Sea Grant Project Progress Report

Project Title: Sex Determination and Reversal in the Black Sea Bass (Centropristis striata).
Principle Investigator: David Berlinsky (Department of Zoology)

Introduction

Due to high consumer demand and limited seasonal availability, black sea bass (Centropristis striata) are an important culture candidate on the east coast and limited commercial production has been initiated. Previous Sea Grant research generated methodologies necessary for efficient spawning, larviculture and juvenile production of this species, but additional problems limit their widespread culture. In the wild, black sea bass are protogynous hermaphrodites, initially developing and spawning as females and changing to males at 3-5 years of age. In culture, however, juveniles may differentiate as males precociously, thereby diverting energy away from somatic growth and towards reproductive development. Further, wild-caught broodstock may undergo sex reversal at an accelerated rate in captivity, necessitating frequent broodstock replacement from wild stocks. These problems are currently limiting commercial production of black sea bass in New Hampshire and elsewhere in the US. The goal of this research is to gain understanding of the environmental and social conditions that influence sex differentiation and reversal in black sea bass. To this end, the following objectives were proposed:

Objectives

Objective 1. Determine the age/size at which sex differentiation occurs.

Objective 2. Examine the effect of rearing temperature and population density on sex differentiation

Objective 3. Determine the effect of photoperiod on sex differentiation and rate of sexual development.

Objective 4. Compare the relative growth rates of male and female black sea bass reared in captivity

Objective 5. Determine if social structure or density influences initiation of sex reversal in captive black sea bass females.
Research findings to date

During this first phase of research, experiments were conducted or initiated to address objectives 1, 2, 4 and 5.

Objective 1. Determine the age/size at which sex differentiation occurs.
Objective 2. Examine the effect of rearing temperature and population density on sex differentiation
Objective 4. Compare the relative growth rates of male and female black sea bass reared in captivity

Black sea bass broodstock were induced to spawn at Great Bay Aquaculture (Portsmouth NH) during their annual spawning season (May-June) in 2003. Following hatching, larvae were cultured statically in 78L glass aquaria at 21+/−0.5 °C for 10 days and fed enriched rotifers. At 10 days post hatch (dph), 400 larvae were transferred into each of 6-78 L tanks and maintained at 18 and 21 C (n=3 tanks/temperature).

Fish were raised statically at 18 and 21 C and fed rotifers enriched with a commercial supplement (Selco® !NVE, Salt Lake City, Utah), three times daily to maintain rotifer counts at 10 per mL. Ten liter (10 L) water changes were conducted daily. Fish were gradually weaned to newly hatched Artemia nauplii by day 30 post hatch and external mechanical filters (Whisper® power filter 40s, Blacksburg, VA) were applied to maintain water quality. All tanks were maintained on a 24-hour light:0-hour dark photoperiod (~110 lx).

Upon completion of metamorphosis (25 days), the fish were transferred to three independent recirculating systems containing 3-340 L, blue, polypropylene tanks, ultraviolet (UV) filtration (40 Watt), biological and mechanical filtration. Each system had independent temperature control and were maintained at 18, 21, and 27 C. Prior to transfer, a subsample of fish (n=5) from each of the nine tanks were sacrificed and gonads were examined histologically
for sex determination. Additional subsamples (n=5/tank) were examined 360 DPH and at the conclusion of the experiment (390 DPH).

Upon conclusion of the experiment, all fish were euthanized with an overdose of MS-222, weighed and measured to the nearest 0.1 g and millimeter, respectively. All fish were checked for spermatiation by application of abdominal pressure and the gonads were surgically removed, and preserved in 10 percent formalin for histology. Ten-twenty oocytes in the largest clutch were measured to the nearest micrometer using ImageJ analysis (National Institute of Health, Bethesda, MD).

Results

Unambiguous sex differentiation was not observed in all of the sampled fish until 360 DPH at which point the experiment was terminated. The fish were classified as males or females only if spermatogonia or oogonia were present in histological sections. Fish reared at 18 C developed as: 75% male, 25% female. Fish cultured at 21 C developed as 90% male, 10% female and fish reared at 27 C developed as 16% male, 84% female (Table 1).

<table>
<thead>
<tr>
<th>Temp (C)</th>
<th>Tank</th>
<th>%Males</th>
<th>Wt. Males (g)</th>
<th>Wt. Females (g)</th>
<th>% spermatiating</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1</td>
<td>66.7</td>
<td>69.9 +/- 30</td>
<td>59.4 +/- 22.1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72.9</td>
<td>79.8 +/- 25.1</td>
<td>57.7 +/- 13.8</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>86.1</td>
<td>72.2 +/- 21.0</td>
<td>98.8 +/- 11.2</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.2 +/- 9.9</td>
<td>74.0 +/- 5.2</td>
<td>72 +/- 15.7</td>
<td>53.8 +/- 6.6</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>94.4</td>
<td>62.9 +/- 23.9</td>
<td>98 +/- 32.1</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>92.1</td>
<td>73.5 +/- 23.2</td>
<td>90.2 +/- 27.4</td>
<td>70.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>85.7</td>
<td>65.2 +/- 26.8</td>
<td>58.9 +/- 9.5</td>
<td>94.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.7 +/- 4.5</td>
<td>67.2 +/- 5.6</td>
<td>82.4 +/- 28.7</td>
<td>86 +/- 13.5</td>
</tr>
<tr>
<td>27</td>
<td>7</td>
<td>9.1</td>
<td>115.1 +/- 29.5</td>
<td>67.9 +/- 18.9</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>20.8</td>
<td>100.9 +/- 19.1</td>
<td>87.0 +/- 31.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>19</td>
<td>129.0 +/- 78.5</td>
<td>96.7 +/- 33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.3 +/- 6.3</td>
<td>115.0 +/- 14.1</td>
<td>83.9 +/- 14.6</td>
<td>0</td>
</tr>
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</table>
Male fish reared at 27°C grew significantly greater than females at that temperature and greater than male fish raised at 18 or 21°C. This was likely influenced by the fact that males reared at 27°C were not spermiating and energy was devoted to somatic growth rather than reproduction. These results strongly suggest that black sea bass, like several other species of teleost fish, exhibit temperature-dependent sex determination.

**Objective 5. Determine if social structure or density influences initiation of sex reversal in captive black sea bass females.**

We previously demonstrated that sex change in black sea bass in captivity is influenced by sex ratio. Specifically, sex change was delayed or prevented when male fish were housed with females in small groups (n=8). We have expanded our investigation to determine if fish density as well as sex ratio influences the rate of sex change.

Post-spawning black sea bass were transported from GBA to the UNH Aquaculture Research center and housed in 1600 l tanks incorporated in a recirculating system. The fish were housed in 3 female:1 male ratio with 8 or 24 fish in each tank (n=3/sex ratio). Blood samples and ovarian biopsies were obtained from all females to verify initial reproductive stage and male fish were selected by the presence of expressible milt. The female fish will be bled and biopsied at 2 month intervals to assay for reproductive steroid hormones and follicular development. The experiment will be terminated at the onset of the next spawning season (May-June).

**Publications:**


**Student Involvement:**
Graduate students – Christopher Benton, James DeGraaf, Kate Strait
Undergraduate Students – Michael Ball, Stephanie Hillsgrove, Candice Brown, Jennifer Plouffe, Brian Hoopc, Laura Williams, Rebecca Heuss, Adam Argeropoulos