Aquaculture 420–421 (2014) 89–94

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Stocking density effects on aggressive and cannibalistic behaviors in larval hatchery-reared spotted seatrout, *Cynoscion nebulosus*

Christopher B. Manley *, Chet F. Rakocinski, Phillip G. Lee, Reginald B. Blaylock

Department of Coastal Sciences, The University of Southern Mississippi, Gulf Coast Research Laboratory, 703 East Beach Drive, Ocean Springs, MS 39564, USA

ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form 28 October 2013
Accepted 29 October 2013
Available online 6 November 2013

Keywords:
Stocking density
Aggression
Cannibalism
Cynoscion nebulosus

ABSTRACT

Cannibalism and aggression are major sources of mortality in the larviculture of the spotted seatrout, *Cynoscion nebulosus*. High stocking density can either increase cannibalism by increasing the likelihood of encounters between aggressors and prey or decrease cannibalism by interfering with normal territorial or aggressive behaviors. The goal of this study was to assess the effect of stocking density on cannibalism and aggressive behaviors in hatchery-reared spotted seatrout. Seven-day-old larvae were stocked randomly into three replicates of three different densities (15 (233 total fish), 30 (465 total fish), and 60 (930 total fish) L^{-1}) in 15.5-L aquaria. Feeding was conducted every 8 h based on residual rotifer or *Artemia* counts. Growth was determined 6 days poststocking at the conclusion of the experiment. To quantify cannibalism and aggressive behaviors, three cameras filmed three tanks for 9 h each day. Recorded behaviors were quantified for three selected 30-minute segments per tank per day: 1 h, 4 h, and 7 h post-feeding. Aggressive acts were scored as: nip (aggressor strikes prey causing prey to dart), chase (aggressor moves more than one body length toward prey), and capture (predator captures and holds prey but unable to consume). In all stocking densities there was a significant increase in aggression and cannibalism with time since feeding. Growth was significantly higher in the lower density treatment. An observed density threshold existed at a stocking density of 30 fish L^{-1}, beyond which the intensity of aggressive behaviors did not increase. Based on the results of this study, aggression in early stage hatchery-reared spotted seatrout might be alleviated with increased feeding frequency. Further, spotted seatrout could possibly be cultured at densities higher than the current protocol allows.

1. Introduction

Aquaculture provides a way to supplement the growing demand for overexploited wild fishery stocks; however, marine finfish production is currently limited by the lack of commercial-scale hatcheries with the capacity to provide plentiful finfish juveniles economically (Lee and Ostrowski, 2001). Many factors contribute to this bottleneck including cost of production, successful spawning of broodstock, and, especially, high mortality during the larval rearing stage. The occurrence of high mortality can be attributed to suboptimal nutrition and rearing conditions as well as aggression and cannibalism.

Aggression and cannibalism contribute significantly to mortality in aquaculture settings, even where conditions appear to be ideal (Baras and Jobling, 2002; Hecht and Pienaar, 1993). The prevalence of aggression and cannibalism in an aquaculture setting can be attributed to intrinsic genetic effects that result in heterogeneous growth of larvae in addition to extrinsic factors that mediate the aggressive behavior of larvae (Baras and Jobling, 2002; Hecht and Pienaar, 1993). Because extrinsic factors can be manipulated, current aquaculture research has focused on managing environmental conditions such as stocking density to control aggression and cannibalism.

The influence of stocking density on aggression and cannibalism varies among species depending on the social structure (Hecht and Pienaar, 1993). An increase in density for a territorial or hierarchical species may lead to a reduction of aggression (Baras and Jobling, 2002; Hecht and Pienaar, 1993), as high densities may swamp competitive defenses, thereby making aggression ineffective due to the trade-off in time required for feeding. Previous studies suggest that this is the case for several aquaculture species including Japanese amberjack (*Seriola quinqueradiata*) (Sakakura and Tsukamoto, 2007), greater amberjack (*Seriola dumerili*) (Miki et al., 2011), and yellow perch (*Perca fluviatilis*) (Baras et al., 2003). On the other hand, in some species higher density may translate into more intense aggression and frequent cannibalism. This phenomenon is evident under aquaculture conditions for fat snook (*Centropomus parallelus*) (Corrêa and Cerqueira, 2007) and Nile tilapia (*Oreochromis niloticus*) (Fessehaye et al., 2006). Possible explanations for a positive relationship between density and aggression include enhanced prey encounter frequency for aggressors and increased rates of conspecific interactions at higher densities (Baras and Jobling, 2002; Hecht and Pienaar, 1993).

In Mississippi (USA), the spotted seatrout, *Cynoscion nebulosus* (Cuvier, 1830), the most popular recreational finfish in the Gulf of

* Corresponding author at: University of Southern Mississippi, Gulf Coast Research Laboratory, 703 East Beach Drive, Ocean Springs, MS 39564, USA. Tel.: +1 484 651 0554.
E-mail address: christopher.manley@eagles.usm.edu (C.B. Manley).

0044-8486/$ – see front matter
http://dx.doi.org/10.1016/j.aquaculture.2013.10.040

Mexico, is being considered for stock enhancement as part of a comprehensive management approach. The spotted seatrout is a euryhaline fish that mainly inhabits marshes and estuaries along the Atlantic and Gulf of Mexico coasts from Massachusetts to Texas (Blanchet et al., 2001). Spotted seatrout have been successfully reared in both extensive and semi-intensive systems (Arnold et al., 1978; Colura et al., 1992; Tucker, 1988). However, semi-intensive and intensive systems have proven to be problematic in part due to the high prevalence of cannibalism once larvae reach 10 days post-hatch (Arnold et al., 1978; Tucker, 1988). Large-scale cultivation of seatrout in intensive systems, therefore, requires a better understanding of the behavioral ecology of the species as well as a practical density for optimizing rearing success.

In this study, we 1) use video analysis to elucidate aggressive behaviors shown by larval stages of hatchery-reared spotted seatrout to better understand the behavioral ecology of seatrout and 2) examine the effects of stocking density on the expression of aggressive and cannibalistic behaviors and growth and survival to illuminate ways to increase survival of spotted seatrout in intensive culture conditions.

2. Materials and methods

2.1. Animal husbandry

Seven-day-old spotted seatrout larvae (mean total length 2.9 mm) were obtained on 21 August 2012 from a single cohort cultured at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center. Eggs were obtained from tank spawns of captive adults maintained under photothermal control similar to the methods described in Arnold et al. (1978) and incubated at a density of 1 mL^{-1} at 30 ppt and 30 °C for 24 h. Newly hatched larvae were stocked at 15 L^{-1} into 1500 L tanks and fed beginning on day 1 post-hatch. The larvae were held in the 1500 L tanks until they were moved to the experimental system at 7 days post-hatch. Prior to the experiment, 20 larvae were measured (total length, TL) alive using the ProgRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter, FL, USA) and randomly allocated among three replicates of three different densities (15 (233 total fish), 30 (465 total fish), and 60 (930 total fish) fish L^{-1}). The nine 15.5 L clear, square, aquaria (22.86 cm × 25.4 cm × 26.67 cm) were connected to a recirculating system with a common water supply with a flow rate of 3.3 mL sec^{-1}. Water recirculated through mechanical, carbon, and ultraviolet filters and water temperature was maintained at 30.7 ± 0.34 °C and salinity at 30 ± 0.29 ppt. The experimental system was continuously illuminated by overhead fluorescent lighting at an intensity of 378 lx. White dividers were placed between each tank to ensure that behaviors of fish were not affected by adjacent tanks. Because the tanks were illuminated overhead, the dividers did not have an effect on light intensity between tanks. Temperature, salinity, pH, dissolved oxygen, ammonia, nitrite, nitrate and alkalinity were monitored daily using Hach© (Hach Co., Loveland CO, USA) test strips and a YSI© 566 MPS probe (YSI Inc., Yellow Springs, OH, USA). Dissolved oxygen was maintained above 8 mg L^{-1}, and pH was maintained at 7.66 ± 0.23 throughout the entire experiment. Ammonia ranged from 0.25 to 0.35 ppm, nitrite ranged from 0 to 0.15 ppm, and nitrate ranged from 25 to 50 ppm. Maintenance included daily siphoning and mortality counts. Equivalently aged spotted seatrout from the same cohort were used to replace those that died to maintain the correct densities throughout the experimental period. Daily percent mortality was calculated as the number of mortalities in each tank divided by the original number of larvae in each tank. Daily survival was calculated by subtraction using the daily percent mortality. Overall percent survival was calculated as the average of daily percent survival over 5 experimental days.

Larvae were fed enriched s-type rotifers (*Brachionus* sp.) on days 1–2 and enriched *Artemia* on days 2–5. Rotifers and *Artemia* were cofed on day 2. Feeding was conducted every 8 h. The amount of food added at each feeding interval was based on residual prey densities obtained by collecting three 1 mL water samples from each tank prior to feeding, which is the standard protocol used to determine residual prey densities at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center. The total number of live food items (rotifer or *Artemia*) per milliliter was calculated from each sample using a dissecting microscope to estimate the residual number of live food items per milliliter. The residual number was then subtracted from the target concentration, between 0 and 8 individuals mL^{-1}, depending on days post-hatch (DPH) to determine the adjusted number of live food items per milliliter that needed to be added to each tank. The target concentrations for each day were based on the standard seatrout feeding protocol used at the Thad Cochran Marine Aquaculture Center and were as follows: Day 0–1, 8 rotifers mL^{-1}; Day 2, 6 rotifers mL^{-1} and 1 *Artemia* mL^{-1}; Day 3, 2 *Artemia* mL^{-1}; Day 4, 3 *Artemia* mL^{-1}; and Day 5, 4 *Artemia* mL^{-1}. After five days, 20 fish larvae were randomly selected from each tank and measured (TL) alive using the ProgRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter, FL, USA).

2.2. Experimental design

To quantify aggressive behaviors and mortality, each of the three video cameras (Sony Handycam DCR-SR45) was used to film the nine tanks for 9 h each day. Filming commenced one day after stocking (8 DPH). Footage was analyzed for 3 selected 30 minute segments per tank per day using the Noldus Observer XT program (Noldus Information Technology Inc. Leesburg, VA, USA). The selected observation periods were 1 h after feeding, 4 h after feeding, and 7 h after feeding. Aggressive acts were scored as: nip (aggressor strikes prey causing prey to dart), chase (aggressor moves more than one body length toward prey), and capture (predator captures and holds prey but unable to consume, i.e., Type 1 cannibalism). Total acts were scaled to the number of individuals per treatment to standardize data to the number of acts per individual for each tank at each observation period.

To guard against observer bias, a different investigator scored three randomly selected observations in a blind comparison. At the completion of the blind comparison, the mean for the blind observations was tested against that for the original observations using a *t*-test. No difference in results was obtained between the two observers. In addition, no differences were found when one observer counted the same video multiple times.

2.3. Data analysis

Two-way repeated measures (RM) ANOVA (GLM procedure in SPSS version 15.0.1) tested the response variables: (1) number of nips, (2) number of chases, and (3) number of captures. The two within-subjects factors (Day and Time) accounted for variability and dependence among the five consecutive days and the three consecutive feeding times per day. The between-subjects factor (Density) is represented by Low, Medium, and High density levels as described above. Due to failure of one of the video cameras, missing data for observations of one replicate observation for each of the three density treatments on 1 day were imputed as means of the other two replicates to enable the RM ANOVAs.

As the directionality of expected differences in behavioral frequencies across density levels was unknown a priori, the significance of between-subjects effects was interpreted as a two-tailed hypothesis. The RM ANOVA model included an intercept (overall mean) and two two-way interaction terms (Density × Day, Density × Time). To scale behavioral responses in terms of per capita rates relative to density treatments, frequencies were normalized relative to the number of fish within each of the three density levels. To stabilize variances of those normalized response variables that could greatly exceed one, data were transformed as log ratios [e.g., log(number nips / number fish)]. Accordingly, the number of nips and the number of chases were