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Assessing Larval American Shad Growth and Survival with in situ mesocosm experiments in three differing habitats within a coastal estuary

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INTRODUCTION

Habitat can be defined as the place where the organism lives including all its physical, chemical and biological dimensions (Odum 1971; Hoss and Thayer 1993). These dimensions include water quality, physical structure, flow regime and biotic interaction. Essential fish habitat (EFH) is further defined as "those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity" (Magnuson-Stevens Act, 16 U.S.C. 1801 et seq.) With new mandates to identify and protect EFH for all species managed under fisheries management plans, evaluation of fish habitat has become a priority. The methods used to identify and define essential fish habitat have ranged from intensive microscale sampling to coarse macroscale delineations. Historically, assessment of fish habitat occurred on small scales and addressed water quality, physical structure and prey/predator interactions. With increases in geographic information systems (GIS) capabilities, large-scale depictions of fish distributions have been completed, however, these surveys often lack the detail necessary to describe the processes driving distribution. Research at both scales is necessary to accurately define and describe essential fish habitat. Macroscale assessments of fish distribution, linked with process-oriented experiments will elucidate the driving forces behind distribution and allow for a more complete identification of essential habitat.

The coastal plain estuaries on the East Coast, including the Chesapeake Bay, provide essential spawning and nursery habitat for numerous commercially and recreationally important fish species. The determination of essential larval and juvenile fish habitat in this region offers a sizeable challenge. Without the advantages that historic landings data may allow with adult stages, additional surveys are necessary to first delineate distributions of the early life stages of targeted fish species. Next linkages between specific habitats and early life stage survival and growth must be developed.

Simulated ecosystem studies (mesocosms) are useful methods to evaluate the impacts of habitat (including water quality, prey availability, and habitat structure) on the early life stages of fish. Numerous types of mesocosms, including enclosed containers or mesh bags, in both drifting and stationary deployments, have been utilized to examine larval fish survival and growth (Lafontaine and Leggett 1987a). Within the Chesapeake Bay, mesocosm experiments conducted have typically been closed systems that encapsulate a column of water from the natural system, but do not incorporate varying environmental conditions present throughout the experiment (Cowan, Jr. and Houde 1990; Cowan, Jr., Birdsong, Houde, Priest, Sharp and Mateja 1992; Houde, Gamble, Dorsey and Cowan, Jr. 1994). In order to evaluate the impact of short-term varying environmental parameters on an organism, the ideal enclosure should reproduce the conditions of the system as well as provide a predator barrier for experimental control and manipulation (Lafontaine and Leggett 1987a). Additionally, these systems should be replicated and reproducible for robust statistical analyses, generally requiring that costs per mesocosm be minimal.

We designed a replicated in situ mesocosm system that exposes larval fish to natural varying conditions within an estuary. The study has the potential to offer new insight into how essential fish habitat should be determined by assessing how specific prey resources, and habitat conditions translate into realized benefit for anadromous fish.

Within coastal plain systems, the American shad *Alosa sapidissima*, an anadromous clupeid, is a prime example of a species affected by loss and degradation of habitat. Declines in Atlantic coastal stocks that are attributed to habitat loss and flow alterations have led to fishing moratoria in some areas (Mansueti and Kolb 1953; Walburg and Nichols 1967; Carlson 1968; ASMFC 1999). The American shad fishery peaked in the Chesapeake Bay in the late 1800s and then declined after the turn of the century (Mansueti and Kolb 1953). Stocks continued to decline in the Chesapeake Bay region during the past few decades, as a probable result of overfishing, habitat degradation, and blockage of spawning runs. The in-river fishery was finally closed for shad in Maryland (1980) and Virginia (1994). In Virginia, in addition to moratoria, fish passageways are opening historic spawning grounds on the James and Rappahannock rivers, and hatchery efforts are taking place on the James and York river systems. Unfortunately, much of what is known about natural spawning and early life history is either anecdotal or incompletely described for all stocks (Massmann 1952; Bilkovic et al. 2002a). It has been postulated that the larval stage (4-9 days) is the critical period at which American shad year class strength is established (Crecco et al. 1983); therefore, varying habitat exposures during the early life stages may impact recruitment and the successful restoration of stocks.

Study Objectives

Within the Mattaponi River, a tributary of the York River where American shad spawn, three geomorphologically distinct aquatic habitat types have been noted: the upstream segment with shallow, narrow channels; the mid-river segment with wide, shallow sandbars; and the downstream segment with wide, deep channels (Bilkovic et al. 2002b). Within each of these distinct habitat types, *in-situ* mesocosm experiments were conducted to discern differences in survival rates, food webs and growth of larval American shad (Figure 1).

METHODS

Mesocosm description and deployment

The overall experimental design consisted of eleven cylindrical mesocosms attached to a PVC-rigid platform (2 m X 3 m) in shallow water (< 4 m in depth) in each of the three major habitats in the Mattaponi River.

The mesocosms consist of three segments 1) a main body cylinder of 1 m length and 0.5 m diameter with 800 μ m mesh netting; 2) a top segment (20 cm length, 0.5 m diameter) constructed with nonporous sail cloth that is cinched closed after deployment with floats attached; and 3) a bottom segment (equal proportions to the top segment) with a 1-L codend jar attached to the bottom into which all contents are drained when a mesocosm is harvested. Each mesocosm is supported by an internal frame of thin stainless-steel rods running vertically that are welded to stainless steel rings which mark the beginning of the top and bottom segments (Figures 2-5).

Each mesocosm was attached to a square PVC structure; these individual structures were then linked to create a raft of eleven mesocosms. Floats and styrofoam were attached to the top of the large linked PVC structure along the outer sides. The rigid platform was secured to private piers at the three locations. The mesocosms were deployed, with the top segment closed, approximately 48 hours prior to obtaining fish specimens to assess the effectiveness of the design.

Trials 1 and 2

Two consecutive trials were conducted with American shad larvae grown in meoscosm sets in each of the three habitats. One trial was conducted from April 1-10, 2002 with 75 six-day old larval (10.14 ± 0.37 mm total length) per mesocosm. The second trial with 100 twelve-day old larval (10.25 ± 0.46 mm in length) per mesocosm was run from April 15-26. Larval fish were obtained from two hatchery programs associated with the York River, the USFWS hatchery program and the Harrison Lake Hatchery program. For each trial at each location, larval shad were placed in ten mesocosms (after 20 minutes of tempering to on-site conditions), leaving one mesocosm to be used as a control for zooplankton and water quality sampling (Figs 6-8). Approximate transit time between sites ranged from 20-30 minutes. Mesocosms from each habitat were sampled at regular intervals to measure growth, and survival. Environmental data (temperature, dissolved oxygen, pH, turbidity, flow) were sampled continuously in surrounding waters during the trial periods using an YSI Sonde 6600. Periodic water quality data (dissolved oxygen, pH, temperature, turbidity, flow) was collected in the control mesocosm to compare with ambient conditions. Additionally, depth, width and physical structure (SAV, bottom type and woody debris) were measured at each site. All fish were scored dead or alive at the time of sampling. Those specimens that were retrieved intact from the mesocosm were preserved and measured in the laboratory to the nearest 0.01 mm, and life stage was noted. Twenty-five control fish for each trial were obtained from the hatchery simultaneously with the experimental fish, and were preserved, measured and assessed for life-stage (yolk-sac presence or absence) in the laboratory.

Zooplankton collections occurred in concert with mesocosm sampling within a control mesocosm as well as in the surrounding water outside of the mesocosm platform to ensure that zooplankton quantities and communities are comparable throughout the experimental study area. A hand-held plankton pump was used to extract whole water 16L samples (8 L surface; 8 L at depth) inside the control mesocosm and outside of the

platform. Samples were filtered through a 100µm mesh and preserved in 90% buffered formalin for zooplankton enumeration.

Trial 1

American shad larvae were placed in each mesocosm on Day 1 of the trials. Subsequent removal of one mesocosm occurred on days 2 and 3; two mesocosms were removed on days 5 and 8; and the final four mesocosms were removed on day 10. To evaluate early mortality sampling occurred more frequently at the onset of the experiment. Small non-motorized boats were utilized to reach the mesocosms and complete sampling. Prior to removal of a mesocosm, the surface and water column were searched with a handheld sieve (20 cm diameter, 220 μ m mesh) for evidence of live fish. Sieving was discontinued if no fish were obtained for five consecutive searches throughout the mesocosm. The mesocosm was then removed from the water and the contents rinsed down into the codend; these contents were then filtered with 600 μ m mesh sieve and preserved in 10% phosphate-buffered formalin for further examination. After the net contents were emptied, the mesocosms were cleaned and reattached to the platform for Trial 2.

Trial 2

Slight modifications were made to the methodology for the second trial because of the high mortality noted in the first trial, and difficulties with sampling. Site 1 was moved further offshore, due to the extreme shallowness of the initial location at low tides. The platform was anchored approximately 7 m offshore of the pier. We increased the number of fish per mesocosm to 100, and sampled two nets per site each day due to high early mortality in the first trial. Two mesocosms were removed on days 2, 4, 8, 10, 12. All other protocols were the same as Trial 1.

RESULTS

<u>Survival</u>

There was a trend of higher survival and increased days survived in Sites 2 and 3 as compared to Site 1 in both trials (Table 1; Graph 1). However, the trends were not statistically significantly different based on Kruskal-Wallis nonparametric test of the equality of medians for two or more populations (p = 0.183), possibly due to the large number of zero results (zero live shad in a replicate sampled mesocosm) obtained during the trial. The test did indicate that Site 1 had observations that were lower than the mean rank for all observations (Z=-1.52), while Site 3 had a mean rank that was higher for all observations to the mesocosm design that will enhance survivability and decrease zero values, this may be rectified.

Table 1. American shad survival in Trials 1 and 2. Total retrieved is the number of shad (live and dead) that was retrieved from each mesocosm.								
DATE	<u>SITE</u>	NET	<u># Survived</u>	<u>%Survival</u>	<u>Total retrieved</u>	Days survived		
2-Apr-02	2	3	13	17.3	16	1		
3-Apr-02	2	1	19	25.3	37	2		
5-Apr-02	2	9	10	13.3	15	4		
5-Apr-02	3	9	16	21.3	22	4		
10-Apr-02	3	8	10	13.3	10	9		
16-Apr-02	1	2	2	2.0	48	1		
16-Apr-02	1	4	9	9.0	33	1		
16-Apr-02	2	2	1	1.0	19	1		
16-Apr-02	3	2	13	13.0	76	1		
18-Apr-02	3	1	1	1.0	5	3		
18-Apr-02	3	9	5	5.0	12	3		
22-Apr-02	3	10	1	1.0	1	7		

Table 2. Kruskal-Wallis Test of significant difference between mean ranksof American shad survival in three differing habitats (Sites 1-3).									
<u>Site</u>	<u>N</u>	Median	Ave Rank	<u>Z</u>					
1	10	0.00000E+00	12.1	-1.52					
2	10	0.00000E+00	16.6	0.51					
3	10	5.00000E-01	17.8	1.01					
Overall	30		15.5						
H = 2.39	DF = 2	P = 0.303							
H = 3.40	DF = 2	P = 0.183	(adjusted for	ties)					



Site Number (Downstream--Upstream)

Another apparent trend was survival exclusively occurred in mesocosms located on the outside edge of the platform (Mesocosm numbers: 1, 2, 3, 4, 8, 9, and 10) (Figure 5; Graph 2). While this trend was not statistically significant (Kruskal-Wallis; p = 0.223), mesocosm with ranks lowest (Z= -0.89) compared to all observations were those on the inside of the platform (Mesocosm numbers: 5, 6 and 7) (Table 3). In the next rendition of mesocosm design, we will eliminate the inner mesocosms.

Table 3.	able 3. Kruskal-Wallis Test of significant difference between mean ranks of American shad survival in each mesocosm (Nets 1-10).								
NET	Ν	Median	Ave Rank	Ζ					
1	6	0.00E+00	34.7	0.62					
2	6	5.00E-01	38.8	1.23					
3	6	0.00E+00	30	-0.07					
4	6	0.00E+00	29.4	-0.16					
5	6	0.00E + 00	24.5	-0.89					
6	6	0.00E + 00	24.5	-0.89					
7	6	0.00E+00	24.5	-0.89					
8	6	0.00E+00	29.7	-0.12					
9	6	2.50E+00	40.2	1.43					
10	6	0.00E+00	28.8	-0.26					
Overall	60		30.5						

H = 5.77 DF = 9 P = 0.762

H = 11.83 DF = 9 P = 0.223 (adjusted for ties)



Site Number (Downstream--Upstream)

<u>Growth</u>

Intact specimens were only obtained from sites 1 and 3 for further laboratory analysis. Often live shad were trapped in the mesh of the mesocosm and could not be extracted properly. Proposed modifications of the mesocosm design to address this concern will be discussed later. Of the intact specimens measured in the laboratory for growth analysis, the average growth for surviving American shad varied drastically between each trial. Based on the difference between the average length of the control fish and the surviving shad lengths, an average loss was estimated for Trial 1 and an average growth indicated for Trial 2 (Trial 1: -0.045; Trial 2: 0.278 mm) (Table 4). Growth compared to the minimum length of the control fish measured indicated small growth occurred in both trials (Trial 1: 0.568; Trial 2: 1.128 mm). A preponderance of the control larval shad was in the yolk-sac stage of development for both trials (Trial 1 - 80%; Trial 2 - 60%). American shad that survived until removal were typically in post-yolk sac stages of development (Trial 1- 54%; Trial 2- 61%). This pattern was influenced by the amount of time spent in the mesocosm prior to removal, and the age of the fish at the time of placement in the mesocosm. In Trial 1, more surviving fish remained in the yolk-sac stage up to 9 days after placement in the mesocosm, as opposed to Trial 2 in which older fish were utilized and the only American shad that remained in the yolk-sac stage were retrieved one day after placement in the mesocosm.

Table 4. Lengths (mm) and standard deviation (SD) of surviving American shad by Trial (date), Site and Mesocosm number (Net). Growth is depicted as the difference between the lengths of surviving fish and 1) the average control fish length (Trial 1: 10.14 mm; Trial 2: 10.25 mm), and 2) the minimum control fish length (Trial 1: 9.53 mm; Trial 2: 9.40 mm).

DATE	SITE	NET	# Survived	Length (mm)	<u>SD</u>	Growth (ave)	<u>SD</u>	Growth (min)	<u>SD</u>
10-Apr-02	3	8	10	10.15	0.51	0.007	0.51	0.620	0.51
5-Apr-02	3	9	16	10.05	0.60	-0.098	0.60	0.515	0.60
16-Apr-02	1	2	2	9.82	0.66	-0.434	0.66	0.416	0.66
16-Apr-02	1	4	9	10.21	0.52	-0.043	0.52	0.807	0.52
16-Apr-02	3	2	13	10.37	0.46	0.118	0.46	0.968	0.46
18-Apr-02	3	1	1	11.10		0.845		1.695	
18-Apr-02	3	9	5	10.34	0.61	0.093	0.61	0.943	0.61
22-Apr-02	3	10	1	11.34		1.086		1.936	
Average: Trial 1				10.10	0.56	-0.045	0.56	0.568	0.56
Average: Trial 2			10.53	0.56	0.278	0.56	1.128	0.56	
Overall (ave)				10.42	0.56	0.197	0.56	0.988	0.56

Zooplankton Communities

Total zooplankton mean abundance (#/16L), including copepods, nauplii and cladocerans, was not significantly different inside versus outside (location) of the mesocosm for all three sites and for both trials (Two-Way ANOVA; p = 0.210) (Graph 3). There was significant difference among sites and total zooplankton mean abundance (Two-Way ANOVA; p < 0.000), and there was a significant interaction between location and sites on zooplankton abundance (Two-Way ANOVA; p = 0.011). Site 2 deviated from Sites 1 and 3 due to high zooplankton densities in Trial 2 outside of the mesocosm.

For further assessment of zooplankton community composition among sites, species were grouped into the general categories: 1) Cladoceran (predominately *Bosmina* spp); 2) Calanoid and 3) Cyclopoid copepods; and 4) nauplii and statistically compared across sites and locations (inside or outside mesocosm). There was no significant difference in Cladoceran communities among Sites or Locations (One-way ANOVA; p = 0.114). However, differences existed for Calanoid, Cyclopoid and nauplii communities among Sites and/or Locations. Calanoid average abundance was highest in Sites 1 and 2 regardless of location and lowest in Site 3 (One-way ANOVA; p = 0.005). Cyclopoid average abundance was significantly higher at Site 3, inside the mesocosm, then all other Sites/Locations (One-way ANOVA; p < 0.000). Nauplii average abundance was significantly higher at Site 2, outside the mesocosm, than all other Sites/Locations (One-way ANOVA; p < 0.000) (Table 6, Graph 4). Lastly, amphipod species were loosely grouped for analysis and had a significantly higher average abundance inside mesocosms, as opposed to outside at all three sites (One-way ANOVA; p < 0.000) (Table 6).



Table 5. Results of Two-way Analysis of Variance comparing mean zooplankton abundance among Sites (1-3) and between locations (inside (1) versus outside (2) of the mesocosms).

Site	Mean	P-value
1	265	0.000**
2	237	
3	62	
Location	Mean	P-value
1	167	0.210
2	210	



Graph 4. Zooplankton Assemblage in Three Sites on the Mattaponi River

Table 6. Average Abundance of zooplankton groups by Site and Location.

		Mean Abundance							
Site	Location	Cladoceran	Calanoid	Cyclopoid	Nauplii	Amphipod			
1	Inside	70.1	45.8	32.2	105.1	6.8			
1	Outside	90.4	45.9	17.7	122.9	0.4			
2	Inside	15.8	30.9	34.5	64.6	31.2			
2	Outside	31.8	54.8	13.7	227.9	0.5			
3	Inside	16.2	5.3	65.5	13.7	28.7			
3	Outside	6.2	2.3	9.8	5.7	1.6			

Water Quality

Water quality conditions (dissolved oxygen, specific conductivity, salinity, turbidity (secchi depth), and temperature) within the mesocosm and outside of the mesocosm were not significantly different at all three sites (Graphs 5-7). However, water quality conditions varied among sites. Sites 2 and 3, where the highest survivability of larval American shad occurred, had significantly lower turbidity (and higher secchi depth), salinity, conductivity and pH than Site 1 (One-way ANOVA; p < 0.000) (Table 7).

	Site 1			Site 2				Site 3		
Parameter	Ave	Min	Max	 Ave	Min	Max	Ave	Min	Max	
Temp C	17.09	4.25	23.63	18.02	4.42	25.60	16.76	2.35	24.74	
SpCond (ns)	0.54	0.02	2.44	0.08	0.00	0.09	0.07	0.00	16.77	
DO (mg/L)	9.13	3.86	14.45	7.88	5.30	13.02	4.96	4.76	9.65	
рН	6.67	4.87	7.10	6.58	4.46	8.31	6.61	6.28	7.71	
Depth (m)	0.87	0.00	1.87	0.77	0.00	1.61	3.88	0.00	6.65	
Turbidity (NTU)	107.8	0.0	1693.4	24.8	1.1	1419.4	46.9	0.0	1062.7	
Secchi Depth (cm)	35	20	45	67	60	80	170	80	210	
Salinity (ppt)	0.26	0.01	1.26	0.03	0.00	0.04	0.03	0.00	0.04	

Table 7. Water Quality measured parameters from 27 March – 26 April 2002, with Average (Ave), Minimum (Min) and Maximum (Max) values estimated for each site.

Graph 5. Continuous water quality conditions at Site 1 (Wakema) on the Mattaponi River.





Graph 6. Continuous water quality conditions at Site 2 (Walkerton) on the Mattaponi River.

Graph 7. Continuous water quality conditions at Site 3 (Aylett) on the Mattaponi River.



SHAD: Site 3

Discussion

Survival and growth of larval American shad during both trials was minimal. There were several contributing factors that may have led to this result. Examination of mesocosm contents prior to and after mesocosm removal indicated that amphipod abundance was significantly higher inside the mesocosms as opposed to outside. Based on evidence of intensive macroinvertebrate (including amphipods and copepods) predation on larval fish in experimental situations (Lafontaine and Leggett 1987b); predation impacts on the early larvae (average length = 10.4 mm) were potentially high. Unpredictably, the highest amphipod abundances occurred at Sites 2 and 3, where American shad survival was the greatest, thus a consistent relationship between predator abundance and larval fish mortality could not be established. To reduce the potential predation impact by macroinvertebrate predators that can gain access to the mesocosm through mesh openings, older and larger fish should be used to further assess habitat and prey density impacts on American shad.

The benefits of utilizing older and larger fish (post-yolk-sac stage) in an *in-situ* mesocosm experiment include the reduction of predation, and the elimination of mortality due to first-feeding failure. American shad that are approximately one month old (past yolk-sac stage and near metamorphosis), will be less susceptible to temperature differences between hatchery and natural conditions, predation by small predators able to gain access to the mesocosm; and will allow for a more accurate depiction of feeding and growth differences between sites (habitats). During our initial trials, feeding/prey impacts could not be accurately depicted, since the fish had yolk-sac stores that encouraged survival in absence of feeding. Also, an unknown and possibly high mortality may have occurred at the critical first feeding stage.

Problems with the initial mesocosm design were observed in the first trials. Too much silt deposited against and within the mesocosms due to high silt levels in the river system and in shore location of the experiment. While removing each mesocosm, large quantities of silt predominated the lower segment of the mesocosm, which caused damage to specimens. We also experienced difficulties in retrieving and observing live fish in the mesocosm prior to removal, due in part to the small size of the fish and the siltiness of the water column.

To create an optimum experimental design, we propose to replace the existing nonpermeable top and bottom segments of the mesocosm with mesh, to allow for further elimination of silt during the experiment. Additionally an open mesh top segment will more closely mimic natural sunlight exposures. We will also explore the possibility of anchoring mesocosms slightly offshore from the initial locations to further reduce silt capture. To reduce labor intensity and capture early mortality more effectively, we will shorten the time of each trial to one week. We will reduce the number of mesocosms from eleven to five (four experimental, one control) to allow for greater flow between mesocosms; and we will place all the mesocosms on the outer edges of the platform, since American shad mortality was greatest in the inner mesocosms (Figure 9). This will also reduce the necessary labor, while still allowing for replication at each site. We expect that the use of older and larger fish will reduce the difficulties in retrieving and observing live fish at the experiment end. We will monitor the water quality within (every other day) and outside of mesocosms (continuously), as well as assess current flow conditions utilizing a Sontek Handheld Advanced Flowtracker.

Overall, the American shad mesocosm protocol was an effective tool to capture varying zooplankton abundances and water quality within the estuary, since there was no significant difference in zooplankton or water quality conditions inside the mesocosms or in the surrounding waters. The experimental design more closely resembled real-world exposures than enclosed mesocosms, thus extrapolations to natural environment habitat influences on the early life stages of American shad may be possible. Since distinct differences in water quality conditions and habitat descriptors (e.g. slope, woody debris, turbidity, sediment, depth shoreline) exist among the sites sampled in the initial trials, the further use of these three sites/areas is valid for discerning habitat influences within an estuary system. Because of the replication in the experimental design, statistical robustness was increased. This protocol was the first to attempt to experimentally characterize the impact of natural varying environmental conditions on American shad larvae within coastal freshwater tidal tributaries. Thus, with some modification to the initial trials, this method may be used to discern the influence of habitat on American shad growth and survival.

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Appendix 1. Photographs And Diagrams Of The Mesocosm Design And Implementation.



Figure 3. Attachment of individual mesocosms to Platform





Figure 5. Diagram of Mesocosm layout



Figure 6. Deployment of Mesocosms at Site 1 (Wakema, Mattaponi River)



Figure 7. Deployment of Mesocosms at Site 2 (Walkerton, Mattaponi River)





Figure 9. Proposed Modified Mesocosm Design

