Report for 2005KY52B: Development and Immunity in Dragonfly (Odonata: Anisoptera): Indicators of Water Quality

Publications

- Conference Proceedings:
    - Harris, Shawna, Claire Fuller, and Howard Whiteman, 2006, Environmental factors affecting dragonfly immunity, in Proceedings of Annual Meeting of the American Society of Biologists, Gatlinburg, Tennessee.

Report Follows

Problem and Research Objectives

Historically, one of the most widely-used indicators of water quality has been the presence or absence of certain organisms, particularly fish and aquatic insects. Such assays have proven very useful for monitoring, yet may fail if the environmental perturbation reducing diversity in these systems cannot be removed by natural or anthropogenic restoration, thus allowing natural recolonization. Scientists thus need an early-warning system that could identify environmentally-stressed animals before the stressor causes population and/or regional harm, i.e., one which is more sensitive than presence-absence data. Such an indicator should be able to measure stress-induced effects before drastic changes take place that would subsequently decrease the organism's survival and reproductive abilities. We proposed to correlate biological indicators (development and immunity) with important water chemistry and habitat variables known or hypothesized to cause negative consequences in an anisopteran odonate (dragonfly), *Plathemus lydia*. Developmental anomalies may indicate exposure to stress in the past; immunity may indicate the likelihood that organisms can withstand future stress. We related water chemistry variables and our bioindicators across forested, agricultural, and industrial landscapes to understand how current land-use practices are influencing odonate populations. The results of this study provide initial data necessary to evaluate the use of odonates as an early-warning indicator of water quality and environmental degradation. Development of these methods will provide researchers with a sensitive biological indicator of environmental health, which can potentially be used to monitor areas that are susceptible to ecological disturbance or where there are human health concerns.

Specifically our objectives were to:

1) Adapt two immunological techniques for use with dragonfly nymphs to add to techniques already in use in our laboratory.
2) Measure developmental and immune parameters in nymphs from 20 ponds across a N-S transect in Western KY. This transect represents a predicted gradient of water pollution due to wind-borne contaminants from Murray, Mayfield, Paducah, and Calvert City, Kentucky). Measurements were planned for a spring and late summer collection.
3) Measure water quality parameters in the same 20 ponds during each collection period.

This research is important to both species conservation and environmental monitoring. The data we collected using USGS funds will allow us to write a larger proposal at the national level (in progress). Such data are necessary since federal funding for ecological research is limited and extremely competitive.

Methodology

Changes to methodology from our initial proposal are indicated in **bold**.

Ponds. We identified over 40 ponds along a N-S gradient in Western KY. Twenty of these ponds were initially selected as potentially useful for our study. **However, six ponds dried during the summer, leaving only 14 for sampling during the fall.**

Time of year for collections. We proposed to collect in spring and late summer, during cool and hot temperatures, respectively (to test the hypothesis that temperature affects development and immunity). **Because of the grant start date, we were unable to begin sampling until the early fall, in 14 ponds. We have identified an additional seven ponds to use in the spring 2006 survey.**

Collections. We proposed to collect ten animals from each pond by scooping leaf litter and sediment into a net. The contents of the net are examined and nymphs gently transferred to a minnow bucket supplied with water and leafy debris from their pond. Animals are transported to the lab for measurement of Fluctuating Asymmetry (FA), immunity assays, and preservation (see below). **We increased the sample size by collecting 10 to 25 animals. Up to 20 of these animals were used for 3 of the immunity assays and FA measurements. Five were used in two additional immunity assays (encapsulation and post-challenge PO activity).**

Fluctuating asymmetry. After capture, individuals were transported in buckets back to Murray State University for digital imaging. Each individual was initially to be anesthetized by chilling on ice for 5 minutes and then photographed with a Pixera Professional digital camera connected to a PC. **However, animals were first used for immunological analyses (except encapsulation because that assay can damage traits measured for FA), then preserved in 95% ETOH, and subsequently photographed.** Digital imagery reduces measurement error considerably when compared to other techniques (Whiteman et al. in prep). In addition, digital imagery allows us to easily take multiple measurements at a later time, rather than immediately while the animal is in the laboratory. Measurements of FA concentrate on major morphological structures associated with the head, mouth, legs, and torso. Each individual was measured three separate times in order to statistically analyze measurement error (Palmer 1994).

Immunological Assays. We originally proposed to conduct the following immunological assays: encapsulation, phenoloxidase (PO) in unchallenged animals, hemolymph protein levels and lytic activity. **We were unable to measure lytic activity. However, we added prophenoloxidase (PPO) as another assay. We also measured PO in animals that were immunologically challenged prior to assessment. To do this, we inserted an inert object (monofilament line) into the hemocoel and measured PO activity 24 hrs later. (Note: the monofilament line also serves as the object for encapsulation in the encapsulation assay).**

Principal Findings and Significance

All water quality measurements (except for organics) for the fall samples have been made and entered into a data base. Organic samples have been prepared for analysis. We photographed and measured all animals captured during the fall and data analysis has begun. We have finished all of the immunological assays (PO, PPO and protein levels) for the fall sampling period except for the measurements of encapsulation (all encapsulation immunological assays have been conducted, however, the animals with their encapsulated monofilmament have been preserved in ETOH and are awaiting measurement). We have conducted preliminary analyses to determine the relationship between these parameters and the following water quality indicators: temperature, pH, conductivity, dissolved oxygen, ORP, and turbidity. We have arranged a meeting with a statistician for help conducting additional statistical analyses. Preliminary

analyses show a number of statistically significant relationships (Table 1). Overall, PPO activity was related to temperature, but this relationship was not significant (Backward stepwise ANCOVA, N = 92, $R^2 = 0.356$). However, a number of environmental variables were significantly related to PO activity (Table 1; Backward stepwise ANCOVA, N = 79, $R^2 = 0.935$). Protein levels were significantly related to one environmental variable, turbidity (Table 1; Backward stepwise ANCOVA, N = 63, $R^2 = 0.547$)

These preliminary analyses of immunity and water quality suggest a number of strong relationships, particularly between PPO and temperature, conductivity, pH and ORP. In addition, we found a significant relationship between hemolymph protein levels and temperature. Thus, we have found at least one potential easy-to-measure early indicator of animal health (PPO). Our next step is to determine experimentally, which of these environmental parameters have the most impact on immunity. In addition, we will experimentally examine susceptibility to disease by exposing odonates to pathogens.

Table 1. The results of ANCOVA analyses examining the relationship between measures of immunity (PO, PPO and protein) and environmental parameters. Only significant or nearly significant values are included in the table.

Dependent Variable	**Independent Environmental Variable**	**df**	**F**	**P**
Phenoloxidase (PO)	Temperature	13, 75	1.550	0.120
ProPhenoloxidase (PPO)	Temperature	4, 63	138.4	< 0.001
	Conductivity	2, 63	13.5	< 0.001
	Ph	2, 63	21.7	< 0.001
	ORP	3, 63	16.9	< 0.001
Hemolymph protein	Turbidity	7, 52	9.74	< 0.001