**Klibansky and Scharf batch fecundity methods**

**Abstract**

Batch fecundity data in the 2011 SEDAR for black sea bass (*Centropristis striata*) come from two separate sources and thus have been collected by different methods. This working paper documents methods by which batch fecundity data have been collected by Klibansky and Scharf. All samples were collected in Onslow in black sea bass traps with commercial fisherman Tom Burgess aboard the FV Barbara Lynne. Fishing was conducted on three main ledges 0.3-1.2m in height, in 12-16m, 18-22m, and 27-31m depths, from approximately 8-56km from shore. Fish were selected uniformly from the entire size range of the catch and females confirmed histologically to be ripe and running were assessed for fecundity analysis. Batch fecundity was determined using image analysis. Data from this research are being combined with data collected at SCDNR and thus will be included in a separate document.

**Methods**

***Sample collection***

Batch fecundity data in the 2011 SEDAR for black sea bass (*Centropristis striata*) come from research at the College of Charleston (Danson 2009), and also from ongoing research on reproductive biology of *C. striata* being conducted by Klibansky and Scharf at UNCW. The data will be combined but have been collected by different methods. This working paper documents methods by which batch fecundity data have been collected by Klibansky and Scharf.

Samples were collected in Onslow Bay, North Carolina near the northern end of the south Atlantic region in two consecutive years, over a period of six months encompassing the entire spawning season. Port sampling was began in January and occurred on a bi-weekly basis until developing fish (Table 1, code L) were abundant in catches indicating that spawning was soon to begin. At this point, sampling at sea began through cooperation with local sea bass fisherman Tom Burgess (TB) at intervals of one to two weeks throughout each spawning season. Once evidence of spawning activity in the catches was minimal, sampling at sea ceased and port sampling resumed until two consecutive sampling dates showed that no spawning was occurring.

The overall sampling plan was adapted to maximize spatial and temporal resolution given the resources at hand. Lab observations (Cerda et al. 1996) and field observations (McGovern et al. 2002) show that *C. striata* appear to begin spawning around sunset. Since batch fecundity is estimated from specimens in which a full batch of hydrated oocytes can be counted, samples must be collected after oocytes are hydrated but before spawning begins thus sampling was focused during the day. Because the daily timing of spawning in past studies represents an average over many dates, and is variable, it is not likely to be a perfect estimator for any one date. Therefore, sampling was conducted for as long as possible on each trip to sea to maximize chances of collecting samples that were at the correct point in the maturity cycle to use for batch fecundity estimation.

Though sampling over a long period during the day is important, it is also valuable to collect samples over some spatial range. The south Atlantic stock is distributed over very large area, but resources only allowed for sampling to take place over a small portion of this area. After consulting with TB about logistical constraints, we decided that the maximum spatial range we could achieve was about 56km. *C. striata* are known throughout their range to be strongly associated with structure, and in Onslow Bay, the primary structures fished by TB are ledges 0.3-

1.2m in height running roughly parallel to shore. To achieve the best spatial/bathymetric contrast, samples were collected from three main ledges 0.3-1.2m in height, in 12-16m, 18-22m, and 27-31m depths.

On any sampling date, *C. striata* were caught using black sea bass traps (Fig. 6) baited with menhaden (*Brevoortia tyrannus*) or occasionally bluefish (*Pomatomus saltatrix*). On each date at each ledge, a group of 10 traps were deployed, spread out along a 1.8-3.7km section of the ledge. To ensure that a sufficient number of fish are caught, these traps were deployed not randomly, but at locations along a ledge where depth sounder readings suggested localized concentrations of fish. *C. striata* were selected uniformly from entire size range of the catch. In the lab all fish collected were macroscopically staged and ripe females (Table 1, code R) were set aside for possible use in batch fecundity estimates. All of these females were then processed and staged using histological methods following Harris and McGovern (1997). Only fish that were histologically classified as ripe and running (Table 2, code 3), and did not exhibit new postovulatory follicles were used to estimate batch fecundity.

***Estimating batch fecundity***

*Sub-sampling ovaries for fecundity*

The most intact lobe of a formalin preserved ovary was blotted dry with a paper towel until not visibly wet. Once ovary was dry a sample was removed from the posterior third (location 1) and the anterior third (location 3) of the ovary. The preserved ovary was sliced along a transverse plane, and 0.2 mL of ovarian material drawn up with 1 mL syringe, modified so that the tip has been cut off at the zero mark. The material was weighed to 0.0001g precision and stored in formalin.

*Scanning ovary sub-samples for image analysis*

Counting and measuring oocytes was accomplished using image analysis software with digital images captured using an Epson flatbed scanner. Samples were broken up by shaking and repeated pipetting. Once the oocytes were well separated, they were emptied into the Petri dish with formalin and swirled around to maximize dispersion. Then Petri dish was then placed in the center of the scanner glass, marked by a template, and scan an image using predefined settings (8.9 X 8.9 cm, 8-bit grayscale at 2400dpi, 8400 X 8400 pixels, uncompressed TIFF, 67.2 MB file size).

*Counting and measuring hydrated oocytes with digital image analysis*

The freeware program ImageJ was used to count and measure hydrated oocytes, however, images were first resized to 1200dpi using the photo editing program Picture Window Pro 4.0. Image analysis was accomplished by first writing three macros using the macro recorder feature in ImageJ, and adding them as buttons in the toolbar for fast and convenient use. The first macro did three main things: 1. inform ImageJ that the scale of the image is 1200 dpi and set the unit of measurement to microns based on a ratio of 0.047244 pixels/micron, 2. select a circular area inside the Petri dish and set the area outside that selection to the background color (black), 3. open the threshold window.

Thresholding is the process of choosing a range of grey values that will be used to distinguish objects of interest from the background and convert the grayscale image into a black and white, binary image. In this case thresholding was used to separate pale oocytes from the black background. Once the threshold range was chosen, the image was converted to binary.

Next a second macro was run which does five main things: 1. run the analyze particles program measuring particles with diameters of 800-1500 microns and circularity 0.8-1.0, 2. produce a data table of various measurements of particles analyzed and saves it in a text file, 3. make a composite image showing outlines of particles that were measured overlaid onto the original image, 4. save the composite image, 5. open the Cell Counter window. The image was then initialized in the Cell Counter window and a third macro was run which will did three things: 1. zoom in on the upper left corner of the image at 100% magnification, 2. close the Results window, 3. select the Hand tool. Image J counts were often far from perfect, so the Cell Counter feature was used to adjust the automated count.

Hydrated oocyte (HO) density was calculated for each sample at the ratio of HO count to sub-sample weight. The coefficient of variation (CV) was then calculated for the two samples, and following McIntyre and Hutchings (2003) only samples with a CV less that 10 were used in further analysis.

*Calculating batch fecundity*

Batch fecundity for an individual fish was estimated from two tissue samples as

$$f_b = \frac{\Sigma \frac{n_i}{w_i}}{2} w$$

where $n_i$ is the number of hydrated oocytes counted and $w_i$ is the formalin wet weight of the $i$th tissue sample, and $w$ is the formalin wet weight of both ovaries.

## Literature Cited

Cerda J, Selman K, Wallace RA (1996) Observations on oocyte maturation and hydration in vitro in the black sea bass, *Centropristis striata* (Serranidae). 9:325-335

Danson BL (2009) Estimating reef fish reproductive productivity on artificial and natural reefs off the Atlantic coast of the southeastern United States. MS Thesis, The College of Charleston

Harris PJ, McGovern JC (1997) Changes in the life history of red porgy, *Pagrus pagrus*, from the southeastern United States, 1972-1994. 95:732-747

Hunter JR, Macewicz BJ, Sibert JR (1986) The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the south Pacific. Fish Bull 84:895-903

McGovern JC, Collins MR, Pashuk O, Meister HS (2002) Temporal and spatial differences in life history parameters of black sea bass in the southeastern United States. 22:1151-1163

McIntyre TM, Hutchings JA (2003) Small-scale temporal and spatial variation in Atlantic cod (*Gadus morhua*) life history. Can J Fish Aquat Sci 60:1111-1121

Moe MA, Jr. (1969) Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico. Report No. 10, p 95

Wallace RA, Selman K (1981) Cellular and dynamic aspects of oocyte growth in Teleosts. 21:325-343

Wenner CA, Roumillat WA, Waltz CW (1986) Contributions to the life history of black sea bass, *Centropristis striata*, off the southeastern United States. 84:723-741

**Table 1.**
Macroscopic sex and maturity staging criteria developed for *C. striata* and *P. pagrus*, modified from species-general key used for northwest Atlantic groundfish species aboard NOAA survey vessels. Revised August 2010.

| Stage | Code | Description and Criteria |
|---|---|---|
| | | **Females** |
| Immature | I | The ovaries are very small but distinct as paired organs joined along the posterior third. The shape is more tube-like than fusiform. The membrane is thin and easily torn with forceps, The content is clear to pink gel or thick fluid, and lacks grainy appearance or distinct oocytes. |
| Early Developing | E | The ovaries may appear as in the I-stage though are generally larger and begin to develop a yellow to yellow-orange tint. The membrane is still thin and mostly transparent with a few fine or no visible blood vessels. The content is less transparent than the I-stage, beginning to cloud and/or granulate. The color is sometimes more pink to red than yellow to orange, but content remains translucent and membrane remains transparent. |
| Late Developing | L | The ovaries are distinctly opaque and grainy as the cream-yellow to yellow-orange opaque oocytes that fill the ovary are visible through the nearly transparent membrane, which is now thicker and more resistant to tearing then in the E-stage. They appear plump and swollen. Major blood vessels are thicker and more apparent while minor blood vessels may remain faint. |
| Ripe | R | The ovaries appear very similar to the L-stage but transparent oocytes are apparent as dark colorless cells among cream-yellow to yellow-orange opaque oocytes. The ovaries may be pink to red but should still be plump with a transparent membrane. The ovaries are often not larger than in the L-stage. |
| Ripe and Running | U | This stage is only identified in fresh specimens prior to cutting open the body cavity. Hydrated oocytes flow freely from the vent with little or no pressure on the abdomen. When the fish is cut open, the ovaries look as in the R-stage. |
| Spent | S | The ovaries have a grainy appearance and patchy coloring that may vary through a range of pink, red, orange, brown, grey, or a combination of the above. They may appear partially deflated or flaccid. They contain oocytes, both opaque and transparent, of variable size and irregular shape, and fine irregular, pale, particles much smaller than the oocytes. The membrane generally remains transparent but occasionally becomes translucent. |
| Resting | T | This stage is very similar to the E-stage except that the contents are usually cloudy and the membrane is often thickened and translucent. |
| Transitional | X | Gonads are still greater than 50% ovarian tissue appearing as in E, S, or T, but with some amount of whitish grey male tissue appearing along the ventral edge of the gonads. |

**Table 2.**
Histological criteria [developed and used by the South Carolina Department of Natural Resources] used to determine reproductive stage in hermaphrodites (see Moe (1969); Wallace and Selman (1981); Hunter et al.(1986); Wenner et al. (1986); Harris and McGovern ; (1997) Roumillat, W. (Pers. comm.). Revised 8 August 2002.

| Stage | Code | Description and Criteria |
|---|---|---|
| | | **Females** |
| Uncertain maturity | 0 | Inactive ovaries with primary growth oocytes only; unable to assess maturity; stage=1 or 5. |
| Immature (virgin) | 1 | Primary growth oocytes only, no evidence of atresia. In comparison to resting female, most primary growth oocytes < 80 um (size is species dependent), area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, oogonia abundant along margin of lamellae, ovary wall is thinner. |
| Early developing, cortical alveoli | 2E | Most advanced oocytes in cortical-alveoli stage. |
| Developing, vitellogenesis | 2F | Most advanced oocytes in yolk-granule or yolk-globule stage. |
| Developing, final oocyte maturation | 2G | Most advanced oocytes in migratory-nucleus stage; partial coalescence of yolk globules possible. |
| Running Ripe | 3 | Completion of yolk coalescence and hydration in most advanced oocytes; zona radiata becomes thinner. |
| Spent | 4 | More than 50% of vitellogenic oocytes in alpha or beta stage of atresia. |
| Resting | 5 | Primary growth oocytes only with traces of atresia possible. In comparison to immature female, most primary growth oocytes > 80 um (size is species dependent), area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongate and convoluted, oogonia less abundant along margin of lamellae, ovarian wall is thicker and exhibits varying degrees of expansion due to previous spawning. |
| Transitional, protandry | 6 | Ovarian proliferation in spent or resting testes. |
| Developing recent spawn | 7B | Vitellogenic oocytes and postovulatory follicles < 12 h old* |
| Developing recent spawn | 7C | Vitellogenic oocytes and postovulatory follicles 12-24 h old* |
| Developing recent spawn | 7D | Vitellogenic oocytes and postovulatory follicles > 24 h old* |
| Mature specimen, stage unknown | 8 | Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive stage. |
| Unknown | 9 | Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive stage. |

*(sensu Hunter et al. (1986))