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Abundance and Spawning Site Utilization of *Fundulus heteroclitus* at the Virginia Coast Reserve

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ABSTRACT

Abundance and spawning site utilization in a population of the mummichog, (*Fundulus heteroclitus*), were compared at regularly and irregularly-flooded mainland salt marshes at the Virginia Coast Reserve from April - November 1992. Mummichog abundance was greatest in June. Mummichogs comprised 83% of fishes collected on intertidal marsh surfaces. Significantly more mummichogs were collected at the regularly flooded marsh (ANOVA, $p = 0.007$). Young-of-the-year represented a greater proportion of total mummichogs collected (72%) at the regularly flooded marsh in comparison to the irregularly flooded marsh (61%). Mummichogs were more abundant in the lower intertidal zone relative to the upper intertidal. Mummichogs utilize empty shells of the ribbed mussel, (*Guekensia demissa*), as egg deposition sites in Virginia Coast Reserve marshes; however, egg distribution is patchy, and patterns were not readily discerned. These results support the contention that large-scale, intensive sampling is necessary to accurately quantify spawning site utilization in salt marsh populations of *F. heteroclitus*.

Key Words: Mummichogs, *Fundulus heteroclitus*, ribbed mussels, *Guekensia demissa*, salt marshes, Virginia Coast Reserve

INTRODUCTION

The mummichog (*Fundulus heteroclitus*), is a ubiquitous component of salt marsh nekton communities along the Mid-Atlantic coast. Production of this species in mid-Atlantic salt marshes is among the highest reported for fishes (> 40.7 g m^{-2} year^{-1}) and sub-adults may account for approximately 80% of total annual mummichog production (Merideth and Lotrich, 1979).

In the mid-Atlantic region, *F. heteroclitus* spawns in conjunction with spring tides, depositing its dessication - resistant eggs in empty shells of the ribbed mussel (*Guekensia demissa*) or attaching them to stems and leaves of *Spartina alterniflora* (Taylor and DiMichele, 1983; Able, 1984). Reproductive condition is highest for several days coincident with full or new moons (Taylor and DiMichele, 1980). This tidal synchrony ensures deposition of eggs in the upper intertidal zone, where they

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are least likely to be removed by tidal currents (Taylor et al.,1979). Egg hatching is triggered by appropriate conditions of submergence and low dissolved oxygen levels, but eggs may remain viable for up to 1 month exposed to air (Taylor et al., 1977; DiMichele and Taylor, 1980). Using *in situ* manipulations, Kneib (1993) demonstrated that growth rate was positively associated and mortality rate negatively associated with tidal flooding for successive cohorts of *F. heteroclitus* larvae in a Sapelo Island, Georgia salt marsh.

The primary objective of this study was to document relative abundance and distribution patterns of *F. heteroclitus* (primarily young-of-the-year) on the surface of two mainland salt marshes varying in hydroperiod (regular vs. irregular flooding) at the Virginia Coast Reserve Long-Term Ecological Research Site (VCR-LTER). A second objective was to quantify distribution and abundance of *F. heteroclitus* eggs on the surface of salt marshes within the VCR-LTER in order to determine if spawning site utilization within mainland marshes of the VCR-LTER was similar to that observed in other mid-Atlantic populations of *F. heteroclitus*.

SITE DESCRIPTION

Two salt marsh sites at the Virginia Coast Reserve were selected for study (Figure 1). The two marshes differed in surface topography and flooding regime. Site 1 was located along a 2nd order tributary of Phillips Creek. Vegetation type was typical of mid-Atlantic high marsh environments with *Salicornia virginica* and *Distichlis spicata* dominating from the forested upland boundary to the mid-marsh. From the mid-marsh to the creekbank, the short-form of *Spartina alterniflora* occurred. Medium to tall *S. alterniflora* occurred only in a narrow fringe surrounding intertidal rivulets at this site. Maximum flooding depth was generally < 10-15 cm and the upper marsh was flooded only during spring tides. The second site was located along a 1st order tributary of an unnamed tidal gut and was separated from Phillips Creek by a man-made causeway. This site, adjacent to a wooded area known locally as "The Hammocks" (hereafter referred to as "Hammocks Marsh"), flooded regularly in excess of 30 cm depth. At this site, *S. virginica* and *D. spicata* were restricted to a narrow band adjacent to the upland boundary. Short-form *S. alterniflora* progressively graded to tall form in the mid-low marsh.

METHODS

Abundance Patterns

Pit traps (Kneib 1984; Talbot and Able 1984) were used to collect mummichogs and other marsh-resident nekton at four stations along elevational transects at Phillips Creek Marsh and Hammocks Marsh. An individual trap consisted of an 11.4 liter plastic container placed into a pit dug into the marsh substrate. A 0.9 x 1.2 m length of 1.6 mm nylon mesh netting was placed into the trap as a removable liner. Four 85 gm pyramid sinkers were attached to the net in order to conform the liner to the bottom of the trap. Two 1.2 m lengths of 1.9 cm diameter PVC pipe were attached lengthwise to the mesh liner and used as brails to purse the net when removing the sample.

Three replicate traps, installed at each topographic level along a transect at each site, were sampled monthly at maximum predicted spring low tides from April through November, 1992. Organisms collected in traps were placed on ice

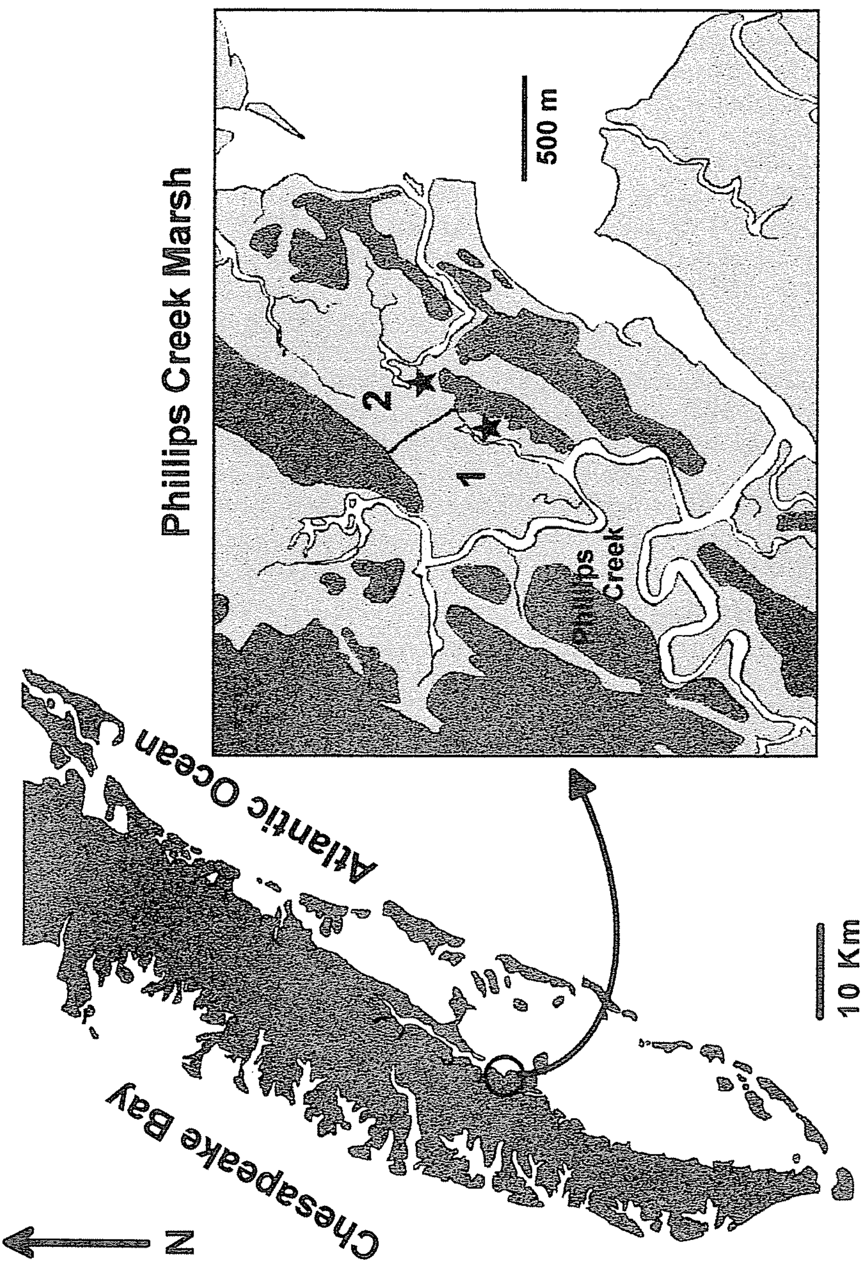

FIGURE 1. Map of the lower Delmarva peninsula and marsh study areas.

and returned to the laboratory for analysis. Unpreserved samples were sorted immediately following collection. All marsh-surface nekton (fishes and decapod crustaceans, excluding fiddler crabs (*Uca pugnax*) and marsh crabs (*Sesarma reticulatum*) were identified, counted, and preserved in 10% buffered formalin. Mummichogs were counted and measured to total length (mm).

Differences in abundance of mummichogs between the two study sites and between sampling stations within sites were tested by a repeated measures analysis of variance (ANOVA) model with MARSH and LEVEL as between subjects factors and MONTH as a within subjects factor. Abundance data were normalized using a log $(y + 1)$ transformation (Sokal and Rohlf, 1981). *A priori* paired contrasts were used to compare selected means when significant differences were specified ($\alpha = 0.05$).

The relative contribution of two age classes (young-of-the-year, adult) was determined by generation of frequency tables. Age/size class assignments were based on literature reported sizes for *F. heteroclitus* (Kneib and Stiven, 1978; Talbot and Able, 1984). Statistical analyses were performed using SuperANOVA and Statview II software for the Macintosh PC (Abacus Concepts, 1989).

Measurements of physico-chemical parameters (salinity, temperature, dissolved oxygen, pH) within marsh surface waters were taken on each sampling date at all sites using a temperature compensated refractometer, a stem thermometer, a YSI Model 57 Oxygen meter, and a Hanna portable pH meter.

Spawning Site Utilization

Permanent 100 m longitudinal transects were established within each of the four elevational strata at Phillips Creek Marsh and Hammocks Marsh. Two spawning substrates were examined for the presence of *F. heteroclitus* eggs. Empty ribbed mussel shells were collected without replacement from randomly selected 1 m^2 sample plots along each transect on June 4, June 15, July 2 and August 4. Shell width (length of long axis, in mm) was measured for each empty shell (Taylor and DiMichele, 1983). Live mussels were censused from each sample plot in order to determine the relative availability of mussel shells as spawning sites at each topographic stratum. Six plots per transect were sampled on June 4 and June 15 and sampling effort was increased to 8 plots per transect for the latter two sampling dates due to the patchy distribution of empty shells. Additional mussel shells were collected on June 5 from 100 m^2 permanent plots located at the upper boundary of the low marsh at each site. In addition, *Spartina alterniflora* stems were harvested from each 1 m^2 plot on all sampling dates. In the laboratory, *S. alterniflora* stems and contents of mussel shells were carefully rinsed onto a # 60 (250 μm) brass soil sieve and examined for *F. heteroclitus* eggs. All eggs collected were preserved in 10% buffered formalin. Stem density of emergent vegetation (primarily *S. alterniflora*) was measured along all transects on June 8.

RESULTS AND DISCUSSION

Abundance Patterns

Fundulus heteroclitus comprised 83% of all fishes collected. Additional marsh resident fish species collected in traps included spotfin killifish (*Fundulus luciae*) and naked gobies (*Gobiosoma bosci*). Daggerblade grass shrimp (*Palaemonetes *pugio*), juvenile blue crabs (*Callinectes sapidus*), and juvenile big-clawed snapping shrimp (*Alpheus heterochaelis*) also frequently occurred in pit traps.

A total of 634 mummichogs were collected in pit traps from April through November, 1992. In general, mean abundance was greatest at creekbank and low marsh stations, with decreasing abundance at high marsh and marsh/upland interface stations (Figure 2). Abundance peaked in June, when large numbers of post-larvae and early juveniles were present on the marsh surface. Overall, young-of-the-year comprised 72% of all mummichogs collected at Hammocks Marsh, and 61% of mummichogs collected at Phillips Creek Marsh. Significantly more mummichogs (66% of total) were collected at Hammocks Marsh (ANOVA, $p = 0.007$), many of which (37% of total) were collected from the low marsh station (Table 1). At Hammocks Marsh, significant differences were observed between the marsh/upland interface, where relatively few fishes were collected, and all other stations (Table 2). At Phillips Creek Marsh, significant differences in abundance were observed between the marsh/upland boundary and the high marsh and creekbank stations. Relatively few mummichogs were collected in the low marsh at Phillips Creek. Recruitment to the marsh surface and/or spawning activity appears to have occurred earlier at Hammocks Marsh, as indicated by greater abundance of YOY in April - June. After July, however, mean monthly abundance was slightly greater at Phillips Creek Marsh.

Surface water physico-chemical parameters did not differ markedly between the two study sites (Table 3). The major difference between the two sites was variation in hydroperiod. Our observations indicate that Hammocks Marsh flooded regularly to a depth of approximately 30 cm. In contrast, Phillips Creek Marsh was generally flooded only on spring tides and average flooding depth was relatively low ($\approx$ 10 - 15 cm). The two marshes were subsequently instrumented with Qualimetrics Richards-Type water level recorders. Measurements obtained during the following field season (August - December, 1993) confirm our earlier observations on flooding frequency/depth at these locations (Yozzo, 1994) . Kneib (1993) experimentally determined that growth rate was positively correlated with flooding duration in a Sapelo Island, Georgia population of *F. heteroclitus*. Our abundance and size-distribution data indicate greater recruitment and survivorship at Hammocks Marsh, a regularly flooded site. Differential patterns of habitat use by larval and juvenile *Fundulus* spp. were described by Yozzo et al., (1994) in a comparison of mainland (Phillips Creek Marsh) and back-barrier marshes at the VCR. Observed patterns of sub-adult finfish abundance were attributed to variation in hydroperiod and the relative availability of high marsh nursery habitat.

Spawning Site Utilization

A total of 1706 *F. heteroclitus* eggs were collected from transects and 100 m^2 mid-marsh plots, and although eggs were collected at both sites, abundance was extremely patchy, and often a single shell would yield large numbers of eggs. Previous workers (Able and Castagna, 1975; Taylor and DiMichele, 1983) have reported that individual ribbed mussel shells may be utilized as egg deposition sites by multiple spawning females .

Live mussels were most abundant at the low marsh and creekbank transects at Phillips Creek. At the Hammocks, mussels were most abundant in the high marsh

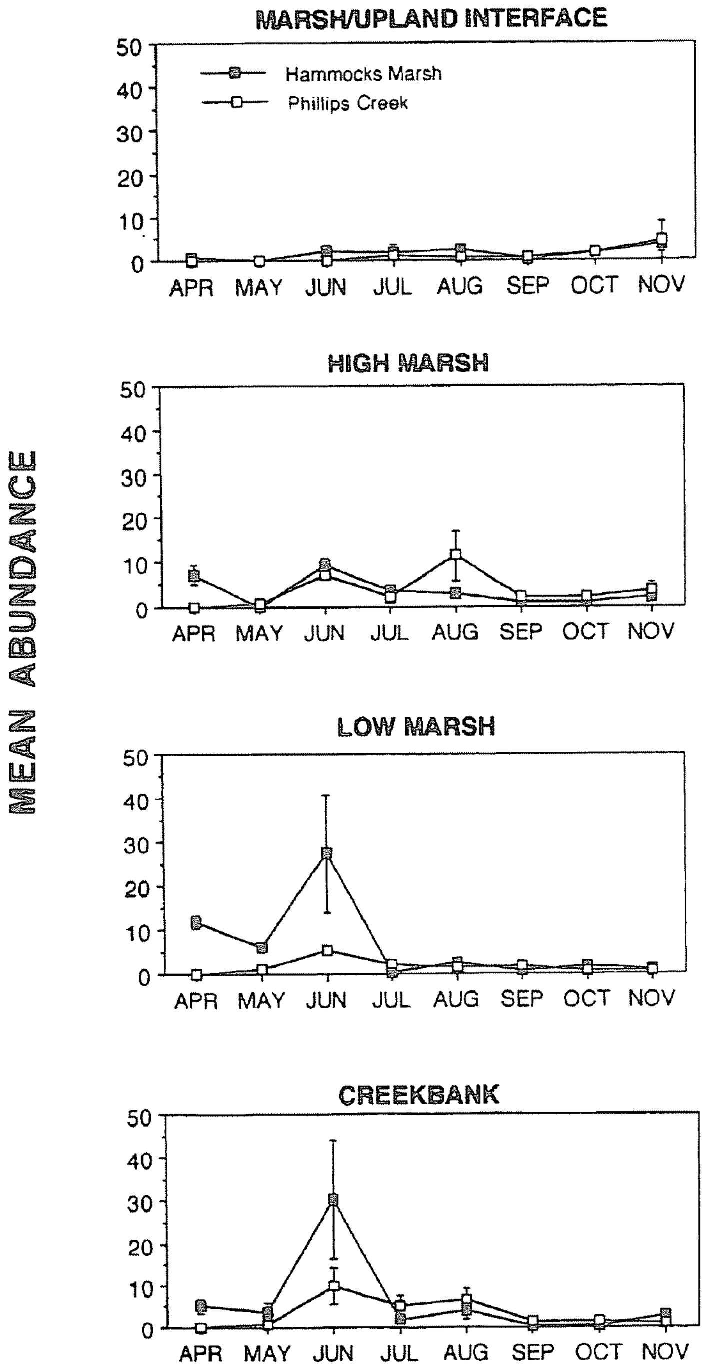

FIGURE 2. Mean monthly abundance of *Fundulus heteroclitus* on the surface of Hammocks Marsh and Phillips Creek Marsh; April - November, 1992.

TABLE 1. Repeated Measures ANOVA comparing *Fundulus heteroclitus* abundance between marsh sites and topographic levels.

Source	SS	df	MS	F	p
Marsh	0.636	1	0.636	9.541	0.0070
Level	2.163	3	0.721	10.822	0.0004
Marsh x Level	0.410	3	0.137	2.052	0.1471
Month	6.990	7	0.999	21.994	0.0001
Month x Marsh	4.271	7	0.610	13.440	0.0001
Month x Level	4.759	21	0.227	4.992	0.0001
Month x Marsh x Level	2.372	21	0.113	2.488	0.0011

TABLE 2. Results of *a priori* ANOVA paired contrasts comparing abundance of *Fundulus heteroclitus* between the Marsh/Upland Interface and other topographic levels (High Marsh, Low Marsh, Creekbank) at Phillips Creek Marsh (PC) and Hammocks Marsh (HAM) *Significant at = 0.05.

		p
PC	MUI x HM	0.0006*
	MUI x LM	0.1098
	MUI x CBK	0.0015*
HAM	MUI x HM	0.0133*
	MUI x LM	0.0029*
	MUI x CBK	0.0082*

TABLE 3. Ranges and means of physico-chemical parameters from Phillips Creek Marsh and Hammocks Marsh, April - November, 1992.

	Salinity (ppt)	Temp. (°C)	pH	DO (mg/l)
Phillips Creek Marsh	30 - 45 (38)	17 - 35 (25)	7.9 - 8.6 (8.1)	2.8 - 13.3 (8.6)
Hammocks Marsh	30 - 38 (34)	13 - 34 (23)	6.8 - 8.6 (7.7)	2.3 - 12.1 (8.4)

and low marsh zones. Empty shells were scarce in transect plots (n = 48), however, all transects yielded empty shells except for the upland boundary at Phillips Creek. Thirteen eggs were found in shells from the creekbank zone at Hammocks Marsh. All of the eggs collected from the low marsh at Phillips Creek (n = 657) were from a single shell (Table 4).

Few eggs attached to *Spartina alterniflora* stems were collected in the intertidal zone; four were collected at the marsh/upland interface at Phillips Creek, where

TABLE 4. Abundance and distribution of live ribbed mussels, total abundance and mean width (mm ±1SE) of empty mussel shells, and total abundance of *Fundulus heteroclitus* eggs at Phillips Creek Marsh and Hammocks Marsh, June - August 1992*.

Site	Total Live Mussels	Total Empty Shells	Mean Shell Width (mm)	Total Eggs
Phillips Creek Marsh (irreg. fl.)				
Marsh-Upland Interface	0	0	0	4 **
High Marsh	36	2	72.5 ±14.5	0
Low Marsh	174	7	76.4 ±7.4	657
Creekbank	167	9	89.6 ±4.9	0
Hammocks Marsh (reg. fl.)				
Marsh-Upland Interface	19	4	85.0 ±4.4	0
High Marsh	232	15	82.1 ±5.0	0
Low Marsh	299	5	91.8 ±8.6	1 **
Creekbank	172	6	86.2 ±6.6	13

* sum of collections made on June 4, July 2, July 15, and August 4.
** eggs found attached to *S. alterniflora* stems

G. demissa does not occur. Mean stem density was highest at this location (Table 5). A single egg was collected in the low marsh transect at Hammocks Marsh.

Collections at the Hammocks Marsh 100 m^2 plot on June 5 yielded 29 empty shells; only 4 empty shells were recovered from the Phillips Creek Marsh plot. Shell width at the Phillips Creek Marsh plot ranged from 75 - 102 mm (mean = 90.0 ±5.5). Shell width ranged from 57 - 106 mm (mean = 89.0 ±1.8) at Hammocks Marsh. A total of 1031 eggs were found in empty shells from the Hammocks Marsh plot. Number of eggs per shell ranged from 0 - 613 (mean = 35.5 ±22.8). No eggs were found in mussel shells from the Phillips Creek Marsh 100 m^2 plot.

Ripe egg counts from female mummichogs further indicate that individual mussel shells may be used as a substrate by multiple females during spawning events (Taylor and DiMichele, 1983). A mature female may produce 100 - 300 eggs per day early in the spawning season (Taylor, 1986).We have observed a maximum of 254 mature ova in a single female (97 mm TL) from > 150 individuals examined at the Virginia Coast Reserve in 1991-92. Hildebrand and Schroeder (1928) counted 460 mature ova in a 98 mm specimen from Chesapeake Bay. Taylor and DiMichele (1983) and Able and Castagna (1975) reported maximum egg counts of 630 and 718 in a single shell, respectively. We occasionally observed > 600 eggs in individual shells from both marsh sites. Additional collections from back-barrier salt marshes at the Virginia Coast Reserve have yielded extremely high densities (> 2500 eggs per shell). These observations suggest a greater degree of *G. demissa* shell utilization at back-barrier marsh sites relative to mainland sites.

TABLE 5. Emergent stem densities (mean no. stems m^2 1SE) at Phillips Creek Marsh and Hammocks Marsh transects, June 4, 1992.

Site	stem density (no. stems m^2)
Phillips Creek Marsh (irreg. fl.)	
Marsh-Upland Interface	714.7 ± 312.8
High Marsh	298.7 ± 29.7
Low Marsh	192.0 ± 40.3
Creekbank	192.0 ± 42.3
Hammocks Marsh (reg. fl.)	
Marsh-Upland Interface	437.3 ± 190.3
High Marsh	189.3 ± 25.4
Low Marsh	78.7 ± 27.6
Creekbank	86.7 ± 3.5

Previous investigators have reported that selection of suitable shells for egg deposition depends on orientation and gape width; eggs are deposited only in shells oriented vertically with a gape width of 0.5 - 5 mm, thereby affording protection to fertilized eggs from dessication and predation/cannibalism (Able, 1984; Taylor, 1986). Although not measured in this study, variation in gape width may account for the observed lack of eggs in otherwise suitable shells. Previous workers (Able and Castagna, 1975) reported that egg deposition was likely to occur only in shells > 60 mm in width. Shells < 50 mm were never utilized as egg deposition sites in Delaware marshes (Taylor and DiMichele, 1983) or in Virginia mainland marshes.

Results presented here are similar to those reported by Taylor and DiMichele (1983) in their investigation of spawning site utilization by *F. heteroclitus* in a Delaware salt marsh. They surmised that *S. alterniflora* stems were probably used as a secondary spawning site by *F. heteroclitus*, and that egg deposition on *S. alterniflora* stems and leaves was likely to be of greater significance in low salinity coastal marshes where *G. demissa* did not occur. In a related study, we have observed widespread deposition of eggs at the base of Arrow-arum (*Peltandra virginica*) stems in a tidal freshwater population of *F. heteroclitus* residing in marshes contiguous with the Chickahominy River, Virginia.

CONCLUSIONS

Fundulus heteroclitus was significantly more abundant at The Hammocks Marsh site, where regular tidal flooding may enhance survival and growth of marsh-resident nekton. Relative abundance of mummichogs (primarily YOY) was greater at creekbank and low marsh stations.

Mummichogs preferentially utilize empty shells of *Guekensia demissa*, which are abundant and widely distributed on the surface of VCR salt marshes, as egg deposition sites. As documented elsewhere, occurrence of eggs on the marsh surface is patchy, and patterns of distribution and abundance are not readily discerned.

The results of this study concur with those of previous workers (Taylor and DiMichele, 1983) and support the recommendation that large-scale, intensive sampling is necessary to accurately identify patterns of egg deposition by *F. heteroclitus* on the surface of salt marshes.

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LITERATURE CITED

Abacus Concepts. 1989. SuperANOVA. Abacus Concepts, Inc. Berkeley, CA.

Able, K.W. 1984. Variation in spawning site selection of the mummichog, *Fundulus heteroclitus*. Copeia 1984:522-525.

Able, K. W. and M. Castagna. 1975. Aspects of an undescribed reproductive behavior in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) from Virginia. Ches. Sci. 16: 282-284.

DiMichele, L. and M.H. Taylor. 1980. The environmental control of hatching in *Fundulus heteroclitus*. J. Exp. Zool. 214:181-187.

Hildebrand, S.F. and W.C. Schroeder. 1928. (Reprinted 1972). Fishes of Chesapeake Bay. Smithsonian Institution Press, Washington, D.C. 366 pp.

Kneib, R.T. 1984. Patterns in the utilization of the intertidal salt marsh by larvae and juveniles of *Fundulus heteroclitus* (Linnaeus) and *Fundulus luciae* (Baird). J. Exp.Mar. Biol. Ecol. 83:41-51.

Kneib, R. T. 1993. Growth and mortality in successive cohorts of fish larvae within an estuarine nursery. Mar. Ecol. Prog. Ser. 94:115-127.

Kneib, R.T. and A.E. Stiven. 1978. Growth, reproduction and feeding of *Fundulus heteroclitus* (L.) on a North Carolina salt marsh. J. Exp. Mar. Biol. Ecol. 31:121-140.

Meredith, W.H. and V.A. Lotrich. 1979. Production dynamics of a tidal creek population of *Fundulus heteroclitus* (Linnaeus). Est. Coast. Mar. Sci. 8:99-118.

Sokal, R.R. & J. Rohlf. 1981. Biometry. 2nd. Ed. W.H. Freeman and Co. New York, 859 pp.

Talbot, C.W. & K.W. Able. 1984. Composition and distribution of larval fishes in New Jersey high marshes. Estuaries 7:434-443.

Taylor, M.H. 1986. Environmental and endocrine influences on reproduction of *Fundulus heteroclitus*. Amer. Zool. 26:159-171.

Taylor, M.H. and L. DiMichele. 1980. Ovarian changes during the lunar spawning cycle of *Fundulus heteroclitus*. Copeia 1980:118-125.

Taylor, M.H. and L. DiMichele. 1983. Spawning site utilization in a Delaware population of *Fundulus heteroclitus* (Pisces: Cyprinodontidae). Copeia 1983:719-725.

Taylor, M.H., L. DiMichele and G.J. Leach. 1977. Egg stranding in the life cycle of the mummichog, *Fundulus heteroclitus*. Copeia 1977:397-399.

Taylor, M.H., G.J. Leach, L. DiMichele, W.H. Levitan, W.F. Jacob. 1979. Lunar spawning cycle in the mummichog, *Fundulus heteroclitus* (Pisces: Cyprinodontidae). Copeia 1979:291-297.

Yozzo, D.J. 1994. Patterns of habitat use by sub-adult marsh nekton: comparison between tidal freshwater and salt marshes. Doctoral Dissertation, University of Virginia, Charlottesville, VA. 163 pp.