# **IDENTIFICATION AND CHARACTERIZATION OF NURSERY HABITAT FOR** JUVENILE SOUTHERN FLOUNDER, PARALICHTHYS LETHOSTIGMA, IN **ARANSAS BAY, TEXAS**

by

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#### Abstract

Southern flounder *Paralichthys lethostigma* populations in Texas have been in steady decline over the last 25 years. Despite the economic importance of this species, little is known about their juvenile habitat requirements. The goal of this study was to determine temporal and spatial habitat use patterns for juvenile southern flounder and characterize these patterns in terms of habitat selection. Monthly sampling was conducted over a two-year recruitment period (January-April 2004, and January-March 2005) in the Aransas-Copano estuaries on the Texas coast. The bay complex was divided into three zones based on a decreasing salinity gradient and increasing distances from Aransas Pass. Replicate estuarine habitat types were sampled in each of these zones. Triplicate samples were taken using a beam trawl in different habitats, seagrass (Halodule wrightii), marsh (Spartina alterniflora), and open-water (nonvegetated bottom), at each of nine sampling sites within each zone. Catch data indicated distinct habitat distribution patterns. Highest densities occurred closest to Aransas Pass in vegetated, sandy bottom areas. Lowest densities occurred in nonvegetated, muddy bottom areas farthest from the pass. Habitat selection patterns for southern flounder were examined using experimental mesocosms. Since wild fish occurred at low densities, hatchery-reared fish were used. Four common natural habitat types were simulated in twelve 38-L glass tanks: (1) oyster reef, (2) salt marsh (Spartina alterniflora) (3) seagrass (Halodule wrightii), and (4) nonvegetated, sand bottom. Mesocosms were divided in half with each containing a different constructed habitat type. Selection patterns were compared for all possible combinations of habitat pairings. Habitat selection experiments showed juvenile southern flounder selected nonvegetated sand bottom over structured

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habitat (vegetated and oyster habitats) and selected seagrass over all other structured habitats when nonvegetated sand bottom was not available for selection. Habitat use patterns show vegetated habitats near a tidal inlet serve as important nursery grounds for juvenile southern flounder. Habitat selection experiments indicated juvenile flounder select to settle in nonvegetated sand bottom habitats near or in vegetated areas. A 25year bag seine data set from Texas Parks and Wildlife Department (TPWD) was analyzed to assess long-term spatial and temporal patterns. Bag seine data were interpreted using Arc GIS 9.1 to calculate southern flounder catch per hectare (catch per unit effort) during the peak recruitment period (December-April). Data maps showed high numbers of flounder near tidal inlets, with the highest number of flounder collected during April. These observations were similar to field observations and support the importance of vegetated habitats near tidal passes as nursery areas.

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#### Introduction

Shallow estuaries are some of the most productive marine ecosystems. This productivity stems from the abundance of estuarine habitats such as seagrass beds, salt marshes, and nonvegetated bottom (Carr and Adams 1973; Weinstein 1979; Rozas and Minello 1998). A variety of nekton species use shallow estuarine areas as "nursery" habitat. Such areas have been termed nurseries because they are often associated with high survival and fast growth rates for young fish (Heck and Thoman 1984; Kneib 1984; Baltz et al. 1993; Rozas and Minello 1998; Stunz et al. 2002). Structured habitats aid in predator avoidance and high food availability (Sogard 1992; Stunz and Minello 2001). Varying nekton density among these productive estuarine habitats is influenced by such factors as habitat complexity and habitat selection (Heck and Orth 1980; Baltz et al. 1993; Levin and Hay 1996; Rooker et al. 1998). Assessing density patterns of fishes in these ecosystems is important to the management of fish stocks and is essential to the conservation of critical nursery habitats used by them.

Information about specific habitat-related densities are critical for assessing essential fish habitat (Beck et al. 2001; Rose et al. 2001). These patterns can serve as indicators of habitat value (Weinstein 1979; Doherty 1982; Baltz et al. 1993; Rozas and Minello 1998; Minello 1999). Specific biotic and abiotic factors contribute to the differences in habitat use among habitat types in these shallow estuaries. For example, many estuarine species show a physiological response to salinity changes within an estuary (Christensen et al. 1997). Proximity to open-water has also affected habitat use in intertidal marsh habitats (Rozas and Odum 1988; Minello et al. 1994; Peterson and Turner 1994). Differences in sediment composition in nonvegetated habitats also

influence fish distributions (Keefe and Able 1994; Moles and Norcross 1995). Therefore, habitats exhibiting suitable biotic and abiotic characteristics will be of more importance or more utilized by fish populations during particular life stages compared to habitats not meeting specific criteria (Zimmerman et al. 1990).

The importance of identifying and quantifying nursery habitats became evident with the amendment to the Magnuson-Stevens Fishery Conservation and Management Act and the Sustainable Fisheries Act (SFA) in 1996. The SFA requires identification of essential fish habitat (EFH) and assessment of habitat quality and quantity (Fletcher and O'Shea 2000). Essential fish habitat is defined as "those waters and substrate necessary to fish for spawning, breeding, feeding, and/or growth to maturity." Identifying these types of estuarine nursery habitats is necessary for assessing growth and recruitment of juvenile fishes, as well as executing effective management and protection measures for EFH.

Southern flounder *Paralichthys lethostigma* supports an important commercial and recreational fishery throughout the Gulf of Mexico (GSMFC 2000). This species is one of the primary commercial and recreational flatfishes landed in the Gulf of Mexico (GSMFC 2000) and accounts for more than 95% of the total harvest of flounder (Stokes 1977). Between 1970 and 1997, annual Texas landings of flounder in Texas averaged over 137,000 kg with a dockside value of \$267,000. The value peaked at \$521,000 in 1982 due do the 1981 ban on the sale of red drum and spotted seatrout. A steady increase in Gulfwide nominal ex-vessel prices (\$/kg) was noted between 1970 and 1990, making the price of flounder in Texas second only to that of red snapper (*Lutjanus campechanus*). Southern flounder is also an important recreational species. From 1976

to 1998, recreational landings by Texas anglers averaged over 195,000 flounder per year. Flounder popularity is primarily due to the quality of the meat, and its easy accessibility to anglers; thereby, making it a highly sought after fish by both bank and boat fishermen.

Southern flounder is an estuarine-dependent species distributed from North Carolina to Florida on the Atlantic coast and from Florida to Texas and Mexico along the Gulf coast (Ginsburg 1952; Gilbert 1986; Wenner et al. 1990). As seen in southern flounder populations in North Carolina, emigration to offshore spawning sites in Texas populations occurs during the period of October through December (Deubler 1958; Stokes 1977; Burke et al. 1998). Immigration back into Texas estuaries begins in January with peak recruitment periods in February and March (Stokes 1977). However, little is known as to the specific habitat use and spatial distribution of juvenile southern flounder once they enter the estuaries.

Texas Parks and Wildlife fishery-independent monitoring gill net data has shown statewide southern flounder populations steadily decreasing during the last 25 years (TPWD 2003). This was seen in mean catch rates of southern flounder in routine gill net surveys conducted in Aransas Bay, Texas from 1978 to 2000 (TPWD 2003). Overfishing, bycatch, and declines in nursery habitat quality and quantity are possible reasons for this decline. However, the extent to which these individual factors contribute to flounder abundance is unknown. The Gulf States Marine Fisheries Commission (GSMFC 2000) has also expressed concern regarding southern flounder populations in Gulf states. However, limited data on the dynamics of this fishery, especially data on essential nursery habitat requirements for juveniles, makes stock assessment difficult.

Despite the economic importance of the flounder fishery, few studies have assessed habitat requirements for young juveniles. Stokes (1977) found that immigration of southern flounder juveniles into Aransas Bay began when temperatures reached approximately 13.8 C and peaked when temperatures ranged from 16.0-16.2 C. January and February were periods of peak recruitment with highest densities of juvenile southern flounder occurring in Redfish Bay, a primary bay near the pass, as compared to more remote bays (i.e. Copano Bay and St. Charles Bay). King (1971) also found similar recruitment patterns associated with Cedar Bayou with a greater abundance of larval and juvenile southern flounder from January through April. In North Carolina, early juveniles prefer low salinity (2-11 ‰) areas (Powell and Schwartz 1977; Allen and Baltz 1997; Walsh et al. 1999) and move down to higher salinity areas as they mature. Juvenile southern flounder recruitment in Barataria Bay, Louisiana also appeared to be dependent on factors related to salinity (Allen and Baltz 1997). These studies suggest that southern flounder select for shallow waters with low salinities, high dissolved oxygen levels, and low temperatures. Higher abundances of this species also appear to be associated with muddy bottom substrates composed primarily of silt and clay sediments (Powell and Schwartz 1977; Stokes 1977; Burke et al. 1991). Clearly, more detailed data is necessary to determine the habitat use of young juvenile stages, particularly in south Texas estuaries.

Habitat selection is a critical factor determining recruitment and growth of fish populations (Connell and Jones 1991; Eggleston 1995). There is ample evidence suggesting that fish select for specific habitats (Sogard 1992; Stunz et al. 2001), and that these selection patterns are not random but dependent on various abiotic and biotic

factors (Jones 1988; Doherty 1991; McConnaughey and Smith 2000). For example, various species show a preference for structurally complex habitats because these habitats usually provide an abundance of food resources and shelter from predators (Levin and Hay 1996; Levin et al. 1997). Selection for habitats that enhance survival has also been shown for some flatfish species when selection is based on structural complexity, salinity gradient, and substrate composition (Powell and Schwartz 1977; Miller et al. 1991; Allen and Baltz 1997). Understanding these selection patterns and the factors contributing to the variations in distribution and recruitment can be examined by conducting both field studies and laboratory experiments. Specifically, experimental laboratory mesocosms can provide a useful tool, in addition to field studies, for determining what habitats fish are selecting when presented with a choice (Moles and Norcross 1995). Results from such experiments can be used to predict distribution of fish in natural habitats and provide vital information for conserving EFH.

Long-term monitoring studies are useful tools used by fisheries managers to effectively manage finfish populations. Texas Parks and Wildlife conducts annual studies on stock densities, habitat quantity and quality, as well as other factors affecting commercially and recreationally important finfish and shellfish populations in Texas waters. Over the past 25 years, this long-term monitoring program has produced an extensive database of fisheries-independent data with the potential utility in detecting changes or fluctuations in relative abundances of commercially and recreationally important fishery populations. Specifically, analysis of this data set can be used to make predictions about temporal and spatial distributions, migration patterns, and general habitat use.

The primary goal of this study was to identify the nursery habitat for juvenile southern flounder in Aransas Bay, Texas and assess temporal and spatial patterns of habitat use. A secondary goal was to experimentally characterize habitat use in terms of habitat selection for juvenile southern flounder. Finally, this study also examined the temporal and spatial distribution of southern flounder in the Aransas-Copano Bay complex by assessing the 25-year bag seine data set from TPWD.

## **Objectives**

1. Characterize spatial habitat use patterns of juvenile southern flounder in Aransas Bay, Texas.

 $H_{01}$ : Southern flounder do not show specific temporal and spatial distribution patterns in Aransas Bay, Texas.

# $H_{AI}$ : Southern flounder show distinct temporal and spatial distribution patterns in Aransas Bay, Texas.

2. Determine habitat selection preferences of hatchery-reared juvenile southern flounder among habitat types in experimental mesocosms.

 $H_{01}$ : Hatchery-reared juvenile southern flounder do not show specific habitat selection patterns in experimental mesocosms.

# $H_{A1}$ : Hatchery-reared juvenile southern flounder show specific habitat selection patterns in experimental mesocosms.

3. Analyze historical trends of southern flounder habitat use in the Aransas-Copano Bay complex from Texas Parks and Wildlife's 25-year fisheries-independent monitoring bag seine data.

## Methods

### STUDY LOCATION

This study was conducted in the Aransas Bay system (40 km x 8 km) which is

situated on the Texas Gulf coast between the Corpus Christi and San Antonio Bay

complexes (Fig. 1). The bay system is primarily comprised of Aransas Bay and Copano Bay. Secondary bays in the system include Mission Bay and St. Charles Bay. Saltwater exchange takes place at the mouth of the bay via the Aransas Pass tidal inlet; however, some water exchange occurs via Cedar Bayou, a small natural tidal inlet (USEPA 1999). Freshwater inflow comes from the head of the bay by way of the Aransas and Mission Rivers and Copano Creek (Armstrong 1987).



Figure 1. Map of the Aransas Bay system on the Texas coast showing all sampling sites: Zone 1-seagrass (Z1-SG1,SG2, SG3), Zone 1-marsh edge (Z1-MS1, MS2, MS3), Zone 1 open-water (Z1-OP1, OP2, OP3), Zone 2-seagrass (Z2-SG1, SG2, SG3), Zone 2-marsh

edge (Z2-MS1, MS2, MS3), Zone 2 open-water (Z2-OP1, OP2, OP3), Zone 3-seagrass (Z3-SG1, SG2, SG3), Zone 3-marsh edge (Z3-MS1, MS2, MS3), Zone 3 open-water (Z3-OP1, OP2, OP3). Map by Cameron Pratt.

Similar to other Texas bays, the Aransas Bay system is shallow with a mean depth of 3.0 m. Bay margins slope gently for a distance of about 0.8 km into the deeper central bay. Sediment composition along the bay margins consists primarily of sand-sized grains with small amounts of silt and clay (Britton and Morton 1989).

# LARGE-SCALE PATTERNS OF HABITAT USE

Spatial patterns of habitat use by newly settled ( $\leq$  40 mm SL) southern flounder were assessed on a wide spatial scale in the Aransas-Copano Bay complex. The bay system was divided into three zones representing increasing distances from Aransas Pass and following a decreasing salinity gradient. Replicate estuarine habitat types were sampled in each of these zones. Triplicate samples were taken using a beam trawl in different habitats, seagrass (*Halodule wrightii*), marsh (*Spartina alterniflora*), and openwater (nonvegetated bottom), at each of nine sampling sites within each zone (Figure 1). Sampling was conducted during peak recruitment period (January-June) for juvenile southern flounder during 2004 and 2005. Each zone was sampled every six weeks during January-June 2004 and monthly during January-March 2005. A total of 81 samples per sampling event, 27 samples from each zone, were collected. A total of 567 samples were collected over the two-year sampling period.

A beam trawl with a 1 m x 0.22 m opening and 3-mm mesh net was used to collect newly settled southern flounder. Open-water sampling was conducted by towing the beam trawl by boat for 100 m at 4 kt covering 100 m<sup>2</sup> of bottom as determined by a

WAAS enabled GPS. Seagrass meadows and marsh edge were sampled by placing the beam trawl on the bottom and walking in a semi-circle route around the sampling site to minimize disturbance. For seagrass beds, the beam trawl was pulled 20 m in a random location in seagrass beds at each site covering 20 m<sup>2</sup>. For marsh edge sites, the beam trawl was pulled along the edge of the marsh, no more than 1 m from the marsh habitat, covering 20 m<sup>2</sup>. Samples were rough-sorted in the field to remove excess seagrass and algae, and the remaining sample was fixed in 10% formalin. All collections were returned to the laboratory for further sorting and identification of all flatfish to the species level. All flatfish were measured to the nearest 0.1 mm standard length (SL) and preserved in 70% ethanol. Water quality parameters, salinity (‰), temperature (°C), dissolved oxygen (mg/L) and depth (m), were taken using a YSI 6-series datasonde at each site.

#### SEDIMENT GRAIN SIZE ANALYSIS

Sediment grain size was analyzed to determine percent sand, rubble, silt, and clay at each sample site within each zone. A total of 81 sediment samples, 27 samples from each zone, were collected near flounder collection sites in June 2004. A benthic sediment sampler was used to collect sediment cores. Following collection, all samples were placed into a labeled plastic bag and temporarily stored in an ice chest.

Laboratory analysis was conducted in the benthic ecology lab at the University of Texas Marine Science Institute (UTMSI) using a technique modified from Folk (1980). A 20-cc homogenized sub-sample was extracted core using a wide mouth syringe. Samples were placed in glass beakers with 50 ml of hydrogen peroxide, to digest organics in the sample, and 75 ml of distilled water. Samples remained in the beakers for

one week or until the liquid cleared. Sediments were separated using a vacuum pump with a Millipore Hydrosol SST filter holder with a 62-µm screen and stainless steel filter. Rubble and sand-sized sediments were placed into pre-weighed labeled aluminum weighing pans and oven dried for at least 24 hrs. Mud fraction (silt /clay) filtrate was put into a 1000-ml graduated cylinder with 10 ml of 10% calgon dispersant. Two 20-ml withdrawals, one to determine percent silt and the other to determine percent clay, were taken and placed into pre-weighed, labeled beakers. Beakers were oven dried for at least 24 hrs. All weights and percentages were calculated and recorded to the nearest 0.001 g.

# HABITAT SELECTION PATTERNS

Habitat selection patterns of hatchery-reared southern flounder were examined using experimental mesocosms. Selection patterns of wild-caught juvenile southern flounder were not examined because low numbers of juveniles captured in the field did not provide the number of experimental organisms needed. Experimental flounder (32 days old) were obtained from captive-induced spawns from the University of Texas Marine Science Institute Fisheries and Mariculture Lab. Flounder were maintained in the laboratory in glass fish tanks and were fed to satiation with brine shrimp. Fish were not fed during experimental trials.

Twelve 38-L glass tanks (50.8 cm x 25.4 cm x 30 cm) were constructed to simulate natural estuarine habitat types. Each mesocosm was constructed by placing 2 cm of washed beach sand on the bottom of each tank overlain with plastic mesh (5-mm) and then another 2-cm layer of washed beach sand. Each tank was filled to a depth of 25 cm with seawater (28.7  $\% \pm 0.09$ ). Water temperatures in mesocosms were maintained at 25.7 °C ± 0.02, and oxygen levels were maintained at 6.2 mg/L ± 0.02 using airstones.

Four common natural habitat types were simulated in the experimental mesocosms: (1) oyster reef, (2) salt marsh (3) seagrass, and (4) non-vegetated bottom. Only the structure of the habitats was simulated in the experimental mesocosms (i.e. there was no associated flora or fauna), and each habitat was randomly assigned to each replicate tank (N = 12). Nonvegetated mesocosms were constructed by adding additional washed beach sand to the bottom. Oyster reef habitat was built by placing 1.5 L of prewashed and dried oyster shells (*Crassostrea virginica*) along the bottom of the tank. Simulation of marsh habitat was constructed with Spartina alterniflora stems cut from Aransas Bay salt marsh and sun dried for 14 d. Once dried, salt marsh stems were inserted into the plastic mesh and pressed into the sand bottom and arranged to represent densities of marsh found in Aransas Bay. Seagrass (Halodule wrightii) was collected from Aransas Bay using a sediment core sampler. Cores were washed before transferring them into the mesocosms and were arranged to simulate densities of seagrass beds in Aransas Bay. Each mesocosm was divided in half with each half containing a different constructed habitat type. All possible combinations of habitat pairings were used (a total of 6 possible pairings).

Three hatchery-reared southern flounder were placed in the center of the water column and monitored for 12 hrs. Following the acclimation period, air stones were removed, and each flounder was visually located in the mesocosm. The habitat type selected by each flounder was recorded hourly for 8 hrs. Percent occurrence of southern flounder in each habitat type was calculated based on 24 observations (3 fish per mesocosm x 8 hourly observations). This procedure was repeated for all twelve replicate paired combinations. Because of the limited supply of hatchery-reared southern flounder,

fish were reused in experimental trials by pooling them in a common holding tank and randomly reselecting fish for use before each observation trial.

#### TEXAS PARKS AND WILDLIFE DATA ANALYSIS

Maps for the 25-year Texas Parks and Wildlife bag seine data set were produced using Environmental Systems Research Institute, Arc GIS 9.1. Samples were collected by TPWD personnel with an 18.3-m bag seine (1.8 m deep) with a 1.3-cm stretched nylon multifilament mesh in the central bag and a 1.9-cm stretched mesh in the remaining net. Bag seines were pulled for 15.2 m, and the surface area sampled (estimated to 0.01 ha but standardized to 0.03 ha in 1984) was estimated by using the distance pulled and the length of the extension of the bag seine (Martinez-Andrade et al. 2005). Prior to 1984, sites for monthly sampling with bag seines were randomly selected from ~100 stations in the bay system (McEachron and Green 1985). Prior to October 1981, only six bag seine samples were collected each month in the bay system with no samples taken in June 1978. Monthly sampling effort steadily increased over time with 10 bag seine samples collected between 1981-1984, 12 samples collected between 1989-1990, 16 samples collected between 1990-1992, and 20 samples collected from 1992-present.

Only southern flounder  $\leq$  50 mm SL collected with a bay bag seine from the months of December through April (1977-2004) in Aransas Bay, Texas, were used to calculate total catch and catch per ha. Sampling intensity was calculated by totaling all sampling events at each sampling site from the months of December through April (1977-2004). Catch per unit effort (catch per ha) was calculated by dividing the total number of flounder by the surface area covered. Total overall catch for all years was

calculated by adding all flounder captured each month. All maps were geographically referenced with coordinates provided in the TPWD data set.

# STATISTICAL ANALYSES

Large-scale patterns of habitat use by southern flounder were analyzed using an Analysis of Variance (ANOVA) during the peak juvenile southern flounder recruitment months of January through March. A one-way ANOVA ( $\alpha = 0.05$ ) was used to analyze mean lengths of juveniles. A one-way ANOVA was used to examine the effects of zone and habitat type on flounder densities. All densities (fish/m<sup>2</sup>) were log (x+1) transformed before analysis was conducted to minimize heteroscedasticity. Significant results were further analyzed using a Tukey's post hoc test ( $\alpha = 0.05$ ) (Day and Quinn 1989).

Sediment samples for all zones and habitat types were also analyzed using an ANOVA ( $\alpha = 0.05$ ). A one-way ANOVA was used to examine differences in sediment composition among zones and habitat types. All sediment percentages were arcsine transformed before analysis was conducted. Significant findings were further analyzed using a Tukey's post hoc test ( $\alpha = 0.05$ ).

For selection patterns in experimental mesocosms, percent occurrence in each habitat for each comparison was determined for all replicates. Experimental habitat selection data was arcsine transformed, and Paired student's t-tests ( $\alpha = 0.05$ ) were used to determine differences between habitat types in each experimental mesocosm.

## Results

# LARGE-SCALE PATTERNS OF HABITAT USE

Juvenile southern flounder were captured in beam trawl samples only during the months of January through March (Fig. 2). No flounder were collected in the April through July samples in 2004. In 2004, young southern flounder were first caught at 8 mm SL. Highest catches were of 9-11 mm SL fish. Early juveniles were present at lengths to 32 mm SL (Fig. 3). In 2005, young flounder were captured at 9 mm SL. Highest catches were of 10 mm SL fish. Flounder were present up to 36 mm SL (Fig. 4).



Figure 2. Mean density ( $\pm$  SE) of newly settled southern flounder collected with a beam trawl from Aransas-Copano Bay during the 2004 and 2005 flounder recruitment season (N = 81). \* Indicates months that were not sampled during each sampling year.



Figure 3. Length-frequency distribution of newly settled southern flounder (N=113) collected with a beam trawl from Aransas Bay during the 2004 flounder recruitment season.



Figure 4. Length-frequency distribution of newly settled southern flounder (N=56) collected with a beam trawl from Aransas Bay during the 2005 flounder recruitment season.

Mean densities of southern flounder were significantly higher in zones near the pass (Zones 1 and Zone 2) and lowest in Zone 3, the sampling location farthest from the pass (df = 2, F = 7.788, P < 0.001, N = 135) (Fig. 5). Figure 6 shows the mean ( $\pm$  SE) length of flounder varied among zones with significantly larger southern flounder in Zone 2 (16.2  $\pm$  0.81 mm) and Zone 3 (17.3  $\pm$  1.83 mm), and smaller juveniles (12.7  $\pm$  0.54) occurring in Zone 1 (df = 2, F = 7.991, P < 0.001, N = 135).



Figure 5. Mean density ( $\pm$  SE) of newly settled southern flounder collected with a beam trawl from Zone 1, Zone 2, and Zone 3 from all samples collected in the Aransas-Copano Bay complex during the recruitment period (January-March) in 2004 and 2005. Horizontal lines below the bars show differences among locations. Bars sharing lines are not significantly different (Tukey's post hoc test  $\alpha = 0.05$ ).



Figure 6. Mean length (mm SL  $\pm$  SE) of newly settled southern flounder collected with a beam trawl from Zone 1, Zone 2, and Zone 3 from all samples collected in the Aransas-Copano Bay complex during the recruitment period (January-March) in 2004 and 2005. Number of fish captured per zone is indicated at the base of bars. Horizontal lines below the bars show differences among locations, and the bars sharing lines are not significantly different (Tukey's post hoc test  $\alpha = 0.05$ ).

Vegetated habitats also exhibited the highest densities of newly settled flounder compared to nonvegetated habitats (df = 2, F = 11.295, P < 0.001) (Fig. 7). Significantly larger flounder (17.2 ± 0.76 mm) occurred in marsh edge, intermediate mean size (12.6 ± 0.5 mm) in seagrass, and smaller mean size (10.0 ± 0.32 mm) in nonvegetated habitat (df = 2, F = 11.295, P < 0.001) (Fig. 8). No significant interaction between zone and habitat type was observed (df = 4, F = 2.378, P = 0.051).



Figure 7. Mean density ( $\pm$  SE) of newly settled southern flounder collected with a beam trawl from marsh edge, seagrass, and open-water, nonvegetated habitat types from all samples collected in the Aransas-Copano Bay complex during the recruitment period (January-March) in 2004 and 2005. Horizontal lines below the bars show differences among habitat types, and the bars sharing lines are not significantly different (Tukey's post hoc test  $\alpha = 0.05$ ).



Figure 8. Mean length (mm SL  $\pm$  SE) of newly settled southern flounder collected with a beam trawl from marsh edge, seagrass, and open water nonvegetated habitat types from all samples collected in the Aransas-Copano Bay complex during the recruitment period (January-March) in 2004 and 2005. Number of fish captured per habitat type is indicated at the base of bars. Horizontal lines below the bars show differences among habitat types (Tukey's post hoc test  $\alpha = 0.05$ ).

## ENVIRONMENTAL FACTORS

Zone salinities differed between the 2004 and 2005 sampling periods. In general, higher salinities were observed for zones during 2004 as compared to those observed during 2005 (df = 1, F = 47.486, P < 0.001) sampling period (Fig. 9). Mean ( $\pm$ SE) salinities among zones were also consistent with the decreasing salinity gradient integrated into the sampling design, with highest mean salinities (21.2 ‰  $\pm$  0.91, N = 39) in Zone 1, intermediate salinities (15.5 ‰  $\pm$  0.92, N = 45) in Zone 2, and the lowest mean salinities (9.5  $\% \pm 0.59$ , N = 45) in Zone 3 (df = 2, F = 50.835, P < 0.001). No differences in salinities among habitat type were found (df = 2, F = 0.157, P = 0.855). Other environmental factors including mean temperature (°C), dissolved oxygen (‰), and mean depth (m) (±SE) are reported by sampling year, zone, and habitat type (Table 2).



Figure 9. Mean salinities (± SE) at Zone 1, Zone 2, and Zone 3 from all samples collected in the Aransas-Copano Bay complex during January-March in 2004 and 2005.

Table 1. Mean (± SE) of environmental conditions and depth for year, Zone, and habitat for southern flounder sampling areas in Aransas-Copano Bay. Environmental conditions, salinity (‰), water temperature (°C), and dissolved oxygen (mg/L), and depth (m) were recorded for every sampling event conducted January and March, 2004, and January-March, 2005.

			Dissolved	
	Salinity	Temperature	Oxygen	Depth
	(‰)	(°C)	(mg/L)	(m)
2004	19.9 ± 0.8	$17.5 \pm 0.4$	9.5 ± 0.2	0.8 ± 0.1
2005	$12.3 \pm 0.7$	$18.9\pm0.5$	$8.9 \pm 0.3$	$0.9 \pm 0.1$
Zone 1	$21.2\pm0.9$	$18.5\pm0.6$	$9.3 \pm 0.3$	$0.9\pm0.1$
Zone 2	$15.4 \pm 0.9$	$18.1\pm0.6$	$8.9\pm0.4$	$1.1 \pm 0.2$
Zone 3	$9.5\pm0.6$	$18.5\pm0.6$	$9.1\pm0.2$	$0.7\pm0.1$
Marsh	$15.0 \pm 1.1$	$19.4\pm0.6$	$9.9\pm0.3$	$0.2\pm0.01$
Seagrass	$14.7 \pm 1.1$	$18.4\pm0.6$	$9.5\pm0.2$	$0.6\pm0.02$
Nonvegetated	$15.7 \pm 1.1$	$17.3\pm0.4$	$8.0\pm0.4$	$2.5\pm0.09$

#### SEDIMENT GRAIN SIZE ANALYSIS

Analysis of sediment grain size for all sampling sites revealed no variation in sediment composition among zones but did show a difference in sediment types between vegetated and nonvegetated habitat types (Fig. 10). No significant difference among percent rubble, clay, silt, or sand among zones was observed (Table 2). However, a significant higher percentage of sand was found in marsh edge and seagrass habitats, while a higher percentage of silt and clay observed in open-water, nonvegetated habitats.



Figure 10. Mean percent ( $\pm$  SE) sediment composition (percent rubble, clay, silt, and sand) for all zones (Zone 1, Zone 2, and Zone 3) and all habitat types (marsh edge, seagrass, and open-water, nonvegetated bottom) collected at flounder sampling sites in Aransas-Copano Bay in June 2004 (N = 81).

Table 2. Analysis of variance table for percent sediment composition in flounder sampling sites in Aransas-Copano Bay. Main effects are percent rubble, clay, silt, and sand for all zones (Zone 1, Zone 2, and Zone 3) and all habitat types (marsh edge, seagrass, and open-water, nonvegetated bottom).

Source	df	SS	F	Р
Zone				
% Rubble	2	450.645	3.408	0.05
% Clay	2	469.228	2.023	0.154
% Silt	2	825.081	1.893	0.173
% Sand	2	1661.385	1.680	0.208
Habitat type	2	45 983	0 161	0 852
% Rubble	2	20.188	0.120	0.887
70 KUUUIC	2	20.188	0.120	0.887
% Clay	2	1701.814	13.171	< 0.001
% Silt	2	3052.55	12.194	< 0.001
% Sand	2	6372.886	10.687	< 0.001

#### HABITAT SELECTION PATTERNS

Distinct habitat selection patterns were observed for hatchery-reared southern flounder in experimental mesocosms (Fig. 11). Specifically, flounder selected for nonvegetated, sand bottom over structured habitats and preferred seagrass habitats when nonvegetated, sand bottom was not available. Comparisons between habitats revealed that hatchery-reared fish selected sand over oyster (t = -5.460, df = 22, P < 0.001), marsh (t = -5.609, df = 22, P < 0.001), and seagrass (t = -2.933, df = 22, P = 0.008). They selected seagrass over marsh (t = 5.692, df = 22, P < 0.001) and oyster (t = 2.815, df = 22, P = 0.010). No selection for marsh over oyster was observed (t = 0.199, df = 22, P = 0.084). Overall, significant differences showed juvenile flounder selected for sand bottom habitats.



Figure 11. Mean percent occurrence ( $\pm$  SE) of hatchery-reared southern flounder in each habitat type (nonvegetated, sand bottom, seagrass, marsh, and oyster) for all possible habitat comparisons. Each comparison represents twelve replicate experimental mesocosms. Significant results from paired Student's t-tests are indicated by \* (P < 0.05), \*\* (P < 0.01), and \*\*\* (P < 0.001).

# TEXAS PARKS AND WILDLIFE DATA ANALYSIS

Texas Parks and Wildlife bag seine data showed both spatial and temporal distribution patterns for southern flounder. Intense sampling of areas along the shorelines of Aransas and Copano Bays was conducted by TPWD (Fig. 12). Total catch showed the

highest number of southern flounder ( $\leq$  50 mm SL) captured in areas near the tidal inlets (within 12 km of Aransas Pass and Cedar Bayou) and indicated relatively low densities of southern flounder in Copano Bay (Fig. 13). Southern flounder first appeared in low abundances in December with the highest catch per hectare occurring during March and April (Figs. 14-18). Results for April indicated southern flounder distributed throughout Aransas and Copano Bay and revealed the highest catch per ha (particularly near Cedar Bayou) among all months analyzed (Fig. 18). Spatial and temporal patterns of southern flounder recruitment mirrored field collection observations in this study.



Figure 12. Map of the Aransas Bay system on the Texas coast showing bag seine sampling intensity (total count of sampling events per site) conducted by Texas Parks and Wildlife from all December, January, February, March, and April months (1977-2004).



Figure 13. Map of the Aransas Bay system showing total catch ( $\geq 1$  fish caught) of southern flounder ( $\leq 50$  mm SL) captured with a bag seine at each site sampled by Texas Parks and Wildlife. Samples were taken from all December data from 1977 through 2004.



Figure 14. Map of the Aransas Bay system on the Texas coast showing catch per hectare of southern flounder ( $\leq$  50 mm SL) captured with a bag seine at each station sampled by Texas Parks and Wildlife. Data were taken from all December months from 1977 through 2004.



Figure 15. Map of the Aransas Bay system on the Texas coast showing catch per hectare of southern flounder ( $\leq$  50 mm SL) captured with a bag seine at each station sampled by Texas Parks and Wildlife. Data were taken from all January months from 1977 through 2004.



Figure 16. Map of the Aransas Bay system on the Texas coast showing catch per hectare of southern flounder ( $\leq$  50 mm SL) captured with a bag seine at each station sampled by Texas Parks and Wildlife. Data were taken from all February months from 1977 through 2004.



Figure 17. Map of the Aransas Bay system on the Texas coast showing catch per hectare of southern flounder ( $\leq$  50 mm SL) captured with a bag seine at each station sampled by Texas Parks and Wildlife. Data were taken from all March months from 1977 through 2004.



Figure 18. Map of the Aransas Bay system on the Texas coast showing catch per hectare of southern flounder ( $\leq$  50 mm SL) captured with a bag seine at each station sampled by Texas Parks and Wildlife. Data were taken from all April months from 1977 through 2004.

#### Discussion

Newly settled southern flounder first appeared in January and were captured throughout March. This pattern corresponds to the peak recruitment period reported by King (1971) and Stokes (1977) in Texas, and Burke et al. (1991) in North Carolina. Flounder were captured in January at around 9-10 mm SL, with few flounder > 30 mm captured in March. Similar temporal size patterns were observed in North Carolina (Burke et al. 1991; Powell and Schwartz 1977). No flounder were captured in our samples taken in April through June 2004. Larger fish may use alternate or unsampled habitats or they may be able to avoid capture by the gear (Wennhage et al. 1997)

Spatial distribution of newly settled southern flounder was related to distance from the tidal pass. Highest densities of juvenile flounders were in higher salinity areas near the pass, within the primary bay. Lowest densities were observed in lower salinity areas farthest from the pass. Similar patterns in Texas estuaries were reported by Stokes (1977) where southern flounder immigrated and settled into areas within the primary bay near the pass, as opposed to more remote bays farther from the pass. Flounder studies in North Carolina Louisiana reported salinity may play a role in flounder distribution, with juveniles seeking out low salinity, upper-bay regions (Burke et al. 1991). One reason flounder migrate toward lower salinity areas may be because predators may not be as abundant at these sites (Rozas and Hackney 1984). This study did not coincide with this pattern, but results did show that areas near the pass are more important recruitment locations for southern flounder regardless of salinity.

Densities of newly settled southern flounder were also influenced by habitat type. Seagrass meadows and marsh edge habitats supported much higher densities compared to

open-water, nonvegetated habitat types. Based on density patterns of juveniles in vegetated habitat types, data suggest these habitat types are functioning as nursery areas for newly settled southern flounder. Similar patterns of southern flounder distribution for Aransas Bay were also reported by Stokes (1977) where higher numbers of southern flounder were associated with vegetated habitats. Vegetated habitats, such as seagrass beds and marshes, clearly support higher densities of fishes compared to open water habitats (Bell et al. 1988; Boehlert and Mundy 1988; Rozas and Minello 1997; Heck and Orth 2003). Vegetated habitats provide complex structure aiding in the avoidance of predators, while supplying an abundance of accessible prey which help increase survival and growth rates of fish species; thus, indicating southern flounder also use complex habitat as juveniles (Rozas and Odum 1988; Minello and Zimmerman 1992; Sogard 1992; Rooker et al. 1998).

The mean size of individuals varied among zones. Smaller fish were captured in areas closest to the pass and were progressively larger with increasing distance from the pass suggesting variable growth rates among zones. Smaller mean size in areas near the pass may be attributed to newer, smaller recruits migrating into the estuaries from offshore spawning sites (King 1971; Brown et al. 2004), while older recruits are moving and growing as they migrate farther and disperse throughout the estuary. Another explanation for differences in mean size may be related to differences in mean salinity among zones. Other flatfish, such as the winter founder (*Psuedopleuronectes americanus*), were reported to have increased or faster growth in low salinities (Manderson et al. 2002). Such low salinity areas may provide refuge from stenohaline

marine predators (Rozas and Hackney 1984) increasing survival and growth and contributing to an overall higher mean size of flounder in these areas.

Mean size of juvenile flounder was largest in vegetated habitats, where density was also highest. Differences in mean size between vegetated and nonvegetated sites are an indication of differential growth rates or survival among these habitats. Vegetated habitats are structurally complex providing abundant prey and refuge from predators (Rozas and Odum 1988; Sogard 1992; Kneib 1993; Rooker et al. 1998). The quality and quantity of these complex habitats may lead to faster growth rates and lower mortality (subsequently better growth and survival), that are important to successful recruitment into adult populations (Houde 1987; Sogard 1992; Kneib 1993; Gibson 1994; Levin 1994). Results also showed differences in mean size between vegetated habitat types, with marsh edge having a larger mean size. This difference may be attributed to marshes potentially having a higher prey abundance (Teal 1962; Boesch and Turner 1984; Rozas and LaSalle 1990; Minello and Zimmerman 1992) thus, contributing to faster growth rates. Walsh et al. (1999) also suggested that the characteristic flat body shape and benthic lifestyle of flatfish makes it difficult to maneuver through seagrass beds while foraging. Since this study did not examine growth as an indicator of "good" quality habitat, further research is needed to examine the difference in growth rates among different shallow estuarine habitat types.

Sediment composition was also examined as a factor affecting the distribution of southern flounder. There was no association between sediment composition and flounder distribution among zones; therefore, no relationship between density patterns and sediments was observed. The only difference among sediment types occurred in the

substrates of vegetated and nonvegetated habitat types. Sediments in seagrass and marsh habitats were primarily composed of sand with only small amounts of silt and clay, while nonvegetated habitats exhibited a muddy substrate (silt/clay composition with small amounts of sand). Although these results were consistent with the substrate composition reported in Texas bays (Britton and Morton 1989), it did not support studies in Texas, North Carolina, or Louisiana where sediment grain size played an important role in southern flounder distribution and abundance (Powell and Schwartz 1977; Stokes 1977; Benson 1982; Gibson 1994; Allen and Baltz 1997). Studies reported southern flounder settled on muddy substrates (silt/clay) (Burke et al. 1991; Burke et al. 1998; Powell and Schwartz 1977) because muddy bottom habitats provide a more suitable substrate, as opposed to a sandy bottom habitat, in which flatfish can easily bury, camouflage, and protect their flat bodies against predators (Tanada 1990; Moles and Norcross 1995; Nasir and Poxton 2001). This study did not show a connection between sediment grain size and southern flounder distribution.

Hatchery-reared juvenile southern flounder showed distinct habitat selection patterns. Although a comparison with wild-caught flounder was not possible due to the low availability of wild fish collected, results still support other research reporting distribution and abundance of demersal marine fishes is influenced via selection for specific habitat types (Bell et al. 1987; Levin et al. 1997; Valle et al. 1999). Overall, hatchery-reared southern flounder preferred nonvegetated habitat over structured habitat, selecting sand over marsh, seagrass, and oyster habitat and selecting seagrass only when nonvegetated, sand bottom was not available. These patterns suggest hatchery-reared flounder prefer to settle in sand or near seagrass beds. Although Heck and Orth (1980)

suggested predation rates of newly recruited fishes decreases as the structural complexity of habitat increases, this may not necessarily take into account the benthic lifestyle of flatfishes. Flatfish are "evolutionarily specialized for benthic existence" (Kellison et al. 2000) and spend the majority of the time feeding on the bottom or buried/camouflaged in the sediment to avoid predation (Ansell and Gibson 1993; Howell and Baynes 1993). Flatfishes may be able to use these non-structured habitats, such as nonvegetated, sand bottom in the experimental mesocosms, in a way that other fish can not use them. Nonvegetated bottom habitats, particularly when associated with vegetated habitats, may also be an important nursery component in juvenile southern flounder habitat selection.

Hatchery fish also exhibited behaviors not associated with the typical benthic lifestyle of flatfish species. Fish usually remained in the water column for a few minutes, swimming around the tank before they settled. Flounder were also observed swimming in the water column when their location was visually assessed in the tank. These behaviors may be a consequence of the conditions under which the experimental fish were reared (Stunz et al. 2001). Fish were conditioned to feed in the water column in confined tanks with no exposure to natural conditions (habitat structures, substrates, or predators). This may explain why flounder did not immediately seek shelter or bury in the substrate. Although these behaviors may not be conducive to survival in the wild, Seikai (1985) and Ellis et al. (1997) suggest these behaviors can be diminished by introducing or exposing hatchery-reared fish to natural conditions or stimuli like fluctuations in salinity or temperature. Further research is necessary to examine and compare habitat preference patterns of wild-caught flounder with the patterns found with

hatchery-reared fish. This information could provide a more realistic basis for predicting southern flounder habitat selection in the wild.

Long-term monitoring studies by Texas Parks and Wildlife Department were useful in detecting changes in southern flounder abundance over large spatial and temporal scales. There were distinct distribution patterns for southern flounder in Aransas Bay. High densities were collected during the March through April peak recruitment period for southern flounder. Results support similar recruitment periods observed by King (1971) and Stokes (1977) in Texas and Burke et al. (1991) in North Carolina. Even though relatively low numbers of southern flounder were distributed throughout the bay system, TPWD's long-term data set suggest areas near the pass serve as important nursery grounds for juvenile southern flounder.

This study provides valuable data on southern flounder distribution and habitat selection patterns. This information can be used to assess the relative value of juvenile southern flounder nursery habitat, as well as supply fisheries managers with essential data needed to help protect and conserve essential fish habitat. Additionally, more research is needed to evaluate the link between the patterns observed and other factors affecting southern flounder recruitment in Texas estuaries. By examining the functional relationships between habitat and fish productivity in terms of survival and growth, fisheries managers will be able to further determine the habitats that are actually accounting for the productivity of southern flounder populations.

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