

Budget Justification – Year 1

Development of a stock enhancement program for the southern flounder *Paralichthys lethostigma*

a. Personnel (\$132,232)

Salary (\$99,817)

Name	Salary/mo.	Months	Total
Reginald Blaylock, PI	\$11,333	2.0	\$22,665
Eric Saillant, Co-PI	\$8,441	1.0	\$8,441
Angelos Apeitos, Co-PI	\$5,794	3.0	\$17,381
Brooke Doucet, Lab Manager	\$3,657	3.0	\$10,972
Kevin Bishop, Biologist II Finfish	\$3,657	3.0	\$10,972
Andrew Gima, Systems Specialists	\$5,167	0.5	\$2,584
Aquaculture Technician II	\$2,567	6.0	\$15,402
Graduate Students	\$1,900	6.0	\$11,400

Fringe (\$32,417)

Name	%	Yr 1
Reginald Blaylock, PI	30.1028%	\$6,823
Eric Saillant, Co-PI	33.3523%	\$2,815
Angelos Apeitos, Co-PI	34.0362%	\$5,916
Brooke Doucet, Lab Manager	38.7344%	\$4,250
Kevin Bishop, Biologist II Finfish	38.7347%	\$4,250
Andrew Gima, Systems Specialists	35.0110%	\$905
Aquaculture Technician II	44.1483%	\$6,800
Graduate Students	5.7746%	\$658

The fringe benefit rate for full-time employees is calculated as a percentage of salary. The benefit includes health insurance \$4,536/year or \$378/mo), life insurance (\$0.09/\$1,000 per mo [\$30,000 min/\$100,000 max]), retirement (\$265,000 maximum gross salary), FICA social security (maximum taxable earnings is \$132,900) FICA medicare (1.45%; unlimited), unemployment (0.09%; \$85 per person per year), and worker’s compensation (nonhazardous 0.7%, hazardous 5%; averages).

b. Travel (\$3,500)

Travel includes meetings (air [500], hotel [600], per diem [225], registration [425]) = \$1750 per person for two people.

c. Equipment (\$36,814)

Aluminum trailer for securing the live hauler. Aluminum hauler for hauling juveniles to the release site. YSI ProDDS Multiparameter device for water quality.

d. Supplies (\$39,541)

Culture supplies: Feed, seed, salt, chemicals, gases, feeders, plumbing, glassware, diagnostics, water quality meters/strips, small tanks, pumps, filters, nets, instruments, etc. pumps, growout gear, algae commodities, ploidy commodities; Repro assays, genetic typing, histology supplies; Class Supplies

e. Contractual costs (\$5,000)

Boat charters for brood fish collection.

f. Other costs (\$43,990)

1. Communications (\$1,500)

Shipping expenses

2. Rents (\$1,800)

USM Boat Rentals.

3. Participant costs (\$0)

4. Professional Services (\$20,000)

Array development and assays, health checks.

5. Tuition (\$13,990)

6. Subcontracts (\$0)

g. Indirects (\$96,851)

The USM federally approved indirect charge rate is 48% and is applied to the direct cost base minus value of Equipment, Rents, and Tuition [$\$254,377 - \$52,604 * 0.48 = \$351,228$]. A negotiated rate agreement is included in this package.

TOTAL (Year 1) = \$351,228

Budget Justification – Year 2

Marine Aquaculture Demonstration Center for Economic Development

a. Personnel (\$129,701)

Salary (\$98,082)

Name	Salary/mo.	Months	Total
Reginald Blaylock, PI	\$11,333	2.0	\$23,118
Eric Saillant, Co-PI	\$8,441	1.0	\$8,610
Angelos Apeitos, Co-PI	\$5,794	3.0	\$17,729
Brooke Doucet, Lab Manager	\$3,657	2.0	\$7,461
Kevin Bishop, Biologist II Finfish	\$3,657	3.0	\$11,191
Andrew Gima, Systems Specialists	\$5,167	0.5	\$2,635
Aquaculture Technician II	\$2,567	6.0	\$15,710
Graduate Students	\$1,900	6.0	\$11,628

Fringe (\$31,620)

Name	%	Yr 1
Reginald Blaylock, PI	30.1028%	\$6,959
Eric Saillant, Co-PI	33.3523%	\$2,872
Angelos Apeitos, Co-PI	34.0362%	\$6,034
Brooke Doucet, Lab Manager	38.7344%	\$2,890
Kevin Bishop, Biologist II Finfish	38.7347%	\$4,335
Andrew Gima, Systems Specialists	35.0110%	\$923
Aquaculture Technician II	44.1483%	\$6,936
Graduate Students	5.7746%	\$671

The fringe benefit rate for full-time employees is calculated as a percentage of salary. The benefit includes health insurance \$4,536/year or \$378/mo), life insurance (\$0.09/\$1,000 per mo [\$30,000 min/\$100,000 max]), retirement (\$265,000 maximum gross salary), FICA social security (maximum taxable earnings is \$132,900) FICA medicare (1.45%; unlimited), unemployment (0.09%; \$85 per person per year), and worker's compensation (nonhazardous 0.7%, hazardous 5%; averages).

b. Travel (\$5,212)

Travel includes meetings (air [700], hotel [550], per diem [500]) = \$1750 per person for two people x two trips. Travel for extension & training (air [500], hotel [300], per diem [56] = \$856 per person for two people.

c. Equipment (\$0)

d. Supplies (\$38,301)

Culture supplies: Feed, seed, salt, chemicals, gases, feeders, plumbing, glassware, diagnostics, water quality meters/strips, small tanks, pumps, filters, nets, instruments, etc. pumps, growout gear, algae commodities, ploidy commodities; Repro assays, genetic typing, histology supplies; Class Supplies

e. Contractual costs (\$5,000)

Boat charters for brood fish collection.

f. Other costs (\$32,690)

1. Communications (\$1,500)

Shipping expenses

2. Rents (\$1,800)

USM Boat Rentals.

3. Participant costs (\$4,400)

Travel for participants to attend workshop at TCMAC (average milage (\$600) or air (\$450) plus one night lodging (\$100) plus 1 day per diem (\$56) x 10 people. Participant costs to attend workshops (extension), food for Scoping Meeting (\$15) per person (lunch) plus (\$30) per person (reception) x 20 people.

4. Professional Services (\$16,000)

Array development and assays, health checks.

5. Tuition (\$14,690)

6. Subcontracts (\$0)

g. Indirects (\$93,943)

The USM federally approved indirect charge rate is 48% and is applied to the direct cost base minus value of Equipment, Rents, and Tuition [$\$216,604 - \$20,889 * 0.48 = \$310,547$]. A negotiated rate agreement is included in this package.

TOTAL (Year 2) = \$310,547

Budget Justification – Year 3

Marine Aquaculture Demonstration Center for Economic Development

a. Personnel (\$132,297)

Salary (\$100,044)

Name	Salary/mo.	Months	Total
Reginald Blaylock, PI	\$11,333	2.0	\$23,581
Eric Saillant, Co-PI	\$8,441	1.0	\$8,782
Angelos Apeitos, Co-PI	\$5,794	3.0	\$18,083
Brooke Doucet, Lab Manager	\$3,657	2.0	\$7,610
Kevin Bishop, Biologist II Finfish	\$3,657	3.0	\$11,415
Andrew Gima, Systems Specialists	\$5,167	0.5	\$2,688
Aquaculture Technician II	\$2,567	6.0	\$16,024
Graduate Students	\$1,900	6.0	\$11,861

Fringe (\$32,253)

Name	%	Yr 3
Reginald Blaylock, PI	30.1028%	\$7,099
Eric Saillant, Co-PI	33.3523%	\$2,929
Angelos Apeitos, Co-PI	34.0362%	\$6,155
Brooke Doucet, Lab Manager	38.7344%	\$2,948
Kevin Bishop, Biologist II Finfish	38.7347%	\$4,422
Andrew Gima, Systems Specialists	35.0110%	\$941
Aquaculture Technician II	44.1483%	\$7,074
Graduate Students	5.7746%	\$685

The fringe benefit rate for full-time employees is calculated as a percentage of salary. The benefit includes health insurance \$4,536/year or \$378/mo), life insurance (\$0.09/\$1,000 per mo [\$30,000 min/\$100,000 max]), retirement (\$265,000 maximum gross salary), FICA social security (maximum taxable earnings is \$132,900) FICA Medicare (1.45%; unlimited), unemployment (0.09%; \$85 per person per year), and worker’s compensation (nonhazardous 0.7%, hazardous 5%; averages).

b. Travel (\$5,212)

Travel includes meetings (air [700], hotel [550], per diem [500]) = \$1750 per person for two people x two trips. Travel for extension & training (air [500], hotel [300], per diem [56] = \$856 per person for two people.

c. Equipment (\$0)

d. Supplies (\$42,101)

Culture supplies: Feed, seed, salt, chemicals, gases, feeders, plumbing, glassware, diagnostics, water quality meters/strips, small tanks, pumps, filters, nets, instruments, etc. pumps, growout gear, algae commodities, ploidy commodities; Repro assays, genetic typing, histology supplies; Class Supplies

e. Contractual costs (\$5,000)

Boat charters for brood fish collection.

f. Other costs (\$29,131)

1. Communications (\$1,500)

Shipping expenses

2. Rents (\$1,800)

USM Boat Rentals

3. Participant costs (\$4,400)

Travel for participants to attend workshop at TCMAC (average milage (\$600) or air (\$450) plus one night lodging (\$100) plus 1 day per diem (\$56) x 10 people. Participant costs to attend workshops (extension), food for Scoping Meeting (\$15) per person (lunch) plus (\$30) per person (reception) x 20 people.

4. Professional Services (\$16,000)

Array development and assays, health checks.

5. Tuition (\$15,424)

6. Subcontracts (\$0)

g. Indirects (\$97,013)

The USM federally approved indirect charge rate is 48% and is applied to the direct cost base minus value of Equipment, Rents, and Tuition [$\$223,734 - \$21,624 * 0.48 = \$97,013$]. A negotiated rate agreement is included in this package.

TOTAL (Year 3) = \$320,747

TOTAL PROJECT COST = \$982,522

PI: Reg Blaylock
Co-PIs: Saillant, Apeitos
Proposal Title: Development of a stock enhancement program for the southern flounder *Paralichthys lethostigma*
Agency: GOMESA
Start Date: 1-Jul-25
End Date: 30-Jun-28

	Monthly Pay Rate	Year 1		Year 2		Year 3		CUMMULATIVE	
		Agency	USM	Agency	USM	Agency	USM	Agency	USM
SALARY (SALARY)									
Reginald Blaylock, PI	11333	2.0	22,665	2.0	23,118	2.0	23,581	69,364	
Eric Saillant, Co-PI	8441	1.0	8,441	1.0	8,610	1.0	8,782	25,833	
Angelos Apeitos, Co-PI	5794	3.0	17,381	3.0	17,729	3.0	18,083	53,193	
Brooke Doucet, Lab Manager	3657	3.0	10,972	2.0	7,461	2.0	7,610	26,043	
Kevin Bishop, Biologist II Finfish	3657	3.0	10,972	3.0	11,191	3.0	11,415	33,578	
Andrew Gima, Systems Specialist	5167	0.5	2,584	0.5	2,635	0.5	2,688	7,907	
Aquaculture Technician II	2567	6.0	15,402	6.0	15,710	6.0	16,024	47,136	
Graduate Student	1900	6.0	11,400	6.0	11,628	6.0	11,861	34,889	
New Employee	0	0.0	0	1.5	0	3.0	0	0	
Subtotal			99,817	24.0	98,082	24.0	100,044	297,943	
FRINGE (FRINGE)									
Reginald Blaylock, PI	30.1028%		6,823		6,959		7,099	20,881	
Eric Saillant, Co-PI	33.3523%		2,815		2,872		2,929	8,616	
Angelos Apeitos, Co-PI	34.0362%		5,916		6,034		6,155	18,105	
Brooke Doucet	38.7344%		4,250		2,890		2,948	10,088	
Kevin Bishop	38.7347%		4,250		4,335		4,422	13,007	
Andrew Gima	35.0110%		905		923		941	2,769	
Aquaculture Technician II	44.1483%		6,800		6,936		7,074	20,810	
Graduate Student	5.7746%		658		671		685	2,014	
New Employee	0.0000%		0		0		0	0	
Subtotal			32,417		31,620		32,253	96,290	
TOTAL PERSONNEL			132,232		129,701		132,297	394,230	
COMMODITIES (COMMOD)			39,541		38,301		42,101	119,943	
Salt	\$0.08/ liter for 150,450 liters in Y-1, 72,200 Liters in Y-2 and 72,200 L in Y-3		12,036		6,276		6,276	24,588	
Feed (brood raw diet)	Shrimp@\$4.88/ lb, Squid @ \$4.88/ lb, Cigar Minnows @ \$2.98/ lb, Vitamins @ \$200 annually , gelatin @ \$200 annually Nutrient concentrate @ \$30.59/Liter for 50 Liters (\$2,529.50), Artemia cysts @ \$4.00/can for 90 cans (\$4,860), enrichments @ \$0.95/g for 1,620 g (\$1,539), Bleach @ \$2.99/gal for 40 gal.(\$119.60), Sodium Hydroxide @ \$0.183/g for 5000 g (\$915.00), Sodium thiosulfate @ \$4.47/kg for 10 Kg (\$44.70), defoamer for \$9.91/L for 2 liters (\$19.82)		4,375		4,375		4,375	13,125	
Live Feeds	Otohime diet (A2, 1 kg @ \$67.00, B1, 2 kg @ \$51.50, B2, 4kg @ \$103.00, C1, 6 kg @ 124.50, C2, 10 kg @ \$415.00, S2, 10 kg @ \$415.00, EP1, 20 kg @ \$210.00) Ammonia @ \$34.00/25 strips (\$538.30 for 16 containers), Nitrite @ \$31.50/25 strips (\$538.30 for 17 containers) and Alkalinity @ \$18.30/25 strips (\$538.30 for 29 containers)		10,029		10,029		10,029	30,087	
Feed (Larval and nursery dry diet)	pH 4 @ \$30.00/liter (\$60.00 for 2 liters), pH 7 @ \$30.00/liter (\$270.00 for 9 liters), pH 10 @ \$30.00/liter (\$270.00 for 9 liters)		1,386		1,386		1,386	4,158	
Test Strips	Extraction and DNA evaluation @\$5/sample, 100 samples in yr 1, 1,500 samples in yrs 2 and 3		1,615		1,615		1,615	4,845	
Calibration Solutions	Library preparation @\$30 per sample		600		600		600	1,800	
Genetics Commodities	ELISA 11-KT kits, tubes, solvents for extraction		500		7,500		7,500	15,500	
Sexing of broodstock	Anesthetic, Copper sulphate, citric Acid		2,500		320		320	2,500	
Chemicals	Biological titer media, bacteria for system maturation, Ammonium chloride for system maturation, tank service and cleaning supplies, water quality testing commodities		3,000		3,000		3,000	9,000	
Miscellaneous commodities			3,000		2,000		2,000	7,000	
Nets			0		200		0	200	
Belt Feeders			0		1,000		0	1,000	
Workshop Cost (Extension)	training materials		0		5,000		5,000	5,000	
COMMUNICATIONS (COMCAT)			1,500		0		1,500	4,500	
shipping			1,500		1,500		1,500	4,500	
CONTRACTUAL SERVICES (OTCSVC)			5,000		5,000		5,000	15,000	
Charters	brood fish collection		5,000		5,000		5,000	15,000	
PROFESSIONAL FEES (PROFES)			20,000		16,000		16,000	52,000	
Array development and assays			20,000		15,000		15,000	50,000	
Health checks			0		1,000		1,000	1,000	
TRAVEL (TRAVEL)			3,500		5,212		5,212	13,924	
conferences/meetings	conferences/meetings (air[500], hotel[600], per diem[225], registration[425]) = 1750 per person (air \$500, hotel\$300, per diem\$56, \$856 per person x2 people)		3,500		3,500		3,500	10,500	
Travel (Extension/ training)			0		1,712		1,712	1,712	
EQUIPMENT (EQUIP) (>\$5,000)			36,814		0		0	36,814	
Aluminum Trailer	For securing the live hauler		14,000		0		0	14,000	
Aluminum Hauler	For hauling juveniles to the release site		16,000		0		0	16,000	
Water quality	YSI ProDDS Multiparameter		6,814		0		0	6,814	
PARTICIPANT COSTS (PARTIC)			0		4,400		4,400	8,800	
Travel for participants to attend workshop at TCMAC	average mileage (\$600) or air (\$450) + 1 night lodging (\$100) + 1 day per diem (\$56) x 10 people		0		3,500		3,500	3,500	
Participant Cost to attend workshop (Extension)	food for Scoping Meeting - \$15/person (Lunch) + \$30/person (reception) x 20		0		900		900	900	
RENTS (RENTS)			1,800		1,800		1,800	5,400	
TCMAC vessel	Collections, assessments and releases		1,800		1,800		1,800	5,400	
SUBCONTRACTS (SUBCON) (F&A charged on first \$25K/sub)			0		0		0	0	
TUITION (SCHOL)			13,990		14,690		15,424	44,103	
(AY20-21: \$10,250 (in-state) + 5% increase/yr; add \$2,000 for OOS, if needed)			13,990		14,690		15,424	44,103	
TOTAL DIRECT COSTS			254,377		216,604		223,734	694,715	
MTDC			201,773		195,715		202,110	599,598	
F&A (INDIRT) MTDC	Rate* =		48% 96,851		48% 93,943		48% 97,013	287,807	
	*Adjust % as needed								
TOTAL PROJECTS COSTS			351,228		310,547		320,747	982,522	

PROJECT NAME: Development of a stock enhancement program for the Southern Flounder, *Paralichthys lethostigma*

CONTACT: The University of Southern Mississippi, Reginald Blaylock, PhD.,
Reg.blaylock@usm.edu, 703 East Beach Dr., Ocean Springs, MS, 228-818-8003

PROJECT LOCATION: Thad Cochran Marine Aquaculture Center (TCMAC), University of Southern Mississippi, Gulf Coast Research Laboratory, Cedar Point Campus, Ocean Springs, Jackson County, Mississippi

AMOUNTED REQUESTED: \$981,857

PROJECT PERIOD: July 1st, 2025 – June 30th, 2028

PROJECT DESCRIPTION:

Human population growth exerts pressure on marine fisheries resources through habitat destruction and demand for food and recreation. Such pressure ultimately may result in depletion of some of the fisheries species that support Mississippi's fishing industry. The Southern Flounder (*Paralichthys lethostigma*) is one of the most popular coastal fishes in Mississippi. It is highly sought after by recreational anglers due to its fine flesh quality. Data on the abundance of Southern Flounder in the north central Gulf of Mexico including Mississippi are limited and insufficient in most areas, but a recent assessment conducted in Alabama (Powers et al. 2018) indicated that, while the stock was not yet overfished, overfishing was occurring. In Mississippi, recreational and commercial landings fluctuate annually, however the trend since 2012 suggests that the fishery is in a state of decline (Burriss, R. personal communication, June 23rd, 2022, Waters, 2022). The Southern Flounder's popularity as a recreational fish combined with its dependence on inshore habitats affected by environmental stressors such as pollution, temperature rise, and extended low salinity stress make it potentially vulnerable to depletion. Fluctuating abundance leads to an unreliable market supply and negatively impacts recreational fishing opportunities. Aquaculture could contribute to addressing the insufficiencies and fluctuations of the resource by providing an independent source of fingerlings to support recruitment in a stock enhancement program where Southern Flounder juveniles produced in aquaculture are released in Mississippi coastal habitats. Stock enhancement will provide managers with an additional tool, alongside traditional fishing regulation approaches, to manage and maintain a sustainable fishery for this species. This project thus aims to establish the culture of juvenile Southern Flounder in Mississippi as a source of seeds for stock enhancement. This will involve acquiring a captive broodstock for seed supply, developing effective spawning protocols, optimizing larval culture techniques to produce weaned juveniles for release, selecting appropriate sites for release, optimizing tagging and release protocols, and quantifying success of releases, and growout. Methods and technologies developed for both culture and stocking will be available for transfer to potential industry partners interested in producing Southern Flounder, through sponsored onsite and off-site hands-on workshops thereby mitigating the risk of poor performance in the existing industry and creating the potential for new job opportunities and workforce development.

JUSTIFICATION:

GOMESA AUTHORIZED USES:

(2) Mitigation of damage to fish, wildlife or natural resources:

Population abundance data for Southern Flounder are limited. However, a recent assessment conducted in Alabama indicated that overfishing is on-going even though the stock is not yet overfished. In Mississippi, data collected from commercial and recreational landings suggests that the fishery has been experiencing a decline since 2016. Southern Flounder use coastal habitats that are subjected to environmental fluctuations potentially leading to variation in cohort strength, which can contribute to population instability, a hypothesis also supported by the commercial and recreational landings data from the past seven years (Burris, R. personal communication, June 23rd, 2022, Waters, 2022). Environmental factors that may affect flounder post-larvae and juveniles include increasing temperature, increasingly long and severe low salinity events (e.g., when spillways are open), and increased pollution. Aquaculture-based stock enhancement could stabilize recruitment by complementing wild recruits in low abundance years and mitigating the effects of these environmental stressors on the stock. Genetic management to maintain genetic diversity in stocked fish will contribute to the long-term sustainability of the stock.

(3) Implementation of federally approved marine coastal or conservation management plan: This project addresses goals outlined in the federally approved “Coast Ecosystem Restoration Council Comprehensive Plan, Restoring the Gulf Coast’s Ecosystem and Economy.” Specifically, this project addresses Goal 4, Enhance Community Resilience and Goal 5, Restore and Revitalize the Gulf Economy. Recreational fishing has been impacted by freshwater flooding, hurricanes, habitat degradation, and the Deepwater Horizon oil spill. Stock enhancement will improve the availability and consistency of an important fishery resource (Southern Flounder) and thereby facilitate resilience in the recreational fishing community. Increased opportunities for recreational fishing resulting from the project will enhance economic activity by stimulating the use of working waterfronts and fishing-related economic activities such as restaurants, fuel, boat, fishing tackle, and repair businesses. Establishment of aquaculture techniques for Southern Flounder could facilitate commercialization that would contribute to revitalization of the Gulf economy through establishment of a new industry. The hatchery methods will ensure a source of seeds for growers interested in growing Southern Flounder locally, and technology transfer will enable the establishment of commercial hatcheries in the long term.

GOALS AND OBJECTIVES:

The goal of this project is to establish the production of juvenile Southern Flounder on the Mississippi Gulf Coast for stock enhancement by developing a captive broodstock, optimizing methods to induce spawning and production of high quality eggs using natural conditioning or hormonal induction, optimizing technologies to culture larvae and juveniles of the species in large numbers to support state stock enhancement efforts, and developing the tools needed to tag and release fish as well as monitor the effectiveness of stock enhancement.

Objective 1: Develop a captive broodstock population- Year 1-3

- a. Collect adult flounder from Mississippi jurisdictional waters (Year 1-3). TCMAC will collect 80-100 adult flounders and transport them back to TCMAC. Upon arrival, brood fish will be processed and quarantined for a period of no less than 30 days using the collection and quarantine protocols used for other species at USM. During the quarantine period, fish will be acclimated to the controlled environment and fed a diet

providing adequate nutrition for the reproductive maturation and production of high-quality eggs following established brood husbandry protocols developed at USM for captive brood fish of other species in recirculating systems (Bardon-Albaret and Saillant 2017, Blaylock et al. 2021, Adams et al. 2021). Upon completion of the quarantine period, fin clips will be collected from all fish to be used as broodstock and genotyped at molecular markers to build a database of parental genetic profiles that can be used to identify released fish using parentage analysis ('genetic tagging').

- b. Develop a method to identify the phenotypic sex of candidate brooders (Year 1-3). This method will allow stocking of maturation and spawning systems with appropriate numbers of males and females. Because the phenotypic sex in this species can be influenced by environmental factors, phenotypic sexing based on circulating levels of sex steroids will be used as in other fish species (Saillant et al. 2021) instead of a genetic method. The sex of each fish will be identified, and males and females will be stocked in maturation systems after tagging with a passive integrated transponder (PIT) tag. We will target maintenance of 50 males and 50 females through the project.
- c. Develop volitional spawning techniques (Year 1-3). Broodstock will be conditioned by applying a natural photo-thermal cycle reflecting conditions in Mississippi coastal habitats to induce gamete maturation and spontaneous spawning in captive conditions. Individuals will be held in two 14,000 liter, 5-foot deep and two 36,000 liter, 10-foot-deep recirculating aquaculture systems at a 1:1 (M: F) sex ratio. The cycle will be shifted for a subset of brooders during the second part of the project to demonstrate year-round production. Methods will adapt protocols available for Southern Flounder (e.g., Watanabe 2006). The effect of varying sex ratios in spawning tanks on maturation and spawning success will be evaluated in controlled experiments.
- d. Develop alternative spawning techniques (Year 1-3). Hormonal induction also will be examined to control the contribution of candidate spawners and maximize the genetic diversity present in larval cohorts destined to be released for stock enhancement. Hormonal induction will employ Gonadotropin Releasing Hormone implants, and single injections and will be guided by protocols used successfully in other species at TCMAC. Egg collection will proceed following volitional release (tank spawning) or will involve manual strip-spawning. In the latter case, protocols for artificial fertilization of eggs will be optimized to enable generation of large numbers of controlled crosses by fertilizing the eggs of multiple females with the sperm of several males to increase genetic diversity among offspring. Alternative protocols will be evaluated considering egg quality criteria and success initiating larviculture runs with high initial survival with produced eggs.

Objective 2: Produce juveniles in recirculating systems- Year 1-3

- a. Optimize larval feeding protocols (Year 1-3). Methods will utilize established protocols for this species (Benetti et al. 2001) and feeding protocols used successfully at TCMAC for other species (e.g., Lemus et al. 2014, Blaylock et al. 2021, Saillant et al. 2021) to determine the best protocol to rear flounder from a yolk-sac stage to an appropriately sized juvenile for release or seeding growout culture units. Experiments will be designed to test feeding regimes (frequency, quantity per feeding, prey transition dates/rates and

augmented nutrition options) in protocols incorporating live prey (rotifers and *Artemia* sp. instar 2 nauplii) as initial feeds. Protocols to wean larvae to dry diets at early stages also will be optimized. Transition to a micropellet diet may not be important for small juveniles intended for immediate release, however, micropellet diets may lead to significant reductions in the cost to culture fish to a size appropriate for tagging or stocking in commercial production units for growout.

- b. Optimize Post-larval husbandry Protocols (Year 2-3). To maximize survival and fitness of post-larvae, experiments will test different system effects (tank color, water currents, water depth, exchange rates) as well as different management strategies (stocking density, weaning timing and duration, photoperiods, size grading).

Objective 3: Develop tools for stock enhancement and implement pilot releases – Year 2-3

- a. Optimize transport and release protocols (Year 2-3). Methods developed for other species will be adapted to accommodate Southern Flounder stocking. Handling and transport of juveniles have been shown to induce stress (e.g., Sulikowski et al. 2006 in winter flounder) and are expected to impact post-release survival. Protocols for transport and deployment on site will be evaluated using stress indicators (behavioral and physiological) and survival as metrics. Predation occurring immediately post-release on naïve juveniles is a major factor affecting survival.
- b. Identify the best candidate locations for releases (Year 2-3). The environmental conditions and habitat characteristics suitable for flounder juveniles will be determined based on available data on life history and coordinating with the USM's Center of Fisheries Research and Development. The obtained habitat parameters will be used to identify candidate sites for releases in Mississippi coastal waters used in pilot short-term releases.
- c. Develop tools for large scale genetic tagging and monitoring of stock enhancement and domestication of Southern Flounder (Year 1-2). The brooders used to produce released offspring will be genetically characterized using fin tissue samples collected in Objective 1-b above and the database obtained used to identify recaptured fish by matching them to parental profiles (Saillant et al. 2009). A panel of Single Nucleotide Polymorphism (SNP) markers suitable for monitoring genetic diversity and identification of released fish through parentage assignment analysis will be used in characterization. The panel will be developed based on published SNP loci positioned on the Southern Flounder linkage map (O'Leary et al. 2018) and accounting for the assignment power of candidate markers during selection of the loci.
- d. Conduct pilot short term releases of juveniles (Year 1-3). Small-scale releases will be conducted on release sites identified in 3b and monitored periodically post-release by seining in conjunction with MDMR. Tissues from juvenile Southern Flounder collected will be sampled for genetic identification. The experiment will allow validating tagging methods and evaluating survival and fitness of released fish. Releases will employ the most promising protocols identified in 3a. The survival and fitness of fish post-release will be monitored to evaluate the success of alternative procedures. Acoustic tagging also will be used to monitor movement and long-term residency of individual fish of sufficiently large size for this type of tagging.

Objective 4: Workforce development and technology transfer (Year1- 3)

TCMAC will work with USM’s Blue Technology accelerator to identify individuals and companies with an interest in Southern Flounder aquaculture. We will coordinate training and technology transfer with these companies through sponsored workshops at TCMAC and farm visits to facilitate the development of a diverse, equitable and inclusive industry and create STEM employment opportunities. The project will lead to the training of graduate, undergraduate and high school CTE program students. Technology transfer for broodstock management and hatchery culture will include development of an outreach fact sheet publication and the first iteration of a culture manual for this species. Personnel at MDMR involved in stock enhancement will be trained in culture techniques and in the release procedure including selection of sites, the release process, and post-release monitoring.

Timeline

OBJECTIVE	YEAR 1												YEAR 2												YEAR 3											
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
1-a Collect broodstock	x	x	x	x	x								x	x	x	x	x								x	x	x	x	x							
1-b Genotype broodstock							x	x										x	x	x	x									x	x	x	x			
1-c Broodstock conditioning					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
1-d Induction and spawn							x	x	x	x									x	x	x	x									x	x	x	x		
2-a Test Juvenile production protocols									x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2-b Optimize juvenile production																				x	x	x	x	x									x	x	x	x
3-a Optimize transport and release																					x	x	x	x										x	x	x
3-b Identify Candidate Release site																			x	x	x													x	x	x
3-c Develop genetic tagging					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x												
3-d Small scale releases									x	x	x										x	x	x											x	x	x
4- Technology Transfer							x	x	x	x	x	x							x	x	x	x	x	x							x	x	x	x	x	x

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