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**Previous exposure to novel prey improves the feeding success of hatchery-reared spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830) within habitat structure**

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**Abstract** The feeding success of naïve hatchery-reared (HR) and experienced HR spotted seatrout on novel live prey (*Palaemonetes*) was compared both in the presence and absence of habitat structure (*Spartina*) in a mesocosm experiment. Naïve fish readily consumed novel live prey in the absence of habitat structure. Both naïve and experienced fish consumed significantly fewer and showed higher relative variance in prey consumption (CV) in the presence of habitat structure than in its absence. Moreover, a significantly lower CV for experienced fish implied they more frequently consumed at least one prey (i.e., fewer empty guts) when initially exposed to habitat structure. The difference in the relative variance of prey consumed was significant between feeding groups for the *Spartina* treatment, but not for the No *Spartina* treatment. Thus, previous experience of HR fish with novel live prey in the absence of habitat structure may facilitate initial feeding success within complex habitat upon release. Digestion of prey appeared less complete in fish from the No *Spartina* treatment, suggesting prey had been consumed over a longer period in the presence of habitat structure. The present study and other ecological experiments with HR fish contribute to an expanding knowledge base about the behavioral plasticity of HR fish, and the formulation of pre-release exposure protocols.

## SHORT COMMUNICATION

### Effects of previous exposure to novel prey on the feeding success of hatchery-reared spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830) within a group setting

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**Running Head:** Previous exposure to novel prey

**Keywords:** novel prey; habitat structure; feeding success

As a popular recreational sportfish in the Gulf of Mexico, the spotted seatrout (*Cynoscion nebulosus*) is probably being managed at close to its maximum sustainable level (Fulford & Hendon 2010). Consequently, the feasibility of stock enhancement is being evaluated in Mississippi as part of a comprehensive management strategy for this species. The success of stock enhancement depends on the ability of newly released hatchery-reared (HR) fish to acquire the necessary survival skills in terms of feeding, habitat use and predator avoidance (Leber 2004; Huntingford 2004; Liao 2004). As the hatchery setting is devoid of natural stimuli, the ability of HR fish to acclimate to the natural setting requires critical assessments (Olla *et al.* 1998; LeVay *et al.* 2007). Thus, the role of complex habitat structure should also be considered when evaluating foraging abilities of HR fish (Salvanes & Braithwaite 2005, 2006).

Unfamiliarity with natural prey and habitat may impede the survival of HR spotted seatrout by hindering their feeding success upon release into the wild. Prior exposure to natural prey may

1 alleviate unfamiliarity with foraging under natural conditions, including structured habitat. To  
2 assess this possibility, the effect of previous exposure to novel natural prey on the feeding  
3 success of HR fish was evaluated as a treatment effect, crossed with the presence or absence of  
4 structured habitat. Our objective was to assess the feeding successs of juvenile HR spotted  
5 seatrout on novel live prey (grass shrimp; *Palaemonetes* spp.) both in the presence and absence  
6 of emergent *Spartina alterniflora* within the context of a mesocosm experiment.

7 Juvenile spotted seatrout were reared in a recirculating system at the USM GCRL Thad  
8 Cochran Marine Aquaculture Center where they had been maintained on a pelleted diet. All  
9 experimental fish came from the same hatchery cohort and were 163 days old (mean TL and Wt,  
10 17.5 cm and 58.22 g). A predominant food item in the wild, grass shrimp (*Palaemonetes* spp.)  
11 (Jackson et al. 2013) was used as prey in this study. Grass shrimp were collected from local  
12 marsh edge habitat for the experiment. Carapace lengths of one-hundred of 720 grass shrimp  
13 used averaged  $22.84 \pm 3.72$  SD mm.

14 The experiment was conducted outdoors under a 5m high, 55% shade cloth, and maintained at  
15 ambient photoperiod and temperature. The design comprised twelve 680 L round fiberglass  
16 tanks, each filled with ~500 L of saltwater. To account for any systematic spatial variation, the  
17 spatial layout was subdivided into three randomized blocks (i.e., four tanks representing all four  
18 treatments). Tanks within each block were randomly assigned to one of the four treatment  
19 combinations: (1) experienced fish with no *Spartina*, (2) experienced fish with *Spartina*, (3)  
20 naïve fish with no *Spartina*, or (4) naïve fish with *Spartina*, thus providing three replicates of  
21 each treatment combination. Experienced and naïve HR fish groups were defined by different  
22 feeding regimes while segregated into circular 1000 L holding tanks filled with approximately

750 L of artificial seawater (at  $\sim 24^{\circ}\text{C}$  and 25 psu), and equipped with biological trickle filters and aeration.

Experienced fish ( $n = 37$ ) were fed grass shrimp at a density of three prey per fish ( $n = 111$  grass shrimp) twice per day (10:30 and 16:30) for a period of five days prior to the experiment. Pilot studies indicated that five days constituted an adequate training period. The naïve group ( $n = 36$  fish) was fed the normal pelleted diet (standard 4.0 mm pellets at  $\sim 1\%$  b.w., or 25 g per feeding event) over the same period. Following the training period, six randomly selected fish were transferred to each mesocosm and allowed to acclimate for 66 h without feeding prior to initiating the experiment. On day 9, *Spartina* stems were placed into designated tanks: 80-cm<sup>2</sup> frames covered with 2-cm mesh polypropylene netting were placed over tanks several centimeters above the water line as a support grid for holding 50 (density of 63 stems per m<sup>2</sup>) regularly spaced dried (30+ d) *Spartina* stems which were weighted at the bottom to keep them vertical in the water column (Fig. 1). To control for any shading effects, frames with netting were placed over all tanks.

On day 10 at 08:30, 60 randomly selected grass shrimp (10 per fish) were evenly dispersed in each mesocosm by pouring them over the water surface, and left for 7.75 hr. Preliminary runs indicated that  $\sim 8$  hrs was an appropriate feeding period at the experimental temperature. Aeration was maintained during the experiment. Upon termination, fish were removed, anesthetized with MS-222 and preserved in 10% formalin. Any remaining grass shrimp were recovered from the mesocosms. Each fish was measured, weighed, and the number of prey within its gut noted. The mean and coefficient of variation (CV) in the number of prey were determined for each experimental unit. As an index of how recently prey had been eaten, their

1 level of digestion (ID) was coded from 1 to 5 for each fish, with 1 representing completely intact  
2 and 5 representing well-digested.

3 Dependent variables within separate Two-Way ANOVA's included the mean number of prey,  
4 the coefficient of variation in the number of prey (CV; i.e., measure of dispersion relative to the  
5 mean) (Zar 1984), and the Index of Digestion (ID). Response variables all conformed to the  
6 normal distribution (One-Sample Kolmogorov-Smirnov tests). The ANOVA model incorporated  
7 training (i.e., experienced fish vs. naïve fish relative to novel prey) and habitat (i.e., *Spartina*  
8 presence or absence) as fixed factors (GLM procedure in SPSS Ver. 15.0.1). Because it was  
9 always non-significant ( $F=0.21 - 0.3.83$ ;  $P = 0.084 - 0.82$ ), the randomized block term was  
10 dropped from the model. The final model included terms for an intercept (overall mean),  
11 training, habitat, and the interaction between training and habitat. Because the interaction was  
12 non-significant for all three feeding metrics (Table 1), this term will not be discussed further.  
13 Levene's tests confirmed homogeneous error variances of the dependent variables across groups  
14 for all three response variables. Special contrasts assessed whether or not experienced fish  
15 differed from naïve fish, both in the presence and in the absence of habitat.

16 Although the mean number of prey consumed was visibly higher for experienced fish (Fig  
17 2a), the difference in the mean number of prey was non-significant between training groups ( $F =$   
18  $1.137$ ,  $P = 0.317$ ). However, this test also exhibited low power (Table 1). Clearly, naïve fish  
19 readily consumed novel live prey within the No *Spartina* treatment. In a previous study, we  
20 found that smaller (i.e.,  $\sim 10$  cm TL) solitary juvenile HR spotted seatrout required multiple  
21 exposures to live novel prey in order to acquire effective foraging skills (Jackson et al. 2013). In  
22 the present study, HR fish were considerably larger (i.e.,  $\sim 17.5$  cm TL) and foraged within a  
23 group setting, which can hasten learning due to cues from conspecifics (Suboski & Templeton

1989, Sundström & Johnsson 2001). HR Atlantic salmon also quickly learn to accept novel prey and improve their foraging skills in the presence of conspecifics (Brown *et al.* 2003). Naïve HR spotted seatrout may be predisposed to social facilitation of feeding as a result of being raised within a group setting.

A major challenge facing newly released HR fishes involves having to cope with complex feeding habitats while avoiding predators. Clearly, both treatment groups consumed significantly fewer (mean;  $F = 14.195$ ,  $P = 0.005$ ), but also more relatively variable (CV;  $F = 6.843$ ,  $P = 0.031$ ) numbers of prey in the presence of complex habitat structure than in its absence (Table 1). Aquatic vegetation is generally known to impede predation rates relative to non-vegetated habitats (Heck & Thoman 1981). In contrast to the present study, feeding rates of wild spotted seatrout on juvenile brown shrimp did not differ relative to the presence of *Spartina* within mesocosms (Minello & Zimmerman 1983). The observed discrepancy in feeding success of HR spotted seatrout within structured habitat underscores the importance of foraging experience under natural conditions.

In contrast to naïve fish, experienced fish more frequently contained at least one grass shrimp (i.e., fewer empty stomachs), as indicated by a significantly lower CV in the number of prey ( $F = 6.561$ ,  $P = 0.034$ ) (Fig. 2b) (Table 1). Indeed, differences in the frequency of empty guts support the interpretation of CV as an indicator of feeding success. Overall, twice as many naïve fish had empty guts (15 naïve vs. 7 experienced fish). Moreover, the significantly higher CV between training groups for the *Spartina* treatment vs. the No *Spartina* treatment ( $F = 6.843$ ,  $P = 0.031$ ) implied experienced fish from the habitat treatment more frequently contained at least one prey item. Again, 61% (11) of the guts of naïve fish vs. only 28% (5) of the guts of experienced fish were empty within *Spartina*. More specifically, the CV in the number of prey was significantly

different between training groups within the *Spartina* treatment ( $F = 7.626$ ,  $P = 0.025$ ), but not within the No *Spartina* treatment ( $F = 0.741$ ,  $P = 0.414$ ). Thus, previous experience with novel live prey in the absence of habitat structure facilitated feeding success of fish when faced with complex habitat structure for the first time. Salvanes & Braithwaite (2005) noted that exposing HR fish to novel habitats and prey types promotes easier transition to live prey. Any pre-release procedure that improves the feeding success of HR fish within structured habitat should help alleviate the survival bottleneck of newly released HR fish.

Digestion of prey was generally less complete in fish from the No *Spartina* treatment, suggesting prey had been consumed earlier in the presence of habitat structure (ID;  $F = 35.557$ ,  $P < 0.001$ ) (Table 1). In addition, the ID was nearly significantly higher for experienced than for naïve fish ( $F = 4.570$ ,  $P = 0.065$ ). Habitat structure can mediate feeding efficiency through effects on the inter-capture interval and the total time spent feeding (Warburton 2003). However, interpretation of the ID is not entirely straight forward, because the consumption of fewer prey within *Spartina* may have protracted the gut passage rate.

Insights from the present study and other ecological experiments with HR fish contribute to an expanding knowledge base about behavioral plasticity of HR fish (Jackson et al. 2013), and the formulation of pre-release exposure protocols (Ellis et al. 2002; Brown et al. 2003).

Foraging behavior of juvenile spotted seatrout has been shown to be plastic both in the present study as well as in an earlier study (Jackson et al. 2013). Grass shrimp can be easily obtained; however, costs and benefits in terms of necessary labor and facilities would have to be further assessed before implementing pre-release exposures to grass shrimp on a large scale. Future research should establish performance capabilities in light of ecological requirements of HR fish,



as well as exposure protocols involving prey, habitat, and predators for promoting the survival of HR fish in the wild.

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**Figure Legends**

**Figure 1.** Experimental layout showing mesocosms with and without *Spartina* as habitat structure.

**Figure 2A.** Mean Number of prey consumed (mean  $\pm$  1 se) by experienced and naïve HR spotted seatrout, both in the presence (non-shaded bars) and absence (shaded bars) of habitat structure.

**B.** Coefficient of Variation (CV) in the number of prey consumed (mean  $\pm$  1 se) by experienced and naïve HR spotted seatrout, both in the presence (non-shaded bars) and absence (shaded bars) of habitat structure.

For Review Only

**Table 1.** Two-Way Analysis of Variance of feeding response metrics for experienced and naïve HR spotted seatrout (TRAINING) in the presence and absence of habitat structure (STRUCTURE).

Variable	Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Power
Mean Number of Prey	TRAINING	4.688	1	4.688	1.137	0.317	0.124	0.156
	STRUCTURE	58.521	1	58.521	14.195	0.005	0.640	0.908
	TRAIN * STRUC	2.836	1	2.836	0.688	0.431	0.079	0.114
	ERROR	32.981	8	4.123				
	TOTAL	237.972	12					
Standard Deviation Number of Prey	TRAINING	0.412	1	0.412	0.336	0.578	0.040	0.081
	STRUCTURE	19.439	1	19.439	15.840	0.004	0.664	0.935
	TRAIN * STRUC	2.124	1	2.124	1.731	0.225	0.178	0.213
	ERROR	9.817	8	1.227				
	TOTAL	127.033	12					
Coefficient of Variation Number of Prey	TRAINING	0.657	1	0.657	6.561	0.034	0.451	0.614
	STRUCTURE	0.685	1	0.685	6.843	0.031	0.461	0.632
	TRAIN * STRUC	0.181	1	0.181	1.806	0.216	0.184	0.220
	ERROR	0.801	8	0.100				
	TOTAL	16.614	12					
Index of Digestion	TRAINING	0.535	1	0.535	4.570	<u>0.065</u>	0.364	0.468
	STRUCTURE	4.161	1	4.161	35.557	0.000	0.816	0.999
	TRAIN * STRUC	0.009	1	0.009	0.079	0.786	0.010	0.057
	ERROR	0.936	8	0.117				
	TOTAL	175.393	12					

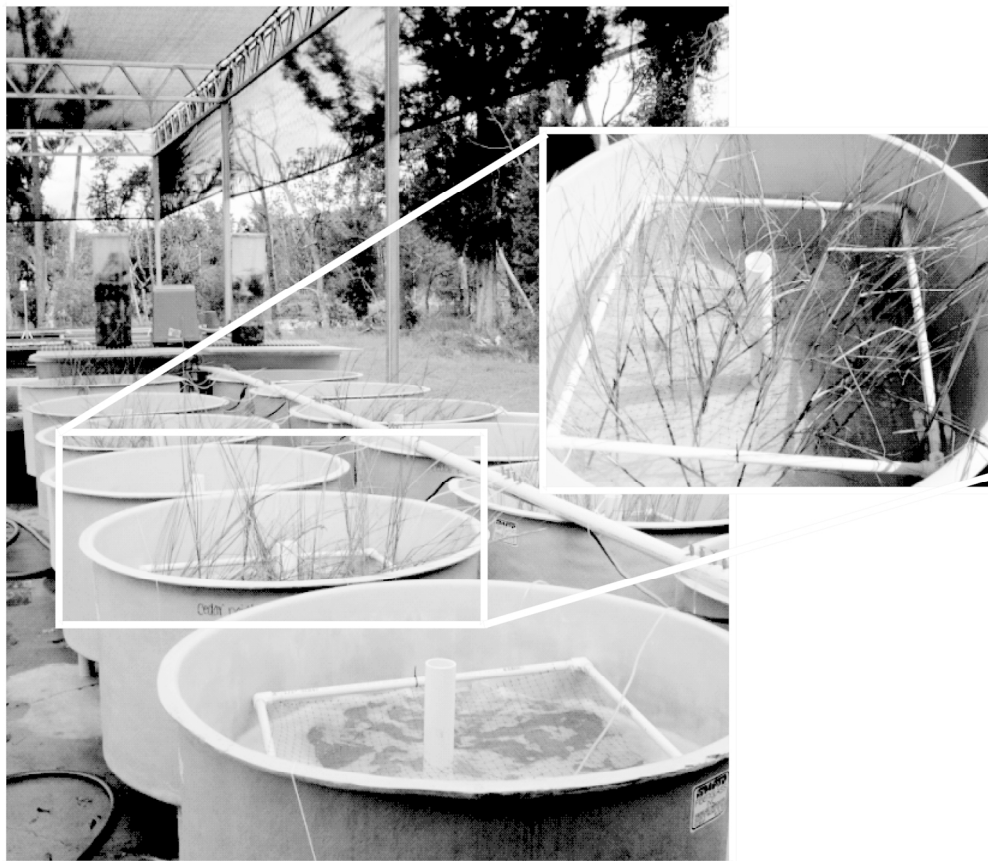


Figure 1. Experimental layout showing mesocosms with and without *Spartina* as habitat structure.  
147x127mm (300 x 300 DPI)

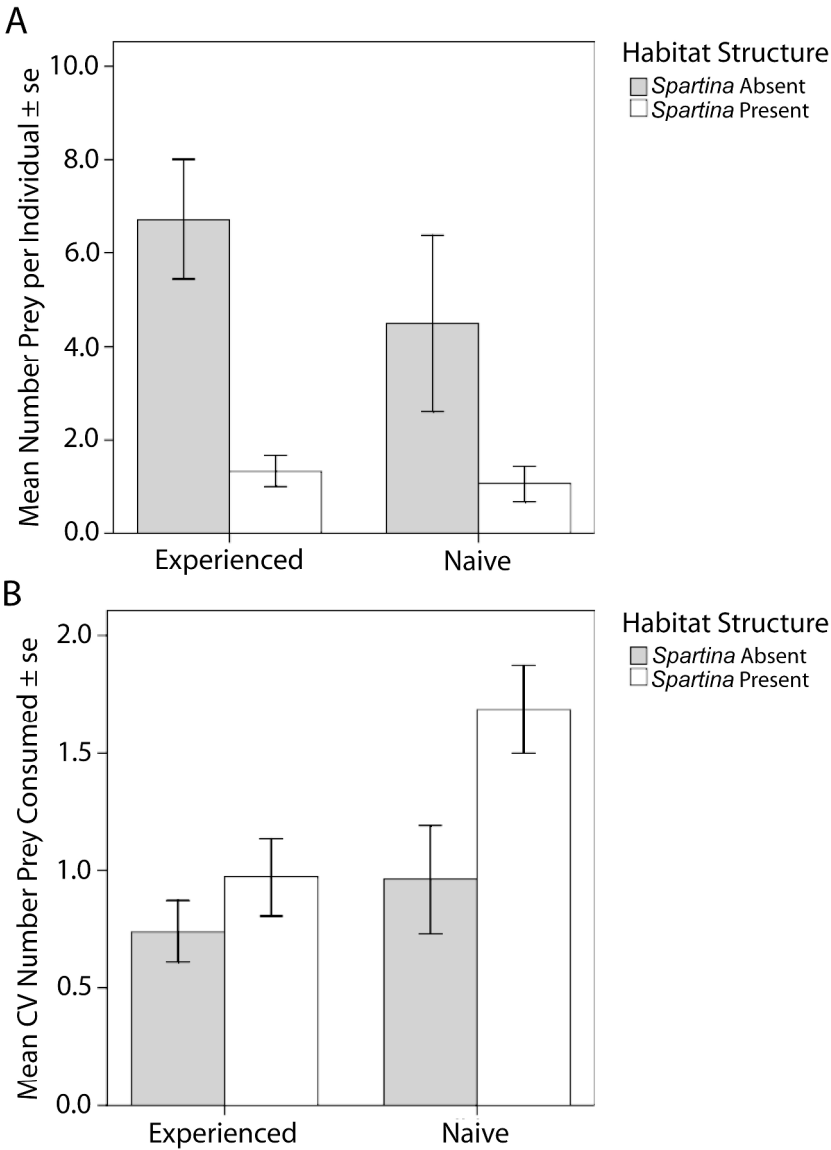


Figure 2A. Mean Number of prey consumed (mean  $\pm$  1 se) by experienced and naïve HR spotted seatrout, both in the presence (non-shaded bars) and absence (shaded bars) of habitat structure.  
B. Coefficient of Variation (CV) in the number of prey consumed (mean  $\pm$  1 se) by experienced and naïve HR spotted seatrout, both in the presence (non-shaded bars) and absence (shaded bars) of habitat structure.

262x381mm (300 x 300 DPI)