Methods of domestic striped bass (*Morone saxatilis*) spawning that do not require the use of any hormone induction

Andersen, L.K.[1], Clark, R.W.[2], Hopper, M.S.[2], Hodson, R.G.[1], Schilling, J.[1], Daniels, H.V.[1], Woods III, L.C.[3], Kovach, A.I.[4], Berlinsky, D.L.[5], Kenter, L.W.[5], McGinty, A.S.[2], Reading, B.J.[1,2*]

[1]North Carolina State University, Department of Applied Ecology, 100 Eugene Brooks Avenue, Raleigh, North Carolina, 27695, USA

[2]North Carolina State University, Pamlico Aquaculture Field Laboratory, 2002 Hickory Point Road, Aurora, North Carolina, 27806, USA

[3]University of Maryland, Crane Aquaculture Facility, 8705 Greenmead Drive College Park, Maryland, 20742, USA

[4]University of New Hampshire, Department of Natural Resources and the Environment, Durham, New Hampshire, USA

[5]University of New Hampshire, Department of Agriculture, Nutrition, and Food Systems, Durham, New Hampshire, USA

***Corresponding Author:** Benjamin J. Reading, bjreadin@ncsu.edu, +1 (919) 515-3830, North Carolina State University Department of Applied Ecology 100 Eugene Brooks Avenue Box 7617 Raleigh, NC 27695

Keywords: batch spawning; genotyping; aquaculture

Abstract

Nineteen batch spawning trials were conducted using 5th and 6th generation domestic striped bass (*Morone saxatilis*) to demonstrate the ability of these fish to volitionally spawn in large tanks to produce larvae using only photothermal and salinity conditioning. The findings described are the first report of multiple striped bass successfully batch spawning in captivity without exogenous hormone administration. The results of these trials indicate that an approximately 1:1 ratio of female to male striped bass in a single batch spawning unit is more favorable for production, that a minimum of at least 10 fish of each sex is required to elicit this particular spawning behavior, and that using 25 fish of each sex will yield commercially scalable larval production. This batch spawning method has been employed to effectively and consistently spawn over half of the female striped bass in the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* (N = 202 of 334 female fish over five years) to produce 44,608,181 swim-up larvae (26.6% hatching rate). Microsatellite genotyping and parentage assignment demonstrates that females will reproduce with between 2 and 18 males and that males will reproduce with between 1 and 6 females. Moreover, the effective broodstock size (N_b) of these batch spawning units is 33 and when accounting for multiple partners and unequal family sizes (N_b^v) is 28. Lastly, the reported results include the successful spawning of female striped bass staged at and beyond 15 Bayless hours, or those that would have previously been considered ineligible for spawning even with the use of exogenous hormone treatment.

1. Introduction

Farming of sunshine bass (hybrid bass produced from a cross of female white bass *Morone chrysops* x male striped bass *M. saxatilis*) is presently the fourth largest finfish aquaculture industry in the United States, behind only catfish, salmon, and trout, with over 14 million pounds produced annually at a farm-gate value of approximately $50 million USD (Reading et al. 2018; USDA 2019). It is estimated that as much as 90% of the sunshine bass raised in the United States from 2015-2019 were produced using domesticated male striped bass broodstock from the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* housed at the North Carolina State University Pamlico Aquaculture Field Laboratory (Aurora, NC, USA). Fish from this program are bred annually and male striped bass broodstock are disseminated to fingerling producers throughout the country. Optimizing domestic striped bass spawning would greatly enhance the capacity and efficiency to produce broodstock for the hybrid striped bass industry, may open the door to tank spawning production of hybrid striped bass or true-breeding hybrid striped bass, and will provide a means for consistent larval fish (fry) production for an emergent striped bass aquaculture or mariculture industry (McCraren 1984; Hallerman 1994; Reading et al. 2018). Additionally, the value of domestic striped bass broodstock from *The National Breeding Program* to the industry may increase overall if they are shown to spawn under greatly simplified culture conditions compared to traditional wild-captured broodstock (Hodson et al. 1999; Woods 2001; Garber and Sullivan 2006).

The current standard method of spawning striped bass is to tank or strip spawn males and females from wild and domestic stocks that have been treated with gonadotropin releasing

hormone analog (GnRHa) implants and/or human chorionic gonadotropin (hCG, Chorulon) injections (Harrell et al. 1990; Hodson and Sullivan 1993; Woods and Sullivan 1993; Harrell 1997; Andersen et al. 2020). Treatment with these exogenous hormone compounds has historically been considered mandatory to induce gamete maturation in female striped bass and to prolong spermiation and milt hydration in male striped bass held in captivity (Hodson and Sullivan 1993; Grizzle et al. 1995; Mylonas et al. 1996; Frankel et al. 2013; Andersen et al. 2020). Although reliance on hormones for commercial aquaculture foodfish production may be useful, there are several considerations such as cost, regulations, handling stress, and public concern (Smith 1989; Hodson and Sullivan 1993; Andersen et al. 2020).

We recently reported the first observations of untreated, domestic striped bass females volitionally spawning in the presence of a hormone-treated female and several males (three to five per tank) to successfully yield fertilized eggs and subsequent fry (Andersen et al. 2020). In this "pace-set" spawning approach, the female striped bass treated with exogenous hormone compound (GnRHa or hCG) is thought to prompt the spawning behavior (i.e., ovulation and release of eggs) of the untreated females and therefore set the pace, or occurrence, of spawning (Andersen et al. 2020). In the majority of those trials (87.5%), the hormone-treated female volitionally spawned, as expected, and in half of the trials this was followed by volitional spawning of the untreated females (Andersen et al. 2020). Prior to the findings reported by Andersen et al. (2020), there was only one published record of a captive female striped bass volitionally releasing eggs in a tank without being treated with exogenous hormone compound (Woods et al. 1990). The reported findings on pace-set spawning suggest that domestic female striped bass do not necessarily require treatment with hormone compounds and may be able to

90 spawn in large groups, or “batches.” The possibility of this is further validated by our
91 observation of untreated domestic female striped bass volitionally releasing their eggs when in
92 the presence of males during routine photothermal conditioning, although the fertilities of these
93 spawns were not verified (R.W. Clark and M.S. Hopper, *unpublished data*).
94
95 Here, we evaluate the efficacy of eliminating traditional hormone induction methods from
96 striped bass spawning protocols (Harrell et al. 1990; Harrell 1997) by manipulating photothermal
97 and salinity parameters to batch spawn between sixteen and seventy-four mixed sex fish in a
98 single tank. Nineteen batch spawning trials were conducted with the following objectives: (1) to
99 determine if domestic female striped bass are capable of volitionally batch spawning *en masse*
100 without hormone treatment (nineteen trials); (2) to determine the optimal sex ratio of domestic
101 striped bass broodstock females and males that results in the most effective spawning success
102 (seven trials); (3) to determine if batch spawning of domestic striped bass is possible without
103 inducing male striped bass with hCG (four trials); (4) to explore the minimum number of
104 domestic striped bass required to elicit hormone-free batch spawning in captivity (four trials); (5)
105 to commercially scale hormone-free batch spawning of domestic striped bass (four trials); (6) to
106 gain insight into the reproductive success of males and females using microsatellite genotyping
107 (one trial); and lastly, (7) to observe the spawning activity of females of different known oocyte
108 stages measured in Bayless hours (five trials) (Bayless 1972; Rees and Harrell 1990; Watson
109 1992).
110
111 **2. Materials and Methods**
112

2.1 Experimental animals

All research was performed under the protocol approved by the Institutional Animal Care and Use Committee of North Carolina State University (Protocol numbers 10-042-A and 19-065-O). The study was carried out in accordance with the recommendations located in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NRC 1996).

All domestic striped bass described here are a part of the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* and were spawned and raised at the North Carolina State University Pamlico Aquaculture Field Laboratory (Aurora, NC, USA). Female fish used in each of the nineteen trials were four years of age and were 5^{th} to 6^{th} generation domestic (bred in captivity; Reading et al. 2018), as trials were conducted over five consecutive annual spring spawning seasons (N = 334 fish; sub-sample (n) of 199 fish weight 4.9 $\pm$ 0.40 kg, all values given as mean $\pm$ SD). Males used in each trial were three years of age and similarly of 5^{th} to 6^{th} generation domestic (N = 429 fish; n = 245 fish weight 2.6 $\pm$ 0.27 kg). Broodstock conditioning was completed for all of the fish included in each of the trials as follows: fish were maintained in freshwater at ambient photothermal cycle during the summer (gonadal recrudescence) through late fall (vitellogenesis). Females were transitioned to a broodstock diet at the onset of vitellogenesis (October) prior to the spring spawning season (females in Trial 1 and Trials 4 through 7 were fed Slow-Sinking Bass Brood feed from Zeigler Bros, Inc., Gardners, PA, USA, 45% protein, 15% lipid; females in Trials 2, 3, and 8 through 19 were fed BioBrood from Bio-Oregon, Westbrook, ME, USA, 45% protein, 20% lipid). In late winter (February), fish were moved from outdoor flow-through pools (38,433 L, 7.3 m diameter)

to separate indoor recirculating systems (3,555 L, 2.2 m diameter, and 7,041 L, 3.0 m diameter, for males and females, respectively), where thermal parameters were maintained at 10-12 degrees Celsius (°C) and 10-12 ppt salinity under ambient photoperiod. Beginning in early spring (April), fish were gradually conditioned (an increase of 1°C and a decrease of 1 ppt salinity per day) to reach spawning temperature (18-20°C) and salinity (0.0-0.5 ppt) under ambient photoperiod (**Figure 1**) (Hodson and Sullivan 1993; Sullivan et al. 2003). Males and females used in each trial were moved separately into the spawning tank within two weeks of warmup beginning. Salinity was temporarily increased by 2 to 5 ppt at the time the females and males were moved into indoor spawning tanks to mitigate impacts of handling stress. The spawning tanks (31,139 L, 6.1 m diameter, unless otherwise stated) were fitted with two upwelling egg collectors (200 L) plumbed into the standpipe drain line (Mullis and Smith 1990) to enable the collection of eggs upon spawning (**Figure 2**).

2.2 Fish handling

Ovarian biopsy was performed on 204 of the striped bass females in the trials indicated below by collecting oocytes with a plastic cannula (outer diameter: 0.3175 cm; inner diameter: 0.2032 cm) inserted into the urogenital opening. Oocytes were examined under a dissecting microscope to determine the Bayless hour stage and eligibility status of females to participate in spawning based on oocyte diameter and ooplasm clearing (Bayless 1972; Kerby 1986; Rees and Harrell 1990; Watson 1992; Chapman et al. 2014; Andersen et al. 2020). The remaining females were not subjected to any measurements or handling other than being transferred into spawning tanks in an attempt to reduce overall handling stress of the broodstock fish (Smith 1989; Hodson and

159 Sullivan 1993). No females were treated with exogenous hormone compounds in any of the trials
160 described. All males were verified to express milt prior to the spawning trials by applying gentle
161 coelomic pressure. Male striped bass in Trials 1 through 9 were anaesthetized in a solution of
162 MS-222 (100 mg/L, Sigma-Aldrich, St. Louis, MO, USA) and injected in the dorsal lymphatic
163 sinus with 165 IU hCG/kg body weight (Chorulon®, Merck Animal Health, USA). Males in
164 Trials 10 through 19 were not treated with any exogenous hormone compound.
165
166 Fish were left undisturbed during the course of the trials (up to 12 days) and egg collectors were
167 checked periodically for the presence of spawned eggs during daylight (every one to two hours
168 between 0530-2100). Eggs from each spawn were harvested from collectors and the settled
169 volume of eggs (mL), eggs/mL, and total eggs produced were calculated at each harvest. Total
170 eggs produced was calculated as the settled volume of eggs (mL) collected in a single harvest
171 multiplied by the average number eggs/mL enumerated from random triplicate samples (1.0 mL
172 each) of harvested eggs (Rees and Harrell 1990). The time that a spawning event occurred was
173 recorded as the time when eggs were first observed in collectors, which was confirmed by
174 embryo staging (Rees and Harrell 1990). Embryo staging was also used to estimate the time that
175 a spawning event occurred when hatchery personnel were not immediately present to harvest the
176 spawn. Embryo viability (fertility) was assessed at between 4 and 10 hours post-fertilization, as
177 most striped bass embryo mortality has been shown to occur within the first 4 hours post-
178 fertilization (Chapman et al. 2014). Eggs from each spawn were incubated separately in
179 McDonald hatching jars and fry were hatched into separate aquaria (Mullis and Smith 1990).
180 Number of fry produced was enumerated by taking triplicate random samples (40-60 mL each)
181 from each aquarium, counting the fry in each sample, and multiplying the average count by the

known volume of each aquarium. Male striped bass were verified to be spermiating at the conclusion of the trials as they had been at the beginning of each trial to confirm spawning competency. Females were checked for the presence of overripe eggs and/or to confirm that they had participated in spawning (i.e., had released their eggs) by applying gentle coelomic pressure or by ovarian biopsy.

2.3 Determination of optimal sex ratio trials (1 – 7)

Seven batch spawning trials were conducted with untreated female striped bass (N = 117 fish; n = 69 fish weight 4.5 $\pm$ 0.47 kg) and hCG-treated males (N = 212 fish; n = 66 fish weight 2.3 $\pm$ 0.15 kg) to determine the optimal sex ratio for volitional *en masse* spawning. Three of these trials were conducted at an approximately 1:1 female to male ratio (Trials 1, 2, and 3; sex ratios 15:19, 22:20, 18:20, F:M respectively), three of the trials were conducted at an approximately 1:2 female to male ratio (Trials 4, 5, and 6; sex ratios 16:31, 15:35, 13:31, F:M respectively), and the seventh trial at an approximately 1:3 female to male ratio (Trial 7; sex ratio 18:56 F:M). Only the females in Trials 2, 3, 4, and 6 were weighed, measured, and subjected to ovarian biopsy as described above.

Three batch spawning metrics (percent females spawned, total eggs produced, and total fry produced) were compared between the groups of trials that were conducted at an approximate 1:1 sex ratio (Trials 1 through 3) and those trials that were not conducted at a 1:1 sex ratio (i.e., approximate 1:2 sex ratio, Trials 4 through 6, and approximate 1:3 sex ratio, Trial 7). Data for the batch spawning metrics were examined for normality using the Shapiro-Wilks test. In the

205 case that the data did not fit a normal distribution, a Wilcoxon Exact test was used to determine
206 significant differences between groups; otherwise data were analyzed using a Student's *t*-test.
207 The nominal level of significance accepted for all statistical tests *a priori* was alpha = 0.05
208 (JMP® Pro v. 14.0.0, SAS Institute Inc., Cary, NC, USA).
209
210 Female striped bass that had not released eggs by the conclusion of Trials 2 and 3 and that were
211 staged between 9 and 15 Bayless hours were treated with 330 IU/kg of hCG and separately tank
212 spawned with two to four hCG-treated males using the methodologies described in Harrell et al.
213 (1990), Harrell (1997), and Andersen et al. (2020). Briefly, hormone-treated male and female
214 striped bass were moved into individual, indoor spawning tanks (2,400 L, 1.8 m diameter)
215 equipped with an external egg collector plumbed into the drain line (Smith and Whitehurst
216 1990). Egg collection, embryo staging, verification of male spermiation and female spawning,
217 and enumeration of harvested eggs and fry produced were completed in the same manner as
218 described above.
219
220 *2.4 Requirement of male hormone induction trials (8 - 11)*
221
222 Four batch spawning trials were conducted at an approximately 1:1 female to male sex ratio
223 (Trials 8, 9, 10, and 11; sex ratios 19:20, 21:20, 19:19, 20:20, F:M respectively) to determine if
224 batch spawning domestic striped bass is possible without inducing male striped bass with
225 exogenous hormone. All females were untreated (N = 79 fish; n = 40 fish weight 4.7 $\pm$ 0.51 kg).
226 Two of the spawning trials (Trials 8 and 9) included males treated with hCG as described above
227 and the remaining two spawning trials (Trials 10 and 11) included untreated males (N = 79 fish;

228 n = 59 weight 2.5 ± 0.27 kg). Only the females in Trials 9 and 10 were weighed, measured, and
229 subjected to ovarian biopsy. The percent of females spawned, total eggs produced, and total fry
230 produced between treatment groups were reported for comparison; statistical tests were not
231 conducted as there were only two replicate observations in both treatment groups.
232
233 Female striped bass that had not released eggs by the conclusion of Trials 8, 9, and 10 and that
234 were staged between 9 and 15 Bayless hours were treated with 330 IU/kg of hCG and tank
235 spawned as described above. Female striped bass that had not released eggs by the conclusion of
236 Trial 11 and that were staged between 9 and 15 Bayless hours were treated with 330 IU/kg of
237 hCG and batch spawned with twenty hCG-treated males using the described methods.
238
239 *2.5 Determination of critical mass trials (12 - 15)*
240
241 Four batch spawning trials were conducted at 1:1 female to male sex ratio (Trials 12, 13, 14, and
242 15; sex ratios 8:8, 10:10, 10:10, and 10:10, F:M respectively) as described above with the
243 exception that the spawning tanks were 7,041 L and equipped with a single 200 L egg collector.
244 The females (N = 38 fish; n = 28 fish weight 5.1 ± 0.15 kg) and males (N = 38 fish; n = 20 fish
245 weight 2.4 ± 0.21 kg) did not receive any hormone treatment. Only the females in Trials 14 and
246 15 were weighed, measured, and subjected to ovarian biopsy.
247
248 The percent of females spawned, total eggs produced, and total fry produced were reported to
249 evaluate the critical mass required to elicit batch spawning behavior of striped bass. Statistical

tests were not conducted to compare the batch spawning metrics for this group of trials as there was only a single replicate observation for the 8:8 sex ratio trial (Trial 12).

2.6 Scalability of production trials (16 - 19)

Four batch spawning trials were conducted at 1:1 female to male sex ratio (Trials 16 through 19; all sex ratios 25:25 F:M). The females (N = 100 fish; n = 75 fish weight 5.1 $\pm$ 0.25 kg) and males (N = 100 fish; weight was 2.8 $\pm$ 0.28 kg) did not receive any hormone treatment. The females in Trials 16, 17, and 18 were weighed, measured, and subjected to ovarian biopsy. The percent of females spawned, total eggs produced, and total fry produced were reported to evaluate the efficacy of scaling these protocols for commercial production. No statistical tests are required as the data correspond to production metrics.

2.7 Microsatellite genotyping

Caudal fin clips from adult broodstock (N = 47 fish) and a sample of fry (N = 119 fry) used for and produced from Trial 4 were collected into 100% ethanol, and shipped to the University of New Hampshire (Durham, NH, USA) for microsatellite genotyping and pedigree assignment as according to Kenter et al. (2018). Briefly, the Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen Inc. Hilden, Germany) and manufacturer's protocol was followed for genomic DNA extraction. Microsatellite markers MSM 1144, MSM 1095, MSM 1096, MSM 1067, MSM 1094, MSM 1168, MSM 1208 and MSM 1243 of Couch et al. (2006) and MSM 1526, MSM 1592 and MSM 1357 of Rexroad et al. (2006) were used for genotyping. Fluorescent-dye-labeled primers (FAM,

HEX, and NED) were used for PCR amplification in 12.5 µl reaction volumes and PCR products were diluted 2:8 in Hi-Di formamide. Fragment analysis was performed at the Yale University DNA Analysis Facility (New Haven, CT, USA) using an automated DNA sequencer (3730xl 96-capillary genetic analyzer; Applied Biosytems, Foster City, CA, USA). Peak scanner (v2.0, Applied Biosystems) was used to manually score peaks and sort raw scores into allelic bins.

To determine male and female reproductive success and the number of mates with which each sex reproduced parentage assignments were reconstructed from the genetic data. Genotypes of the fry were compared to those of the broodstock candidate parents using CERVUS version 3.0.7 software (Marshall et al. 1998). A genotyping error rate of 1% was assumed and the proportion of parents sampled was 100%. For offspring with parental assignment confidence less than 80%, we also conducted sibship assignment using the maximum likelihood method implemented in COLONY version 2.0.6.3 (Jones and Wang 2010). COLONY can determine the number of parents for a set of progeny for which not all parents have been sampled and it can use these estimated parental genotypes to reconstruct sibship groups.

The pedigree data from Trial 4 were used to calculate effective population size (N_e), considered here as effective broodstock size, or N_b (Wright 1933, 1939; Wang et al. 2016):

$$N_b = \frac{4N_mN_f}{N_m + N_f}$$

Where N_b is the effective broodstock size, N_m is the number of breeding males, and N_f is the number of breeding females. The N_m and N_f for each spawn were based on the number of male

296 and female spawning participants determined by parental microsatellite genotypes and sibship
297 assignment of larval offspring.
298
299 When considering the number of potential spawning participants in the batch spawns and the
300 tremendous potential for genetic mixing that may occur (i.e., the assumption that males and
301 females have the potential to spawn with multiple partners and produce unequal family sizes),
302 the N_b was corrected for variance of offspring production using $N_b{}^V$ (Gall 1987):

303
304
$$N_b{}^V = \frac{8(N_b)}{V_m + V_f + 4}$$

305
306 Where V_m is the variance of male offspring production and V_f is the variance of female offspring
307 production.
308
309 *2.8 Stage of ovarian maturation throughout spawning*
310
311 Females in Trials 14 through 18 were tagged with different colored yarn that coordinated to
312 Bayless hour stage determined by ovary biopsy at the beginning of the respective spawning trials
313 (Bayless 1972). Yarn was tied to the dorsal fin of each female through a small puncture (Hodson
314 et al. 1999). Red yarn was used to tag females staged at 10 Bayless hours, orange yarn if 11-12
315 Bayless hours, yellow if 13-14 Bayless hours, green if 15 Bayless hours, and blue if ovarian
316 maturation had not yet been initiated (i.e., those that would be considered ineligible for hCG
317 induction per standard protocol). Fish were regularly (every two hours) inspected throughout the

trials and at the observation and harvest of eggs from collectors to identify the female(s) that had released eggs to record the color of yarn and corresponding Bayless hour stage.

3. Results

A total of 202 female striped bass of the 334 included in all of the trials (60.5% of all female fish) were found to have participated in spawning by the conclusion of all trials (**Table 1**), demonstrating that female striped bass can spawn volitionally without hormone induction. The mean oocyte stage for the striped bass females sampled for ovarian biopsy was 13.4 $\pm$ 0.82 Bayless hours. Spawns were observed in all but one of the trials (Trial 12), which included only eight fish of each sex (i.e., the lowest number of fish in any of the trials). The mean time to first observed spawn for the successful trials was 1.2 $\pm$ 0.77 days (27.7 $\pm$ 18.41 hours) after the fish reached spawning temperature (18-20°C) and 2.3 $\pm$ 0.90 days (54.1 $\pm$ 21.52 hours) after males had been moved into the spawning tank. The eighteen successful batch spawning trials produced a total of 167,735,910 eggs. If only considering the female striped bass that had participated in spawning, the number of eggs produced by each female is calculated as 830,376 (502,203 eggs per female if all females are included; **Figure 3**). The percent fertility of harvested eggs ranged from 0.0% to 76.0% (mean fertility was 31.6 $\pm$ 13.61%). The eggs harvested from these batch spawns yielded 44,608,181 swim-up fry (26.6% hatching rate) and over half of the fry (59.3%; 26,466,026 fry) were produced from spawning trials where neither male or female striped bass were treated with any exogenous hormone compound. When considering all fry produced by the female striped bass that had participated in spawning, these females produced 220,833 fry per female (133,557 fry per female if all females are included; **Figure 3**). When expressed in the

341 context of total female broodstock biomass (mean weight 4.9 kg) this value is 45,068 fry/kg
342 bodyweight of participating females (N = 202 fish) or 27,257 fry/kg bodyweight of all females
343 included in the spawning trials (N = 334 fish).
344
345 The summary data for each of the nineteen batch spawning trials are provided in **Table 1**. The
346 conditioning data alongside egg harvesting data for each group of trials are presented in
347 **Supplementary Figures S.1 through S.4**. Specific conditioning and egg harvesting data for
348 each individual spawning trial are provided in **Supplementary Tables S.1 through S.19**.
349
350 *3.1 Determination of optimal sex ratio trials (1 - 7)*
351
352 The female striped bass participating in Trials 1 through 7 produced a total of 47,762,405 eggs
353 with a range of fertility from 0.8% to 69.1% that resulted in a total of 11,745,957 swim-up fry
354 (**Table 1, Figure 4**). The average percent of participating females in the trials conducted at an
355 approximately 1:1 sex ratio (Trials 1 through 3) was 64.1 $\pm$ 12.18%, which did not significantly
356 differ from the percent of females that spawned in the trials not conducted at an approximately
357 1:1 ratio (45.6 $\pm$ 22.19% of females had spawned in Trials 4 through 7; Student's *t*-test,
358 p=0.1098). The three trials conducted at an approximately 1:1 sex ratio produced an average of
359 9,394,743 $\pm$ 2,349,150 eggs per trial, which was significantly greater than the average eggs
360 produced per trial by those conducted at a 1:2 or 1:3 sex ratio (4,894,560 $\pm$ 1,427,603 eggs per
361 trial; Student's *t*-test p=0.0291). The fry production metrics did not follow a normal distribution
362 (Shapiro-Wilks test, p=0.0305), however, these values did vary significantly between sex ratio

363 treatment groups (3,148,903 ± 1,465,453 fry per trial for 1:1 ratio trials and 574,812 ± 240,775
364 fry per trial for 1:2 and 1:3 ratio trials; Wilcoxon Exact test p=0.0286).
365
366 Nine females were eligible to receive hCG treatment at the conclusion of Trials 2 and 3. These
367 fish were tank spawned to produce a total of 8,186,615 eggs and 1,555,434 swim-up fry.
368 However, one of these nine females was found to have died egg-bound in the tank.
369
370 *3.2 Requirement of male hormone induction trials (8 - 11)*
371
372 The female striped bass participating in Trials 8 through 11 produced a total of 34,461,135 eggs
373 with a range of fertility from 3.8% to 76.0% that resulted in a total of 11,156,191 swim-up fry
374 (**Table 1, Figure 4**). The numbers of eggs and fry produced in these trials are slightly reduced
375 values of the actual production, as there was an egg collector overflow during the spawning and
376 egg harvesting period for Trial 9. A slightly greater percentage of the females included in batches
377 with hCG-treated males (23 of 40 females, 57.5%) were found to have participated in spawning
378 compared to the trials where males were left untreated (17 of 39 females, 43.6%) (**Figure 4**). The
379 trials with hCG-treated males also produced more eggs (18,232,035) and swim-up fry
380 (6,398,198) than the trials without hCG-treated males (these trials produced 16,229,100 eggs and
381 4,757,993 swim-up fry) (**Figure 4**). Statistical comparisons were not conducted as there were
382 only two replicates for each of these treatment groups.
383
384 Nineteen females were eligible to receive hCG treatment at the conclusion of Trials 8 through
385 10. Ten of these females successfully tank spawned to produce a total of 11,260,880 eggs and

2,107,443 swim-up fry; the remaining nine did not produce viable eggs or fry due to being overripe or failure to release eggs. Eight females from Trial 11 were eligible to receive hCG treatment and were batch spawned with twenty hCG-treated males to produce 7,642,500 eggs and 2,519,247 swim-up fry. These data are not included with the data for the nineteen batch spawn trials reported in **Table 1**.

3.3 *Determination of critical mass trials (12 - 15)*

Trial 12 included the fewest number of fish (eight females and eight males) and none of the females had released any eggs by the conclusion of the trial (**Table 1, Figure 4**). The success of spawning participation and viable fry production of the trials conducted at a 10:10 female to male sex ratio (Trials 13 through 15) suggests a critical mass threshold for these batch spawning methods. These trials produced a total of 11,942,625 eggs (range of fertility: 0.0% to 57.4%) that resulted in a total of 1,198,434 swim-up fry (**Table 1, Figure 4**).

3.4 Scalability of production trials (16 - 19)

Trials 16 through 19 produced a total of 73,569,745 eggs (range of fertility: 9.1% to 71.4%) that resulted in a total of 20,509,599 swim-up fry (**Table 1**). This group of trials had the greatest percentage of females that spawned, number of eggs produced, and number of fry produced compared to the other groups (**Figure 4**). The total biomass of females that had participated in these spawning trials (N = 83 of 100 females; mean weight was 5.1 kg) was 423.3 kg and therefore produced an average of 48,447 fry/kg. When considering all of the females included in

409 the four trials (N = 100 females; 510.0 kg total biomass), the average fry produced per kg was
410 40,211 (**Figure 3**).
411
412 *3.5 Microsatellite genotyping*
413
414 Genotyping and parentage assignment of larvae sampled from those produced in Trial 4 (N =
415 119) showed that twenty-seven of the thirty-one males (87.1%) and twelve of the sixteen females
416 (75.0%) included in the trial successfully reproduced and contributed to producing sampled
417 larvae (**Table 1, Figure 5**). Each individual male contributed to 3.7 $\pm$ 2.49% of all larvae
418 sampled on average and each female contributed to an average of 8.3 $\pm$ 5.83% of all larvae
419 sampled (**Figure 5**). The parentage data also showed that male striped bass reproduced with an
420 average of 2.3 $\pm$ 1.41 females with a range between one to six females, and female striped bass
421 successfully reproduced with an average of 3.2 $\pm$ 3.13 males with a range between two to
422 eighteen males. The raw parental contribution to sampled offspring data can be found in
423 **Supplementary Figure S.1 and Tables S.20 and S.21**. Considering only the twenty-seven male
424 and twelve female striped bass individuals identified as having contributed to the sampled
425 offspring, the effective broodstock size (N_b) was found to be 33 (33.23). The effective
426 broodstock size value corrected for the multiple spawning partners and unequal family sizes
427 (N_b^V) was 28 (27.98).
428
429 *3.6 Stage of ovarian maturation throughout batch spawning*
430

The spawning activity of females in Trials 14 through 18 that were tagged with different colored yarn based on ovary stage (N = 95) is shown in **Figure 6**. All females included in these trials that were tagged at the 10 Bayless hour stage (red tags; n = 5) spawned within three days of males being moved into the batch spawning tank. All of the females tagged at the 11-12 Bayless hour stage (orange tags; n = 33) spawned within four days of males being moved into the spawning tank. Just over half of these 11-12 Bayless hour females spawned within the first two days (n = 17 of 33). Most of the females tagged at the 13-14 Bayless hour stage (yellow tags; n = 24) spawned between two and five days after males were moved into the spawning tank; three failed to participate in spawning. Females tagged at the 15 Bayless hour stage (green tags; n = 22) that participated in spawning did so within four to five days after males had been moved into the tank and the remainder failed to participate (n = 12 of 22 failed). Four females tagged as ineligible (blue tags; n = 11) participated in spawning, two within three days of males being moved into the spawning tank (Trial 17) and two within seven days (Trial 18).

4. Discussion

The results of the eighteen successful batch spawning trials are the first reported confirmation that domestic striped bass females and males are able to volitionally spawn in large batch spawning tanks (7,047 L - 31,139 L) and yield appreciable fry production after only photothermal and salinity conditioning. All attempts to spawn female striped bass in captivity without the use of exogenous hormones prior to the reported success by Andersen et al. (2020) had been met with marginal success or failure (Woods et al. 1990; Woods et al. 1995; Sullivan et al. 1997). In the “pace-set” approach described by Andersen et al. (2020), single untreated

454 female striped bass were found to volitionally spawn when in the presence of several male
455 striped bass and one female striped bass that had been treated with exogenous hormone
456 compound (hCG or GnRHa) was thought to be the "pace-setter" of spawning behavior. The
457 successful spawning of these untreated female striped bass was attributed in part to behavioral
458 and/or pheromone cues that promote completion of ovarian maturation and/or ovulation in
459 captivity, as described in other fish species such as the goldfish (*Carassius auratus*) (Dulka et al.
460 1987; Stacey et al. 2003).
461
462 The second group of spawning trials (Trials 8 through 11) demonstrate that domestic male
463 striped bass are able to participate in volitional batch spawning without receiving any exogenous
464 hormone treatment. The ability of both male and female striped bass to do so is similar to that for
465 Atlantic cod (*Gadus morhua*), where untreated males and females volitionally spawned in a large
466 batch after being conditioned by photoperiod regime (Herlin et al. 2008). The previous
467 contentions on using hCG to treat male striped bass for spawning were that the hormone may
468 induce spawning behavior and support the hydration of milt, and was thus believed to aid in
469 fertilization rate during tank spawning (Rees and Harrell 1990; Woods et al. 1992). However, the
470 batch spawns where male striped bass did not receive hCG treatment did not yield dramatic
471 differences in egg or fry production compared to those trials with male striped bass that did
472 receive treatment. The elimination of hCG use during spawning comes with a cost benefit of
473 approximately $4.30 per female and $2.00 per male fish based upon hormone dose per average
474 body weight (Andersen et al. 2020).
475

476 The observed spawning behavior of untreated striped bass may have been in part due to
477 domestication (Andersen et al. 2020). The striped bass included in this study have been bred in
478 captivity for five or more generations; a feature of such domestication is the directed, active
479 selection of individuals that perform well in culture and passive selection out of individuals that
480 may not adapt to culture conditions (Reading et al. 2018). Therefore it is possible that the
481 domestication of these fish has led to an increased tolerance of culture conditions and subsequent
482 improved ability to reproduce in captivity and without induction by exogenous hormone
483 compound. Although additional studies are required for confirmation, if proven true, this trait of
484 domesticated striped bass broodstock may further facilitate the transfer of these fish from the
485 breeding program to industry stakeholders as this reproductive characteristic may increase the
486 demand for and value of the fish (Reading et al. 2018). However, it is still ultimately uncertain
487 whether the volitional tank spawning behavior is a function of husbandry, domestication, or a
488 combination of both. Further research will need to be conducted using wild-captured striped bass
489 broodstock and these methods described here to determine if striped bass from wild populations
490 are similarly able to batch spawn without exogenous hormone compound treatments.
491
492 The findings of these spawning trials provide a framework for optimizing commercial scale
493 production of striped bass fry that is more consistent (i.e., reliable) and less labor intensive.
494 Chiefly, the spawning activity observed in the trials serves to validate the generalized
495 conditioning protocol presented in **Figure 1**, as this activity occurred shortly after target
496 temperature and salinity were reached (these data are presented in **Supplementary Figures S.1**
497 **through S.4 and Tables S.1 through S.19**). The first group of trials (Trials 1 through 7) suggest
498 that a favorable sex ratio for batch spawning is one female to one male, as these spawns

499 generally produced a greater number of eggs and fry. This finding suggests that reproductive
500 behaviors of striped bass may be negatively impacted by a male-biased sex ratio, or, conversely,
501 are positively impacted by an approximately equal sex ratio. Similar trends favoring 1:1 sex
502 ratios have been observed in other fish species when sex ratios biased towards one sex shift
503 competition and mate availability dynamics, among others (Maskill et al. 2017). For example,
504 male-biased sex ratios resulted in increased competitive male behavior and decreased responses
505 from females in sand gobies (*Pomatoschistus minutus*) and guppies (*Poecilia reticulata*),
506 respectively (Kvarnemo et al. 1995; Jirotkul 1999). Additionally, using an approximately 1:1 sex
507 ratio would limit the costs required to maintain a greater number of male striped bass broodstock
508 for a male-biased sex ratio.
509
510 The results of the third group of trials (Trials 12 through 15) suggest that the inclusion of at least
511 ten fish of each sex may be required to elicit volitional spawning of striped bass in batch
512 spawning conditions without exogenous hormone induction. It is possible that the minimum
513 required number of striped bass of each sex present in a batch spawning system is fewer than ten,
514 as only a single trial, Trial 12, included fewer fish of each sex (eight of each) and, as such,
515 additional trials will be required to verify this (e.g., additional replicates conducted at 8:8 female
516 to male ratio or spawning trials conducted at 9:9 female to male ratio). The results of Trials 1
517 through 11 indicate that including around twenty fish of each sex typically results in at least half
518 of the females spawning to produce between one and nearly four million fry (**Table 1**). In Trials
519 16 through 19 it was shown that increasing this number slightly to twenty-five fish of each sex
520 can greatly increase production and spawning participation by females, such that nearly twice as
521 many eggs are ovulated and two to three times (200.0% to 300.0%) as many fry are produced

522 (**Table 1**). This remarkable scaling indicates that commercial fry production using these methods
523 is possible and may only require fifty broodstock fish for each spawning unit. A continuation of
524 this study would be to include more fish in each reproductive batch spawning unit (i.e., thirty of
525 each sex or more). However, if those spawning units produce a much greater number of fry per
526 unit of broodstock biomass this effort would likely be well above the present industry demand
527 for domestic striped bass larval production.
528
529 The microsatellite genotyping and parentage assignment of the fish and offspring included in and
530 produced from Trial 4 (**Figure 5**) demonstrate that the striped bass are promiscuous (i.e., will
531 reproduce with many partners; females with between two and eighteen males and males with
532 between one and six females) and that the male striped bass may contribute to multiple, distinct
533 spawning events over a given time period when under the conditions described here
534 (**Supplementary Figure S.5**). This also suggests that wild spawning populations of striped bass
535 are capable of maintaining considerable genetic diversity, as this captive batch spawning
536 behavior likely reflects what they are doing in the wild (e.g., spawning aggregations) (Salek et al.
537 2001). The determined effective broodstock size (N_b) and the corrected value ($N_b{}^V$) that accounts
538 for the multiple spawning partners observed with unequal family sizes is 33 and 28, respectively.
539 This batch spawning trial (Trial 4) included 16 females and 31 males (an approximate 1:2 female
540 to male sex ratio) and the N_b and $N_b{}^V$ reported represent high genetic diversity among offspring.
541 These values are similar to the N_b reported by Andersen et al. (2020), which was 29 (28.89)
542 when ten tank spawning units each including one female and three male striped bass were
543 considered as a single unit (10 females and 30 males, a 1:3 female to male sex ratio). The N_b and
544 $N_b{}^V$ values are more sensitive to the number of broodstock fish in the tank rather than sex ratio

545 adjustments. For example, *The National Breeding Program* utilizes a mass selection spawning
546 strategy whereby maximum genetic diversity is desired and then selection pressure is applied for
547 a given trait(s) (e.g., growth performance) (Reading et al., 2018). Increasing the number of
548 broodstock fish while maintaining an approximate 1:1 sex ratio is optimal for the mass selection
549 strategy, however, manipulating the sex ratio (e.g., by increasing the number of males) may be
550 more appropriate for commercial production where genetic diversity of a single year class is not
551 a chief concern. Manipulating sex ratios would be similarly useful if a future strain selection
552 strategy was employed in the breeding program.
553
554 When all females included in the trials here are considered (N = 334 females), these fish
555 produced 27,257 fry/kg female biomass, which increased to 45,068 fry/kg female when only
556 those females found to have participated were considered (N = 202 females). The latter value is
557 comparable to that found by Hodson et al. (1999), who report that the successful spawning of
558 hormone-treated striped bass females yielded 43,480 fry/kg female biomass. However, Andersen
559 et al. (2020) found that traditional tank spawning methods of striped bass (one female and three
560 males, all treated with exogenous hormone) produced 10,788 fry/kg female biomass and 13,520
561 fry/kg female biomass when the included females were treated with GnRHa and hCG,
562 respectively. Ranges of egg fertility in the present and these previous studies were quite broad
563 and hatching rates were variable, which is characteristic of striped bass females generally
564 (Chapman et al. 2014). However, the overall hatching rate of 26.6% reported here is considered
565 quite reasonable for striped bass spawning, as the range of hatching rates observed from
566 hormone-treated striped bass that were tank spawned by Andersen et al. (2020) was 6.7 to 21.4%
567 and those tank spawned by Hodson and Sullivan (1993) was 14.5 to 76.1%. The “pace-set” trials

568 described by Andersen et al. (2020) produced slightly more fry/kg female biomass than the
569 traditional tank spawning trials, where the trials including a pace-set female treated with GnRHa
570 produced 26,126 fry/kg and those trials including a pace-set female treated with hCG produced
571 54,068 fry/kg. If employing the batch spawning strategy described here, we estimate using ~
572 40.0 kg of four year old domestic striped bass female broodstock (~ 8 female fish at
573 approximately 4.9 kg/fish) for every one million fry desired for production.
574
575 The findings herein support integrating batch spawn methods into protocols for commercial
576 production of domestic striped bass. In addition, our findings support the inclusion of female
577 striped bass that would have previously been deemed "ineligible" for spawning (i.e., staged at
578 greater than 15 Bayless hours), even with exogenous hormone treatment (Grizzle et al. 1995;
579 Bayless, 1972; Rees and Harrell 1990). This was demonstrated in the spawning trials in which
580 female fish were tagged with different colored yarn according to their Bayless hour stage at the
581 start of the spawning trials (**Figure 6**). These trials showed that those fish which were further
582 along in ovarian maturation were those that typically spawned earliest in each batch spawn as
583 expected. Remarkably however, the trials also showed that there were instances of volitional
584 releases of eggs from those female fish deemed "ineligible" ($\geq$ 15 hour Bayless stage) for
585 spawning according to previously established protocols. This is the first report of successful
586 spawning of untreated female striped bass that were deemed ineligible to respond to hCG
587 (Hodson and Sullivan 1993). Fish designated as ineligible typically proceed through ovarian
588 maturation to the 10 to 12 Bayless hour stage where they cease further progression. The cause of
589 this failure to progress through ovarian maturation is unknown, although it may be related to
590 stress or other factors. However, the findings reported here suggest that it may be possible to

591 spawn these particular female fish at greater frequency in trials of longer duration or according to
592 the pace-set spawning method described in Andersen et al. (2020). Additionally, these previously
593 ineligible fish are potentially optimal candidates for hCG-induced strip spawning to produce
594 original cross hybrid striped bass.
595
596 The findings reported here support the use of a generalized thermal and salinity cycle as shown
597 in **Figure 1** to successfully elicit spawning behavior in domestic striped bass males and females
598 without any use of hCG or GnRHa when at least ten fish of each sex are placed together in large
599 batch spawning tanks. If these protocols are followed correctly, the domestic striped bass
600 broodstock should volitionally spawn without any use of hCG or GnRHa. This has been
601 demonstrated annually since 2014 by the practices used in the *National Program for Genetic*
602 *Improvement and Selective Breeding for the Hybrid Striped Bass Industry*, whereby striped bass
603 have been effectively and consistently spawned using the batch spawning protocols described
604 here, such that most recently all of the fish were spawned without any exogenous hormone
605 treatment (**Figure 7**). In addition to simplifying husbandry practices and eliminating the use of
606 hormones, these spawning practices show an increased efficiency of fry production compared to
607 traditional methods that require hormone induction procedures of a similar number of broodstock
608 animals. The technical difficulty in effectively and reliably reproducing striped bass females in
609 captivity to produce commercially-scalable numbers of fry has been a major limitation in the
610 establishment of a striped bass aquaculture industry. The combinatorial approach of batch
611 spawning and later treating any remaining eligible female fish with exogenous hormone
612 compound also lends itself to an assembly line of production for the hybrid striped bass industry.
613 Domestication may have contributed to correcting this problem along with development of this

614 batch spawning protocol described here. Further attention should also be paid to synchronizing
615 the fish or reducing the timeframe to spawning and the key to this may be in understanding the
616 behavioral and/or pheromone cues required to induce this volitional batch spawning behavior.

Acknowledgements

The authors thank Dr. Craig V. Sullivan for his longstanding contributions to the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry*. The authors would also like to thank B.J. Runde for providing graphical consultation. This work was supported by funding provided from the following sources: The United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), Northeastern Regional Aquaculture Center [USDA 14-078], Foundation for Food and Agriculture Research New Innovator Award (FFAR), and North Carolina Sea Grant (R/MG-1411). The striped bass is a priority species for the USDA National Research Support Project 8 (NRSP-8; National Animal Genome Project) and funding to support the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* was provided by the NRSP-8 and the USDA NIFA (Hatch Multistate Project), the USDA Agricultural Research Service (ARS, SNARC), the North Carolina College of Agriculture and Life Sciences, the North Carolina Agricultural Foundation William White Endowment, and various industry stakeholders including Premex, ADM, Clay Chappell, Locals Seafood, and several others who wish to remain anonymous. This is publication number 121 from the North Carolina State University Pamlico Aquaculture Field Laboratory.

Declaration of Competing Interest

The authors declare that there is no conflict of interest in this work.

640 **References**

641

642 Andersen, L.K., R.W. Clark, A.S. McGinty, M.S. Hopper, L.W. Kenter, S.A. Salger, J. Schilling,
643 R.G. Hodson, A.I. Kovach, D.L. Berlinsky, B.J. Reading. 2020. Volitional tank spawning of
644 domestic striped bass (*Morone saxatilis*) using human chorionic gonadotropin (hCG) and
645 gonadotropin releasing hormone analogue (GnRHa)- induced 'pace-setting' females.
646 Aquaculture, *in press*. https://doi.org/10.1016/j.aquaculture.2020.735967.

647

648 Bayless, J.D. 1972. Artificial propagation and hybridization of striped bass, *Morone saxatilis*
649 (Walbaum). South Carolina Wildlife Resources Department.

650

651 Chapman, R.W., B.J. Reading, C.V. Sullivan. 2014. Ovary transcriptome profiling via artificial
652 intelligence reveals a transcriptomic fingerprint predicting egg quality in striped bass, *Morone*
653 *saxatilis*. PLoS One, *9*(5):e96818. https://doi.org/10.1371/journal.pone.0096818.

654

655 Couch, C.R., A.F. Garber, C.E. Rexroad III, J.M. Abrams, J.A. Stannard, M.E. Westerman, C.V.
656 Sullivan. 2006. Isolation and characterization of 149 novel microsatellite DNA markers for
657 striped bass, *Morone saxatilis*, and cross-species amplification in white bass, *Morone chrysops*,
658 and their hybrid. Molecular Ecology Notes, 6(3):667-669. https://doi.org/10.1111/j.1471-
659 8286.2006.01292.x.

660

661 Dulka, J.G., N.E. Stacey, P.W. Sorensen, G.J. Van Der Kraak. 1987. A steroid sex pheromone
662 synchronizes male–female spawning readiness in goldfish. Nature, 325(6101):251.
663 https://doi.org/10.1038/325251a0.
664
665 Frankel, T.E., D.D. Theisen, H.D. Guthrie, G.R. Welch, L.C. Woods III. 2013. The effect of
666 freezing rate on the quality of striped bass sperm. Theriogenology, 79(6):940-945.
667 https://doi.org/10.1016/j.theriogenology.2013.01.009.
668
669 Gall, G.A.E. 1987. Inbreeding. Pages 47-87 *in* N. Ryman, F. Utter, editors. Population genetics
670 and fishery management. University of Washington Press, Seattle, Washington, United States.
671
672 Garber, A.F., C.V. Sullivan. 2006. Selective breeding for the hybrid striped bass (*Morone*
673 *chrysops*, Rafinesque × *M. saxatilis*, Walbaum) industry: status and perspectives. Aquaculture
674 Research, 37(4):319-338. https://doi.org/10.1111/j.1365-2109.2005.01439.x.
675
676 Grizzle, J.M., D. Xu, W.A. Rogers. 1995. Efficacy of human chorionic gonadotropin for
677 spawning striped bass and white bass. Proceedings of the Southeastern Association of Fish and
678 Wildlife Agencies, (49):88-96.
679
680 Hallerman, E.M. 1994. Toward coordination and funding of long-term genetic improvement
681 programs for striped and hybrid bass *Morone* sp. Journal of the World Aquaculture
682 Society, 25(3):360-365. https://doi.org/10.1111/j.1749-7345.1994.tb00219.x.
683

684 Harrell, R.M. (Editor). 1997. Striped bass and other *Morone* culture. Developments in
685 Aquaculture and Fisheries Science (30). Elsevier, Amsterdam, The Netherlands.
686
687 Harrell, R.M., J.H. Kerby, R.V. Minton (Editors). 1990. Culture and propagation of striped bass
688 and its hybrids. American Fisheries Society, Bethesda, Maryland, United States.
689
690 Herlin, M., M. Delghandi, M. Wesmajervi, J.B. Taggart, B.J. McAndrew, D.J. Penman. 2008.
691 Analysis of the parental contribution to a group of fry from a single day of spawning from a
692 commercial Atlantic cod (*Gadus morhua*) breeding tank. Aquaculture, 274(2-4):218-224.
693 https://doi.org/10.1016/j.aquaculture.2007.11.034.
694
695 Hodson, R.G., R.W. Clark, M.S. Hopper, A.S. McGinty, G.M. Weber, C.V. Sullivan. 1999.
696 Reproduction of domesticated striped bass: commercial mass production of
697 fingerlings. Proceedings of the United States Japan Natural Resources (UJNR) Panel on
698 Aquaculture, (28):23-32.
699
700 Hodson, R.G., C.V. Sullivan. 1993. Induced maturation and spawning of domestic and wild
701 striped bass, *Morone saxatilis* (Walbaum), broodstock with implanted GnRH analogue and
702 injected hCG. Aquaculture Research, 24(3):389-398. https://doi.org/10.1111/j.1365-
703 2109.1993.tb00562.x.
704
705 Jirotkul, M. 1999. Operational sex ratio influences female preference and male–male competition
706 in guppies. Animal Behaviour, 58(2):287-294. https://doi.org/10.1006/anbe.1999.1149.

707

708 Jones, O.R., J. Wang. 2010. COLONY: a program for parentage and sibship inference from
709 multilocus genotype data. Molecular Ecology Resources, 10(3):551-555.
710 https://doi.org/10.1111/j.1755-0998.2009.02787.x.
711
712 Kenter, L.W., A.I. Kovach, L.C. Woods III, B.J. Reading, D.L. Berlinsky. 2018. Strain
713 evaluation of striped bass (*Morone saxatilis*) cultured at different salinities. Aquaculture, 492:
714 215-225. https://doi.org/10.1016/j.aquaculture.2018.04.017.
715
716 Kerby, J.H. 1986. Striped bass and striped bass hybrids. Pages 127-147 *in* R.R. Stickney, editor.
717 Culture of nonsalmonid freshwater fishes. CRC Press LLC, Boca Raton, Florida, United States.
718
719 Kvarnemo, C., E. Forsgren, C. Magnhagen. 1995. Effects of sex ratio on intra- and inter-sexual
720 behaviour in sand gobies. Animal Behaviour, 50(6):1455-1461.
721 https://doi.org/10.1016/0003-3472(95)80002-6.
722
723 Marshall, T.C., J.B.K.E. Slate, L.E.B. Kruuk, J.M. Pemberton. 1998. Statistical confidence for
724 likelihood-based paternity inference in natural populations. Molecular Ecology, 7(5):639-655.
725 https://doi.org/10.1046/j.1365-294x.1998.00374.x.
726
727 Maskill, P.A., I.R. Miller, L.J. Halvorson, H.B. Treanor, C.W. Fraser, M.A. Webb. 2017. Role of
728 sex ratio and density on fertilization success of intensively cultured endangered woundfin.

729 Journal of Fish and Wildlife Management, 8(1):249-254. https://doi.org/10.3996/062016-JFWM-
730 048.
731
732 McCraren, J.P. (Editor). 1984. The aquaculture of striped bass: a proceedings. University of
733 Maryland Sea Grant Publication, College Park, Maryland, United States.
734
735 Mullis, A.W., J.M. Smith. 1990. Artificial spawning and fry production of striped bass and in
736 hybrids. Pages 7-16 *in* R.M. Harrell, J.H. Kerby, R.V. Minton, editors. Culture and propagation
737 of striped bass and its hybrids. American Fisheries Society, Bethesda, Maryland, United States.
738
739 Mylonas, C.C., Y. Magnus, A. Gissis, Y. Klebanov, Y. Zohar. 1996. Application of controlled-
740 release, GnRHa-delivery systems in commercial production of white bass x striped bass hybrids
741 (sunshine bass), using captive broodstocks. Aquaculture, 140(3):265-280.
742 https://doi.org/10.1016/0044-8486(95)01178-1.
743
744 National Research Council (NRC). 1996. Guide for the care and use of laboratory animals. The
745 National Academies Press, Washington, DC, United States. https://doi.org/10.17226/5140.
746
747 Reading, B.J., A.S. McGinty, R.W. Clark, M.S. Hopper, L.C. Woods III, D.A. Baltzegar. 2018.
748 Genomic enablement of temperate bass aquaculture (family *Moronidae*). *In* H. Wang, X. Liang,
749 editors. Breeding and culture of perch and bass. Science China Press (Chinese Academy of
750 Sciences).
751

752 Rees, R.A., R.M. Harrell. 1990. Artificial spawning and fry production of striped bass and in
753 hybrids. Pages 43-72 *in* R.M. Harrell, J.H. Kerby, R.V. Minton, editors. Culture and propagation
754 of striped bass and its hybrids. American Fisheries Society, Bethesda, Maryland, United States.
755
756 Rexroad, C., R. Vallejo, I. Coulibaly, C. Couch, A. Garber, M. Westerman, C.V. Sullivan. 2006.
757 Identification and characterization of microsatellites for striped bass from repeat-enriched
758 libraries. Conservation Genetics, 7(6):971-982. https://doi.org/10.1007/s10592-006-9122-0.
759
760 Salek, S.J., J. Godwin, C.V. Sullivan, N.E. Stacey. 2001. Courtship and tank spawning behavior
761 of temperate basses (Genus *Morone*). Transactions of the American Fisheries Society,
762 130(5):833-847. https://doi.org/10.1577/1548-8659(2001)130<0833:CATSBO>2.0.CO;2.
763
764 Smith, T.I.J. 1989. The culture potential of striped bass and its hybrids. Journal of the World
765 Aquaculture Society, 20:32-38.
766
767 Smith, J.M., D.K. Whitehurst. 1990. Tank spawning methodology for the production of striped
768 bass. Pages 73-78 *in* R.M. Harrell, J.H. Kerby, R.V. Minton, editors. Culture and propagation of
769 striped bass and its hybrids. American Fisheries Society, Bethesda, Maryland, United States.
770
771 Stacey, N., A. Chojnacki, A. Narayanan, T. Cole, C. Murphy. 2003. Hormonally derived sex
772 pheromones in fish: exogenous cues and signals from gonad to brain. Canadian Journal of
773 Physiology and Pharmacology, 81(4):329-341. https://doi.org/10.1139/y03-024.
774

775 Sullivan, C.V., D.L. Berlinsky, R.G. Hodson. 1997. Chapter 2, Reproduction. Pages 11-74 *in*
776 R.M. Harrell, editor. Striped bass and other *Morone* culture. Elsevier Science Press, Amsterdam,
777 Netherlands.
778
779 Sullivan, C.V., N. Hiramatsu, A.M. Kennedy, R.W. Clark, G.M. Weber, T. Matsubara, A. Hara.
780 2003. Induced maturation and spawning: opportunities and applications for research on
781 oogenesis. Fish Physiology and Biochemistry, 28(1-4):481-486.
782 https://doi.org/10.1023/B:FISH.0000030635.92568.0a.
783
784 United States Department of Agriculture (USDA). 2019. 2017 Census of Agriculture, 2018
785 Census of Aquaculture, 3(2), Publication AC-17-SS-2.
786
787 Wang, J., E. Santiago, A. Caballero. 2016. Prediction and estimation of effective population
788 size. Heredity, 117(4):193-206. https://doi.org/10.1038/hdy.2016.43.
789
790 Watson, L.C. 1992. Temperature-modified prediction of ovulation for striped bass. The
791 Progressive Fish-Culturist, 54(3):189-195. https://doi.org/10.1577/1548-
792 8640(1992)054<0189:TMPOOF>2.3.CO;2.
793
794 Woods III, L. C. 2001. Domestication and strain evaluation of striped bass (*Morone*
795 *saxatilis*). Aquaculture, 202(3-4):343-350. https://doi.org/10.1016/S0044-8486(01)00783-9.
796

797 Woods III, L.C., R.O. Bennett, C.V. Sullivan. 1992. Reproduction of a domestic striped bass
798 brood stock. The Progressive Fish-Culturist, 54(3):184-188. https://doi.org/10.1577/1548-
799 8640(1992)054<0184:ROADSB>2.3.CO;2.
800
801 Woods III, L.C., C.C. Kohler, R.J. Sheehan, C.V. Sullivan. 1995. Volitional tank spawning of
802 female striped bass with male white bass produces hybrid offspring. Transactions of the
803 American Fisheries Society, 124(4):628-632. https://doi.org/10.1577/1548-
804 8659(1995)124<0628:VTSOFS>2.3.CO;2.
805
806 Woods III, L.C., C.V. Sullivan. 1993. Reproduction of striped bass, *Morone saxatilis*
807 (Walbaum), broodstock: monitoring maturation and hormonal induction of spawning.
808 Aquaculture Research, 24(2):211-222. https://doi.org/10.1111/j.1365-2109.1993.tb00543.x.
809
810 Woods III, L.C., J.G. Woiwode, M.A. McCarthy, D.D. Theisen, R.O. Bennett. 1990. Technical
811 notes: noninduced spawning of captive striped bass in tanks. The Progressive Fish-Culturist,
812 52(3):201-202. https://doi.org/10.1577/1548-8640(1990)052<0201:TNNSOC>2.3.CO;2.
813
814 Wright S. 1933. Inbreeding and homozygosis. Proceedings of the National Academy of Sciences
815 of the United States of America, 19:411–420.
816
817 Wright S. 1939. Statistical genetics in relation to evolution. Exposés de Biométrie et de
818 Statistique Biologique. Herman & Cie: Paris, France.
819

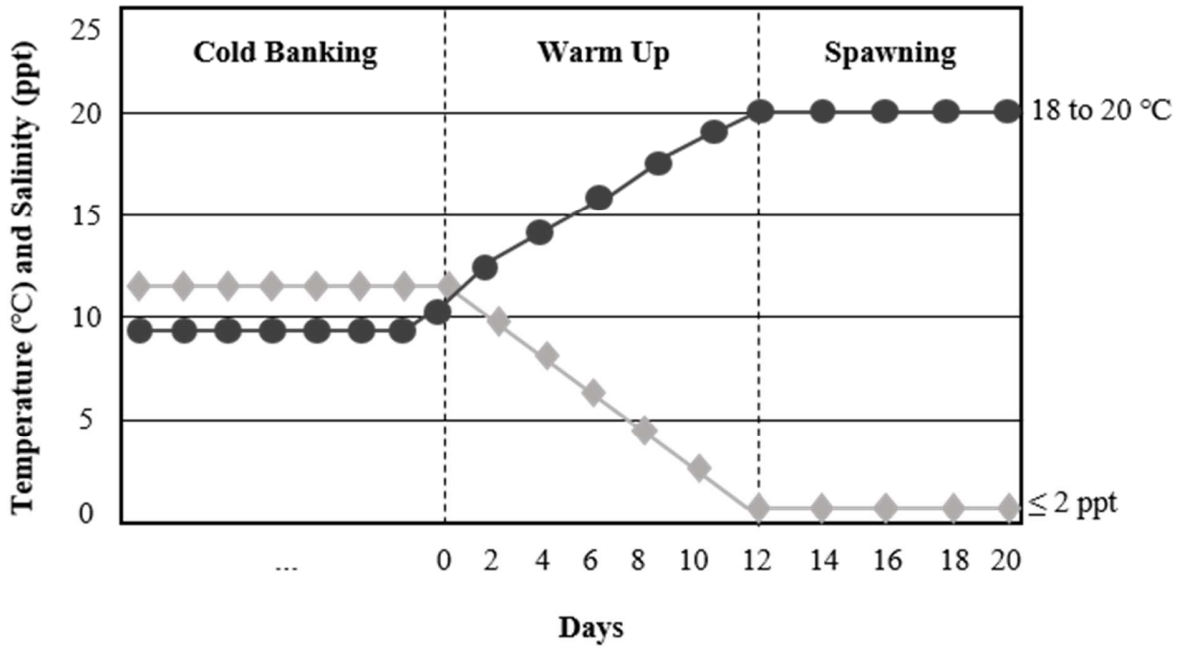
820 **Table 1.** Summary data for striped bass batch spawning trials. Trials 1 through 7 were designed
821 to determine the optimal sex ratios of fish, Trials 8 through 11 were to determine the requirement
822 of treating male striped bass with exogenous hormone compound, Trials 12 through 13 were to
823 determine the minimum number of striped bass required to elicit volitional spawning using the
824 batch spawn protocol, and Trials 16 through 19 were designed to evaluate commercial scalability
825 of these methods. Weights and Bayless hour stages of oocytes are reported as the mean ± SD
826 standard deviation if measured.
827
828 **Figure 1.** Generalized thermal and salinity parameters, including cold-banking (simulated winter
829 at 10-12°C), for striped bass batch spawn conditioning. Temperature is increased by 1°C per day
830 and salinity is decreased by 1 ppt per day to elicit volitional spawning behavior by striped bass
831 included in each batch spawning unit. Spawning is anticipated within 1 to 7 days after optimal
832 temperature and salinity are reached (indicated at the right).
833
834 **Figure 2.** Batch spawning tank system for striped bass. **(A)** The building that houses **(B)** two
835 31,139 L tanks equipped for batch spawning with the following: **(C)** an outer collar standpipe
836 (PVC, 203.2 mm diameter), **(D)** an inner standpipe with two rings of holes (20-30 mm in
837 diameter) to allow eggs to pass through to the outflow (PVC, 101.6 mm diameter), **(E)** three to
838 four air diffusers per tank, **(F)** dual egg collectors (200 L) each with **(G)** filter screens (500-1,000
839 μm) that enable egg collection by prohibiting passage through the outflow.
840
841 **Figure 3. (A)** Eggs produced per kilogram of female striped bass body weight in each batch
842 spawning trial (trial number and grouping are along the top and bottom, respectively). **(B)** Fry

843 produced per kilogram of female striped bass body weight in each batch spawning trial. Black
844 points in panels A and B represent the eggs and fry produced per female body weight when all
845 the females in a given trial are included and white points (outlined in black) represent the eggs
846 and fry produced per female body weight when only the females that were found to have
847 participated in spawning were included in the calculation of these metrics. In trials where
848 individual females were not weighed (*see*: Table 1), the reported average weight of the all
849 weighed females (n = 199 of 334 total female striped bass weight 4.9 $\pm$ 0.40 kg, values given as
850 mean $\pm$ SD) was used to calculate eggs and fry per kilogram of female body weight. Statistical
851 analyses were not conducted on these metrics.
852
853 **Figure 4.** Metrics used to evaluate batch spawning trials of striped bass by trial group and within
854 group treatment: **(A)** the percent of females that had participated in spawning; **(B)** the number of
855 eggs produced from each group or subgroup of trials; and **(C)** the number of fry produced.
856 Statistical comparisons were made between trials that were conducted at a 1:1 female to male sex
857 ratio and those that were not (1:2 or 1:3 female to male ratio) in the "Optimal Sex Ratio" group
858 and differing letters denote significant differences between groups (alpha = 0.05; Student's *t*-test
859 was used for metrics shown in panels **A** and **B**, the Wilcoxon Exact test was used to compare
860 metrics in panel **C**, as these data did not fit a normal distribution as determined by the Shapiro-
861 Wilks test). Statistical comparisons were not made within other trial groups as sample sizes did
862 not adequately permit doing so.
863
864 **Figure 5.** Percent contribution of the domestic striped bass males (outer circle) and females
865 (inner circle) included in Trial 4 to the larval offspring sampled (N = 119). Twenty-seven of the

866 thirty-one males and twelve of the sixteen females included in the trial were identified as having
867 contributed to the sampled larvae. The spawning data for Trial 4 can be found in **Table 1**. The
868 specific reproductive partners identified for each participating parental fish can be found in
869 **Supporting Information Figure S.1** and the raw percent contribution data for each parent can
870 be found in **Supporting Information Tables S.20** and **S.21.**
871
872 **Figure 6. (A)** The range (high and low) of temperature and salinity over time for striped bass
873 batch spawning Trials 14 through 18, in which female fish were tagged with colored yarn
874 according to their stage of ovarian maturation at the beginning of each trial and measured as
875 Bayless hour stage (Bayless 1972). The exact temperature and salinity for each day of the
876 spawning trials can be found in the **Supporting Information (S.14-18)**. **(B)** Timeline and
877 observed spawning of domestic female striped bass in each trial. Each trial was conducted at a
878 1:1 female to male sex ratio and none of the male or female fish were treated with exogenous
879 hormone compounds. Each individual arrow represents the spawning of a single female and the
880 color corresponds to the yarn tag given based on Bayless hour stage. **(C-I)** are representative
881 images of Bayless hour stages where **(C)** is 10 Bayless hours (red), **(D)** and **(E)** are 11 and 12
882 Bayless hours (orange), respectfully, **(F)** and **(G)** are 13 and 14 Bayless hours (yellow),
883 respectfully, **(H)** is 15 Bayless hours (green), and **(I)** is ineligible (blue).
884
885 **Figure 7.** Number of domestic striped bass fry produced (in millions) in the *National Program*
886 *for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* between
887 2014 and 2018 from parental fish that had been treated with gonadotropin releasing hormone

888 analog (GnRHa; dark grey), human chorionic gonadotropin (hCG) hormone (grey), or left
889 untreated with exogenous hormone compounds (light grey).
890

891



892

893 **Figure 1.**

894
895 **Figure 2.**

896

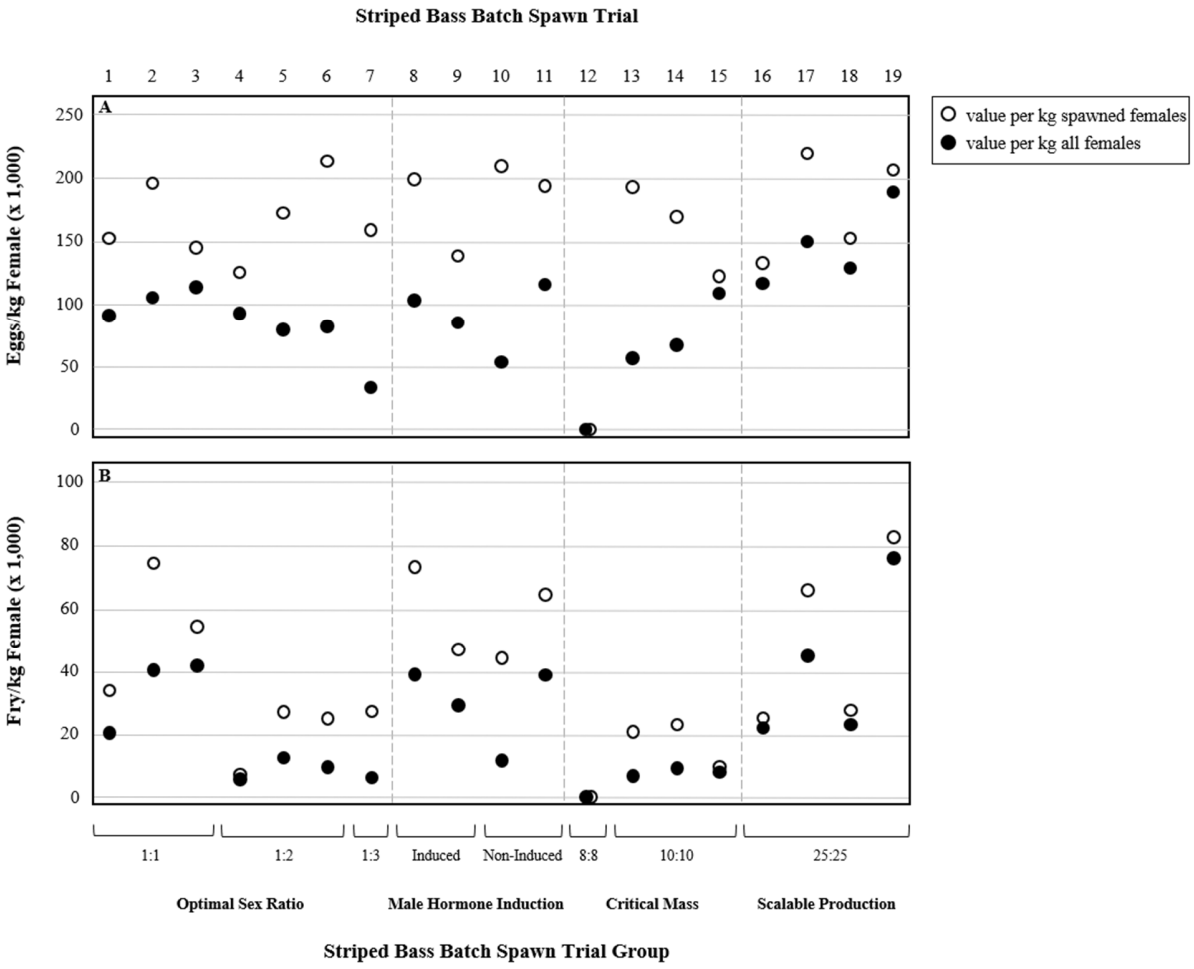

Striped Bass Batch Spawn Trial
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
A
B
Eggs/kg Female (x 1,000)
Fry/kg Female (x 1,000)
250
200
150
100
50
0
100
80
60
40
20
0
value per kg spawned females
value per kg all females
1:1
1:2
1:3
Induced
Non-Induced
8:8
10:10
25:25
Optimal Sex Ratio
Male Hormone Induction
Critical Mass
Scalable Production
Striped Bass Batch Spawn Trial Group

Figure 3.

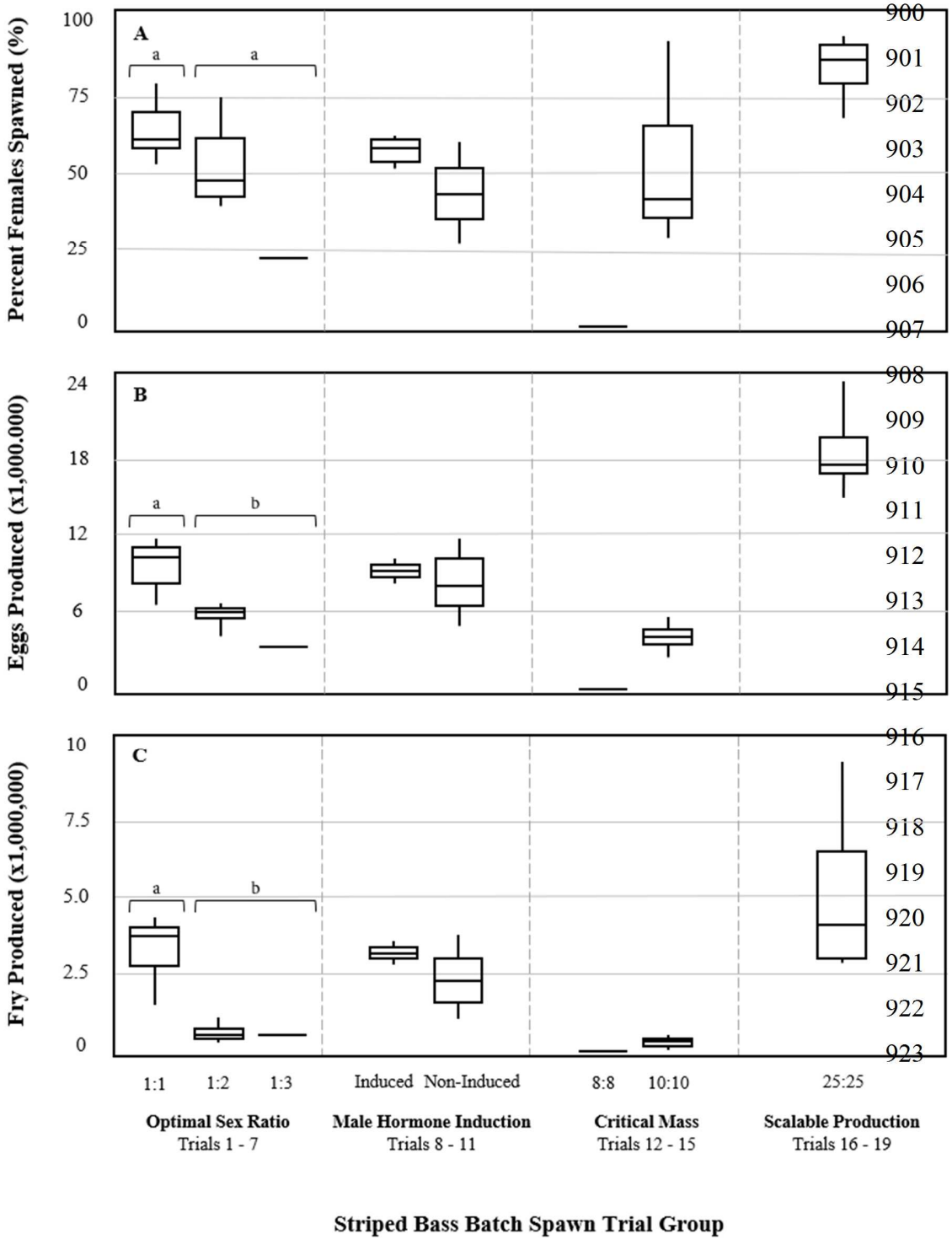
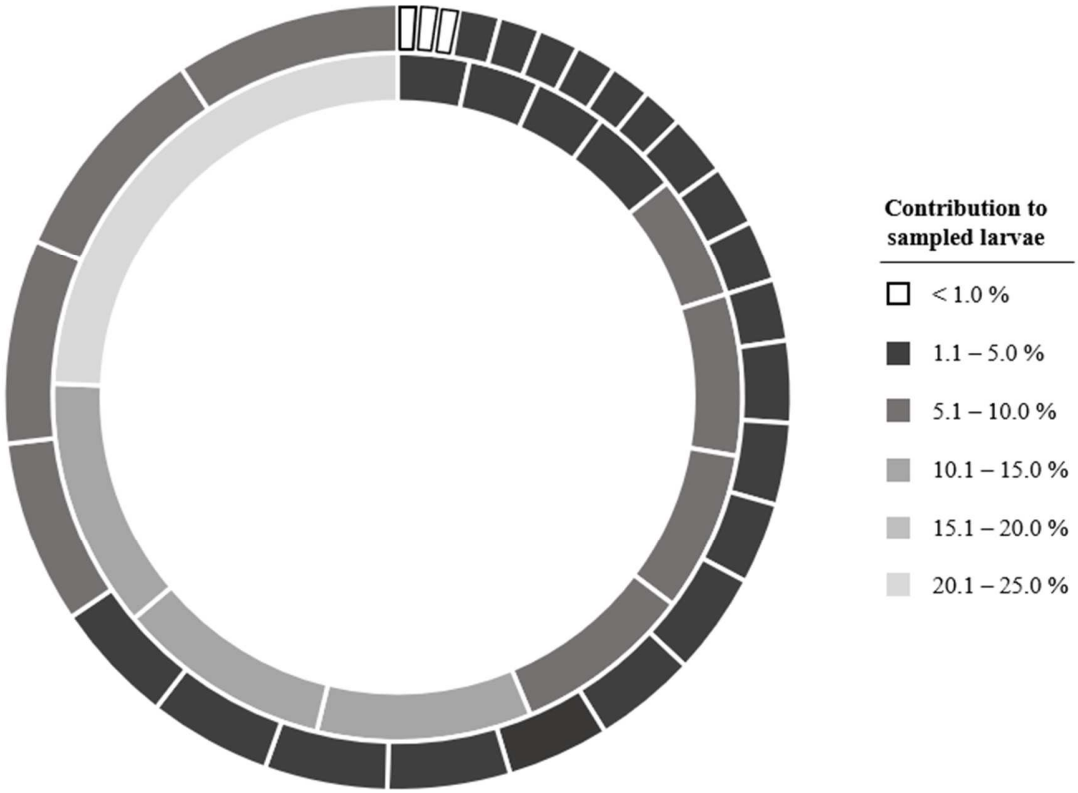

A
B
C
Percent Females Spawned (%)
Eggs Produced (x1,000,000)
Fry Produced (x1,000,000)
100
75
50
25
0
24
18
12
6
0
10
7.5
5.0
2.5
0
a
a
a
b
a
b
1:1
1:2
1:3
Induced
Non-Induced
8:8
10:10
25:25
Optimal Sex Ratio
Trials 1 - 7
Male Hormone Induction
Trials 8 - 11
Critical Mass
Trials 12 - 15
Scalable Production
Trials 16 - 19
Striped Bass Batch Spawn Trial Group

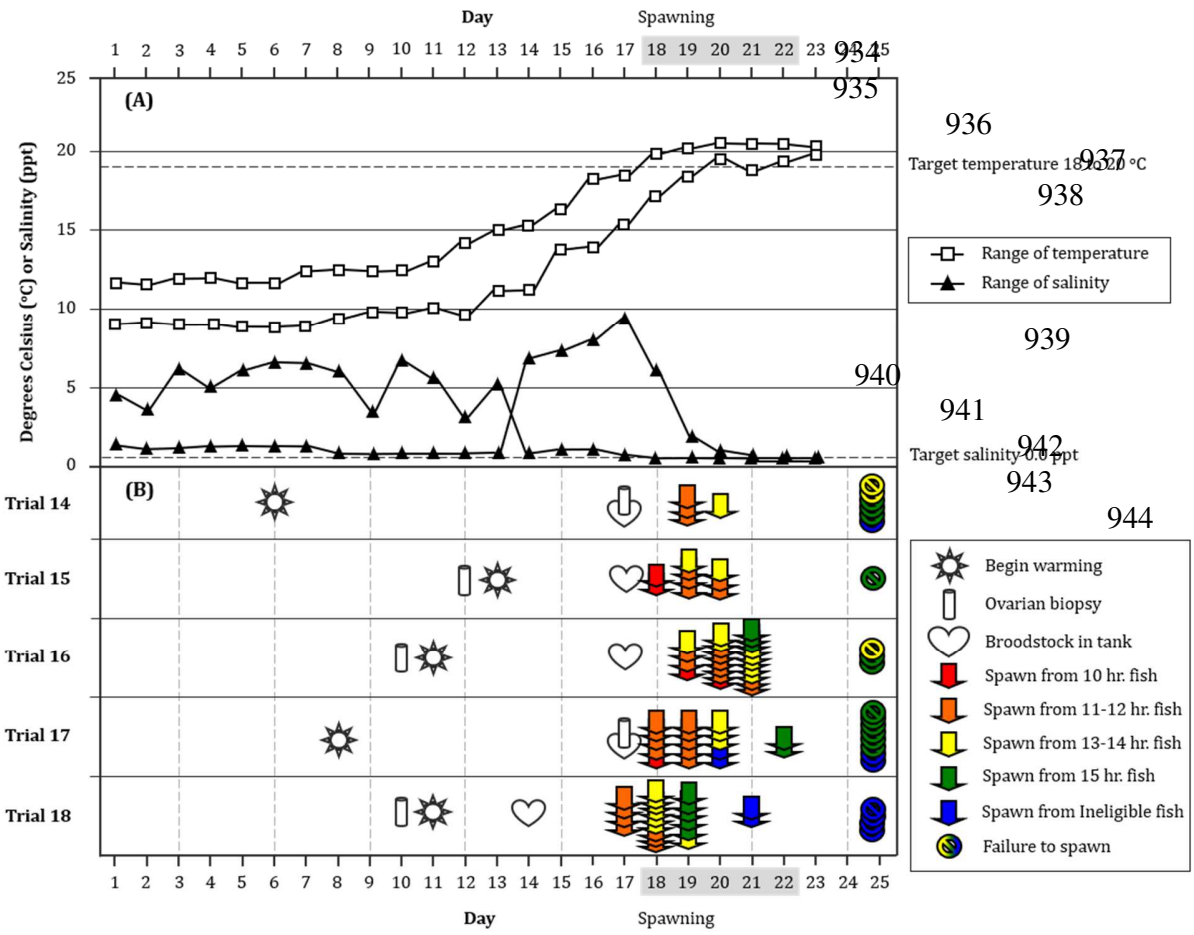
Figure 4.



930

931 **Figure 5.**

932


Day
Spawning
(A)
Degrees Celsius (°C) or Salinity (ppt)
Target temperature 18 to 20 °C
Range of temperature
Range of salinity
Target salinity 0.0 ppt
(B)
Trial 14
Trial 15
Trial 16
Trial 17
Trial 18
Begin warming
Ovarian biopsy
Broodstock in tank
Spawn from 10 hr. fish
Spawn from 11-12 hr. fish
Spawn from 13-14 hr. fish
Spawn from 15 hr. fish
Spawn from Ineligible fish
Failure to spawn
Day
Spawning

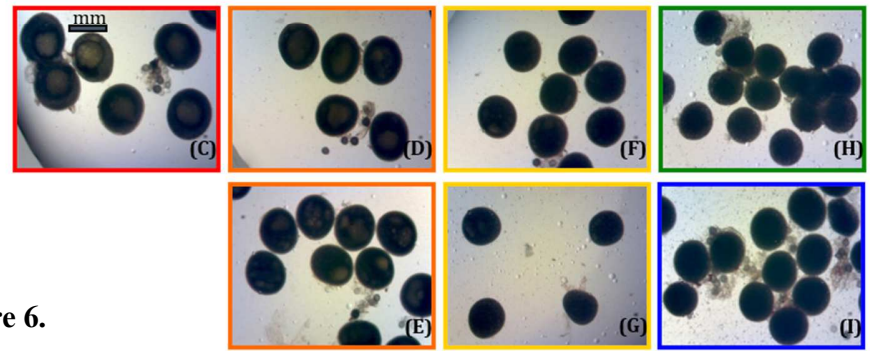
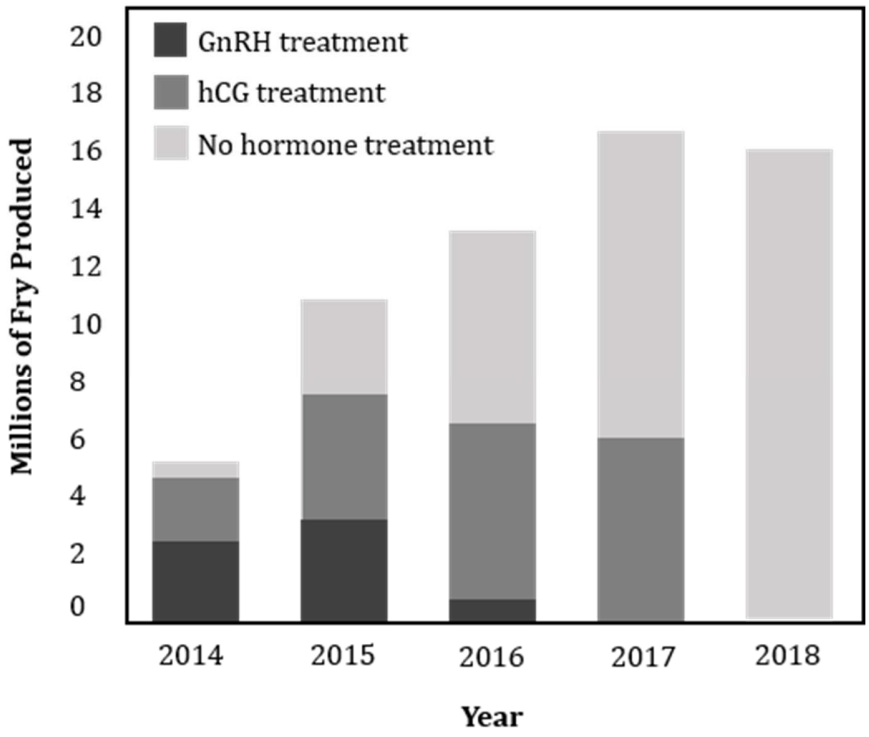

mm
(C)
(D)
(F)
(H)
(E)
(G)
(I)

Figure 6.



958
959 **Figure 7.**