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Abstract - *Paralichthys lethostigma* (Southern Flounder) inhabits the continental shelf and estuarine waters of the Gulf of Mexico and the east coast of the North Atlantic, from peninsular Florida to Albemarle Sound in North Carolina. Between 30 May and 20 August 2012, we collected 15 juvenile (71–192 mm) Southern Flounder in fyke nets in the Mattaponi River, a tributary of the York River, in southeastern Virginia. This is the first known documentation of juvenile Southern Flounder in any tributary of Chesapeake Bay. We confirmed our identification of the specimens as Southern Flounder morphologically and genetically by counting gill rakers and sequencing cytochrome oxidase subunit I, respectively.

Introduction

Paralichthys lethostigma Jordan and Gilbert (Southern Flounder) is a member of the family Paralichthyidae, the left-eyed flounders. It is characterized by extreme lateral compression, with both eyes located on the left side of the body, and dark brown mottled pigmentation on the left side of the body; it can be distinguished from other members of the family through a combination of meristic data (e.g., fin ray and gill-raker counts). This carnivorous species inhabits the continental shelf and estuarine waters of the western North Atlantic (McEachran and Fechhelm 2005, Murdy and Musick 2013, Murdy et al. 1997). Adults migrate to the continental shelf to spawn in the fall (Gi1bert 1986), and once eggs have hatched, larvae are carried to estuaries and coastal rivers by inshore currents (Guindon and Miller 1995). Juvenile Southern Flounder live in estuaries and rivers for approximately 2 years (Daniels 2000), feeding primarily on plankton, macroinvertebrates, and small fishes, after which they reach spawning age (Van Maaren and Daniels 2008). Southern flounder are sought by both commercial and recreational fishermen along the Gulf and eastern coasts of the US (Stokes 1977) and are a managed species throughout their range.

Southern Flounder are distributed along the coast of the Gulf of Mexico, from the southern tip of Texas to Caloosahatchee River in Florida, and on the Atlantic coast from the Loxahatchee River in Florida to Albemarle Sound in North Carolina (Fischer and Thompson 2004). This species inhabits shallow bays, estuaries, and rivers that are characterized by mud and silt substrates. Adult Southern Flounder are known to stray into the southern regions of Chesapeake Bay during late summer and

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early fall (Murdy and Musick 2013). Several individuals have been encountered between August and November (2007–2009) during electroshocking surveys in the Pamunkey, James, and Chickahominy rivers (Virginia) conducted by the Virginia Department of Game and Inland Fisheries (VDGIF) (B. Greenlee, VDGIF, Charles City, VA, pers. comm.), as well as the Virginia Institute of Marine Science (VIMS) Juvenile Fish and Blue Crab Trawl Survey (VIMS 4750; collected at the mouth of the James River in August 2009). Between May and August 2012, researchers with the American Shad Monitoring Program at VIMS collected several juvenile Southern Flounder in fyke nets in the tidal freshwater reaches of the Mattaponi River, a tributary of the York River in Virginia. The purpose of this paper is to document and discuss the occurrence of this southern species in tributaries of Chesapeake Bay.

Field Station Description

The Mattaponi River joins the Pamunkey River at West Point, VA, to form the York River, and collectively the 3 rivers form the York River System (YRS). We sampled in the Mattaponi River between Melrose Landing and Walkerton, VA, in the river's numerous tidal freshwater marshes. Sediments in tidal freshwater marshes in this area are dominated by silt, with vegetation comprising mainly *Peltandra virginica* (L.) Schott (Green Arrow-arum), *Leersia oryzoides* (L.) Sw. (Rice Cutgrass), and *Bidens* spp. (beggarticks) (Priest et al 1987).

Our sampling stations were as follows: station 1 (Walkerton Landing) river mile (RM) 53 (37°43.4' N, 77°01.0'W); station 2 (upriver of Garnetts Creek), RM 49 (37°41.4' N, 76°57.5'W); station 3 (Sandy Point), RM 46 (37°40.9'N, 76°55.0'W); station 4 (Wakema), RM 43 (37°39.3' N, 76°53.5'W); and station 5 (Melrose Landing), RM 41 (37°38.6' N, 77°52'W) (Fig. 1). Station 1 is located on the southern bank of an island closest to the northern bank of the river and experiences a major growth of *Hydrilla verticillata* (L.f.) Royle (Hydrilla) and other submerged aquatic vegetation (SAV) in mid- to late summer. Station 2 is located on the southern bank of the river near the mouth of a large creek, but there is little or no SAV growth. Station 3 is located on the northern bank of the river on a small flat just above a steep drop-off. Station 4 is located close to the mouth of a creek, and station 5 is located on the southern bank of the river on the northern bank of a small island; this fifth station experiences little to no SAV. Typical of freshwater tidal marshes in the region, all stations have a silt-dominated substrate.

Methods

Sampling

We sampled for 15 weeks during 15 May–21 August 2012 by setting fyke nets at the 5 stations. Electroshocking and trawling were used sporadically to supplement the fyke nets at the 5 stations and to sample different portions of the river that contained suitable habitat for Southern Flounder (i.e., silt and mud substrates associated with areas of aquatic vegetation). We constructed fyke nets out of 6.35-mm (0.25-inch)-mesh netting. Each net consisted of a 15.2-m leader, two 7.6-m wings,

4 hoops, 2 throats, and 1 cab. Each fyke net was set perpendicular to the shore with the leader running on shore and wings running at a 45° angle from the leader. We set all nets at low tide and fished during the first low tide of the following day (tides and weather permitting) to complete a 24-hour set; nets were removed after fishing (n = 5 fyke nets deployed). We identified all species collected in the fyke nets and transported Southern Flounder to the laboratory for further processing. We recorded surface water temperature (°C) and salinity (ppm) at each of the 5 stations on the day the fyke nets were deployed and on the day the fyke nets were fished.

We conducted electroshocking on 12 July 2012 in the Mattaponi River and 20 June 2012 on Back Bay in southeastern Virginia. We employed a 5.59-m (19') aluminum Clark electrofishing boat (Clark Boat Company, Bellevue, IA) equipped with a double-electrode array, each with 6 droppers. Our pulse box was a Smith Root 9.0 GPP (Generator Powered Pulsator, Smith Root International, Vancouver, WA) that was set to maximum output at 680v, with 120 pulses per second at 20 amps. The sampling crew consisted of a driver, a spotter, and 2 netters. Our electroshocking effort varied with location and depended on the size of the area we deemed as suitable Southern Flounder habitat. We used a standard otter-trawl with a 4.88 m (16') headrope, 3.81 cm (1.5") stretch-bar body, 1.9 cm (0.75") stretch-bar cod-end, 0.63 cm (0.25") cod-end liner, and a 22.86 m (75') rope bridle; tow speeds were 1–3 knots. We conducted nine 5-minute trawls and

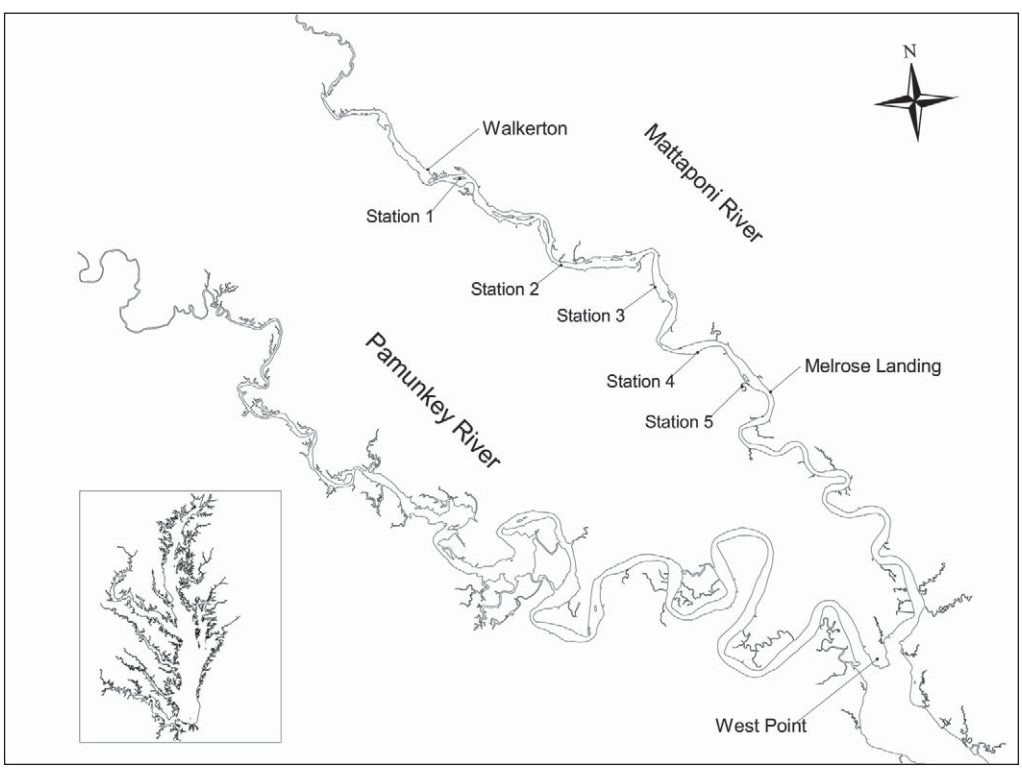

Figure 1. Map of fyke-net sampling-stations along the Mattaponi River in southeastern Virginia.

one 10-minute trawl on 11 June 2012, and ten 5-minute trawls on 18 June 2012 (total = 20 trawls).

Specimen processing

We counted the total number of gill rakers on the first arch of all individuals and identified fish with 10–14 gill rakers as Southern Flounder; the sympatric species *Paralichthys dentatus* L. (Summer Flounder) has 16–24 gill rakers on the first arch (Murdy et al. 1997), a diference that allowed us to make positive identifications. We recorded total length (TL; mm) and weight (g) of all Southern Flounder specimens and removed sagittal otoliths for age estimation; a tissue sample was removed and stored in 95% ethanol for genetic analysis. We preserved all specimens in formalin and stored them in the VIMS ichthyology collection. Multiple people collected, prepared, and read otoliths. However, we found that daily age estimates derived from these reads were not reliable due to lack of clarity; therefore, age data are not reported here.

Genetic analysis

We analyzed tissue from 6 of the individuals collected in the Mattaponi River for a genetic identification; we included an adult specimen collected in Back Bay, VA (VIMS 13589) in our analysis. DNA was extracted using a QIAGEN DNeasy kit (QIAGEN, Inc., Valencia, CA) following the manufacturer's protocol. We amplified cytochrome oxidase subunit I (COI) using PCR on a BIORAD S1000 thermal cycler (Bio Rad, Hercules, CA). PCR reactions (25 µl) were completed using 13.3 µl water, 5 µl of 5x PCR Buffer, 3 mM MgCl, 0.5 µl of 200-µM dNTPs, 1 µl each of the COI primers HCOI 5' TAAACTTCAGGGTGACCAAAAAATCA 3', and LCOI 5' GGTCAACAAATCATAAAGATATTG 3' (Folmer et al. 1994) for a concentration of 0.4 µM , 1 unit Taq polymerase (Promega Corporation, Madison, WI), and 1 µl DNA. We used the following temperature cycle: initial denaturing at 94 °C for 4 minutes followed by 45 cycles of denaturation at 94 °C for 1 minute, annealing at 45 °C for 1 minute, and extending at 72 °C for 1 minute; final extension was at 72 °C for 10 minutes. We screened PCR products for successful amplification on a 1% agarose gel, excised the COI amplicon, and purified it using the QIAGEN gel-extraction protocol. Sequencing reactions used ABI's BigDye (Life Technologies, Carlsbad, CA) chemistry and were completed by denaturing at 96 °C for 1 minute followed by 25 cycles of 96 °C for 1 minute, 50 °C for 1 second, and 60 °C for 5 seconds. DNA was precipitated using ethanol/sodium acetate following the manufacturer's protocol. We ran out sequences on an ABI 3130 genetic analyzer (Life Technologies, Carlsbad, CA), and used Sequencher V.4.10.1 (Gene Codes Corporation, Ann Arbor, MI) to visualize chromatograms, create contigs, edit the forward and reverse sequences. We compared sequences to most closely related sequences using a BLAST search of GenBank (http://www.ncbi.nim.nih.gov/genbank).

Results

During 16 June–28 August 2012, we collected 21,923 individual fishes (51 species) in fyke nets. The average water temperature for the period was 27.5 °C

(Table 1). We collected a total of 15 juvenile Southern Flounder from fyke-net sampling; these individuals weighed 2.56–84.0 g and were 71–192 mm TL (Fig. 2). We collected Southern Flounder at all 5 stations, with most individuals captured at station 1 (n = 6). A single Southern Flounder (84.0 g, 190 mm TL) was collected during electroshocking. Our gill-raker counts confirmed that all specimens collected were Southern Flounder (Table 2). We were able to obtain usable sequencing results from 3 of the 6 tissue samples collected from the Mattaponi River for genetic confirmation. BLAST-search results for those 3 samples matched Southern Flounder sequences generated by Weigt (2012).

Table 1. Catch data from fyke-net sampling in the Mattaponi River.

	Total number collected				Water
Date	Fishes	Species	Families	Southern Flounder	temp. (°C)
5/16/2012	1873	25	14	0	22.15
5/22/2012	1781	26	18	0	21.87
5/30/2012	1671	32	17	3	26.37
6/5/2012	1431	28	13	1	24.20
6/12/2012	1369	31	16	5	25.50
6/19/2012	3291	27	15	2	24.24
6/26/2012	1327	32	16	1	27.82
7/3/2012	1127	26	17	2	29.66
7/10/2012	563	24	15	0	30.91
7/18/2012	1943	36	15	0	30.54
7/24/2012	846	30	17	1	29.77
7/31/2012	1800	30	13	0	30.43
8/7/2012	1731	40	16	0	30.67
8/15/2012	1005	28	16	0	29.11
8/21/2012	556	32	16	0	26.52

Table 2. Data from specimens of Southern Flounder collected in the Mattaponi River.

Specimen #	Date collected	Station	Gear type	TL (mm)	Weight (g)	Gill-raker count
VIMS 13578a	5/30/2012	4	Fyke net	75	3.54	12
VIMS 13578b	5/30/2012	4	Fyke net	71	2.56	12
VIMS 13579	5/30/2012	1	Fyke net	92	5.84	12
VIMS 13588	6/5/2012	3	Fyke net	81	3.28	10
VIMS 13580a	6/13/2012	1	Fyke net	112	13.92	12
VIMS 13584	6/13/2012	3	Fyke net	118	15.34	11
VIMS 13580b	6/13/2012	1	Fyke net	117	15.84	13
VIMS 13580c	6/13/2012	1	Fyke net	127	19.92	13
VIMS 13580d	6/13/2012	1	Fyke net	129	20.71	13
VIMS 13586	6/19/2012	5	Fyke net	108	11.90	10
VIMS 13583	6/26/2012	2	Fyke net	93	6.94	13
VIMS 13581	7/3/2012	1	Fyke net	157	36.14	12
VIMS 13587	7/3/2012	5	Fyke net	149	30.86	12
VIMS 13582	7/12/2012	Near 1	Electroshocking	190	84.00	12
VIMS 13585	7/24/2012	3	Fyke net	192	71.30	11

Discussion

Morphological and genetic analyses confirmed that Southern Flounder were present in the Mattaponi River in the summer of 2012. Wenner et al. (1990) found that male and female Southern Flounder reach maturity at 230 mm and 320 mm, respectively. All individuals collected in the present study were significantly smaller than 230 mm; therefore, we identified them as juveniles. The Mattaponi River is ~200 miles north of the northernmost reported spawning population of Southern Flounder (Blandon et al. 2001, Enge and Mulholland 1985, Gilbert 1986). Although adult individuals are known to enter the lower Chesapeake Bay in late summer and fall (Murdy et al. 1997; B. Greenlee, pers. comm.), the occurrence of juveniles has not been recorded. It is possible that the presence of juvenile Southern Flounder in the Mattaponi River is a rare event; there may have been a slight alteration of offshore ocean currents that carried larvae up the Atlantic coast and deposited them in the lower Chesapeake Bay. These larvae ingressed into the Mattaponi River in early spring, likely as a result of the North Carolina population of Southern

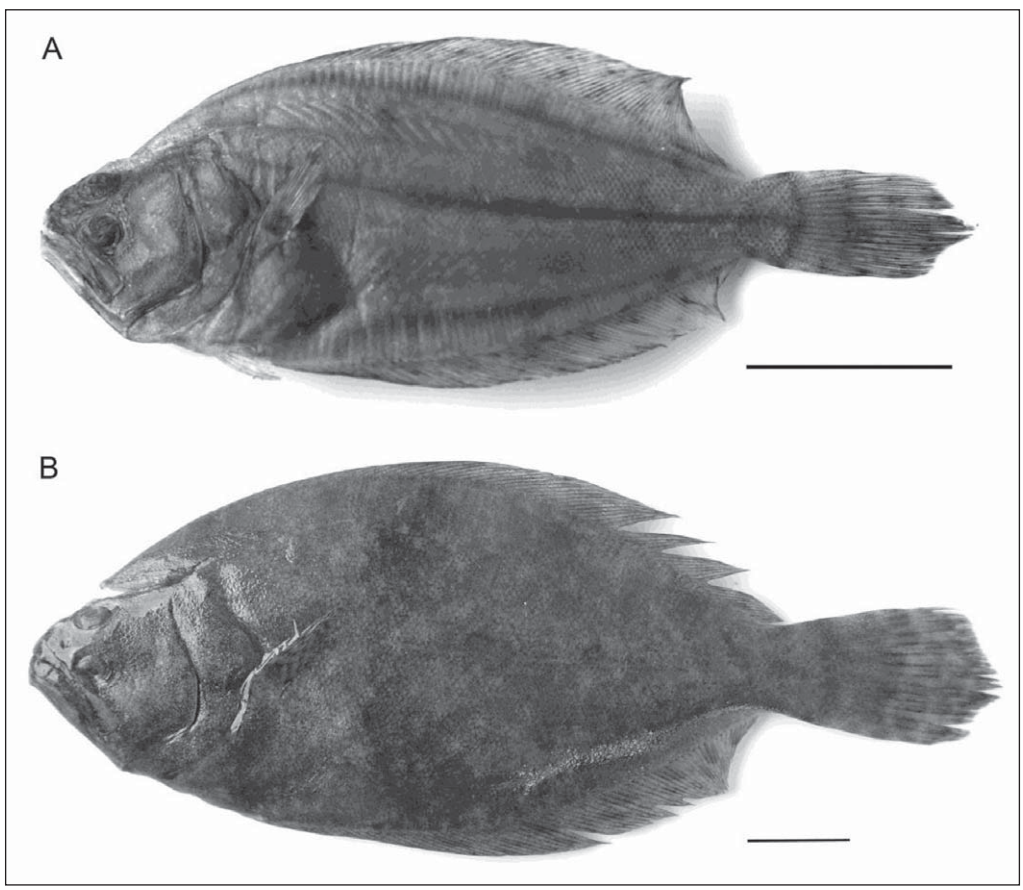

Figure 2. Juvenile *Paralichthys lethostigma* (Southern Flounder) from the Mattaponi River. A) VIMS 13579, caught 30 May 2012 (92 mm TL; fixed in formalin prior to photo). B) VIMS 13585, caught 24 July 2012 (192 mm TL). Scale bars equal 20 mm.

Flounder spawning farther north than they have historically, perhaps due to warming coastal temperatures. Cheung et al. (2013) recently suggested that there is a strong correlation between rising ocean temperature and an increase in the presence of warm-water fishes in higher latitudes. An additional study looking at the correlation between Chesapeake Bay winter water temperatures and the survival rate of juvenile Southern Flounder in the bay would be invaluable in determining how this species responds to changing ecological environments.

Gibson (1994) found that temperature, and to a lesser extent salinity, is the most important abiotic factor for optimal survival of juvenile flatfishes. Daniels et al. (1996) found that pre-metamorphosis, Southern Flounder suffered 100% mortality when exposed to salinities of 0 parts per thousand (ppt). Daily salinity recorded at all stations sampled was less than 1 ppt. Therefore, the individuals collected in our sampling likely metamorphosed prior to entering the freshwater reaches of the Mattaponi River, probably in either the main stem Chesapeake Bay or in the lower York River system. This pattern of migration from saline to fresh waters, including substantial use of freshwater habitats, was described by Lowe et al. (2011), who reported that juvenile Southern Flounder select freshwater habitats (typically estuaries) based on biological, chemical, and physical characteristics. Additionally, they suggest that juvenile Southern Flounder use these freshwater habitats as a nursery area before they mature and migrate back to the continental shelf to spawn.

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