

# Distribution, condition, and growth of newly settled southern flounder (*Paralichthys lethostigma*) in the Galveston Bay Estuary, TX

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## Abstract

Several flatfish species, including southern flounder (*Paralichthys lethostigma*) recruit to estuaries during early life. Therefore, evaluation of estuarine sites and habitats that serve as nurseries is critical to conservation and management. The present study used density data in conjunction with biochemical condition and growth measurements to evaluate settlement sites used by southern flounder in the Galveston Bay Estuary (GBE). In 2005, beam-trawl collections were made in three major sections of the GBE (East Bay, Galveston Bay, West Bay). Three sites were sampled in each bay. Within each sampling site, replicate collections were taken from three habitats: 1) marsh edge (<1 m depth), 2) intermediate zone (10–20 m from marsh interface; ~1 m depth), and 3) bay zone (typically >100 m from marsh interface; depth >1 m). Average size of southern flounder collected was 12–19 mm standard length, and peak densities occurred in January and February. Catch data indicated that densities of southern flounder were significantly greater in East Bay (2.75 per 100 m<sup>2</sup>) than in Galveston Bay (0.91 per 100 m<sup>2</sup>) or in West Bay (0.45 per 100 m<sup>2</sup>). Densities were statistically similar among habitats. Otolith-based estimates of age indicated that the majority of southern flounder collected were 35–45 days old and derived from early December to early January hatch-dates. Growth rates were similar among bays and among habitats, with the average growth rate being 0.40 mm day<sup>-1</sup> (range: 0.21–0.76 mm day<sup>-1</sup>). RNA:DNA was above the established baseline value for nutritional stress, indicating that newly settled southern flounder in the GBE were in relatively high condition. Habitat-specific differences in RNA:DNA ratios were not observed; however, ratios were significantly lower in West Bay (average 8.0) than in East Bay (average 9.5) or in Galveston Bay (average 9.8), suggesting the condition of new recruits may vary spatially within the GBE. Findings from the current study suggest density and condition of newly settled southern flounder vary at the bay scale, suggesting that parts of GBE do not function equally as nurseries.

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## 1. Introduction

Settlement patterns of estuary-dependent flatfishes can vary both temporally (several influxes of larvae in a season) and spatially (occurrence in different areas), and nursery areas are often separated from spawning grounds

(Burke et al., 1991; Miller et al., 1991; Gibson, 1994; Cowan and Shaw, 2002). Variation in the settlement of flatfishes within an estuary occurs at several spatial scales, and factors that affect recruitment change with the scale of observation (Burke et al., 1991; Miller et al., 1991; Gibson, 1994; Burke, 1995). Similar to other estuarine-dependent taxa, large-scale variation in flatfish settlement is often linked to larval supply (Burke et al., 1998), which is regulated to some degree by hydrographic processes (e.g. tidal forcing, river flow, wind driven circulation). In addition to having a direct impact on transport of larvae, hydrography within the estuary also affects environmental and physicochemical conditions, and these changes can influence growth and survival of new recruits (Cowan, 1985; Jenkins et al., 1997). At smaller scales (e.g. within nursery or bay), variation in the distribution and abundance of new recruits is often linked to factors such as substrates (Burke et al., 1991; Malloy et al., 1996; McConnaughey and Smith, 2000; Stoner et al., 2001) or water quality parameters (Miller et al., 1991; Gibson, 1994), as well as the presence or absence of competitors (Burke et al., 1991).

In the northwestern Gulf of Mexico, southern flounder (*Paralichthys lethostigma*) are the dominant paralichthyid that use estuaries as nursery habitat (Hoese and Moore, 1998; McEachran and Fechhelm, 2006). Adults leave estuaries and coastal waters to spawn off shore in late fall and early winter, eggs are fertilized in pelagic waters, and larvae are eventually transported to the estuaries by ocean currents (GSMFC, 2000). Since many estuaries in the Gulf are heavily impacted by anthropogenic activities (Hanson et al., 1993; Morse et al., 1993; Santschi et al., 2001), there is a need to evaluate the role estuaries play in fisheries productivity. In particular, there is a need to identify habitats and environmental conditions that favor the settlement and growth of southern flounder during early life. Assuming the quality of habitats varies spatially, important nursery areas of southern flounder should foster settlement and rapid growth through a combination of factors, the most important being high-quality prey resources, refuge from predators, and suitable physicochemical conditions (e.g. temperature, salinity, dissolved oxygen) (Gibson, 1994; Beck et al., 2001).

The purpose of the present study was to examine large-scale (site-specific) and small-scale (habitat-specific) variation in density of newly settled southern flounder and associated flatfishes in the largest estuary in the northwestern Gulf of Mexico, the Galveston Bay Estuary (GBE). Since condition and growth are linked to early life survival and recruitment potential (Buckley, 1984; Ferron and Leggett, 1994; Able and Fahay, 1998; Beck et al., 2001), the quality of different settlement sites was assessed

by measuring the biochemical condition (RNA:DNA) and growth (otolith microstructure analysis) of new settlers. These two measures were used in conjunction with density data to evaluate the relative value of areas occupied by newly settled southern flounder.

## 2. Methods

The GBE was separated into three main areas for study: East Bay, Galveston Bay, and West Bay (Fig. 1). East Bay is connected to the Gulf of Mexico at Rollover Pass and Bolivar Roads (main entrance to estuary and shipping lanes). Galveston Bay is in the central portion of the estuary, with water flow through Bolivar Roads. West Bay is located in the far western portion of the GBE, direct input from the Gulf of Mexico by San Luis Pass is impeded due to extensive marsh area at the mouth of West Bay. Preliminary sampling in 2004 was conducted during the last week of February through the first week of March, the third week of April, and the second week June. Regular monthly sampling was conducted the second week of January through April in 2005. The same sampling design (described below) was used in each year at the same selected sites; due to differences in sampling frequency, approximately 28% more area was sampled in 2005 (total area covered 2004=10.01 km<sup>2</sup>, area covered 2005=12.78 km<sup>2</sup>). Due differences in sampling regularity and the small number of newly settled southern flounder collected in 2004 ( $n=30$ , density=0.3 per 100 m<sup>2</sup> pooled across bays/sites), further description and analysis of southern flounder data will be based entirely on collections from 2005.

Sampling targeted the primary recruitment period of southern flounder, January to May as reported by Stokes (1977) and Burke et al. (1991); exploratory sampling was conducted in December of 2004, but no southern flounder were collected. Three sites in each designated bay were selected such that they were interspersed throughout the bays, and each site contained adjacent marsh habitat and deeper areas where a trawl could operate. Upper eastern reaches of the GBE were sampled preliminarily, but no newly settled southern flounder were found. Data from a Texas Parks and Wildlife Department 25 year bag-seine study (Martinez-Andrade et al, 2005) indicated greater numbers of southern flounder were found more proximal to the tidal passes, in addition, a study by Stokes (1977) found southern flounder settlement patterns correlated with nearness to passes. Therefore to maximize effort and limited resources, our sites focused on bays nearer to tidal passes through which larvae would be transported. Three habitat types were sampled in replicates of three: 1) marsh edge; depth < 1 m, 2) intermediate zone (10–20 m from

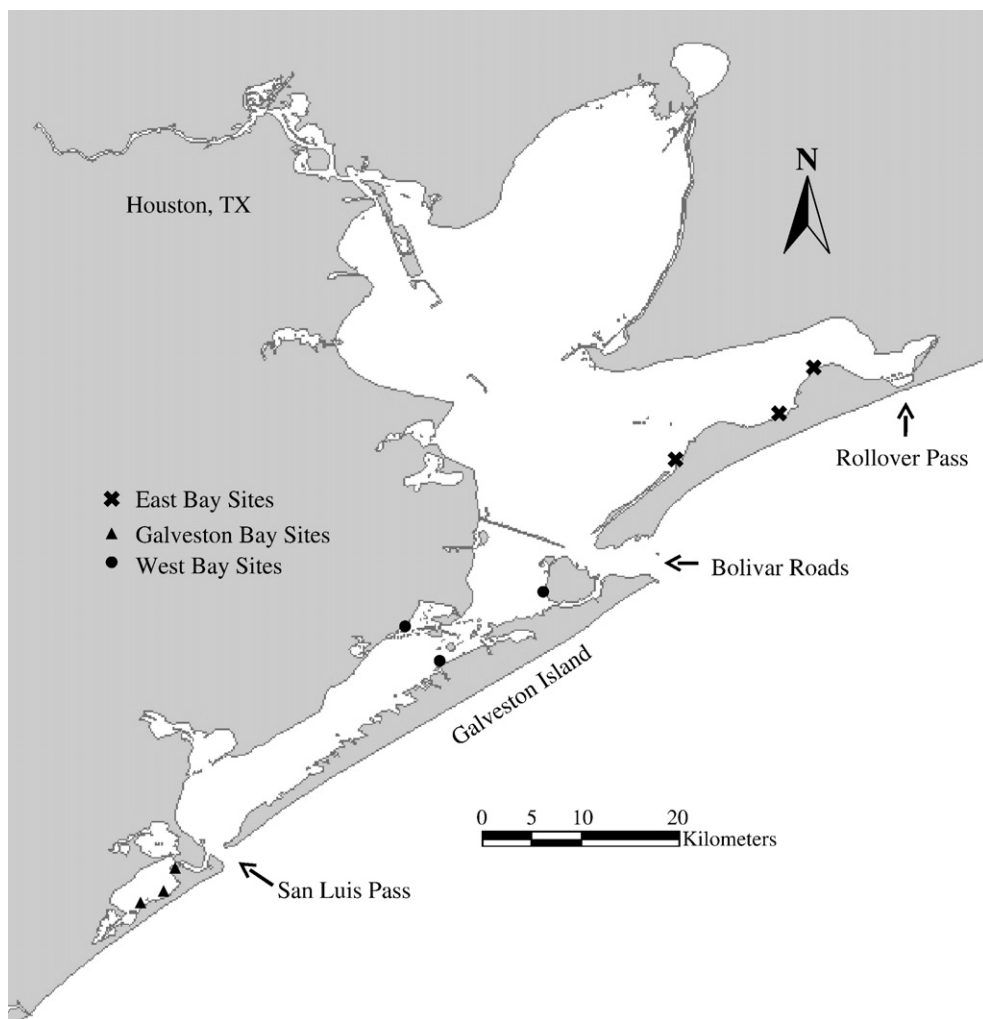


Fig. 1. Map of study sites in the Galveston Bay Estuary.

marsh interface; ~1 m depth), and 3) bay zone (typically >100 m from marsh interface; depth >1 m) for a total of 27 samples per bay each monthly sampling round. We used a beam trawl (opening 1 m wide by 0.5 m tall with 1-mm mesh net) operated by hand with 20-m lines in the marsh edge and intermediate zones, and in the bay zone, we towed the net connected to a 10-m bridle rope with a small boat at approximately the same speed as trawls pulled by hand. Area swept was estimated either by direct measurement in the marsh edge and intermediate zones (trawl pulled length of rope) or from start and finish locations determined with a WAAS-enabled GPS (wide area augmentation system; global positioning system) unit for the bay zone samples. Bottom complexity was similar among habitats sampled and any differences in gear efficiency across habitats was likely negligible. Several flatfish studies using similar beam trawls found no significant effect on mean size collected and no

significant differences in efficiency between beach or marsh habitat (Wennhage et al., 1997; Kellison et al., 2003). Densities were calculated as number of fish per 100 m<sup>2</sup>. Sampling was conducted during daytime, and temperature, salinity, and dissolved oxygen (DO) were recorded for each sample near the bottom with an YSI 55. Any southern flounder captured in the trawls were preserved on dry ice in the field and later stored in a -80 °C freezer. Other collected flatfishes were preserved in ethanol in the field.

### 2.1. Condition

Sections of trunk muscle tissue were extracted from frozen newly settled southern flounder for RNA:DNA analysis. Only a subset ( $n=49$ ) of southern flounder (SL ~10–23 mm) were analyzed from one sampling period (month: February) to reduce potential effects of temporal

variation. Analysis followed the ethidium bromide (EB) fluorometric procedures described by Westerman and Holt (1988). Two replicates of individual trunk muscle samples were homogenized, and aliquots of homogenates (25–100  $\mu$ l) were used to estimate DNA and RNA concentrations. Calculations were based on comparisons with DNA-EB and RNA-EB calibration curves from known standards [calf thymus DNA and yeast RNA (type III)]. Sample order was randomized and standards were analyzed at the beginning and end of readings of each replicate set. Instrument drift was negligible.

## 2.2. Age and growth

To determine daily age, sagittal otoliths from all collected newly settled southern flounder were removed and cleaned with one of the pair randomly selected for aging. The otolith was mounted on a slide and polished to the core on each side using Buehler Carbimet paper discs and 0.3  $\mu$ m alumina polishing compound following the procedure outlined by McCurdy et al. (2002). Polished otoliths were examined using transmitted light with a compound microscope with analysis aided by image analysis software (Image Pro Plus 4.5). Daily growth increments were counted by one reader with a subset of otoliths counted by a second reader for quality control. Fitzhugh and Rice (1995) determined that southern flounder produce daily otolith growth increments, and thus those increments seen under the microscope were considered daily increments. Otolith counts that were not within 10% agreement between readers were recounted by both readers. If agreement between the second set of counts did not meet the criteria for agreement, the sample was removed from the

analysis. The final count assigned to an otolith was an average between the two counts. Otoliths that were considered unreadable (due to cracking, polishing error, or position in epoxy) and otoliths ruined by epoxy drying were also discarded. Of all southern flounder collected ( $n=184$ ), 54% were assigned age ( $n=100$ ), 23% were deemed unreadable due to polishing error (including cracking that might have occurred during polishing), 13% were unreadable or lost due to epoxy problems, and 10% were deemed unusable due to structure of otolith or count disagreement. Growth rates (daily instantaneous) were determined by fitting an exponential model:

$$L_t = L_0 e^{gt}$$

where  $L_t$ =length (mm standard length) at time  $t$ ,  $L_0$ =estimated length at hatching,  $g$ =instantaneous growth coefficient, and  $t$ =estimated age (days after hatching). Data were log-transformed so that model parameters could be estimated using linear regression.

## 2.3. Data analysis

Analysis of variance (ANOVA) was used to examine the effects of bay, habitat, and month on environmental variables (salinity, temperature, dissolved oxygen). ANOVA of summer flounder density was also conducted with the main effects of bay and habitat type and month as a blocking factor. April was excluded from the analysis because only one southern flounder was caught during this period. Density data were log-transformed to minimize heteroscedasticity. Significant ANOVA results were further examined with Tukey's

Table 1  
Environmental parameters (salinity, temperature, and dissolved oxygen) from three regions of the Galveston Bay Estuary in 2005

	January	February	March	April
<i>Salinity (ppt)</i>				
East Bay	12( $\pm$ 0.61)	13( $\pm$ 0.76)	9 ( $\pm$ 0.0.40)	13 ( $\pm$ 1.42)
Galveston Bay	19( $\pm$ 0.68)	16( $\pm$ 1.63)	15( $\pm$ 1.21)	25( $\pm$ 1.29)
West Bay	25 ( $\pm$ 0.58)	22 ( $\pm$ 0.33)	20 ( $\pm$ 0.50)	31 ( $\pm$ 0.40)
<i>Temperature (<math>^{\circ}</math>C)</i>				
East Bay	13.4 ( $\pm$ 0.99)	15.1 ( $\pm$ 0.50)	16.8 ( $\pm$ 1.65)	21.9 ( $\pm$ 0.78)
Galveston Bay	17.1 ( $\pm$ 0.92)	18.1 ( $\pm$ 0.53)	16.6 ( $\pm$ 0.84)	22.7 ( $\pm$ 0.42)
West Bay	18.9 ( $\pm$ 0.66)	13.1 ( $\pm$ 0.22)	18.3 ( $\pm$ 0.36)	23.7 ( $\pm$ 0.28)
<i>Dissolved oxygen (mg L<sup>-1</sup>)</i>				
East Bay	9.86 ( $\pm$ 0.25)	8.95 ( $\pm$ 0.67)	7.97 ( $\pm$ 0.47)	7.57 ( $\pm$ 0.46)
Galveston Bay	10.02 ( $\pm$ 0.58)	9.92 ( $\pm$ 2.29)	9.78 ( $\pm$ 0.45)	7.87 ( $\pm$ 0.54)
West Bay	9.93 ( $\pm$ 0.44)	9.53 ( $\pm$ 1.06)	8.88 ( $\pm$ 0.58)	8.03 ( $\pm$ 0.66)

Estimates are mean value ( $\pm$ 1 SE) of the three sites surveyed within each bay. Salinity is reported in ppt, temperature in  $^{\circ}$ C, and dissolved oxygen in mg L<sup>-1</sup>.

honestly significant difference (HSD) test to determine which factor levels of the main effects differed significantly. Analysis of covariance (ANCOVA) was used to investigate effects of bay and habitat on RNA:DNA ratios and growth (exponential model). The covariate in all ANCOVA models was standard length, and an alpha level of 0.05 was chosen prior to analysis.

### 3. Results

#### 3.1. Environmental conditions

Salinity varied both spatially and temporally within the GBE in 2005. Mesohaline conditions were typically observed in East Bay (mean range: 9–13 ppt), but conditions within the central portion of Galveston Bay (15–25 ppt) and West Bay (20–31 ppt) were essentially polyhaline (Table 1). Significant differences in salinity occurred among bays (ANOVA,  $F_{2, 44}=171.1, p<0.001$ ) and among months (ANOVA,  $F_{3, 44}=41.0, p<0.001$ ) with

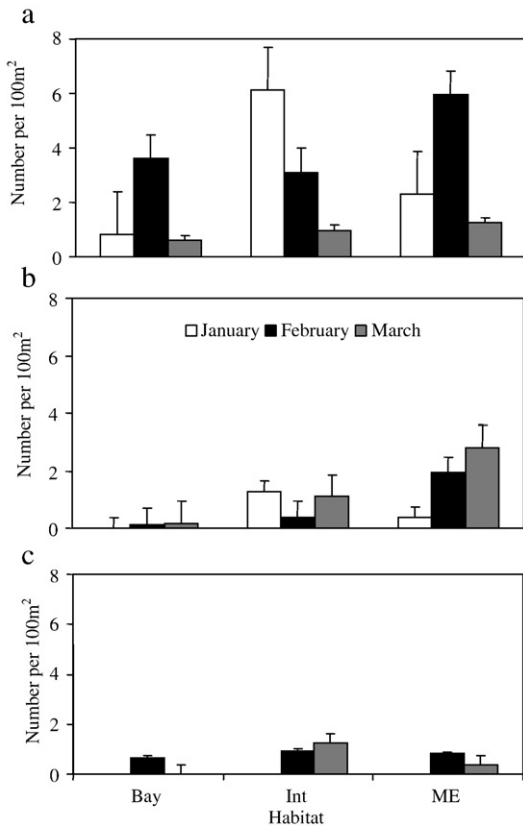


Fig. 2. Densities (number per 100 m<sup>2</sup>) of newly settled southern flounder from the Galveston Bay Estuary in 2005. Estimates of density are given by habitat and data are divided by bay: a) East Bay, b) Galveston Bay, and c) West Bay. Error bars represent 1 SE. Habitat codes: Bay=bay zone, Int=intermediate zone, ME=marsh edge.

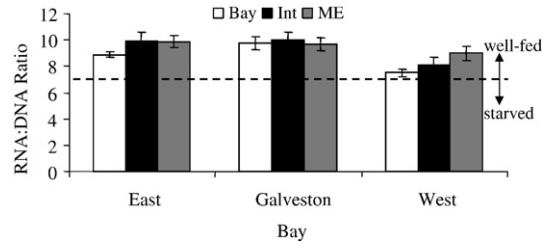


Fig. 3. Mean RNA:DNA for newly settled southern flounder from the Galveston Bay Estuary in 2005. Ratios are given by bay and habitat. Error bars represent 1 SE. Habitat codes: Bay=bay zone, Int=intermediate zone, ME=marsh edge. RNA:DNA above dashed line are in a “well-fed” range versus below the line in a “starved” range as found in laboratory studies.

a significant bay\*month interaction (ANOVA,  $F_{6, 44}=5.4, p<0.001$ ); however, salinity did not vary among habitat types within bay (ANOVA,  $F_{1, 44}=0.1, p=0.735$ ). Tukey’s HSD test indicated that East Bay had significantly lower salinity than both Galveston Bay and West Bay and that all bays were significantly different from one another. Aside from an expected overall seasonal warming trend, there was no consistent pattern of temperature across bays as indicated by significant main and interaction effects (bays:  $F_{2, 45}=6.8, p=0.003$ ; months:  $F_{3, 45}=36.3, p<0.001$ ; bay\*month,  $F_{6, 45}=5.3, p<0.001$ ). Further, temperature did not vary as a function of habitat type within bays (ANOVA,  $F_{2, 45}=0.2, p=0.632$ ). Minimum (13.1 °C in February) and maximum (23.7 °C in April) observed temperatures occurred in West Bay. Dissolved oxygen (mg/L) levels were not significantly different among bays (ANOVA,  $F_{2, 44}=0.5, p=0.582$ ), but were significantly different among months (ANOVA,  $F_{3, 44}=5.3, p=0.003$ ) with no bay\*month interaction (ANOVA,  $F_{6, 44}=0.7, p=0.061$ ). Tukey’s HSD test indicated that DO levels in January (9.94 mg L<sup>-1</sup>) and February (9.41 mg L<sup>-1</sup>) were higher than April (7.82 mg L<sup>-1</sup>). In contrast to salinity and temperature, dissolved oxygen varied among habitat types in the GBE (ANOVA,  $F_{1, 44}=19.6, p<0.001$ ) with higher concentrations in the marsh edge and intermediate zones (mean=10.14 mg L<sup>-1</sup>, both) than in the bay zone (mean=8.37 mg L<sup>-1</sup>), while there is variation these concentrations are all well above hypoxic levels (2 mg L<sup>-1</sup>).

#### 3.2. Distribution and abundance

Overall five species of flatfish were collected: southern flounder ( $n=184$ ), bay whiff (*Citharichthys spilopterus*) ( $n=355$ ), blackcheek tonguefish (*Symphurus plagiusa*) ( $n=50$ ), fringed flounder (*Etropus crossotus*) ( $n=4$ ), and hogchoker (*Trinectes maculatus*) ( $n=1$ ). Newly settled southern flounder were collected in all bays sampled in 2005, southern flounder made up 54% of



all flatfish collected in East Bay ( $n=142$ ), 28% in Galveston Bay ( $n=27$ ), and 7% in West Bay ( $n=17$ ). Across the 2005 sampling period, southern flounder were the dominant flatfish collected in January and February in all three bays, whereas bay whiff was the main flatfish species collected in March and April.

Density of newly settled southern flounder in the GBE varied significantly by month and location in 2005, with a maximum of 21.7 per 100 m<sup>2</sup> observed in East Bay. The most prominent pattern was significantly higher densities of southern flounder in East Bay such that rank order of bay density was East Bay > Galveston Bay = West Bay (ANOVA,  $F_{2, 94}=5.4$ ,  $p=0.006$ ; Fig. 2). Sample numbers in East Bay were sufficient to enable investigation of specific patterns of habitat use; due to low numbers in other bays, interaction effects between habitat and bay were not explored. Although no significant differences were detected between habitat types in East Bay (ANOVA,  $F_{2, 33}=0.9$ ,  $p=0.404$ ), the value of densities in the bay zone (1.7 per 100 m<sup>2</sup>) were lower than in the intermediate zone (3.4 per 100 m<sup>2</sup>) or marsh edge (3.2 per 100 m<sup>2</sup>). Monthly variation was not significant (ANOVA,  $F_{3, 94}=2.5$ ,  $p=0.065$ ), but the value of mean densities were higher in January (1.2 per 100 m<sup>2</sup>) and February (1.9 per 100 m<sup>2</sup>) than in March (0.95 per 100 m<sup>2</sup>).

3.3. Condition

Mean RNA:DNA value for southern flounder was 9.17 (range: 6.34 to 15.19), and did not vary significantly as a function of standard length (ANCOVA,  $p=0.520$ ,  $F=0.4(1, 37)$ ). Based on a previous laboratory validation study that quantified RNA:DNA of well-fed and starved southern flounder reared at a constant temperature of 18 °C and salinity of 31 ppt (G. Joan Holt, unpublished data), 90% of the assayed southern flounder from the GBE matched the “well-fed” category with RNA:DNA > 7.0. Significant differences in RNA:DNA were detected among bays (ANOVA,  $p=0.003$ ), but no significant habitat or habitat\*bay interaction effects were detected (habitat: ANOVA,  $F=2.1(2, 34)$ ,  $p=0.114$ ; interaction: ANOVA,  $F=0.6(4, 34)$ ,  $p=0.632$ ). Southern

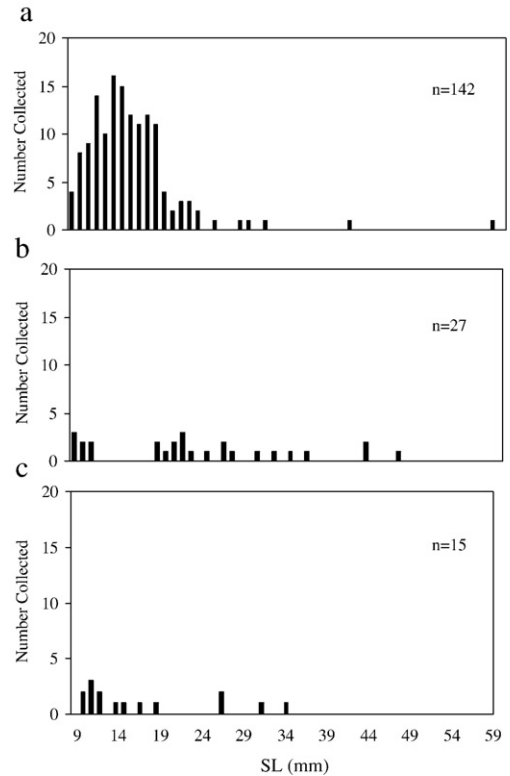


Fig. 5. Length–frequency distributions of newly settled southern flounder from the Galveston Bay Estuary in 2005. Data are divided by bay: a) East Bay, b) Galveston Bay, and c) West Bay.

flounder from West Bay had significantly lower RNA:DNA (mean 8.0 SE±0.3) than East Bay (mean 9.5 SE±0.2) and Galveston Bay (mean 9.8 SE±0.4), with no significant difference in condition between East Bay and Galveston Bay (Tukey’s HSD  $p=0.758$ ; Fig. 3).

3.4. Age and growth

Otolith-based estimates of age indicated that southern flounder collected in the GBE in 2005 ranged in age from 27 to 78 days post hatch. Over half the southern flounder aged ( $n=54$ ) were 35 to 45 days old. Recruitment of southern flounder to the GBE showed a protracted hatch-date distribution, with a peak in hatch-dates from December

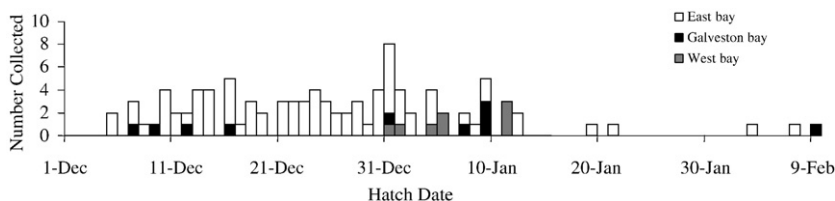


Fig. 4. Hatch-date distribution of newly settled southern flounder from the Galveston Bay Estuary in 2005. Data are coded by bay.

9 to January 12, 2005 (Fig. 4). Hatch-dates of individuals collected in East Bay and Galveston Bay ranged from December 4 to February 9, while hatch-dates of recruits from West Bay ranged from December 31 to January 11 (Fig. 6).

The smallest individuals collected during 2005 sampling were 9 mm, possibly suggesting settlement occurs at this size (Fig. 5). Catches were dominated by individuals between 12 and 19 mm standard length (SL). No significant difference was found in length among habitats, using month as a blocking factor (ANOVA,  $F=2.53_{(2,173)}$ ,  $p=0.082$ ). The blocking factor of month was found to have a significant effect on size with March>February>January (ANOVA,  $F=131.70_{(2,173)}$ ,  $p<0.0001$ ; January =  $12.81 \pm 2.04$ , February =  $16.35 \pm 3.45$ , March =  $30.11 \pm 9.64$ ) with no interaction effect between habitat\*month (ANOVA,  $F=0.14_{(4,173)}$ ,  $p=0.966$ ).

Overall growth of southern flounder (all bays combined) was described by the exponential model:  $SL \text{ (mm)} = 5.519e^{0.0253 * \text{age}}$  ( $R^2=0.6242$ ; Fig. 6), modeling that length increases by 2.5% per day during this period. Linear growth rates increased with age, and are presented here in 10-day groupings for comparison with other published values:  $0.24 \text{ mm d}^{-1}$  (21 to 30 days old),  $0.31 \text{ mm d}^{-1}$  (31 to 40 days old),  $0.40 \text{ mm d}^{-1}$  (41 to 50 days old), and  $0.51 \text{ mm d}^{-1}$  (51 to 60 days old), and, with an overall linear growth rate of  $0.40 \text{ mm d}^{-1}$ . Although sample sizes were small ( $n=36$  for habitat,  $n=100$  for bay), significant habitat- (within East Bay) and bay-specific variability in growth rates was not detected (habitat: ANCOVA,  $F_{2, 77}=0.8$ ,  $p=0.436$ , power=0.189; bay: ANCOVA,  $F_{2, 95}=0.7$ ,  $p=0.514$ , power=0.160). Further, growth rate differences between December and January cohorts (representing hatch-dates for most of the summer flounder in 2005) were not significant (ANCOVA,  $F_{1, 93}=1.9$ ,  $p=0.172$ , power=0.276).

#### 4. Discussion

Differences in settlement numbers of flatfishes at large scales have been reported and are often linked to spatial variation in environmental conditions, with species often more abundant in areas of the estuary where conditions are preferable for growth and survival (Burke et al., 1998). Large-scale spatial variation in densities of newly settled southern flounder was found in the GBE both within years and inter-annually. Low densities in 2004 were followed by greater-than four-fold higher densities during comparable time periods in 2005, and in 2005 southern flounder were more concentrated in one sub-estuary (East Bay) of the GBE. Several studies have determined that salinity is an important environmental parameter for flatfishes,

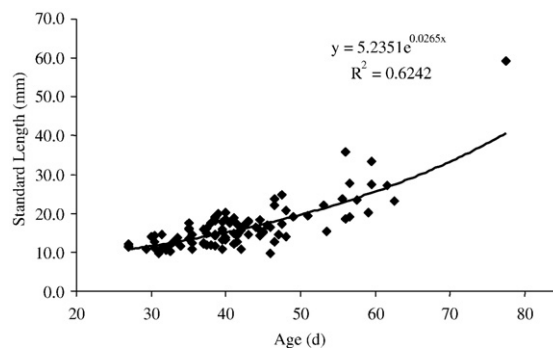


Fig. 6. Size-at-age relationship for newly settled southern flounder from the Galveston Bay Estuary (all sites pooled) in 2005 ( $n=100$ ). Exponential growth equation is given.

particularly during early life (Miller et al., 1991; Gibson, 1994). More specifically, Burke et al. (1991) reported that southern flounder distribution in North Carolina estuaries was determined in part by salinity with highest catches at lower salinities ( $17.5 \text{ ppt} \pm 8.3$ ). Therefore, it is possible that higher numbers of southern flounder observed in East Bay were partly a function of lower salinities there ( $8\text{--}17 \text{ ppt}$ ) than in the other bays. It should be noted that a study on southern flounder habitat use in Aransas Bay, Texas (south-west on the coast from GBE) found greater densities of newly settled southern flounder in higher salinities ( $\sim 22 \text{ ppt}$ ) closer to the tidal pass than in lower salinity areas ( $\sim 10 \text{ ppt}$ ) further away from the tidal pass (Nanez-James, 2003).

Physical processes (e.g. wind driven currents, tidal forcing) can influence larval supply and have been shown to influence patterns of settlement as well as nursery habitat use (Bell and Westoby, 1986; Jenkins et al., 1997; Brown et al., 2005). Jenkins and Black (1994) compared the temporal settlement patterns of King George whiting (*Sillaginodes punctata*) to movements of passive particles in a bay system and determined that settlement was largely influenced by coastal hydrodynamic processes. Similarly, Brown et al. (2005) examined the link between physical processes and larval supply to estuarine nurseries in south Texas using a circulation model coupled with a fixed-depth particle transport model, and found that the proximity of the inlet to the nursery ground and the approach path to the inlet were critical factors that influenced larval supply. Although a comparable model does not exist for the GBE, the high density East Bay sites were closer to a tidal inlet (Rollover Pass) than sites in West Bay (which were blocked by extensive marsh zones). Also, sites in East Bay are connected to coastal spawning areas by two tidal passes (Rollover Pass and Bolivar Roads) rather than one, and this may have increased the supply of larvae, particularly if southern

flounder recruits from two different spawning areas converge on the same nursery habitats in East Bay.

Other abiotic factors (temperature, DO) varied across the bays sampled and may also have influenced observed patterns of habitat use, but these varied temporally within each bay such that clear associations with southern flounder density were not evident.

Small-scale, habitat-specific variation in density of flatfishes has also been reported and often attributed to a variety of environmental factors (Burke et al., 1991; Miller et al., 1991; Gibson, 1994; Guindon and Miller, 1995; Burke et al., 1998). In the present study, no habitat-specific differences in densities were detected, indicating either that responses to conditions across nursery habitats were relatively equal or movement by individuals occurred between habitats. However, power for this test was low and a larger sample size for habitat comparisons might change this result. The apparent lack of a habitat effect is not surprising because environmental qualities (temperature, DO, salinity) and observed substrate condition were consistent among habitat types within the same site. Substrate composition of each site was observed to be dominated by mud and silt with similar sized particles. Nanez-James (2003) found no effect of sediment grain size on settlement of southern flounder in a similar Texas bay. Also, the lack of a co-occurring parichthyid (e.g. Gulf flounder or summer flounder) in the study area may have allowed southern flounder to utilize several habitats rather than partitioning available resources to minimize overlap.

Peak recruitment of southern flounder to nurseries within the GBE occurred in January and February in 2005, with the majority of recruits being derived from early winter (late December to early January) spawning events (based on hatch-date distribution). Findings from the present study are in accord with previous reports of early winter spawning of southern flounder (Ginsburg, 1952; Richards, 2006). The timing of peak recruitment of southern flounder in the GBE was slightly earlier than in North Carolina, which occurred in late February (Burke et al., 1991). Observed differences in recruitment times were relatively small and may have been due to natural variability (inter-annual effect) rather than a regional effect. Nonetheless, it is possible that warmer temperatures in the GBE may have been partly responsible for earlier settlement events. Collection numbers of southern flounder in the GBE decreased in March and only one southern flounder was captured in April. The decline in numbers was probably a function of multiple factors including emigration from settlement habitat(s), increasing avoidance of our sampling gear with size, and mortality (Rogers et al., 1984; Burke, 1995).

RNA:DNA of most southern flounder in our samples was well above a minimum ratio for fed larvae of winter flounder (3.2 to 3.5) and starved Japanese flounder (1.4 to 3.8), and within the ranges found for wild-caught summer flounder (~2.7 to 7.5) (Buckley, 1984; Malloy and Targett, 1994; Gwak and Tanaka, 2001), suggesting food was not a limiting factor in any of the bays or habitats sampled. Moreover, RNA:DNA of the individuals in the present study indicated that over 90% of the southern flounder in the GBE were in the same nutritional condition as well-fed fish from laboratory studies (fed versus starved; G.J. Holt, personal communication). Rooker and Holt (1997) reported RNA:DNA of wild red drum from Texas estuaries and, similar to the present study, they found that the nutritional condition of wild-caught individuals was well above the minimum or starved baseline estimate. This indicates that prey resources were likely adequate for newly settled southern flounder and food was not a limiting factor for new recruits in any of the habitats or bays during the 2005 sampling season.

Spatial variability in RNA:DNA of southern flounder in the GBE was possibly related to temperature differences among the bays. Specifically, water temperature in West Bay at the time of collection was about 2 °C lower than in the other two bays. RNA:DNA was highest for southern flounder from Galveston Bay samples, which had the highest water temperature at time of collection. A positive relationship between water temperature and RNA:DNA is expected as protein synthesis increases with increasing temperature (Fry, 1971). In fact, several studies have reported relationships between temperature and RNA:DNA, including work on flatfishes and other marine teleosts (Buckley, 1984; Ferron and Leggett, 1994; Mathers et al., 1993; Caldaroni et al., 2003; Mercaldo-Allen et al., 2006).

All bays and habitats in the GBE supported equivalent growth, with a mean rate of 0.40 mm d<sup>-1</sup> (range 0.21 to 0.76 mm d<sup>-1</sup>). A confounding factor in growth rate comparisons is the size of the fish being compared. Southern flounder in our study (9 to 57 mm SL) were smaller than southern flounder evaluated in other studies (37 to 90 mm SL) (Reichert and van der Veer, 1991; Fitzhugh and Rice, 1995; Guindon and Miller, 1995). Plus, larger juvenile fish have higher growth rates than smaller juveniles (growth is exponential at small sizes but once fish move into adult size ranges growth slows with ages), thus our lower growth rates could be due to size differences. Despite these factors, our growth estimates were within the ranges reported in other studies of flatfish, including summer flounder 0.11 to 0.27 mm d<sup>-1</sup> (Necaise et al., 2005), Japanese flounder (*Paralichthys olivaceus*) 0.34 to 0.93 mm d<sup>-1</sup> (Gwak et al., 2003), and fourspot



flounder 0.40 to 0.60 mm d<sup>-1</sup> (Reichert and van der Veer, 1991). Also, observed growth rates of southern flounder were in the range reported for caged southern flounder (37 to 70 mm SL) in Pamlico Sound, NC: 0.07 to 0.79 mm d<sup>-1</sup> (Guindon and Miller, 1995). Still, our field estimates are lower than the 1.2 to 1.4 mm d<sup>-1</sup> rate obtained for juvenile southern flounder (79 to 90 mm SL) in laboratory settings (Peters, 1971; Reichert and van der Veer, 1991). Differences in growth between lab and field conditions were likely a function of size, ad libitum feeding and water temperature, which was lower in the field (10–24 °C) than controlled conditions in the lab (30 °C) (Peters, 1971).

Findings from the current study suggest that intra-annual variation in density and condition of newly settled southern flounder is most important at the bay scale, and that different parts of the GBE do not function equally as nurseries. In contrast, at a smaller scale (i.e. habitat-specific), differences in density, condition, and growth were not evident between habitats in close proximity, suggesting a variety of habitats maintained conditions favorable for growth of newly settled southern flounder. The observed differences in density among bays were correlated with salinity and proximity to inlets; therefore, physical processes may play an important role in determining the distribution of newly settled southern flounder (see also Glass, 2003). Evidence of evenly distributed hatch-dates in East Bay may indicate a more stable inflow of recruits to the bay and could explain higher densities. This would indicate exposure to larval supply could be an important factor in habitat quality. The strong inter-annual variability in density for comparable time periods between 2004 and 2005 also indicates the importance of recruitment limiting processes in during the pre-settlement stages. Miller et al. (1991) reasoned that year-class strength of southern flounder and similar flatfishes is primarily determined during the pre-settlement period. While East Bay may support higher densities, wide ranging lower density areas in the rest of the GBE may also contribute significantly to the adult population. Although density and condition in certain areas of the GBE (e.g. East Bay) were higher than others, the actual nursery value of these bays will ultimately be related to the contributions made by each bay to adult populations (Beck et al., 2001).

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