Anthropogenic Effects on Queen Conch Reproductive Development in South Florida

A Final Report

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Executive Summary

From spring 2004 through March 2008, a team of scientists from the Florida Fish and Wildlife Conservation Commission, The University of Florida, The University of Southern Mississippi, and North Carolina State University were engaged in a project to determine the cause(s) of reproductive failure in the sub-population of Florida queen conch (*Strombus gigas*) that is found closest to shore. The primary focus of this project was to isolate the protein biomarker vitellin, which is the major egg yolk protein associated with reproduction in queen conch and to develop antibodies against it to use in analyzing reproductive failure in the nearshore animals. Isolating and identifying vitellin was problematic, but was eventually achieved from gonads. In the process, alternative approaches were investigated to identify the stressor(s) responsible for the deficiencies as well as to determine the underlying impacted physiological processes. The project was multidisciplinary in scope and incorporated histological examinations of the tissues, biomarker development for proteins known to be indicators of stress in organisms, neuropeptide assessments, and genomics. Concentrations of metals and select organic compounds in the environment and in the tissues of conch were also examined. Although we did not ascertain the exact stressor(s) causing the reproductive deficiencies, we were able to make considerable gains in a number of areas that are critical for a comprehensive understanding of conch reproductive impairment. The most significant results of this study are detailed below.

Morphology

- **Verge length is significantly larger in offshore male conch than male conch found nearshore.** An analysis of the penis length of male conch found nearshore indicates that the nearshore conch have deficiencies which may ultimately lead to inefficient or ineffective fertilization.
- **Lip thickness was greater in conch collected offshore** probably due to higher mortality nearshore (*i.e.*, conch do not live long nearshore).
- **There were two instances of imposex in nearshore conch.** One conch had external male sex organs, but female gonadal tissue after histological inspection. The other conch had external female sexual characteristics, but male gonadal tissue after histological inspection.

Histology

- **Neural ganglia tissues are structurally impaired in nearshore conch of both sexes, suggesting the neuropeptides involved in reproduction may be impaired.**
- **Gonadal tissues in nearshore conch have serious deficiencies relative to offshore conch.** This confirms the results of our previous studies.

Chemistry

- **Zinc concntrations in conch tissues were significantly higher in conch found nearshore than offshore.** The differences in tissue concentrations of zinc

between nearshore and offshore conch, the identification of genes coding for zinc exposure, and the literature on reproductive impairments associated with zinc argue for expanded and targeted investigations of the effects of zinc on reproductive impairment in conch.

- **Other metals in the environment that were examined do not appear to be causing the reproductive deficits** because concentrations were higher in tissues of offshore animals.
- **Chlorinated compounds (OCP, PAH, and PCB) in the environment do not appear to be causing the reproductive deficits.**
- **Naled and DDVP (Dichlorvos) pesticides do not appear to be causing the reproductive deficits.** These chemicals were undetectable in sediments and water samples.
- **Estradiol and ethynylestradiol (EE2) were found in measurable quantities both offshore and nearshore.** This implies that sewage enters the offshore zone because EE2 is a synthetic estrogenic prescribed for birth control in humans.

Protein Biomarkers

- **The utility of vitellin (Vtg) as a biomarker is questionable at this point.** Although we isolated Vtg from gonadal tissue, Vtg has questionable utility because it is not found in the hemolymph and because nearshore female conch have no gonadal tissue. Additionally, Vtg is not inducible using 17-β estradiol; however, we do not know if other estrogenics may induce Vtg synthesis.
- **Heat Shock Protein 60 was higher in the neuroganglia of conch nearshore suggesting cellular disruption.** However, other Hsps and protein biomarkers were higher in conch collected offshore than nearshore.

Neuropeptides

- **Egg-laying hormone was higher in offshore female *and* male conch than in both female and male conch found nearshore in a variety of tissues.** This suggests that hormone synthesis is impaired nearshore and is affecting both sexes.
- **AGPWamide was higher in offshore conch of both sexes than those found nearshore.** AGPWamide is a proposed penis morphogenic factor and may be related to the smaller penis sizes found in nearshore conch.

Genomics

- **A library of Expressed Sequence Tags was developed** with over 8,000 well-annotated genes. This library was used to examine gene expression between nearshore and offshore conch.
- **A number of genes coding for basic organismal function were identified** that were differentially expressed between conch in the nearshore and offshore zones.