, feeding, and fertilization strategies for Gulf killifish began in 1976 at the Claude Peteet Mariculture Center at Gulf Shores, Alabama. Since that time, research expanded pond based culture methods including stocking densities, broodfish spawning techniques, and three-phase growing systems (see SRAC Publication No. 1200, *Growing Bull Minnows for Bait).*

Figure 1. Adult female (above) and male (below) Gulf killifish, *Fundulus grandis.* Males are identified by their specked sides and white fin margins while females have an olive drab coloration. Photo Credit: Craig Gothreaux - LSU Agricultural Center

Interest in killifish culture outside of previous pond production scenarios has sparked interest in recent research projects. This factsheet will describe non-pond based approaches for Gulf killifish culture. Since the initial research on pond culture of this species in the 1970's and 1980's, a number of advances in aquaculture technologies have allowed for new opportunities for culturing aquatic species. The application of phase-based systems that segregate reproduction and growth phases include outdoor tanks or indoor recirculation systems that provide improved environmental control, efficient methods for waste removal, and feeding. Additionally, there exists the potential for cross-over in these culture approaches for Gulf killifish to other related species such as the mummichog or Seminole killifish *(F. seminolis).*

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Basic Culture Considerations for Gulf Killifish

Salinity

Gulf killifish occupy coastal marshes where salinity can change dramatically over a short time period. These fish have the ability to live in salinities that range from freshwater (0 ppt) to near double the concentration of seawater (up to 70 ppt) for several days. Sodium and chloride make up the bulk ions contributing to 'salinity' in seawater. However, there are many other ions that these fish use to regulate their internal conditions such as potassium, magnesium, and calcium (Fig. 2). The most common form of salt available to culturists is rock salt, but it is composed primarily of sodium and chloride; and lacks other ions. This lack of ions within these saline solutions has proven to be detrimental to young Gulf killifish with both reduced growth and survival. Marine salts contain a full complement of the ions these fish need, but they can be very costly. Recommendations of ion values for juveniles and adults have not been researched. The use of seawater or natural waters from a bayou, estuary, or pond must be properly sterilized or processed due to the potential for introduction of pathogens/parasites, dissolved organic materials, or other unwanted and detrimental agents when put into a contained system. Make sure to contact local, state, and federal officials regarding water rights, use, discharge permits, and applicable laws regarding any aquaculture operations.

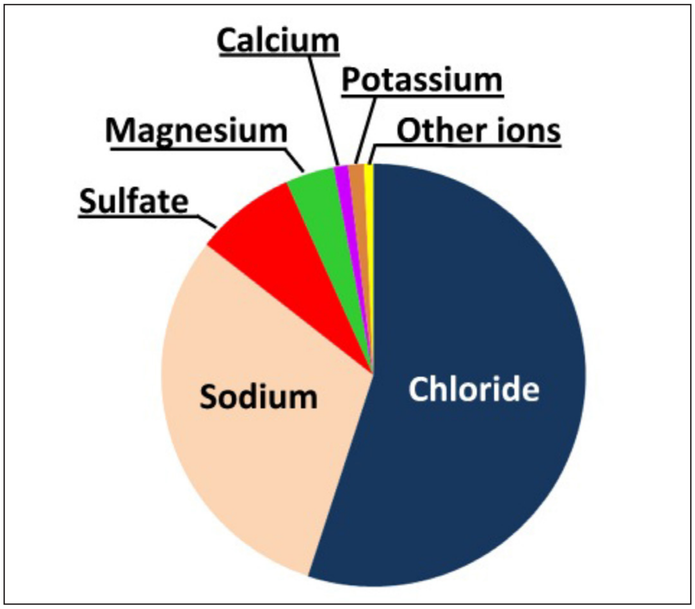

Figure 2. Typical elemental components of ocean salt.

Regardless of water source, an initial water test by a water testing laboratory to characterize the ionic composition of your potential source water will ensure that your specific conditions will be sufficient for culturing killifish. For regular water quality assessments, salinity (not specific ions) can be monitored easily using an inexpensive hydrometer, although more expensive tools such as a refractometer or digital salinity meter will provide more accurate measures.

Salinity plays a large role in the growth of Gulf killifish and can be monitored and altered based on water composition. Juveniles cultured for 12 weeks in experimental recirculation systems grew better at a salinity of 12 ppt when compared to salinities of 8, 5, and 0.5 ppt (Fig. 3). Gulf killifish can survive in low salinity water, such as 0.5 ppt, but it is important to note that both growth and survival will be reduced (60 percent survival over 12 weeks). Ideally salinity should be maintained between 8 and 15 ppt for optimum growth possibly due to this salinity being similar to the fish's internal osmotic conditions. It is possible that the relationship between salinity and growth may result from local environmental factors the fish has previously encountered and thus may differ across the geographic range of this species.

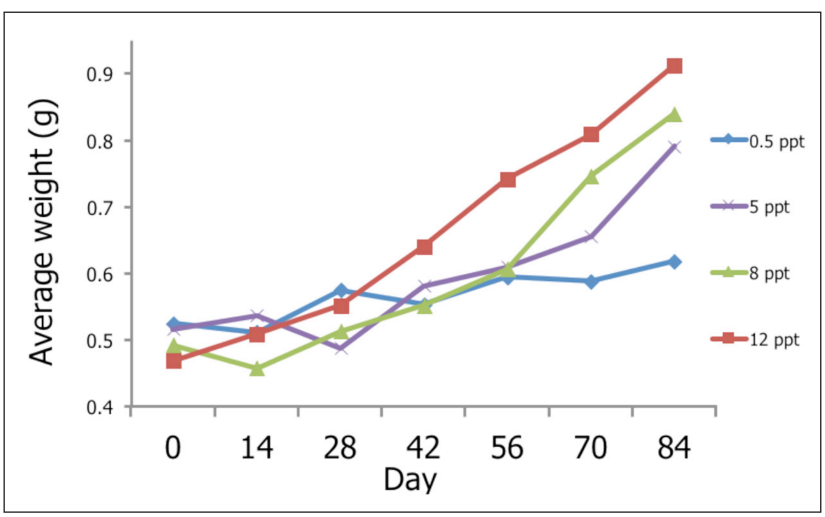

Figure 3. Growth of juvenile Gulf killifish, *Fundulus grandis* over an 84 day (12 week) period reared in recirculation aquaculture systems at salinities of 0.5, 5, 8, and 12 ppt.

At a salinity of 9 ppt obtained solely from the addition of NaCl, Gulf killifish larvae have been cultured in waters modified by the addition of chemicals, i.e., potassium chloride, magnesium chloride, and calcium chloride, in concentrations that provided ions for optimum survival and growth (Table 1). Spawning of Gulf killifish has been accomplished at salinities between 3 and 20 ppt with few reports of some inland populations spawning in freshwater.

Table 1. Composition of critical ions (potassium, magnesium, and calcium) in freshwater and various saline mixtures. The column on the far right represents recommended minimum values of these ions for larval Gulf killifish, *Fundulus grandis* culture.

	Pond (freshwater)	Municipal (freshwater)	Municipal + NaCl	Municipal + synthetic sea salt	Composition for larvae
Salinity (ppt)	—	0.1	9.8	9.8	9.5
K^+ (mg/L)	3.4	1.5	12.7	113.0	90.0
Mg^{2+} (mg/L)	21.6	0.1	1.0	286.0	70.0
Ca^{2+} (mg/L)	29.8	3.0	13.2	93.6	15.0

Water Quality

In the wild, Gulf killifish eggs and adults can survive brief periods of stranding out of water as many marshes are influenced by tides. As a response to harsh estuarine conditions, these fish are adapted to survive dramatic changes in temperature, salinity, dissolved oxygen, and pH. Gulf killifish are tolerant of a range of water quality parameters but optimal water quality ranges should be maintained to promote good health, survival, and growth. Normal pH values for culture in outdoor tanks and recirculation systems range from 7.5 to 9.0; but Gulf killifish can tolerate brief exposures of pH up to 9.5 in outdoor systems with large concentrations of algae and organic matter. Maintaining dissolved oxygen concentrations above 5 mg/L has proven ideal for Gulf killifish in both recirculation and outdoor tanks. Gulf killifish have been spawned successfully in temperatures in excess of 90 °F (32 °C); however, viability of eggs and larvae decrease significantly at these temperatures and above. Although absolute values have not been determined for this hardy baitfish, they appear to be relatively tolerant to high levels of total ammonia nitrogen over a short period of time. Employing best practices to remove and reduce union-ized ammonia in culture systems greatly improve overall health and growth over time.

Culture Units and Scenarios

A three-phase system for pond-based production of Gulf killifish is demonstrated in SRAC Publication No. 1200, *Growing Bull Minnows for Bait.* Similar to production scenarios for freshwater bait, this system utilizes three separate sets of ponds for segregating: 1) broodfish and egg production; 2) hatching fry and larvae; and 3) grow-out. It is possible to use the three-phase production scenario to incorporate non-pond systems. In previous alternative scenarios with one- and two- phases of production there have been reduced numbers of juveniles available for grow-out, probably due to cannibalism.

Tanks

Above ground tanks constructed of fiberglass or polyethylene holding between 500 and 2,500 gallons (2,000 and 10,000 L) have been used for both egg production and grow out of larval Gulf killifish. As in ponds, natural productivity in outdoor tanks is crucial in providing supplemental nutrients. Using tanks for egg production or early larval and fry development has several advantages. Predation from birds and other larger predators can be more easily controlled by placing netting over tanks. Since tanks are contained environments it is easier to observe feeding, mortalities, and spawning. It is also easier to maintain and adjust water quality parameters in comparison to the variability experienced in larger earthen ponds throughout the year. Pond or river silt can be added to each tank to provide a layer of soil in which nitrifying bacteria can become established. Stocking density in tanks is extremely limited so their use is not recommended for grow-out, but rather to hold broodfish for egg production and separate nursery tanks for larval/fry culture.

Recirculation

Recirculating aquaculture systems (RAS) have the potential to dramatically increase the density of fish within a confined volume of water and provide improvements in water quality and waste removal when compared to ponds and outdoor tanks. RAS offer fish producers a method to maximize production with a limited supply of water, allow control over the culture environment to maximize growth parameters, locate production near the appropriate markets, and improve harvest by reducing subsequent stressors of handling. The incorporation of a RAS in Gulf killifish production allows for growth and harvest of market size individuals throughout the year to target months when wild baitfish supplies are low.

In spite of the advantages, using RAS greatly increases the cost of production due to expensive components, infrastructure requirements, and energy costs. All the nutritional requirements of the fish must be fulfilled by a formulated diet rather than primary productivity of a natural system. Studies indicate that larval (newly hatched) to juvenile (~ 0.5 grams and 1.4 inches [35 mm] in length) stocking densities within recirculation systems can range between 7 and 19 individuals per gallon (2 and 5/L) for optimum growth and survival. In addition, based on growth rates from previous pond based studies with this species, the use of a RAS does not result in faster growth but has the potential to greatly increase survival.

Broodfish and Egg Production Management

Gulf killifish held in outdoor tanks maintained at a salinity of approximately 9 ppt will continuously spawn in lunar cycles from March to October, with peaks in late spring and fall when water temperatures are 73 to 82 °F (23 to 28 °C). Egg production for the Gulf killifish on a per female or per weight basis is very low relative to freshwater baitfish species and potentially represents a bottleneck in their production. Fish with a total length between 4 and 4.8 inches (10 and 12 cm; 15 and 20 grams, respectively) can be expected to produce approximately 279 and 558 eggs per month (0.6 to 0.9 eggs/g body weight each day), respectively. The use of smaller individuals as broodfish is not recommended due to large differences in size-based fecundity. If starting with a smaller broodfish between 3.0 to 3.5 inches (7.5 to 9 cm) it is recommended to stock these individuals in February or March before the spawning season to allow them time to grow prior to spawning.

Research on the use of non-pond environments has demonstrated the ability to produce Gulf killifish eggs in contained systems such as above ground tanks and recirculation systems. Optimum densities for broodfish populations in these contained systems (static tank or RAS) indicates that a maximum density of 0.11 to 0.16 fish per gallon of water (30 to 40 fish/m3) produces the largest numbers of eggs while greater densities reduce output (possibly due to negative interactions of dominance behavior or egg predation). Comparisons between a 2:1 and 4:1 female to male sex ratio have resulted in no advantage in egg production and therefore it is recommended that broodfish be stocked at a 2:1 ratio.

There are a number of considerations for increasing egg production outside of sex ratios and stocking density in RAS and outdoor tanks. When using outdoor tanks the use of greenhouse shade cloth over approximately 80 percent of each tank has been demonstrated to increase the number of viable eggs collected during the summer months of egg production. Shade cloth can also be used in conjunction with netting to restrict access to the tanks by birds and other predators. Placement of spawning substrate in these tanks is similar to the placement of spawning mats in ponds. Mats cut to dimensions of 12 × 18 inches (30 cm × 45 cm) made of Spawntex® are held horizontally by floats in the tanks and suspended approximately 8 inches (20 cm) below the surface of the water (Fig. 4). The amount of available substrate influences egg production and it is recommended to place two mats rather than one per tank when using a tank volume greater than 600 gallons (2,200 L). Eggs are deposited on mats everyday by spawning females with a pattern of greater egg deposition during the periods between the new and full moon. It is recommended that mats be collected twice a week.

Figure 4. Spawntex® mat used for Gulf killifish, *Fundulus grandis* egg collection is suspended in tanks using capped PVC as floats. Photo Credit: Craig Gothreaux - LSU Agricultural Center

Egg Incubation and Larval Hatching

Eggs can be easily removed from mats and incubated separately, as it appears that the eggs lose most of their adhesion within 24 hours after spawning. Separating eggs from mats allows for volumetric determination of egg numbers and makes it possible to incubate them in a cleaner environment. The eggs can literally be shaken or gently beaten out of the mat over a collecting tub filled with a small amount of water. The eggs in the tub can be poured into and sieved onto a nylon window screen (1 to 1.5 mm2) to remove unwanted debris. Eggs can be incubated in traditional hatching jars; however, they hatch over a protracted period which increases the differences in larval sizes which can promote cannibalism. As a result of this protracted hatching period, incubation of these eggs in water is not recommended for large collections of Gulf killifish eggs.

Air incubation appears to be a common occurrence in wild Gulf killifish populations whereby females lay their eggs among marsh grasses during the maximum high tides so eggs develop within a humid, but not wet environment. These eggs then hatch when the tides return between 13 to 15 days later. Aquaculturists can take advantage of this unique adaptation within Fundulus spp. by incubating cohorts of collected eggs in a similar way. Batches of eggs can be placed on foam mats (expanded polystyrene or soft hobby foam) that have been previously soaked in 7 to 9 ppt salinity water. Foam of a similar size should be placed on top of a single layer of eggs to form a sandwich that can be placed within a container, e.g., small storage tote (Fig. 5). The salinity of the incubation water used to keep the foam wet (i.e. spray bottles) can be increased (up to 18 ppt) to reduce fungal/mold/bacterial formation on the eggs throughout incubation. Air incubation reduces the protracted hatch time observed in water incubation and reduces the likelihood of larger larvae consuming younger and smaller individuals.

Figure 5. Storage totes containing wetted foam for air incubation of Gulf killifish, *Fundulus grandis* eggs (inset). Photo Credit: Paula Ouder - Louisiana Sea Grant

Another large advantage of air incubation is the ability to alter the developmental rate of a cohort of eggs by incubation at higher or lower temperatures to coordinate hatch times. The temperature should be relatively constant throughout incubation. Incubation of Gulf killifish eggs at approximately 74 °F (23.5 °C) results in embryos that are ready to hatch between 9 and 19 days of incubation. As the embryos continue to 'delay' hatch they consume yolk while still inside the egg, therefore extending incubation can result in hatching larvae that immediately need to receive feed in order to survive. The warmer the temperature the more accelerated the development will be, shortening the incubation period (Table 2). Manipulation of different egg batches with temperature allows for batches of eggs to be hatched at similar times even though they were collected over a protracted period. One way this can be accomplished is by making an incubator out of a small dormitory style refrigerator equipped with an external thermostat to maintain the internal temperature lower than the external air temperature. Once the eggs are ready to hatch, submerge them in water of equivalent temperature. If the incubation temperature is significantly different from the water temperature used for hatching, consider acclimatizing the eggs slowly to reduce the possibility of shocking the embryos. Depending on the incubation temperature, time, and readiness, they will hatch out in minutes to hours.

Table 2. Minimum and maximum number of days for incubation of Gulf killifish, *Fundulus grandis* eggs hatched using air incubation.

Temperature °F (°C)	Minimum number of incubation days	Maximum number of incubation days
86 (30)	5	11
79 (26)	7	15
73 (23)	9	19
68 (20)	11	23

Phases of Growth

Newly hatched Gulf killifish first begin to 'feed' from yolk stores and once the yolk is depleted, artificial feed or a natural food source must be provided. Micro-particulate diets formulated for larval fishes (from vendors such as Otohimetm or Zeiglertm) with a particle size of approximately 250 to 350 μm can be accepted by Gulf killifish at first feeding. Artemia nauplii may also be used at first feeding and will produce slightly larger fry, with no differences in survival.

The density of newly hatched larvae is very important; densities of more than 35 larvae per gallon (9 fish/L) are not recommended. Feed should be provided up to four or more times per day at salinity between 8 to 12 ppt and temperatures of 77 to 83 °F (25 to 28 °C). Cannibalism tends to occur within 4 to 6 weeks of culture and has been reduced when stocking densities are decreased to approximately 15 fish per gallon (4 fish/L). Fish should be expected to reach approximately 1 ¼ inches (30 mm) between 10 and 13 weeks and an approximate weight of 0.35 grams each.

Juveniles have been reared in RAS at densities of 8 and 18 individuals per gallon (2 and 5 fish/L) with survival after 12 weeks of 95 and 84 percent, respectively. Throughout 12 weeks of growth fish should be expected to increase from a mean weight of 0.35 g each to approximately 2 grams each when fed a 0.8 mm diameter feed (40 percent protein, 10 percent lipid) three times daily. Unfortunately, complete recommendations on optimum diet composition (percent protein and lipid) for this species are not complete.

Outdoor tank stocking of fry (5 to 10 mg each) has resulted in fast growth, however, survival is inversely related to density and maximum densities within tanks are limiting. This approach partially takes advantage of the primary productivity within outdoor systems but this effect is reduced as increased stocking densities overtake natural productivity. Water should be exchanged in static tanks weekly or as a function of stocking density and feed inputs due to build-up of waste products that increase primary productivity and increased eutrophication.

Stocking outdoor tanks for grow-out practices has followed previous pond density recommendations of 80,000 and 160,000 per acre (200,000 and 400,000/ha). Tanks filled with 20,000 gallons (75,000 L) of water and maintained at salinities of 5 ppt and stocked with 25 day old Gulf killifish at densities of 80,000 per acre (200,000/ha) produced market sized fish (approximately 4 g each) within 100 days of culture, but survival was approximately 50 percent. Stocking densities of 160,000 and 200,000 per acre (400,000 and 500,000/ha) in tanks have failed to produce market sized fish within 100 to 140 days even when provided a supplemental feed. Given the stocking limitations, growth, and survival results for tank-based growth studies with this species, tanks will not serve as an ideal environment for growing adequate numbers of juvenile Gulf killifish to market sizes.

Combination of Non-Pond and Pond Based Scenarios and Conclusions

Tank and RAS based systems could play a role in the production of Gulf killifish perhaps through their uses with broodfish management and rearing of larvae and juveniles. Direct egg collection and subsequent incubation aids with batch coordination for protracted spawning seasons into the late spring and fall. Although fecundity is low in this species relative to freshwater baitfish, the larvae are well developed at hatch and much larger in size. The ability to present a formulated diet upon first feeding and completely remove predators using an RAS could increase fry production. A large disadvantage of tank systems is in using them for later grow-out phases of production, as it appears stocking density in these contained systems is limited for larger production operations. Relative to water volume and the land needed for ponds, recirculation systems utilize stocking densities far greater than previous pond studies. The loss of killifish growth in the absence of pond productivity and the high start-up costs associated with infrastructure and system components for RAS are significant drawbacks of RAS.

The use of RAS and/or outdoor tanks could be added as a component of Gulf killifish production based on years of research and experiences that demonstrate effective use of ponds in grow-out of this marine baitfish. Closed systems with broodfish and early larval growth could provide larger individuals (0.25 to 0.50 g) for later stocking into productive low salinity ponds. The use of pond productivity to increase growth upon stocking of juveniles has already been demonstrated in the past within proposed multi-phase scenarios.

An enterprise budget analysis of a pond-based culture scenario for Gulf killifish production projected inconsistent economic viability dependent upon scale effects (10 and 2 total acres; in Chapter 13, Cocahoe Minnow Production Manual. Louisiana State University Agricultural Center, Baton Rouge). One type of hypothetical system evaluated was a hybrid system where larvae are produced from captive broodfish maintained and spawned intensively within a captive tank or a RAS system with eggs collected and incubated to coordinate cohorts of larvae. These larvae could then be hatched in either RAS or tanks and fed a formulated diet prior to subsequent stocking in ponds where nutrition is available from natural productivity and supplemented with artificial diets. Budget analysis of a mixed method scenario using tank spawning and pond grow-out resulted in consistent profitability for farms with either 10 or 2 total acres (4 or 0.8 ha). In any potential scenario undertaking a large project such as culturing Gulf killifish (or any baitfish), prior planning must be done in order to guard against costly decisions and failure.

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