Morphometric analysis for nonlethal sex determination in brook trout:

a new tool for research and management

A Final Independent Project Proposal

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Background

Brook trout (*Salvelinus fontinalis*) are an iconic species that has been considered recreationally and aesthetically important throughout their native range for centuries. Brightly-colored and charismatic fish, brook trout are also ecologically important - they are recognized as a bioindicators for water temperature and quality in the southern regions of their historical range (Waco & Taylor 2009). Brook trout require water that is relatively cold (seldom exceeding 25^oC) and well-oxygenated. As such this species typically inhabits streams that are surrounded by forested areas.

Brook trout face many threats and have already been extirpated from many of their native streams in Maryland (Stranko et al. 2008). The only native trout species found in Maryland, they have decreased from a population of millions to a few hundred thousand (MD DNR 2005). Habitat threats include water temperature increases due to climate change, land use changes, run-off (urban, agricultural and mining) and habitat fragmentation (Heft 2006, Letcher et al 2007). Brook trout also face competition and predation by stocking of non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). The western Appalachian region, with its cool streams in less disturbed, mostly forested watersheds, contains most of the state's extant brook trout. Brook trout can be found in other streams throughout Maryland, but those populations are considered greatly reduced (Hudy et al. 2005). However, even strong populations are in danger of decline.

As a species of "Greatest Conservation Need' in Maryland (MD DNR 2005), conservation efforts and management plans for the species have been instituted, including fishing restrictions, conservation assessments, and life history research. Sex-specific life history differences are not known, but are potentially important to effective management. However, there are no proven non-lethal methods for sexing brook trout. Anecdotally, there are distinctive physical differences between male and female brook trout within populations, though this has not been quantified to-date. A non-lethal approach is essential to reduce stress on the threatened native populations of brook trout while obtaining important life history information.

Sexual dimorphism has been studied extensively in fish and, typically, becomes more pronounced with size in sexually mature fishes (Beacham & Murray 1986). In species such as threespine sticklebacks (*Gasterosteus aculeatus*) and Mediterranean blennies (*Blenniidae* sp.), body size has shown significant relationship to dimorphism (Cooper et al 2011, Lengkeek et al. 2008). The relationship between head size and sex was examined in threespine sticklebacks (*Gasterosteus aculeatus*), using a geometric morphometric approach based on anatomical landmarks, most of which were located in the head region of the fish. Through photo-analysis, the authors determined males could be distinguished from females through larger head, eye and mouth size (Cooper et al 2011). For some species, such as the California Sheephead (*Semicossyphus pulcher*), whose life history involves transition from female to male, morphometrics do not provide adequate basis for sex determination (Loke-Smith et al. 2010).

Although sexual dimorphism has not been rigorously examined in brook trout, other salmonids have been studied for morphological differences including Arctic charr (*Salvelinus alpinus*) and Pacific salmon species (*Oncorhynchus* sp.). Jahunen et al. (2009) conducted photo-analysis of Arctic charr based on a truss network of twenty-eight measurements and found that mature males have more robust (greater length and depth) bodies and heads than females and juveniles. Pacific salmon species were shown to exhibit differences in kype presence and adipose fin size compared between sexes (Beacham & Murray 1986). Beacham and Murray (1983) determined length and height of adipose fin was on average 31%-48% (variable between species) larger in males than females and concluded that adipose fin size could be used to externally determine sex in Pacific salmon species. Further work with *Oncorhynchus* species has found that 87-97% (variable between species) of individuals could be correctly sexed using adipose fin size and/or jaw length (Beacham & Murray 1986).

Merz and Merz (2004) specifically studied chinook salmon (*Oncorhynchus tshawytscha*) and found that ratios of snout length to fork length led to 96% accuracy in determining sex in handled fish and ratios of adipose fin length to fork length led to 86% accuracy in determining the sex of fish measured from video images at a fish passage facility. When combining these two ratios with head length measurements, accuracy increased to 92% in determining sex of fish measured through video images (Merz & Merz, 2004). These studies provide precedents for sexual determination in salmonids and support the basis for the research objectives of this project.

Research objectives and hypotheses

Nonlethal approaches to sex determination via morphology have been successfully developed for other salmonids (Beacham & Murray 1986, Merz & Merz 2004). Currently, no such techniques exist for brook trout, and biologists lack effective tools to determine sex. For my independent project, I propose to examine sexual dimorphism in brook trout to identify metrics than can be used for nonlethal sex identification.

Fundamental Research Questions:

1. *Are there distinct and quantifiable morphological differences between male and female brook trout that allow for non-lethal determination of sex?*
2. *Can morphometrics be used to rapidly sex brook trout in the field?*
3. *What metrics are most effective?*
4. *What size range of fish can be sexed using this approach?*

Derived from the above questions, I propose the following hypothesis:

> *Mature male and female brook trout are sexually dimorphic. Based on anecdotal observations, the females will exhibit smaller, rounder heads with blunt snouts while the males will exhibit larger, more angular heads with more acute snouts and kype development in more extreme cases.*

Statement of Purpose

This project will draw upon my coursework completed as a Johns Hopkins AAP Environmental Science and Policy student. Courses that provided the greatest foundation for this project idea and implementation of the project include Freshwater Ecology and Restoration of Aquatic Ecosystems, Ecological Assessment and Field Methods in Ecology. The project will highlight my interest in fish ecology and broaden my knowledge of ecological and resource management while developing an applied tool to address a management need.

Project Development and Implementation Schedule

Spring – Fall 2011

- Discussed project ideas with Dr. David Elbert

August 2011

- Dr. Robert Hilderbrand agreed to be my advisor for this project
- Began developing research ideas

September – November 2010

- Continued to refine research ideas with Dr. Hilderbrand
- Discussed project ideas with MD DNR brook trout specialist (Matt Sell)
- Collected pictures of known sex brook trout during spawning season

December 15th, 2011

- Draft project proposal submitted to Dr. McGurty and Dr. Hilderbrand

January 15th, 2012

- Final project proposal to be submitted to Dr. McGurty and Dr. Hilderbrand
- Project implementation begins

January 15th –March 15th, 2012

- Conduct image analysis
- Analyze and interpret data

March 15th – April 15th, 2012

- Write draft paper

May, 2012

- Submit final paper to Dr. McGurty and Dr. Hilderbrand

Methods

Data Collection

In October 2011, backpack electrofishing was used to collect brook trout in tributaries within the Savage River watershed in western Maryland. This work was conducted in close collaboration with MD DNR and the UMCES Appalachian Laboratory, in support of an ongoing collaborative research project. The collection took place during spawning season when sex of brook trout is most evident and can be accurately determined. The fish were lightly anesthetized with tricaine methanesulfonate (ms-222) buffered with 0.2 mM $NaHCO_3$, pH = 7. The total length (mm) of the fish was measured on a fish ruler board and the width of head (mm) was measured with calipers. The fish were assessed through manual gamete expression to accurately determine sex. They were then placed on a light-colored background and photographed (with camera attached to a tripod at a specified height) on their left side. The target sample size of 25 fish of each sex was calculated based on a two-tailed powered analysis for a moderate effect size. We collected 111 individuals - 36 adult males, 29 adult females, 46 adults of unknown sex (15 only of which full measurements were taken). Individuals <100 mm total length were not used for analysis, as these fish were assumed to be sexually immature and we could not determine their sex.

Image Analysis

I will analyze images of known sex brook trout using ImageJ, an open-source imaging software. I will measure distances between anatomical features in form of a truss network (Figure 1). I will also measure the distances portrayed in Figure 2 and determine if the relationships examined (snout length to fork length ratio, adipose fin length to fork length ratio, and the combination of both ratios with head length) are useful in determining variation in brook trout morphology. These methods have been successfully used to examine morphologic variation in other salmonids (Janhunen 2009, Merz and Merz 2004).

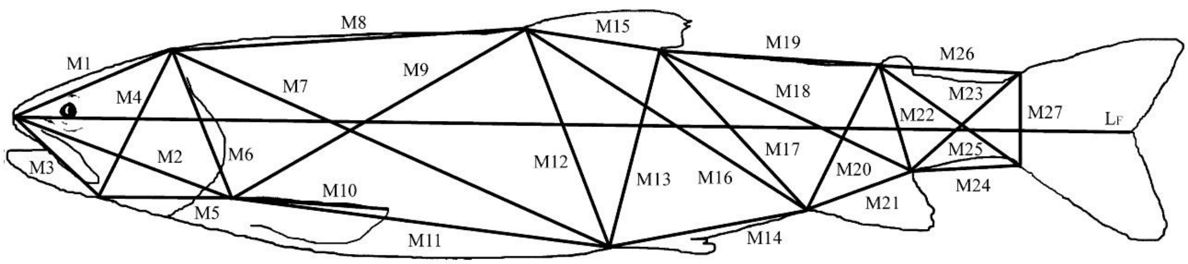

Figure 1: Truss network of morphometrics applied to Arctic charr (*Salvelinus alpinus*) (Janhunen et al. 2009)

Figure 2: Measurements applied to determine sex in Chinook salmon (*Oncorhynchus tschawytscha*) (Merz & Merz 2004)

Statistical Analysis

Once all images have been processed, I will run the following procedures in program R (a free software environment for statistical computing and graphics):

1. Log_{10} transform each measurement and convert to standardized residuals of the relationship between length and the variable (Janhunen et al. 2009).
2. Analysis of variance
    a. Which measures exhibit significant (α=0.05) sexual dimorphism?
    b. And thus, which measures can be used independently to predict sex?
3. Principal component analysis of standardized residuals
    a. Which measures are collinear?
    b. Which measures explain the majority of the variation?
4. Discriminate function analysis
    a. Are principle components derived from morphometrics effective at predicting sex?
    b. Is the accuracy of assignments correlated to total length?
    c. What sex does the discriminant function analysis assign to the unknown individuals?

Implications

Although brook trout are exhibiting widespread declines and are the focus of many conservation efforts, we still lack basic management tools. Development of a nonlethal approach to determine sex via morphology will open new doors to brook trout research and has the potential to improve the biological information on which management decisions are based. Metrics for determining sexual dimorphism could be used to examine sex-specific vital rates, including growth, survival, and movement. Sex ratios may also be evaluated using this approach. This information may have considerable implications for population dynamics, and is currently overlooked by managers. Although this tool is being developed based on individuals collected in a single watershed, the results should be applicable across a much broader geographic area. This project will develop a non-lethal tool for sex identification in brook trout to further basic and applied science and, consequently, aid in the conservation of the species.

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