Effect of Water Quality on Hatching Success of Blueback Herring Eggs in the Chowan River Basin, North Carolina

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Abstract: River herring (alewife [*Alosa pseudoharengus*] and blueback herring [*A. aestivalis*]*)* within the Albemarle Sound basin in North Carolina once supported large commercial fisheries that have declined dramatically since the 1970s. Overfishing, poor water quality, and habitat loss have been suggested as causes of this decline. The objective of this study was to examine the effect of water quality on the hatching success of blueback herring eggs in the Chowan River, a major tributary to Albemarle Sound. We combined eggs and milt obtained from running-ripe fish and placed incubators containing fertilized eggs at 11 sites throughout the basin. Mean hatch rates at field sites ranged from 26% to 89%, compared to a mean of 92% for control trials carried out using distilled water. An analysis of covariance indicated that hatch rates were significantly related to the dissolved oxygen level and were lower at sites on smaller tributaries when compared to sites on the mainstem of the Chowan River. Of the water quality parameters for which published standards exist, dissolved oxygen was the only one not within documented levels for normal development of blueback herring eggs. Given the relatively high hatch rates at most sites, we conclude that mortality of blueback herring eggs due to poor water quality is unlikely to account for the declines in abundance that have been observed.

Key words: herring, water quality, dissolved oxygen, eggs, hatching

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Blueback herring (*Alosa aestivalis*) is an anadromous species ranging from Cape Breton, Nova Scotia, to the St. Johns River, Florida (Loesch 1987). Each spring, adult blueback herring migrate from the Atlantic Ocean into freshwater systems to spawn (Bozeman and Van Den Avyle 1989). During spawning, a female is typically accompanied by several males (Loesch and Lund 1977), and in North Carolina spawning typically occurs in shallow, slow moving water including river edges, streams, and flooded swamps (Loesch 1987, Bozeman and Van den Avyle 1989, Walsh et al. 2005). Fertilized eggs are initially adhesive for about 24 h (Fay et al. 1983) and are then essentially pelagic but are demersal in still water (Jones et al. 1978). Eggs hatch within 2 to 4 d depending on water temperature (Bozeman and Van Den Avyle 1989).

Blueback herring are similar in appearance and in life history to alewife (*Alosa pseudoharengus*) and collectively they are referred to and marketed as river herring (Rulifson 1994). Historically, the spawning migration of river herring supported large commercial fisheries along the Atlantic coast, but many of these fisheries have declined substantially since the 1970s. Within the Albemarle Sound basin in North Carolina, blueback herring runs in all major rivers have been declining since 1980, and a number of tributaries no longer support spawning runs (Rulifson 1994). The blueback herring stock in the Chowan River, a major tributary of Albemarle Sound, is classified as overfished (North Carolina Division of Marine Fisheries [NCDMF] 2007). Poor water quality and habitat loss may also be important factors contributing to the declines in river herring stocks (NCDMF 2007).

The objective of this study was to examine the effect of water quality on the hatching success of blueback herring eggs in the Chowan River basin. River herring have historically spawned throughout the Chowan River basin (Odom et al. 1986, Collier and Odom 1989), and the basin historically accounted for up to 85% of the total Albemarle Sound landings of river herring (Winslow et al. 1983). We focused on blueback herring because it accounted for 41%–98% of the annual river herring landings (1972–2004) from the Chowan River (NCDMF 2007). If specific water quality parameters affecting hatching success could be identified, spawning areas could be targeted for stricter land-use or discharge regulations.

Methods

We selected 11 study sites within the Chowan River basin (Fig. 1) based on the amount of water quality data and the presence or

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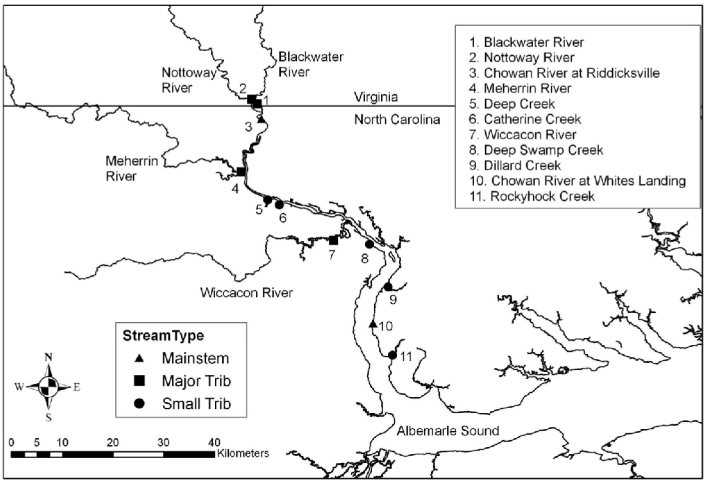

Figure 1. Field sites used for estimating hatching success of blueback herring eggs within the Chowan River drainage.

absence of spawning runs. Water quality data were obtained from the U.S. Geological Survey, Union Camp Corporation, and North Carolina Division of Water Quality. Information on existing and historical spawning runs was obtained from local commercial fishers and biologists as well as published reports. The selected sites were not drawn randomly but are considered representative of the three major types of spawning habitat: mainstem Chowan River, major tributaries (Blackwater, Nottoway, Meherrin, and Wiccacon rivers), and small tributaries. At each location, hatching success was measured in a backwater area (mean surface flow of 0.02 m/sec) that would be typical blueback herring spawning habitat (bald cypress [*Taxodium distichum*] swamps out of the main channel flow). During the 1997 field season, we either observed spawning or collected naturally spawned eggs at all but one field site (Chowan River at Riddicksville).

We used Plexiglas incubators to estimate hatching success of blueback herring eggs. These incubators have been used successfully to evaluate hatching success *in situ* for lake trout (*Salvelinus namaycush)* and lake herring (*Coregonus artedi)* (Manny et al. 1989, Evans et al. 1994, Savino et al. 1994). Our incubators were similar in design and consisted of three Plexiglas plates, each containing 50 holes that were 10 mm in diameter (Waters 1998). The two outer plates had dimensions of 240 x 145 x 3 mm. The center plate was 300 x 145 x 9 mm, so it extended beyond the two outer plates by 30 mm at each end. Four holes on each extension of the center plate were used to attach the incubator to 31.75-mm PVC pipe with cable ties. The PVC pipe, which slid over smaller diameter PVC pipe driven into the substrate, held the incubator perpendicular to the surface of the water about 30 cm off the bottom. A 300-µm mesh screen was inserted between the center plate and each of the outer plates. When assembled, an incubator held one egg in each of the 50 separate 10-x 9-mm compartments. The incubator design allowed each egg to be exposed to ambient water conditions but protected it from predation and the spreading of fungal infections among cells (Manny et al. 1989).

We obtained eggs and milt by stripping (applying slight pressure to the abdomen to expel either eggs or milt) running-ripe fish collected during the late afternoon by gill netting or electrofishing. We used the dry method of fertilization, in which eggs and milt were initially combined in the absence of water (Davis 1967, Shepherd and Bromage 1988). Eggs of each female were stripped into a 1.9-L plastic tray followed by milt from several males. The eggs and milt were combined in the tray and were stirred by finger for 3–5 min to promote fertilization. Distilled water was then added to dilute the mixture, and excess water and milt were decanted from the solution. The dilution process continued until the solution was clear (Davis 1967, Woynarovich and Horvath 1980, Shepherd and Bromage 1988). The solution was then stirred for an additional 15 min to prevent the eggs from sticking (Evans et al. 1994, 1995). The eggs were generally held overnight in aerated water before being deployed at the study sites.

Using a micropipette, one live water-hardened egg was placed in each of the 50 compartments of an incubator. Viability was determined by egg color: dead eggs had a white, opaque appearance whereas live eggs were translucent (Davis 1967). Incubators were stored in aerated water until deployed at the study sites (Evans et al. 1994, 1995). For each trial, all incubators contained eggs from the same female in order to eliminate maternal differences in hatching success among individuals (Kellogg 1982, Jessop 1993).

We deployed one incubator at each site, and two additional incubators were placed in control tanks containing aerated distilled water. One control incubator was placed in distilled water immediately after loading and was not removed until the trial ended. A second control, which was transported in the same manner as other field incubators during deployment and retrieval, was used to determine whether our transport methods had an effect on hatch rate.

In 1996, we conducted two partial trials and three full trials from 15 April to 13 May, and in 1997, we conducted six full trials from 1 April to 3 May. Several unsuccessful trials were the result of stripping females that were not fully ripe in 1996 (Woynarovich and Horvath 1980) and fluctuating water levels (>0.5 m) in 1997. Therefore, we limited our hatch rate analyses to trial 2 from 1996 and trials 1, 4, 5, and 6 from 1997.

Total incubation time (fertilization to trial completion) generally ranged from 77 to 90 h with typical incubator deployment about 48 h at each site. After the incubators were retrieved, we

classified the contents of each incubator using the following categories: dead egg, egg infected by fungus, live egg, dead larvae, or live larvae. The total count was subtracted from 50, and the difference was classified as missing.

To estimate hatch rate, we used the formula (live larvae + dead larvae + live eggs)/50, based on the rationale that live and dead larvae both indicated successful hatching. We also included live eggs in the successful hatch category, under the assumption that those eggs would have hatched if the incubation period had been longer. Fully developed and active larvae were visible in the live eggs. Water temperature and the rate of egg development varied among sites, particularly for the control tanks which were considerably cooler than the field sites. Since the length of a trial was kept about equal among sites, the differences in temperature resulted in some sites having large numbers of live eggs while others had large numbers of dead and live larvae. Finally, we assumed that compartments with missing specimens had contained dead eggs that had decomposed. We consider it unlikely that eggs would have hatched into larvae which then died and decomposed.

We used an analysis of covariance with trial and stream type (mainstem, major tributary, small tributary) as main effects and water quality parameters as covariates. Water temperature, dissolved oxygen, pH and conductivity were measured daily during each trial and we used within-trial averages as covariates. Water temperature regulates the timing of spawning but extreme values can reduce hatching success (Klauda et al. 1991). The dissolved oxygen concentration is a fundamental measure of water quality; values above 5 mg/L are assumed to be suitable for fish (Bain 1999). Extreme pH levels can cause egg or larval mortality (Klauda et al. 1991). Conductivity is related to the ion concentration of water. Higher values may be an indication of biological productivity but can also indicate the presence of a pollutant (Bain 1999).

Because our hatch values were proportions, we used an arcsine square-root transformation of the proportions in order to reduce non-normality and stabilize variances (Sokal and Rohlf 1995). We tested for differences among least squares means for stream type using Tukey's HSD (honestly significant difference; Sokal and Rohlf 1995). All significance tests were based on a Type I (α) error level of 0.05.

We used Geographic Information System methods to determine watershed area for each site. Watershed areas were delineated manually using data layers from BASINS (USEPA 1998) to represent river reaches (RF1 and RF3-Alpha), USGS Hydrologic Unit Boundaries, and elevation (USGS Digital Elevation Model files). A land use data layer from BASINS (USEPA 1998) was used to estimate the percentage of watershed area in agricultural use.

Results

Across all trials, we observed a mean hatch rate of 70% (SE = 17%) for field sites and 92% (SE = 4%) for the controls. Most sites had a high hatch rate for at least one trial, and only one small tributary site (Dillard Creek) had consistently low hatch rates (Table 1). There was a consistent pattern in water temperature among study sites, with lower temperatures for upstream sites and the controls (Table 1). Mean dissolved oxygen levels at field sites ranged from 2.3 to 9.8 for individual trials, with 10 of 55 trial means less than the recommended threshold of 5.0 mg/L. Some of the variability among trials in dissolved oxygen levels was likely due to generally higher temperatures during 1996 (mean = 20.7 C, range 16.7–23.9 C), compared to trials in 1997 (mean = 16.9 C,

Table 1. Mean values among trials (range in parentheses) for water chemistry parameters, percentage of watershed in agricultural use, and hatch rate for mainstem Chowan River (MS), major tributary (MT), and small tributary (ST) sites.

	Water temperature °C	Dissolved oxygen mg/L	pH	Conductivity µS/cm	Agriculture as percentage of watershed	Hatch rate
Blackwater River (MT)	16.9 (14.3–20.8)	5.8 (4.3–6.8)	6.5 (6.3–6.7)	148 (117–330)	10.7	0.69 (0.56–0.90)
Nottoway River (MT)	17.2 (14.3–21.0)	7.0 (5.2–8.4)	6.5 (6.2–6.7)	113 (87–190)	15.1	0.79 (0.58–0.98)
Riddicksville (MS)	17.6 (14.8–20.9)	5.6 (2.3–7.7)	6.5 (6.3–6.8)	117 (104–150)	14.2	0.89 (0.76–1.00)
Meherrin River (MT)	18.5 (16.4–23.0)	6.6 (4.9–7.7)	6.6 (6.3–6.9)	100 (90–130)	25.5	0.86 (0.78–0.98)
Deep Creek (ST)	18.7 (15.0–21.1)	7.5 (5.5–9.0)	7.1 (6.6–7.4)	203 (105–298)	30.4	0.74 (0.52–0.88)
Catherine Creek (ST)	19.7 (15.8–21.3)	5.4 (2.4–7.7)	6.2 (5.8–6.7)	99 (75–135)	4.9	0.64 (0.38–0.88)
Wiccacon River (MT)	17.5 (14.4–21.9)	4.8 (2.3–6.7)	6.3 (5.8–6.8)	108 (92–140)	38.4	0.60 (0.30–0.86)
Deep Swamp Creek (ST)	18.4 (14.5–20.1)	6.1 (3.6–8.9)	6.6 (6.3–7.0)	139 (107–170)	69.3	0.58 (0.08–0.96)
Dillard Creek (ST)	18.7 (16.0–23.0)	4.9 (2.8–7.7)	6.4 (6.1–6.6)	198 (127–400)	68.1	0.26 (0.00–0.52)
Whites Landing (MS)	19.1 (12.5–22.8)	7.6 (4.5–9.8)	6.8 (6.5–7.2)	111 (103–130)	19.3	0.86 (0.62–0.94)
Rockyhock Creek (ST)	19.5 (16.2–23.9)	6.0 (2.8–9.2)	6.6 (6.3–6.9)	186 (128–239)	57.5	0.79 (0.30–0.86)
Control	15.9 (12.1–20.1)	8.7 (5.3–11.1)	6.6 (5.2–7.7)	38 (16–135)	–	0.92 (0.78–1.00)
Transport control	15.8 (12.1–20.5)	8.7 (5.3–11.1)	6.5 (5.2–7.6)	35 (16–115)	–	0.92 (0.88–0.94)

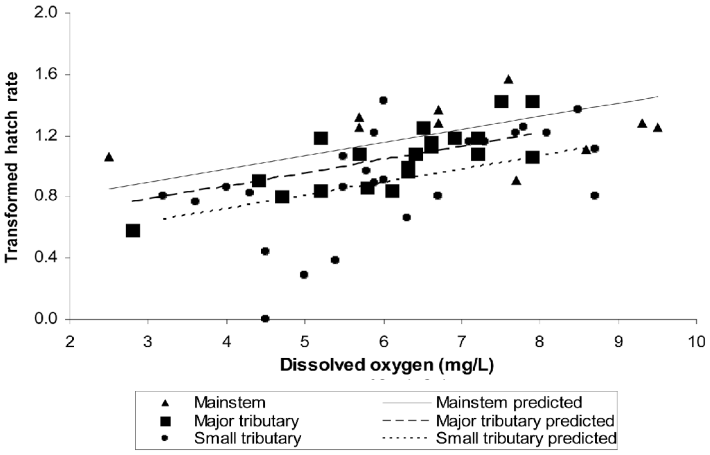

Figure 2. Observed and predicted transformed hatch rate of blueback herring eggs. Predicted values by stream type are from the analysis of covariance model, using average values over the five trials.

range 12.1–21.3 C). Water quality variables were moderately correlated, ranging from –0.49 (temperature and dissolved oxygen) to 0.49 (pH and conductivity). Agriculture as a percentage of each site's watershed was highest for small tributary sites (Table 1).

Stream type ($F_{4,47} = 2.65$, $P = 0.0093$) and trial ($F_{2,47} = 5.18$, $P = 0.0446$) were significant main effects in the analysis of covariance. Dissolved oxygen concentration was a significant covariate (slope = 0.086, SE = 0.026; $F_{1,47} = 10.70$, $P = 0.0020$); no additional covariates were significant when added to the model. Overall, the model accounted for 51% of the variation in hatch rate. There was not a significant interaction between stream type and dissolved oxygen concentration ($F_{2,45} = 2.62$, $P = 0.0841$). A quadratic term for dissolved oxygen concentration was also not significant ($F_{1,46} = 1.03$, $P = 0.3145$), so there was no indication of curvature in the relationship between dissolved oxygen and transformed percent hatch (Fig. 2). A comparison of least squares means by stream type showed that transformed hatch rate was significantly lower for small tributaries than for mainstem sites.

Discussion

The hatch rate of blueback herring eggs varied due to trial and stream type as main effects and dissolved oxygen level as a covariate. Trial as a main effect would account for differences among individual females or due to the timing of each trial. It is not known what caused the differences among stream types, but predicted hatch rate was lowest in small tributaries, intermediate for major tributaries, and highest for mainstem Chowan River sites. Small tributaries often had the lowest dissolved oxygen levels and highest conductivity levels, which suggests that there is a connection between hatching success and water quality. The analysis of covariance should account for a linear effect of any measured water quality variable, but the stream-type effect may be due to a combination of water quality parameters or a nonlinear effect. Although not included here, Waters (1998) also measured nitrates, total ammonia, and total phosphorus for four of the five trials in this study. However, except for the highest nitrate value being coincident with the lowest observed hatch rate, there was no evidence that nutrient levels affected hatch rates (Waters 1998). Waters (1998) also measured contaminant concentrations at each site using low-density polyethylene samplers (Huckins et al. 1996, Hofelt and Shea 1997), but found that the measured levels were generally well below published standards. Water quality in the Chowan River basin is affected mostly by non-point source pollution (McMahon and Lloyd 1995), so smaller streams may be impacted more directly and immediately by agricultural or logging practices. Small tributary sites also had a higher percentage of the watershed in agricultural use than did most major tributary and mainstem sites. Uzee (1993; cited in O'Connell and Angermeier 1999) found an inverse relationship between the proportion of a stream's watershed in agricultural use and the overall tendency of that stream to support river herring runs.

Our results indicate that dissolved oxygen level was the primary water quality variable affecting hatch rate of blueback herring eggs. This is reasonable on biological grounds, in that a developing fish egg uses dissolved oxygen from the environment, and uptake rate increases with egg development. For example, Atlantic herring (*Clupea harengus*) eggs at fertilization require 0.01 µL/h and at hatching 0.07 µL/h (Hempel 1979). Diffusion of oxygen across the chorion is also affected by factors such as temperature and pH. An egg can tolerate anoxic external conditions prior to gastrulation and for a short period in later developmental stages by using oxygen stores in the yolk and perivitelline fluid (Hempel 1979). However, oxygen deficiency over a longer period of time can result in the death of the egg (Hempel 1979).

Very little has been documented about the oxygen requirements of blueback herring eggs except for the reported threshold of 5.0 mg/L (Jones et al. 1978, Piper et al. 1982, Klauda et al. 1991). However, in our study, the hatch rate varied in a linear fashion across dissolved oxygen levels ranging from 2.5 to 9.5. Our lowest hatch rates were observed at dissolved oxygen concentrations below 5.0 mg/L, and daily concentrations were below this proposed threshold 28% of the time, but there was not a clear threshold response.

Water temperatures during trials were typically within the reported ranges required for the normal development of blueback herring eggs. Development of blueback herring eggs can occur between 14 and 26 C, while 20 to 24 C is reportedly optimal (Fay et

al. 1983, Bozeman and Van Den Avyle 1989, Klauda et al. 1991). Water temperature values during 1996 trial 2 and 1997 trials 1, 5, and 6 were between 14 and 26 C 98% of the time. Over both seasons, we encountered running-ripe individuals when water temperatures ranged from 11.9 to 21.2 C, with all but one collected at temperatures above 14.2 C. Observations of spawning at our field sites in 1997 occurred while water temperatures ranged from 14.6 to 17.2 C. Jones et al. (1978) reported that spawning activity for blueback herring occurs between 14.0 and 25.5 C. Winslow et al. (1983) and Bozeman and Van den Avyle (1989) reported a temperature range of 13.0–19.0 C for blueback herring spawning in North Carolina. O'Connell and Angermeier (1999) reported a median water temperature of 17.3 C when both alewife and blueback herring were spawning in a Virginia stream, compared to a median of 18.2 C when only blueback herring were spawning.

We did not detect a relationship between hatch rate and pH, which ranged from 5.8 to 7.4 at field sites. Klauda et al. (1991) reported that pH values for normal egg development were between 5.7 and 8.5 for blueback herring, with a range of 6.0–8.0 defined as optimal. In a Virginia stream supporting good runs of alewife and blueback herring, O'Connell and Angermeier (1999) reported that median pH was 6.4–6.5 during periods when blueback herring were spawning. A potentially important water quality variable that we did not measure was turbidity (or total suspended solids). High levels of suspended solids have been shown to reduce egg survival (Henley et al. 2000) although concentrations less than 1000 mg L did not significantly reduce hatching success of blueback herring eggs (Klauda et al. 1991).

Our overall assessment is that mortality of blueback herring eggs due to poor water quality is not likely to account for the decline in overall herring abundance that has been observed. Our hatch rates averaged 70% across all field sites, and for some sites, rates were similar to those observed for control trials carried out using distilled water. Low hatch rates were observed at some small tributary sites but those habitats probably represents only a small proportion of total spawning habitat. The importance of those sites would depend on the relative amount of spawning in small tributary creeks versus larger tributaries and mainstem rivers. Walsh et al. (2005) reported that spawning of blueback herring occurred in backwater areas, small tributaries, and along the mainstem river edge. They reported generally similar larval densities among the various habitat types.

We recommend several areas for future research. Laboratory studies are needed to better define the effect of dissolved oxygen level on egg hatching success, and to reexamine whether a threshold of 5.0 mg/L is appropriate. Factors resulting in low dissolved oxygen levels within small tributaries need to be investigated. Also, because larvae are more sensitive to poor water quality than eggs (Mohr et al. 1990), a study of the effect of water quality on larval growth and survival should be undertaken.

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