

Final Report
ACES Project Summary: Part 1 Web Summary Report

Period Covered by the Report: 6/01/01 – 12/31/03
Date of Report: 12/31/03
EPA Agreement Number: R-827072-01-1
Title: Effects of Estrogen Pollution on the Reproductive Fitness of the Gulf Pipefish, *Syngnathus scovelli*
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Institution: University of South Alabama¹, Middle Tennessee State University², and US Fish and Wildlife Service³
Research Category: Small Grant for Exploratory Research
Project Period: 06/01/01-12/31/03

Objectives of the Research Project:

Developmental and sex related changes in fish resulting from environmental estrogen exposure have been implicated in causing adverse population level effects. Recent research has focused on the influence of environmental estrogens on adult fish, although it has long been known that juvenile fish will experience sex reversal with hormone treatment at critical stages of development. The influence of estrogens on oviparous fish fecundity and offspring sexual development has been assessed using sentinel species including the fathead minnow, *Pimephales promelas*, the sheepshead minnow, *Cyprinidon variegatus*, and the rainbow trout, *Oncorhynchus mykiss*. The current research assesses the effects of xenoestrogenic exposure on the reproductive fitness of the male-brooding Gulf pipefish, *Syngnathus scovelli*. Such species possess adaptations that may limit the effect environmental estrogens have on their offspring. Baseline studies on untreated fish were used to establish benchmark conditions of various physiologic parameters and for comparison with subsequent exposure derived data. The specific objectives were to:

1. Determine baseline blood and brood pouch fluid osmolality, total glucose, total protein, and plasma vitellogenin, and examine normal brood pouch development, gonad morphology, gonado-somatic and hepato-somatic Indices for reference fish.
2. Determine the sensitivity of *S. scovelli* to estrogenic exposure utilizing vitellogenin induction levels.
3. Examine the effect of estrogen exposure on the liver and gonad morphology, gonado-somatic and hepato-somatic Indices.

Progress Summary/Accomplishments:

Research

The brood cycle of the Gulf pipefish lasts approximately 15 days. Four brood stages have been defined using the development of embryos as markers. These stages are termed: A) no brood stage - the male has not received eggs from the female (0 day); B) pharyngula stage - only embryonic eye pigmentation visible through the brood pouch (~5 day); C) protruding snout stage - all embryonic fins developed, snout only slightly protruding, large amount of yolk still remaining (~10 day); and D) embryonic/juvenile stage – embryos fully developed, little or no yolk remaining, ready for release (~15 day). Baseline structural and physiological data for each stage was collected during the first year of the study. Histological data shows that at the no brood stage, the surface of the brood pouch is flat, smooth, and lined with pavement epithelial cells. During incubation, the pouch inner surface forms shallow depressions with low walls, arranged in

longitudinal rows (pharyngula stage). From the pharyngula through the embryonic/juvenile stage the walls increase in height, particularly the medial walls. The flap shows similar changes and by the protruding snout stage or shortly thereafter, the pouch and flap walls meet, completely separating the embryos. Epithelial cells lining the floor of the depressions differ from those of the wall during incubation, and the floor pouch epithelium appears to be the site of attachment for the egg chorion through the protruding snout stage. These structural changes are paralleled by changes in specific reproductive markers and in the organic and inorganic make up of the blood and brood pouch fluid of male pipefish. GSI values decrease significantly through the protruding snout stage, increasing thereafter. HSI values do not vary with brood stage. Blood and brood pouch fluid protein and glucose values do not vary with the brood cycle. Blood osmolality values stay constant throughout the brood cycle and are hyperosmotic relative to the environment. Brood pouch fluid osmolality is initially higher than blood osmolality, then decreases just prior to juvenile release. Based on differences seen in baseline studies, the focus of xenoestrogen exposure studies focused on comparisons between the no brood and protruding snout stages.

The synthetic estrogen, ethynyl estradiol (EE2) was used for exposure experiments. Preliminary EE2 stability experiments have shown that exposure experiments can be run under the normal light:dark regime for culture of pipefish, but will require daily EE2 renewals. In the original proposal, fish blood samples were to be sent out for vitellogenin analyses. However, Dr. Tim Sherman (University of South Alabama, Biology) offered his assistance in the development of an ELISA based assay for the pipefish samples using antibodies available from Cayman Chemical Company. The results obtained are comparable to those available through the University of Florida, Protein Chemistry and Molecular Biomarkers Laboratory. Vitellogenin levels were below detection limits for all brood stages of male field-collected fish. Those for female fish were comparable to levels found in other species. Exposure of laboratory-cultured fish to EE2 lead to significant increases in vitellogenin concentrations. Exposure to EE2 also lead to increases in HSI values and to a change from normal male pigmentation patterns to female pigmentation patterns. GSI was not affected.

Education

The MS student, Charlyn Partridge, was been supported by this project. She received her MS in August of 2003. Several undergraduates (Jessica Rozelle, Christi Cazalas, Jeremy Roop, Jeff Bolland, and Nobuo Ueda) have been supported by and/or have conducted directed research projects associated with this project. This has provided these students hands-on research experience in both the laboratory and the field. Two of the undergraduates, Jessica Rozelle and Jeff Bolland, conducted Honors Research related to the project. Components related to the current research were also incorporated into the BLY 436 Animal Physiology course as both lecture and laboratory material.

Publications/Presentations:

- Partridge, C., C. Cazalas, J. Rozelle, J. Hemming, and A. Boettcher. In press. Small-scale captive breeding of a euryhaline pipefish. *World Aquaculture*.
- Partridge, C., J. Shardo, and A. Boettcher. In prep. Nutrient and osmoregulatory role of the brood pouch in gulf pipefish, *Syngnathus scovelli*.
- Bolland, J. and A. Boettcher. In prep. Breeding cycles and population survey of euryhaline *Syngnathus scovelli* in a freshwater portion of Mobile Bay.
- Ueda, N., J. Bolland, C. Partridge, J. Hemming, T. Sherman, and A. Boettcher. In prep. Effects of 17 α -ethynylastradiol on male gulf pipefish, *Syngnathus scovelli*.

- Partridge, C. 2001. Understanding a complex male brooding system: Experimental design. Oral Presentation. Dauphin Island Sea Lab Graduate Student Symposia. Dauphin Island, AL. February 2001.
- Cazalas, C., J. Rozelle, C. Partridge, J. Hemming, and A. Boettcher. 2002. Physiological role of the brood pouch in Gulf pipefish, *Syngnathus scovelli*. Poster presentation, 2002 Benthic Ecology Meeting, Orlando, FL. March 2002.
- Partridge, C. and J. Shardo. 2002 Morphological changes in the brood pouch of the Gulf pipefish, *Syngnathus scovelli*, during egg incubation. Poster presentation, 2002 Benthic Ecology Meeting, Orlando, FL. March 2002.
- Cazalas, C., J. Rozelle, C. Partridge, J. Hemming, and A. Boettcher. 2002. Physiological role of the brood pouch in Gulf pipefish, *Syngnathus scovelli*. Poster presentation, University of South Alabama's 9th Annual Research Forum, Mobile, AL. April 2002.
- Partridge, C. and J. Shardo. 2002 Morphological changes in the brood pouch of the Gulf pipefish, *Syngnathus scovelli*, during egg incubation. Poster presentation, University of South Alabama's 9th Annual Research Forum, Mobile, AL. April 2002.
- Rozelle, J., C. Partridge, A. Boettcher, and D. Forbes. 2002. Use of gas chromatography in the elucidation of carbohydrates in the body fluids of the Gulf pipefish, *Syngnathus scovelli*. Poster presentation. University of South Alabama, University Committee for Undergraduate Research 4th Undergraduate Research Week. October 2002.
- Rozelle, J., C. Partridge, A. Boettcher, and D. Forbes. 2003. Use of gas chromatography in the elucidation of carbohydrates in the bodily fluids of the Gulf pipefish, *Syngnathus scovelli*. Poster presentation. 225th American Chemical Society National Meeting, New Orleans, LA.
- Partridge, C.G. and A.A. Boettcher. 2003. Reproductive Physiology of the Gulf pipefish, *Syngnathus scovelli*, brood pouch. Oral Presentation. 32nd Annual Benthic Ecology Meeting, Groton, CT.

Supplemental Keywords:

Xenestrogens, *Syngnathus scovelli*, environmental estrogens, water, risk assessment, sensitive populations, animal, sex, indicators, ecology, gulf coast

Relevant Websites:

<http://www.nwri.ca/links.html>

<http://www.ourstolenfuture.org/index.htm>

<http://www.epa.gov/scipolu/oscpendo/>

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