Immunohistochemical Localization of the Red Tide Organism (*Karenia brevis*) in the Florida Manatee (*Trichechus manatus latirostris*)

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The female Florida manatee (*Trichechus manatus latirostris*) only produces a single calf every 2.5 to 5 years. The current population of manatees is 3,000 with an annual death rate of 400. Consequently, the manatee population may continue to decline until it reaches extinction. One leading factor that decreases the manatee population is the exposure to Florida red tide brevetoxins, produced by the dinoflagellate organism *Karenia brevis*. Sections were made from formalin-preserved paraffin-embedded blocks of manatee tissue. Following incubation with the primary *K. brevis* antibody overnight as well as no antibody as a negative control, the tissue sections were incubated with biotinylated donkey anti-sheep IgG, streptavidin ABC-AP and chromagen AEC for 10 minutes each. The tissue slides were then examined for traces of *K. brevis*, which appeared red on the sections. The organism was concentrated in the mandibular, axillary, retropharyngeal, and T-septal lymph nodes, as well as elements of the mucosal-associated lymphoid tissues (MALT). By successfully developing an immunohistochemical test to determine the presence of *K. brevis* in manatee lymphoid tissues, the pathogenesis of brevetoxin and its mode of entrance into the body can be better understood, revealing the impact of red tide on the survival of the Florida manatee.

**Introduction**

On June 7, 2006, a unanimous vote from the Florida Fish and Wildlife Conservation Commission and the approval of Florida Governor Jeb Bush led the Florida manatee (*Trichechus manatus latirostris*) to be taken off of the endangered species list. Rodney Barreto, the commission chairman, claimed that their reason for removing the species from the list was that with such a great population, “the manatee has recovered.” However, a female manatee produces only one calf every 2.5 to 5 years, with a current population of about 3,000 and a death rate of 400 manatees per year. Thus, the mortality rate of the manatee is likely to exceed the population’s growth rate. The long-term survival of the manatee population is in severe jeopardy.

One of the main causes of the decline in the population is the exposure of the manatees to Florida “red tide” brevetoxins. Red tide is caused by periodic blooms of *Karenia brevis* (formerly known as *Gymnodinium breve* and *Ptychodiscus breve*). *K. brevis* is a single-celled dinoflagellate organism normally restricted to the Gulf of Mexico and the Caribbean often incorrectly described as algae or phytoplankton. As a dinoflagellate, the organism is capable of manufacturing its own food from carbon dioxide, sunlight, nitrogen, and other nutrients. A cyst or resting form may be included in their life cycle, which may transform into the motile phase when environmental conditions are appropriate. The organism normally stays at the surface of the water during the day, then moves to deeper water at night. It is always present at low concentrations off of the Gulf coast of Florida, but under certain conditions *K. brevis* reproduces to such great numbers that the water appears to turn a red color, creating a “red tide.”

Red tides occur almost annually along the west coast of Florida, usually in the late summer and autumn. The most severe events occurred in 1971, 1973 to 1974, 1996, and 2005. Until recently, blooms had only been reported on the west coast of Florida, but harmful blooms were reported on the east coast in September of 2007. Even though these red tide explosions have been observed since the sixteenth century, it is suspected that in recent years an increase in frequency and duration of blooms has been promoted by nutrients from coastal pollution. Red tides created by *K. brevis* have killed millions of fish, manatees, birds, and bottlenose dolphins, and have brought on significant economic damages due to the closing of shellfish beds and decreased tourism.

*K. brevis* produces up to nine toxins, including a neurotoxin called brevetoxin, which can be fatal to fish, birds, and mammals. Brevetoxins are lipid-soluble, polycyclic ethers that are sodium channel activators. PbTx-2 is the brevetoxin produced by *K. brevis* in the greatest concentration, but the small organism also produces PbTx-1 and PbTx-3. However, older algal blooms in the wild sometimes produce the higher concentration of PbTx-3.

Human exposure can occur through ingestion of contaminated shellfish or through inhalation of aerosolized toxins. Neurotoxic Shellfish Poisoning (NSP) is a severe form of food poisoning caused by the ingestion of

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brevetoxin-contaminated shellfish and characterized by gastrointestinal, neurologic, and cardiovascular components. Severe cases of brevetoxicosis occasionally cause paradoxical dysesthesia, or the reversal of temperature discrimination. Brevotoxins often accumulate in fish inhabiting affected areas. A study by Naar and his associates demonstrated that the muscle tissue and viscera of shellfish, pinfish, and croakers exposed to brevetoxin for a two-week period still contained 40% and 15% of the maximum observed concentrations two weeks later. Thus, shellfish exposed to red tides may adversely affect humans even several weeks after the red tide bloom has cleared.

Although the effects of ingestion are more severe, inhalation of brevetoxins is significantly more widespread. The toxins become aerosolized when the fragile organism is broken down by wave action, releasing toxins into the water column. The aerosol is then incorporated into the salt spray and spread inland. The brevetoxin can thus be carried up to a mile inland. Both healthy and asthmatic individuals have reported eye irritation and respiratory symptoms, including upper respiratory tract discomfort and decreased forced expiratory volume per second, after being exposed to Florida red tide toxins. These symptoms were generally eliminated by leaving the beach and entering an air-conditioned area. Asthmatics tend to be more susceptible to respiratory symptoms, showing a statistically significant change in their airflow parameters after a one-hour period of exposure.

Mortality in marine mammals due to brevetoxin is not yet well understood. Manatees probably inhale the brevetoxin when they come up to the surface of the water to breathe, but they also eat many sea grasses that may absorb the toxin. In his study of manatees that had died after a red tide event, Bossart and his colleagues found that the lung pathology of all 149 victims indicated that the brevetoxins had been inhaled. However, a later study of 27 manatees and 36 dolphins that died after a different event observed an absence of this lung pathology and high toxin concentrations in the stomach contents, indicating exposure from ingestion. The stomach contents were composed entirely of sea grass, which was revealed to have high concentrations of brevetoxins, mostly in the epiphytic region. Filter-feeding tunicates were absent, ruling out the possibility that they could have acted as vectors for the toxin.

After the toxin is inhaled or absorbed, it is phagocytosed by macrophages, which remove pathogens from the body and stimulate other immune cells to respond. The toxin may kill the macrophages by denaturing or destroying certain enzymes, which leads to the uptake of the toxin by other macrophages. It causes neurologic signs in manatees, often including an inability to remain upright in the water, which may lead to drowning. Bossart and his associates observed severe cerebral, nasopharyngeal, pulmonary, hepatic, and renal congestion in fatalities of the 1996 epizootic, as well as nasopharyngeal and pulmonary edema and hemorrhaging. Leptomeningitis and hemosiderosis, or bleeding of the lungs, was also observed. Lymphocytes and macrophages were found in the lung, liver, and secondary lymphoid tissues. Lymphocytes and macrophages were also found in areas of the nasopharyngeal mucosa and meninges, and were positive for brevetoxin. Mortality from brevetoxicosis may result after chronic inhalation or ingestion, and is not necessarily due to acute exposure. Brevotoxins may initiate apoptosis or the release of inflammatory mediators that lead to toxic shock and death.

Stressors such as cold weather and boat strikes can affect a manatee’s immunocompetence, leaving it unable to ward off invading brevetoxin. This may result in disease or death. Infectious bronchopneumonia, generalized infectious dermatitis, and enterocolitis often accompany chronic cold stress, with secondary fungal infections of the skin and lungs occurring as it progresses. Mortality due to cold stress can occur when water temperatures drop below 21°C for prolonged periods of time. However, deaths due to brevetoxicosis tend to outnumber those due to cold stress. Few studies have been done to evaluate the immune health of manatees, but those existing demonstrate that immune suppression is a result of dietary restrictions and a possible co-factor in the cause of papilloma virus. Immune competence in dolphins, killer whales, and pilot whales can be evaluated through lymphocyte proliferation assays, and may be used to characterize immune dysfunction due to pollutants in the environment.

In this study, immunohistochemical tests will be used to examine lymphoid specimens from several manatees that have died due to red tide exposure, with the goal of demonstrating exposure of the Florida manatee to red tide by revealing the presence of *K. brevis* in different organs. These specimens will be compared with specimens from other manatees found in the same regions that were determined to have died from other causes, such as cold stress or boat strikes. By examining lymphoid tissues for the presence and intensity of *K. brevis* using immuno-histochemistry, we may come to better understand the pathogenesis of brevetoxin and its relative effects on Florida manatee populations.

**Materials and Methods**

Specimens from over 200 animals were collected from the Marine Mammal Pathology Laboratory of St. Petersburg, Florida, and submitted to the Histotechniques Laboratory of the Department of Small Animal Clinical Sciences at the University of Florida for histopathological evaluation. From the animals available, I chose 5 that had been found on the west coast of Florida whose pathology reports indicated that they had likely died from red tide exposure. From the specimens available from these 5 manatees, I selected mandibular, axillary, retropharyngeal, septal, caudal mesenteric, and anorectal lymph node tissue as well as the spleen, stomach, small and large
intestines, liver, lungs, and mucosal-associated lymphoid tissue (MALT) associated with the nasopharyngeal mucosa and small intestine. The tissue was preserved in formalin and embedded in blocks of paraffin which were used to make 5.0 micrometer (µm) sections. Each tissue slide was deparaffinized in alcohol, exposed to 3.0% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity, and exposed to 5% horse serum for 20 minutes to block fragment crystallizable (Fc) receptors. The slides were then incubated overnight at 4°C and at a 1:100 dilution with the primary K. brevis antibody, provided by Dr. Peter McGuire of the University of Florida College of Medicine. One slide was incubated with no antibody as a negative control. Following a 10-minute incubation with biotinylated donkey anti-sheep immunoglobulin G (Santa Cruz cat. SC-2475) at room temperature, the slides were incubated for 10 minutes at room temperature with streptavidin ABC-AP (HRP-Streptavidin Kit, Zymed, cat. 85-6143), then with cold chromagen AEC (Zymed kit) for about 7 minutes. The tissue slides were then examined by light microscope for traces of K. brevis, which appeared red on the sections.

Results

In the 5 animals observed, the organism itself was infrequently found in the nasal passage as well as within the gut, and no reaction was found in the liver or lungs. Instead, the organism was concentrated in the mandibular (Fig. 1), axillary (Fig. 2), retropharyngeal, and T-septal (Fig. 3) lymph nodes, as well as elements of the MALT (Fig. 4). The areas in these images that are stained a deep red indicate where the K. brevis antibody, and therefore the organism itself, can be found. Thus, the cells affected by the red tide organism and its toxin that are attacked by the immune system can be localized. The red localization is obvious in comparison with the control, which used no antibody and does not display any significant red coloration (Fig. 5). Some reaction was also seen in the spleen (Fig. 6). The organism was found to be present in the lymph nodes of all 5 individuals that had died from red tide exposure.
advancements in red tide research in a variety of areas.

Further studies may be done to examine specimens of manatees found on the east coast. These specimens should not reveal any significant presence of the red tide organism, with the exception of those found after the September of 2007 episode.

Through co-localization immunohistochemistry for *K. brevis* and macrophages within manatee lymphoid tissue, it could be determined if macrophages are concentrated in the same areas as the *K. brevis* antibodies. The same may be done with other components of the immune system, such as B-lymphocytes and T-lymphocytes. With these studies, we may come to better understand the immune response of affected manatees to brevetoxin and the organism that produces it.

As we now have a method to localize the red tide organism, the conjunctival-associated lymphoid tissues (CALT) of manatees can be examined by immunohistochemical localization. If the organism is localized within CALT, it can be concluded that antibodies for it must be expressed in manatee tears. Mammalian eyes do not have any specific immune components, and thus tears must be produced to help prevent the invasion of harmful microbial life. Manatees are thought to have the thickest tear film of any sea mammal\(^\text{22}\), which may be vital in protecting their eyes in murky, pathogen-rich waters. Manatee tears could easily be collected without removing the animal from its habitat by wiping the eye with a small cotton swab, and exposure to brevetoxin could be diagnosed through immunohistochemical localization for *K. brevis* in the tears. This test could eventually be used along with or instead of blood tests for diagnosis. Red tide exposure, as well as other health problems, may be determined in this manner. By using tears to determine if a manatee has been exposed to red tide, the animal can be diagnosed quickly and in a non-invasive manner. This would allow for better rescue and rehabilitation strategies and would allow treatment to be delivered more appropriately and efficiently.

An intensity distribution of the organism within the population, different organ systems, and within the immune system may give an estimation of the relative amounts of exposure to brevetoxins. By comparing results from manatees living in different geographic regions, we may identify the areas most affected by red tide blooms. This information may be valuable to conservationists in taking actions to limit the damages caused by red tides by working to prevent the blooms or protect other marine life. A comparison of the presence and intensity of the organism within each organ system and also of cells within an organ system could be used to better understand the pathogenesis of brevetoxin and to determine its routes of entry into the body. Statistical analyses could be performed to compare the quantitative differences in amounts of chromagen per pixel within the lymphatic tissues.

**Discussion**

Through immunohistochemical localization, the presence of *K. brevis* was revealed in all 5 individuals that had died due to red tide exposure. In a previous study, we found that the organism had also been localized in the respiratory and gastrointestinal tracts of one of these animals. The localization observed is somewhat similar to localization observed for brevetoxin, which was found in inflammatory lesions of the nasopharyngeal mucosa and meninges.

The localization of *K. brevis* in manatee lymphoid tissues has many implications for the survival of manatees as a species. By running these immunohistochemical tests and observing the usual histopathologic signs, brevetoxicosis can be diagnosed as the definite cause of death. Up until this point, brevetoxicosis could only be hypothesized as the cause of death due to pathological findings in many cases. A test for the presence of *K. brevis* will lead to

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**Figure 5**: Axillary lymph node of a manatee without antibody (400x magnification). The tissue slide was incubated overnight with PBS rather than the *K. brevis* antibody, which makes clear the results achieved from immunohistochemical localization. This tissue section was taken from the same specimen that was used for localization in Fig. 2. Without the antibody, the secondary antibody, the streptavidin, and the chromagen were unable to bind, resulting in a lack of localization. The manatee, which is also used in Figure 1, 2, and 3, was found on the west coast of Florida in 2003 and is designated by case number 03R-47.

**Figure 6**: Immunohistochemical localization of *K. brevis* in the spleen of a manatee (250x magnification). While reaction in the spleen was not as strong as that observed in other lymph nodes, it shows the localization of *K. brevis* nonetheless. This tissue was collected on the west coast of Florida in 2006 from a manatee designated as case number 06R-525.
The information gained from this study has opened doors for many other aspects of red tide research. An immunohistochemical test for K. brevis exposure will provide researchers, conservationists, and veterinarians with valuable insight into the impact of red tide and the survival of the Florida manatee.

References

Using Ecological Niche Modeling to Examine the Hypothesis of the Influence of Host Specificity on Species Distribution of the Leaf Beetle *Microrhopala floridana* and Its Host Plant *Pityopsis graminifolia*

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The Florida leaf beetle, *Microrhopala floridana*, is known to be host-specific on narrowleaf silkgass *Pityopsis graminifolia*. The distribution of both beetle and plant includes the state of Florida. To test the hypothesis that the distribution of *M. floridana* is limited to the distribution of its host plant, the niche modeling algorithm Maximum Entropy (MaxEnt) was used to generate hypothesized species distributions for both organisms based on known georeferenced collection sites and correlate these with the presence of various environmental information layers. Statistical measures of model accuracy were better than random and the predicted range of the beetle was smaller than that of the plant. The environmental variables that showed the highest degree of correlation with the known presence of these organisms were vegetation type, soil texture, and surficial geology. Missing environmental data, small sample size, collecting bias, and undetected subpopulations are discussed as possible obstacles to accurately predicting species distributions.

**Introduction**

Maximum Entropy, or MaxEnt, is one of several algorithms that may be applied to ecological niche modeling. This method generates predicted distribution maps from presence-only locality data and “layers” representing environmental variables such as climate, elevation, and vegetation or land cover. The MaxEnt approach discriminates between founded and unfounded constraints on distribution, minimizing the latter by predicting the most uniform distribution possible under available conditions. Simply put, MaxEnt is a useful method for making predictions based on incomplete data (Phillips et al. 2006; Jaynes 1957). MaxEnt’s algorithm produces continuous geographic output, wherein each map pixel is assigned a probability of occurrence between 0 and 100, and is color-coded accordingly (Phillips et al. 2006). Species distribution modeling based on presence-only data has become more useful with developments in database accessibility and environmental data collection. Occurrence data from museums and herbaria are increasingly available through the Internet, as are high-resolution environmental data layers from satellites and other sources (Elith et al. 2006).

*Microrhopala floridana* Schwarz is a metallic, dark-blue beetle, family Chrysomelidae, that is reported to be monophagous on *Pityopsis graminifolia* (Michaux) Small. Larvae of *Microrhopala* are internal leaf miners of Asteraceae, and the adults are external leaf feeders on the same host. *Microrhopala floridana* may be distinguished from other members of its genus by its elongate body form and parallel-sided prothorax. It is host-specific on *P. graminifolia*, with known feeding period from April to August. It has been reported from *Lupinus diffusus* (Fabaceae), but this is usually assumed to be an erroneous record (Clark 1983). *Microrhopala vittata* and *M. xerene* overwinter in the adult stage, which may also be true of *M. floridana*, whose life history is not known. The adults are often found head-down inside leaf axils (Fig. 1.), where they are difficult to collect by sweep netting (C. S. Staines, pers. comm.). The currently described range of *M. floridana* includes parts of North Carolina, Georgia, Alabama, and Florida. In Florida, it has been recorded from Highlands, Indian River, Liberty, Marion, Okaloosa, Orange, Palm Beach, Polk, Putnam, Seminole, Sumter, and Volusia counties (Peck and Thomas 1998; Clark 1983).

![Fig. 1: Microrhopala floridana in leaf whorls of Pityopsis graminifolia. Leaf pulled downward and slightly opened to expose beetles situated near the stem. (Photo courtesy of C. S. Staines, Jr.)](image)

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Pityopsis graminifolia (known as narrowleaf silgrass or grass-leaved goldenaster) is common throughout pine savannas in the southeastern United States (Semple & Bowers 1985). Harms (1969) viewed P. graminifolia (therein as Heterotheca [section Pityopsis] graminifolia) as a species complex with highly variable morphological characteristics. Cronquist (1977) recognized three varieties of P. graminifolia (therein as Chrysopsis graminifolia) as differing in head size and gland appearance. In a recent revision of the genus Pityopsis, Semple and Bowers (1985) recognized five varieties within the species P. graminifolia. Var. graminifolia is reported from northern Florida and other parts of the Southeast. Var. aequifolia Bowers & Semple is endemic to central Florida. Var. tracyi (Small) Semple is found throughout peninsular Florida. Var. tenuifolia (Torr.) Semple & Bowers is common throughout the range of the species. Var. latifolia (Fern.) Semple & Bowers is reported from the southeastern United States as well as Guatemala, Mexico, and Honduras. In this study, we treated P. graminifolia as one species, as this was the taxonomy used by the Florida Museum of Natural History (FLMNH), the source of our herbarium data.

Because M. floridana is poorly known and infrequently collected, it was important to select a modeling method with demonstrated success in producing useful models from extremely small data sets. Hernandez et al. (2006) found that MaxEnt had the strongest performance in a study comparing it with other methods (GARP, Bioclim, and Domain). Its results were the most consistent across a range of sample sizes, and it exhibited especially superior accuracy and spatial concordance using the smallest sample sizes (five and 10 occurrences). Pearson et al. (2007) compared MaxEnt and GARP models of gecko distribution in Madagascar and concluded that MaxEnt can make useful predictions at sample sizes of 5 or more. Using ecological niche modeling (MaxEnt) to generate hypotheses of species distributions, this study will compare the predicted ranges of a monophagous beetle and its host plant. We test the hypothesis that a monophagous insect and its host plant will have similar environmental requirements and concordant ranges. In order to test the hypothesis, distribution maps and accompanying statistical data were produced from museum specimens of M. floridana and P. graminifolia.

Materials and Methods

Specimen label data were collected for P. graminifolia from the FLMNH Herbarium (University of Florida), while label data were collected for M. floridana from the insect collections at Texas A & M University, the University of Georgia, and the University of Kansas, and from Dr. C. S. Staines’ personal collection (Smithsonian Institution). Some of the acquired label data contained GPS coordinates representing collection sites. Other collection sites had to be georeferenced using GoogleEarth (Google 2008) by selecting a point as nearby as possible to the described collection site. Some points were more accurate than others. For example, the Lake Eaton collection site was pinpointed by the collector, Mike Thomas, and the latitude and longitude of Red Hill Loop were recorded by C. S. Staines. The Ocala National Forest and Blackwater State Forest sites were traced using the GoogleEarth ruler feature. Roadside points were selected for the junction of Routes 98 and 370, the middle of Walt Williams Road, and Turkey Creek Plaza. Locality data were assembled from 25 specimens of M. floridana (Appendix A). Of these, 9 labels were detailed enough to georeference with some degree of confidence, and 6 were used to create the model, the others being outside of the range of the available environmental layers (e.g., some Florida counties were missing soil collection data, so collection sites in these counties would not contribute to the calculation of species distribution). Locality data were assembled from 96 specimens of P. graminifolia (Appendix B). Of these, 26 were detailed enough for georeferencing, and 22 of these were used to create the model. Of the 26 plant specimens, 4 were labeled as var. latifolia, 1 as var. aequifolia, 1 as var. stenolepis, and the rest were not identified to variety.

The layers selected for use in our MaxEnt-generated models were elevation, vegetation, geological epoch, surficial (sediment) geology, soil drainage class, soil hydric rating, and soil texture. Elevation was the only continuous variable; all others were categorical. Environmental layers for the state of Florida were compiled from the Florida Geographic Data Library. All environmental layers were converted into ASCII format with a grid size of 100 meters. Layer data were then imported into MaxEnt, which produced individual color-coded (depicting log likelihood of occurrence) distribution maps and jackknife analysis of variables for each species. MaxEnt parameters for both species were set as in Table 1. Using “auto features,” the program was able to select a set of features appropriate to the number of presence records. For M. floridana, the program used linear feature types, and regularization values were set at 1.000 lineardimensional/2-dimensional/3-dimensional, and 0.605 categorical. For P. graminifolia, the feature types were linear quadratic, and regularization values were set at 0.757 linear/quadratic/product, and 0.464 categorical. The species distribution analysis for both species used a grid size of 100 m.

<table>
<thead>
<tr>
<th>Table 1: MaxEnt parameters used in the analysis for both M. floridana and P. graminifolia</th>
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<tbody>
<tr>
<td>Regulization multiplier</td>
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<tr>
<td>Output format</td>
</tr>
<tr>
<td>Output file type</td>
</tr>
<tr>
<td>Maximum iterations</td>
</tr>
<tr>
<td>Convergence threshold</td>
</tr>
<tr>
<td>Random test percentage</td>
</tr>
</tbody>
</table>
Results

On inspection, the resulting Maxent distribution maps predicted a smaller range for *M. floridana* (Fig. 2) than for *P. graminifolia* (Fig. 3). The plant was predicted to occur without the beetle along the Gulf coast and throughout southern Florida including the Keys. In northern Okaloosa and Hamilton counties, the beetle was predicted where the plant was not. Because the output is continuous, there is no absolutely correct way to compare distribution sizes. Using thresholds determined by minimum training presence, 29.2% of the map was predicted to be suitable for the beetle, and 65.8% was suitable for the plant. MaxEnt divides occurrence points into training samples and test samples. Training samples teach the system to make initial predictions, which are then adjusted according to the test samples. Model performance is evaluated separately for test and training data. Thresholds for binomial interpretation depend upon factors related to sample size and thus are not directly comparable (Phillips et al. 2006).

A receiver operating characteristic (ROC) analysis is a threshold-independent method of evaluating model performance. The area under the ROC curve (AUC) serves as a measure of model performance in terms of sensitivity versus specificity. This analysis was performed for both distribution models, *M. floridana* and *P. graminifolia*. For *M. floridana*, AUC values were 0.898 for training data and 0.833 for test. For *P. graminifolia*, AUC values were 0.864 for training data and 0.726 test. The generated distribution models for both species showed a significantly high level of performance when compared to random (AUC = 0.5) (Fig. 4).

![Fig. 2](image2.png)  
**Fig. 2:** Hypothesized distribution of *M. floridana* in Florida generated from a MaxEnt analysis of collection records (*n*=6). Colored dots represent known collection localities; white dots indicate “presence” data used for training the distribution model and violet dots represent localities used to test the distribution model. Colors at far left indicate the predicted likelihood of occurrence across the state of Florida, log scale.

![Fig. 3](image3.png)  
**Fig. 3:** Hypothesized distribution of *P. graminifolia* in Florida generated from a MaxEnt analysis of collection records (*n*=2). Colored dots represent known collection localities; white dots indicate “presence” data used for training the distribution model and violet dots represent localities used to test the distribution model. Colors at far left indicate the predicted likelihood of occurrence across the state of Florida, log scale.

![Fig. 4](image4.png)  
**Fig. 4:** Area under the ROC curve (AUC) for the beetle (left) and its host plant (right). The y-axis represents sensitivity, or true positive rate, meaning the absence of omission error. The x-axis represents the value (1 - specificity), or false positive rate, representing commission error.

The relative importance, i.e., contribution, of the variables used in creating the insect and plant distribution models differed (Table 2). Stronger correlations cause these variables to be weighted more heavily. Surficial geology was most highly correlated with beetle distribution whereas vegetation was most correlated with plant distribution. Each categorical environmental layer includes metadata describing its composition (Table 3). MaxEnt indicates which categories were correlated with probability of occurrence. The two species’ environmental preferences differed in the areas of vegetation, soil texture, and geology.

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>M. floridana</em></th>
<th><em>P. graminifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Surficial geology</td>
<td>32</td>
<td>7.5</td>
</tr>
<tr>
<td>Vegetation</td>
<td>24.7</td>
<td>31.3</td>
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<tr>
<td>Soil texture</td>
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<td>Soil drainage class</td>
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<tr>
<td>Soil hydric rating</td>
<td>9.3</td>
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<tr>
<td>Geological epoch</td>
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<tr>
<td>Elevation</td>
<td>0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 2: Variable contributions by percent
Table 3: Environmental categories preferred by the beetle and plant. Variables are generally listed from most important to least important, based on average percent contribution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>M. floridana</th>
<th>P. graminifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetation</td>
<td>Forests of longleaf pine and xerophytic oaks</td>
<td>Mangrove swamp forests and coastal marshes</td>
</tr>
<tr>
<td></td>
<td>Wet to dry prairie marshes on marl and rockland</td>
<td>Wet to dry prairie marshes on marl and rockland</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Sand</td>
<td>Extremely gravelly loam</td>
</tr>
<tr>
<td></td>
<td>Medium fine sand and silt</td>
<td>Coarse sand</td>
</tr>
<tr>
<td>Soil drainage class</td>
<td>Well drained</td>
<td>Moderately well drained</td>
</tr>
<tr>
<td></td>
<td>Moderately well drained</td>
<td>Excessively well drained</td>
</tr>
<tr>
<td>Geologic epoch</td>
<td>Pliocene/Pleistocene</td>
<td>Pleistocene</td>
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<tr>
<td></td>
<td>Pliocene</td>
<td>Eocene</td>
</tr>
<tr>
<td>Soil hydric rating</td>
<td>Not a hydric soil</td>
<td>Not a hydric soil</td>
</tr>
</tbody>
</table>

Discussion

The analysis suggests that M. floridana prefers drier, sandy soils in pine and hardwood forests, while P. graminifolia is capable of living under these conditions as well as in wetter prairie and coastal marsh habitats. The results do not support the hypothesis, which supposed concordant distributions for the two species. The hypothesized distribution of the beetle was much smaller than that of the host plant. There were several areas in which the beetle was predicted, but the host plant was not. In addition, the distribution model calculated different ecological requirements in the areas of vegetation and soil texture between the two organisms. The accuracy of this study may have been affected by small sample size, collecting bias, or the presence of ecologically specialized subpopulations within P. graminifolia.

Vegetation type and soil texture were the most important variables of the layers used in this study, so it is worthwhile to note the differences between the two species. These may represent actual differences in ecological requirements, or they could be an artefact of imperfect layer compilation. The vegetation layer has particularly low resolution, with large heterogeneous regions of land designated as one type. Both the soil texture and drainage layers have higher resolution, but the system is not capable of recognizing patterns beyond the individual soil texture classifications; to the modeling algorithm, sand and coarse sand are as different from one another as sand and clay loam. Discrepancies and missing sections of county soil maps may have compromised the accuracy of the predicted species distributions. Two specimens of M. floridana were located in the Ocala National Forest, but could not be included in the analysis because soil data were not available for that part of Marion County. This could have skewed the results by omitting important environmental information.

Sample size is very important to niche modeling analyses. Larger sample sizes are ideal for distribution modeling, but specimens of M. floridana were rare, and those with detailed locality data were rarer. Six occurrence points may not have been enough to be representative of the beetle’s range. Every effort was made to access as many specimens as possible from museum holdings from across the southeastern United States (see acknowledgements for collections that provided specimens and/or label data). While we were able to amass a large series of specimens, most did not contain collection locality data that were specific enough for georeferencing and subsequent inclusion in the study. Collecting bias is always a relevant issue and its effects are amplified by small sample sizes. Collectors may favor roadside locations, accessible areas of parks, or certain habitats. This could cause certain environmental variables to be either overemphasized or underrepresented. The usual situation of M. floridana adults occurring inside the bottom of leaf whorls makes this species difficult to collect without specifically looking for it. In addition, collectors in search of this insect are likely to seek it out in “typical habitat.”

The prediction of an apparently monophagous beetle without its host plant in the northern Florida Panhandle raises some interesting questions. This may be due to input problems as described above. Otherwise, it may indicate the presence of an alternate host or ecologically specialized subpopulations of the plant. Certain varieties of the plant may share ecological requirements with the beetle, but the modeling dataset included multiple varieties of the plant without differentiating between them. Further research is needed to determine whether the beetle might feed on alternate species of Asteraceae, as do some other members of its genus.

While this study raises the possibility that M. floridana might not be monophagous, additional research is required to produce a more robust dataset with which to test this hypothesis. These efforts should include collection efforts focused on this beetle, i.e., that they feed largely concealed from view and are not collected with traditional collection methods, such as sweep netting. Additionally, the environmental layers (variables) selected to be incorporated into the model may not have direct or significant relevance to the biology of the beetle or the plant. The beetle sample sizes available for this study were not large enough to robustly determine a significant correlation between known collection location and the environmental variables incorporated into the model. This may be due to collectors targeting collecting efforts in a specific habitat.

Further research is recommended in the area of modeling insect distributions, as many of the comparative modeling
studies have been based solely upon plant and vertebrate animal species. Some methods may prove to be more appropriate to entomological modeling, especially in the case of cryptic or highly vagile taxa.

Acknowledgements

Thanks to Kyle Beucke (University of Florida) for environmental layers, technical support, and statistical help; Mike Thomas (Florida Department of Agriculture, Division of Plant Industries), Ed Riley (Texas A & M University), Zack Falin (University of Kansas), and Kent Perkins (Florida Museum of Natural History – Botany) for collection and herbarium data; Mark Deyrup (Archbold Biological Station, FL) for ecological observations; and Heather McAuslane for commenting on the manuscript. Special thanks to C. S. Staines, Jr. (Smithsonian Institution) for photographs, collection data, and natural history information.

Appendix A : Label data for Microrhopala floridana from the Florida Department of Agriculture, Division of Plant Industry (DPI), the Texas A & M University (TAMU), the University of Kansas (KU), and the collection of C. S. Staines, Jr.

(* = specimens collected from regions of Florida for which environmental layers were missing. These could not be used in the analyses.)

<table>
<thead>
<tr>
<th>County</th>
<th>Locality</th>
<th>Locality</th>
<th>Year</th>
<th>Month</th>
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<th>Latitude</th>
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<th>Collection</th>
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<td>C. Staines</td>
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<td>Red Hill Loop</td>
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<td>2005</td>
<td>March</td>
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<td>C. Staines</td>
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<td>Ocala National Forest</td>
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<td>July</td>
<td>R.E. Woodruff</td>
<td>R.E. Woodruff</td>
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<td>1.7 mi N Holt</td>
<td>Blackwater State Forest</td>
<td>1978</td>
<td>June</td>
<td>L. Stange</td>
<td>C.S. Staines, Jr.</td>
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<td>86.74775</td>
<td>DPI</td>
</tr>
<tr>
<td>Polk</td>
<td>Walt Williams Rd</td>
<td>Lakeland</td>
<td>1939</td>
<td>Aug</td>
<td>R.H. Beamer</td>
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<td>81.926006</td>
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<td>2008</td>
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<td>Deland</td>
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<td>29.040725</td>
<td>81.293744</td>
<td>KU</td>
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Literature Cited


Staines, CS Jr. 2008. Personal communication (e-mail).
Appendix B: Label data for *Pityopsis graminifolia* from the University of Florida Herbarium (FLAS). The standard acronym is a holdover from the Florida Agricultural Experiment Station.

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<th>Locality</th>
<th>Year</th>
<th>Month</th>
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<th>Longitude</th>
<th>Collection</th>
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<td>Airport Industrial Park, NE 49 rd off SR 84</td>
<td>Gainesville</td>
<td>1998</td>
<td>Oct</td>
<td>29.699875</td>
<td>82.261758</td>
<td>FLAS</td>
<td>196664</td>
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<td>Alachua</td>
<td>UF campus, 1900 block of SW 34th St, 180 m SSE of SE corner of 34th and Hull, 25 m E of fence</td>
<td>Gainesville</td>
<td>2002</td>
<td>Oct</td>
<td>29.635961</td>
<td>82.371756</td>
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<td>219891</td>
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<td>Charlotte</td>
<td>Along Fanabee Rd, 0.4 mi E of State Hwy 31, 1.4 mi S of Desoto Co. , 18 mi E of Port Charlotte</td>
<td>Port Charlotte</td>
<td>1975</td>
<td>Nov</td>
<td>27.018347</td>
<td>81.759825</td>
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<tr>
<td>Clay</td>
<td>E side of SR 21, ca 10 mi N of Keystone Hts (includes drawing)</td>
<td></td>
<td>1981</td>
<td>Jul</td>
<td>29.837736</td>
<td>81.955597</td>
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<td>Collier</td>
<td>US Hwy 41 at Collier-Lee county line</td>
<td></td>
<td>1952</td>
<td>Aug</td>
<td>26.316253</td>
<td>81.805536</td>
<td>FLAS</td>
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<tr>
<td>Columbia</td>
<td>see drawing</td>
<td>Ichetucknee Springs State Park</td>
<td>1991</td>
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<td>Hwy 236, opposite N entrance to Park.</td>
<td>Ichetucknee Springs State Park</td>
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<td>29.896264</td>
<td>82.760275</td>
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<td>Dade</td>
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<td>1968</td>
<td>Dec</td>
<td>25.6963</td>
<td>80.309661</td>
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<td>114909</td>
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<tr>
<td>Dade</td>
<td>Low pineland, Old Cutler Rd and SW 144 St, S23 T55S R40E</td>
<td></td>
<td>1968</td>
<td>Sep</td>
<td>25.637408</td>
<td>80.308763</td>
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<tr>
<td>Duval</td>
<td>Sandy low ground along Rte 115; ca 1 mi N of jct with US 1; 10 mi S of Jacksonville city limits</td>
<td>S of Jacksonville</td>
<td>1953</td>
<td>Oct</td>
<td>30.189372</td>
<td>81.551214</td>
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<td>Hillsborough</td>
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<td>1953</td>
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<td>27.694969</td>
<td>82.460808</td>
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<td>Lake</td>
<td>W side of FL 19, ca 0.3 mi S of jct with S US Business 441 (FL500A) in Tavares (0.2 mi N of Dora Canal) s29 T19S R26E</td>
<td>Turkey Oak Woods</td>
<td>1979</td>
<td>Nov</td>
<td>28.803544</td>
<td>81.738225</td>
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<td>Lake</td>
<td>Along State Hwy 50 at Orange-Lake county line. Under a tree near a lake.</td>
<td></td>
<td>1970</td>
<td>Dec</td>
<td>28.545758</td>
<td>81.65805</td>
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<tr>
<td>Lake</td>
<td>Along County Rd 445 at Alexander Springs River; 15 mi NE of Altoona. Dry road side; edge of woods</td>
<td></td>
<td>1973</td>
<td>Dec</td>
<td>29.081186</td>
<td>81.5655</td>
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<tr>
<td>Levy</td>
<td>17 mi SW of jct of Rt 19 and Rt 24, on S side of 24. Dunes.</td>
<td>SW of Bronson</td>
<td>1987</td>
<td>Sep</td>
<td>29.191478</td>
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<td>Levy</td>
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<td>Marion</td>
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<td>1973</td>
<td>Dec</td>
<td>28.928117</td>
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<td>Okeechobee</td>
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<td>Orange</td>
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<td>81.646597</td>
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<td>Palm Beach</td>
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<td>Boynton Beach</td>
<td>1986</td>
<td>Dec</td>
<td>26.572017</td>
<td>80.070667</td>
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<td>171019</td>
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<td>Palm Beach</td>
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<td>N Palm Beach Gardens</td>
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<td>Savannas State Reserve</td>
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<td>1992</td>
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<td>27.298611</td>
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<td>29.151733</td>
<td>81.516836</td>
<td>FLAS</td>
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Native Dragonflies as Pest-Consumers in Organic Gardens and Farms of North-Central Florida: A Preliminary Assessment

Montana Atwater et al.* College of Agricultural and Life Sciences, University of Florida

The goal of this study was to explore the possibility that dragonflies may play positive functional roles in agroecosystems as predators on pest insects. Specific objectives included: 1) to compare species richness of dragonflies in organic farm fields and gardens, 2) to determine patterns of microhabitat use by different species of dragonflies in cropped areas, and 3) to determine prey types taken and foraging behaviors exhibited by the Eastern pondhawk (Erythemis simplicicollis), the most common species observed. A total of 170 observations of dragonfly individuals were made across 4 sampling locations in Alachua County, and 6 species were detected. Species richness of dragonflies was significantly greater in gardens than farm fields. Foraging heights (microhabitats) varied significantly among species, and the abundant Eastern pondhawk was most dominant at the crop level where it commonly used both ground and crop substrates for perching/hunting. Because of its abundance and foraging microhabitat preferences, we conclude that the Eastern pondhawk is most likely to have an effect on pests.

Introduction

Integration of biodiversity conservation on farmlands is a high priority for achieving sustainable food production (Thrup 2000). Greater understanding of native wild species’ positive roles in food production is needed to support integration of biodiversity in farming systems (Vandermeer 1997). Because they are simplified in structure and managed for few plant species, most croplands, by nature, support a low diversity of insects with large populations. Pest insects certainly follow this pattern: their outbreaks in response to the rapid growth of crops that they feed on have been the focus of a great deal of research (Bach 1980, Bezdieck and Granatstein 1989, Gurr et al. 2003). Studies relating to the dynamics of native, locally occurring predators that may feed on pest insects, before or during outbreaks, has not been very extensive, but the research that has occurred suggests native predators may play significant roles in pest control. Native vertebrates such as birds and bats clearly feed on arthropod pests within agroecosystems (Greenberg et al. 2000, Jones et al. 2006, Wickramasinghe et al. 2004). In addition, the economic effects of their consumption of pest prey are beginning to be understood through a variety of experimental approaches. For example, insectivorous birds, by controlling agricultural pests in coffee plantations, can cause significant decreases in frequency of herbivore damage (Greenberg et al. 2000).

Although invertebrate predators have long been considered important parts of biological control and Integrated Pest management (IPM) programs, more attention has been paid to introducing exotic, specialized predators into agroecosystems on an “as needed” basis than has been directed at fostering diverse native invertebrate predators (Elliott et al. 1996, Linteren et al. 2006). Given the hazards of introducing exotic species, there is a need to fully ascertain the potential pest control of native predators in agroecosystems. Even though they are widely recognized for their predatory habits, little attention has been paid to species of the dragonfly families (Smith and Capinera 2005) regarding their potential impact on pests. In a study occurring throughout Thailand, the Philippines, and Hong Kong, dragonflies were found to feed on common pests in rice fields; gleaning prey from the leaves of rice plants and consuming large numbers of stem borers (Cambidae) and leaf hoppers (Jassidae) (Corbet 1999). While the impacts on plant growth arising from dragonfly predation on pests has not been assessed, current evidence suggests that consumption of pests by dragonflies is significant and should be assessed as sustainable (biodiversity-friendly) agricultural practices are developed. In this study, we sought evidence that dragonflies may play important positive functional roles as pest predators in gardens and vegetable farms of North-Central Florida.

Study Design and Methods

Specific objectives of this study were: 1) To determine species richness of dragonflies in organic farms and gardens in Alachua County, Florida, and determine if species richness differs between small gardens (< 1 ha in area) and organic farm fields (> 1 ha in size); 2) To determine the

*Authors: Montana Atwater and Katie E. Sieving, PhD
partitioning patterns of microhabitats by the different species of dragonflies in cropped areas, i.e., airspace and perching substrates; 3) To determine what kinds of prey and foraging behaviors were most often used by the Eastern pondhawk, the most common species observed foraging at the crop level.

We used descriptive and comparative methods to achieve stated goals, observing dragonflies on two organic gardens on the UF campus and on two small organic vegetable farms in Alachua County, during the summer of 2007. We made repeated visits to the study sites between 1200-1700 hours during 4 June 2007–9 August 2007. Within the cropped areas at each site, 182 focal observations were conducted at arrays of 4-6 sampling points. For each site visit, a minimum of 10 to 30 minutes per sampling point was spent observing activity. Observations occurred in a circle of radius 4 m around the observer, generally. For each site, date, time, cloud cover, temperature, and wind speed were recorded. For each sample point, initiation time and duration were recorded. Lastly, for each observation we recorded species, sex, perch height, perch type, and behavior. When necessary, binoculars were used to identify species inside and outside the sample points. The species observed in this study are all classified as perch-hunters, which enhanced identification and ease of observation since individuals remained on or near single perches throughout observation sessions.

Objective 1) Determinations of species richness (total number of species observed in crops) were made by tabulating all species seen in or out of sample circles over the course of all visits to each site. Then we calculated the mean number of species detected for farms and for gardens and compared the total species richness between the two types of sites using a Kruskal-Wallis test.

Objective 2) Each time an animal was observed, the substrate type (ground, on crop plants, posts, edge of crops, or in flight) and height of the perch was recorded. To determine whether different species used different microhabitats, we compared heights of the different species using a 1-way ANOVA and post multiple comparisons tests.

Objective 3) The Eastern pondhawk was the numerically dominant species in the fields, so we focused foraging observations on this species. We calculated summary statistics and present the dominant foraging substrates used by this species in pie diagrams.

Results

Objective 1) A total of 170 observations of dragonfly individuals were made across all 4 locations and 6 species were detected (Fig. 1). Species richness of dragonflies was significantly greater in gardens than farm fields (K-W Chi-square = 19.4, DF = 1, P < 0.001); gardens had a mean of 2.2 species (SD = 1.1, N = 36) and farm fields had a mean of 0.63 species (SD = 0.07, N = 16; Fig. 2).

Objective 2) Foraging heights (or, microhabitats) varied significantly among the 6 species (ANOVA; F = 45.9, P < 0.001, DF = 5; See Fig. 3). Multiple comparisons among the mean perch heights of the different species indicated the following important results (statistical significance at 0.05); Eastern pondhawks foraged significantly lower than 3 species (FSPE, GWSK, and ROSK; see letters in Figure 3). The Four-spotted pennant foraged significantly higher than all 5 other species.
Objective 3) The Eastern pondhawk was most dominant at the crop level. The ground and crop were the most common perch types exhibited by the Eastern pondhawk (see Fig. 4). Feeding by the pondhawk was witnessed twice but no identification of prey could be made. Both prey items were captured on the wing and brought back to a perch to consume.

Discussion

Pest control potential of dragonflies. We determined what species of dragonflies are likely to be common on agro-ecosystems in North-central Florida. Of the 6 species detected, only 2 appeared abundant enough to potentially play significant functional roles on farms; Eastern pondhawk and Roseate skimmer. Of those 2, the Eastern pondhawk was particularly abundant, and we would recommend future research of this kind be focused on that species. Because the Eastern pondhawk nearly always foraged at the crop level, or close to the ground, they are the most likely to have an effect on pests. Among other predatory insects known to operate at the crop level, certain non-web building species of spiders have been noted for their potential to participate in pest control. For example, spiders are known to narrow their feeding niche significantly when a suitable prey species reaches high numbers relative to other prey groups (Nyffeler et al. 1994). This trait is also thought to be exhibited by most dragonfly species; though they are highly opportunistic foragers, they are able to become temporary specialists in response to high availability of one kind of prey (Corbet 1999). Therefore, Eastern pondhawks have a high likelihood of function as pest-controllers in the small cropped areas we studied, when outbreaks of characteristic invertebrate pests occur. As we observed in this study, dragonfly territories and hunting perches are occupied with great consistency throughout the growing season, suggesting that if the prey changed, the dragonflies must change their prey preferences to take advantage of incident prey. Temporary specialization offers the benefit of reducing time devoted to searching and recognition. Therefore, it is quite possible that dragonflies, as generalized predators, may moderate fluctuations of prey populations if, as is likely, they respond to prey in a density-dependent way with shifting search images that are frequency-dependent (Corbet 1999). Though more research is needed, we propose the hypothesis that dragonflies could help contain or prevent pest outbreaks, thus achieving a central aim of integrated pest management.

Although we were not able to effectively identify prey species in the field, it is clear that dragonflies forage in cropped fields and may be foraging on pests. To properly identify prey, future studies should include dragonfly capture and dissection of the gut contents and identification of prey using microscopes. As a first assessment of pest-eating potential of dragonflies in agroecosystems, this study strongly suggests that taking the next steps to begin identifying prey taken and measuring impacts of prey consumption on crops would be a fruitful line of study.

Fostering dragonflies and other forms of biodiversity on farms. The diversity of predatory insects within crop fields is significantly related to vegetative diversity whether from adjacent non-crop habitats or a greater diversity of crop plants (Dennis et al. 1992, Kajak 2007). Fur-

![Figure 3: Mean perching and foraging heights of individuals of the study species in farms and gardens. See Fig. 1 legend for key to species abbreviations. Letters above bars signify significant differences between mean perching heights (bars with the same letters are not significantly different from each other). Error bars represent one SE.](image1)

![Figure 4: Perch types exhibited by the Eastern pondhawk. Numbers indicate the number of observations of individuals using each perch type.](image2)
thermore, increasing vegetative diversity is found to sustain populations of other beneficial animals such as birds, spiders, and even wild pollinating species in agroecosystems (Asteraki et al. 2004, Benjamin et al. 2008, Thomas et al. 1991, Winfree et al. 2008). This study confirms previous work; we found dragonfly diversity to be significantly greater in gardens and, in general, the gardens had a greater diversity of crop plants, and more non-crop habitats than farms. Identifying specific habitat requirements for beneficial invertebrates that are locally occurring could help support integration of biodiversity in management of agricultural landscapes. Further research is needed to refine such strategies across scales and systems for the future of sustainable food production.

Acknowledgments

This study was funded by the University of Florida’s Scholars Program. Thanks go to Pat & Ronald Carlisle - Organic Farm, Alachua, FL, Rosie Koenig of Rosie's Organic Farm, Gainesville, FL; and Seth Bybee, Graduate Research Assistant in the Branham Laboratory of the Department of Entomology and Nematology, University of Florida.

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Acoustic Refraction: A Three-Dimensional Shear Layer Correction for Microphone Measurements in an Open-Jet Acoustic Wind Tunnel

Jeremy Sanford

College of Engineering, University of Florida

Introduction

An anechoic wind tunnel has been in operation at the University of Florida since 2006 (Fig. 1). Its recent construction was prompted by the growing importance of fundamental aeroacoustics and the relative scarcity of suitable academic research facilities (Matthew, 2006). The increased demand for aerodynamic research is driven by the critical economic importance of the aviation, and, increasingly, wind generation industries. These are subject to progressively more stringent noise regulation. The ability to model and predict aerodynamic sources of noise during the design phase has thus become a prerequisite to competing in these industries. The immaturity of the analytical tools developed to date is reflective of the chaotic interaction of the already complex phenomena of acoustic wave propagation and turbulent fluid mechanics. The principal aim of ongoing investigations, therefore, is to provide a controlled experimental foundation for models predicting acoustic performance.

The Anechoic Wind Tunnel. Anechoic chambers are designed to isolate a volume from ambient noise sources and minimize internal reflections over a range of frequencies so that acoustic signals measured in the chamber are only those directly generated by the source. Open-jet wind tunnels provide test sections free of the hard acoustic and aerodynamic boundaries found in the typical closed section arrangement. The University of Florida’s anechoic wind tunnel is a juxtaposition of these two concepts where an anechoic chamber surrounds the test section of an open-jet wind tunnel.

In practice, the rectangular test section is usually bounded by two sidewalls composed of five-inch thick acoustic foam backed by aluminum substructure. The walls help avoid the complications of three-dimensional flows that would accompany airfoil models with free ends. The acoustic foam construction helps maintain the approximate acoustic free-field in the remaining open directions by minimizing interference from reflections. Surrounding the support structure, instrumentation, and the tunnel itself are treated with a combination of Nomex (http://www2.dupont.com/Nomex/en_US/) and acoustic foam to minimize deviation from the ideal anechoic environment. The foundation of that environment is a chamber 6.92 m wide by 9.96 m long by 4.26 m high in outer dimensions with all six interior surfaces covered by fiberglass wedges designed for a 100 Hz cutoff frequency (Matthew, 2006). The wind tunnel test section is 1.8 m long in the flow direction and is sized to accommodate models 1.12 meters wide. Models are subjected to flow velocities up to 75 m/s ($M \approx 0.22$) which is conditioned for low turbulence and hence low self-noise.

Simulating and reliably measuring free-field aeroacoustic behavior is the result of a complex series of compromises in the context of a closed research facility. The complications have led some to full-scale measurements where large outdoor microphone arrays are employed. Facilities like the anechoic wind tunnel cannot ideally simulate this condition, but they do offer enough fidelity to provide invaluable insight with a measure of experimental control and repeatability while requiring fewer resources.

Shear Layers and Acoustic Refraction. A primary advantage of open-jet wind tunnels for acoustic research is the ability to locate microphones outside of the jet where a relative lack of flow-induced background noise may improve signal to noise ratios by up to 9 decibels (Mueller, 2002). Measuring sound which propagates through a convective speed gradient, however, introduces a fundamental experimental challenge. The interface between a jet of viscous fluid and surrounding quiescent medium is known as a shear layer. It influences both the direction and amplitude of a pressure wave that passes through it. The effect is analogous to the well-known phenomena of light changing directions upon passage through materials with different indices of refraction, and it is thus termed acoustic refraction.

A shear layer is a region separating primarily inviscid fluid regimes where viscosity becomes a significant factor governing the fluid dynamics. Its characterization is complicated by the fact that there is no universal standard delineating the boundaries between still-air, shear layer, and the open-jet flow region. The shear layer is also frequently turbulent for typical wind tunnel test conditions and its thickness varies with axial position along the flow direction. An exact description of wave propagation through a shear layer is therefore impractical. Instead, analytic corrections are derived by modeling the boundary as an infinitely thin vortex sheet and further assuming uniform conditions on either side of the boundary. The model has supported the derivation of a correction procedure which is independent of source type by separating direction and amplitude corrections. This approach has provided useful and experimentally validated results which are the basis of the present investigation.
Directional Acoustic Arrays. Multiple measurement locations are required to map the acoustic field when source directivity is to be measured (Pierce, 1991). A modern approach employed at the anechoic wind tunnel for obtaining such measurements is by use of a directional acoustic array. It is composed of multiple microphones that are typically surface mounted to a plate in a fixed geometry. A data-processing technique called beamforming takes advantage of variations in phase and amplitude in the measured signals to electronically steer the array to a point in space (Arnold, 2001). Accuracy, compactness, signal integrity, experimental flexibility, and lack of mechanical complexity all justify the use of phased arrays for aeroacoustic research.

Two circular arrays are currently in use at the UF anechoic wind tunnel: a small aperture directional array (SADA) (Fig. 2) and a large aperture microphone directional array (LAMDA). The SADA is comprised of 33 microphones arranged in a series of concentric circles designed to mimic a NASA design for data set comparison (Humphreys, Brooks, Hunter, & Meadows, 1998). The LAMDA is nearly six feet in diameter and contains a total of 63 microphones in spiral arrangement with maximum microphone to microphone spacing of 44 inches. This array, with its widely spaced microphones spanning much...
of the test section in two dimensions, introduces yet another complication concerning measurements made through a shear layer. The geometric construction of shear layer corrections is most often presented in the form of a ray tracing procedure in two dimensions; that is, the source and observer are assumed to be coplanar and corrections are made in terms of angle definitions. The microphone arrangement of the large array places 63 widely spaced observers in plane with each other but not the source. This adds a third dimension to the ray tracing geometry which is unique to each microphone location.

**Research Objectives.** The ultimate goal of the present investigations in the University of Florida’s anechoic wind tunnel is to identify the range of frequencies, flow velocities, and measurement techniques for which previously developed acoustic refraction theories are valid. Prudence would dictate that prior experimental procedures be emulated to obtain baseline results for comparison to establish facility-unique characteristics. The experiment by Schlinker and Amiet characterizing shear layer refraction is the relevant reference (Schlinker & Amiet, 1980). The investigation would then expand to include conditions specifically relevant to typical facility operation such as the use of laser-initiated microdetonation for a non-intrusive broadband noise source and use of the phased acoustic array for measurements.

Logistical considerations precluded the timely execution of the baseline experiments. Ongoing use of the LAMDA, however, offered an alternate investigative path while narrowing the scope of current research objectives. Array measurements have been obtained where the location of a well characterized source was accurately assessed in the absence of test section flow. Data was also acquired when the same source was active in the presence of a shear layer and the resulting displacement of the acoustic source was evident. If the distortion was primarily the results of shear layer effects, and standard analytical models are valid, the distorted image could be corrected to reproduce the free-field image. Positive results would simultaneously verify several aspects of acoustic refraction behavior in the facility while negative results would indicate the need for further investigation. The goal of this thesis then is to show the development of a general correction algorithm for acoustic refraction in three dimensions and use existing array measurements to assess its suitability and direct future work. The scope of the work is further restricted to directional corrections since amplitude corrections follow directly from the geometry obtained by the directional corrections and are of secondary importance.

**Technical Approach**

The current activity began with the development of the basic two dimensional shear layer correction algorithm (Amiet, 1978) to validate its suitability for use with in-plane measurements in the University of Florida’s anechoic wind tunnel. This was based on a geometric derivation and satisfactory results prompted the development of a more sophisticated correction for a three-dimensional system. The general algorithm was first used to reproduce the earlier two-dimensional results and then used to model actual ray geometry with array-representative observer locations.

**Principles and Assumptions.** R. K. Amiet’s 1978 publication in the Journal of Sound and Vibration (Amiet, 1978) and Sodeman & Allen’s chapter in Aeroacoustic Measurements (Mueller, 2002) describe the most common shear layer corrections employed in modern research facilities. The procedure refined by Amiet was followed by an experimental assessment conducted at United Technologies Research Center in partnership with Robert H. Schlinker (Schlinker & Amiet, 1980). The results confirmed the utility of the model and supporting assumptions, although there remains ambiguity regarding the extent to which some conclusions can be applied to a unique facility. Amiet’s rigorous derivation lent itself to the development of the general correction algorithm and the conventions found there are adopted here. The equivalent treatment by Sodeman & Allen is presented briefly as an alternative construction to help illustrate some important definitions more intuitively (Mueller, 2002).

Nomenclature is the only real distinction between the two approaches as both employ ray acoustics in their construction. A ray path is the line in space traced by a point that moves with velocity such that it is always located on the wavefront (Pierce, 1991). The two conventions are illustrated on equivalent schematics in Fig. 3 and Fig. 4 for reference, and Table 1 summarizes the equivalence of the various definitions in the two constructions.

![Figure 3](image3.png)

**Figure 3:** Amiet naming convention adapted from Amiet (1978)

![Figure 4](image4.png)

**Figure 4:** Sodeman & Allen naming convention adapted from Mueller (2002)
Both conventions define a measured ray as the straight line between source and observer; the path traveled in still air. The path traced by the solid line is the ray from source to observer under the influence of jet convection and shear layer refraction. Additionally, both describe a corrected observer position lying on the path that the solid ray would take in the absence of a shear layer (uniform flow extends to infinity).

It may be tempting to conceptualize the solid ray as equivalent to $r_m (R_m)$ under the influence of the open-jet. This, however, is not stipulated in the definition and is, in fact, not the case. The two paths are physically related only in the sense that they describe the path a ray must travel to join the actual source and observer positions under a given condition. The ray which can conceived as “what happens to” an acoustic ray in quiescent conditions if it is subjected to open-jet conditions is instead represented by the path connecting the corrected observer and retarded source in Fig. 3 or, alternatively, the ray defined by $\Theta'$ in Fig. 4. The physical distinctions are perhaps more apparent in latter construction by Soderman & Allen (Mueller, 2002) and motivated its inclusion in this discussion. With these points illustrated, further geometric descriptions will be restricted to the Amiet convention (Amiet, 1978) where the difference is handled in a mathematical sense by simply noting that $\Theta$ does not equal $\Theta_c$.

The vortex sheet model permits the derivation of wave propagation through the shear layer from the convective wave to standard wave equations and application of pressure and fluid particle-displacement continuity for boundary conditions at the shear layer. The derivation is partially presented in an appendix to Amiet’s journal paper and gives rise to several important assumptions along with some restrictions. The results are independent of source frequency except for the cases where the wavelength approaches characteristic source and observer dimensions. The source does not need to be a point source or even a compact one. All acoustic energy is assumed transmitted without reflection or turbulent scattering. Fluid density changes across the boundary are neglected not out of necessity but due to their secondary importance, which is a reasonable assumption for the low Mach numbers in the facility ($M < 0.22$). Finally, analysis is restricted to the parallel plane shear layer case since that is the one applicable to the anechoic wind tunnel.

### Geometric Relationships

The mathematical correction for the special case of the source and observer in plane can be arrived at with only geometric relationships and use of Snell’s law. Snell’s law is not an arbitrary construct in acoustic refraction but is a consequence of boundary conditions that constrain trace velocities on either side of the vortex sheet to be identical (Mueller, 2002). Ordinarily, the measured angle and distance will be known along with the Mach number and perpendicular distance between source and shear layer. A system of three equations was developed in terms of these known parameters to find the remaining three angles in Fig. 3 needed to fully describe the ray geometry.

Geometric relationships from Amiet yield one independent equation.

$$r_m \cos \theta_m = h \cot \theta_c + (r_m \sin \theta_m - h) \cot \theta$$

Wave refraction at the shear layer is developed from Snell’s law

$$\frac{c}{\cos \theta_r} + U = \frac{c}{\cos \theta}$$

where $c$ is the isentropic speed of sound (constant across boundary) and $U$ is the free stream velocity. Normalizing equation (2) by the speed of sound gives

$$\frac{1}{\cos \theta_r} + M = \frac{1}{\cos \theta}$$

which, after rearranging, gives the second independent equation.

$$\cos \theta_r = \frac{\cos \theta}{1 - M \cos \theta}$$

Equation (5) is developed from the velocity triangle for the convected wave and the law of sines.

$$\frac{c}{\sin \theta_c} = \frac{U}{\sin(\theta_r - \theta_c)}$$

Normalizing by the speed of sound and invoking a trigonometric identity yields

$$\frac{1}{\sin \theta_c} = \frac{M}{\sin \theta_r \cos \theta_c - \cos \theta_r \sin \theta_c}$$

Rearranging equation (6) provides the third independent relationship.

$$M = \sin \theta_r \cot \theta_c - \cos \theta_r$$

$$\implies \cot \theta_c = \frac{M + \cos \theta_r}{\sin \theta_r}$$
The angle definitions are useful for intuitive geometric derivation of ray relationships and additional angles could be used to describe the general case of non-coplanar source and observer. In this case, however, angle definitions complicate the conceptual picture. Instead a vector-based approach was employed making direct use of relationship developed by Amiet in Cartesian systems. The original angle definitions are shown again in Fig. 5 for comparison with the similar geometry in Fig. 6 which is defined in Cartesian coordinates.

The additional parameter “d” in Fig. 6 is included for later use with the general correction algorithm where the observer (microphone) to shear layer distance is fixed and “h” is allowed to vary. Additionally, the corrected observer position is translated along $r_c$ to illustrate that it can lie at any point along $r_1$. The origin of the x-y-z coordinate system is fixed at the source location forming the primary reference for ray geometry in the Cartesian construction. The positive x-direction is coincident with the flow direction, the positive z-direction is normal to the shear layer pointing in the direction of the observer, and the positive y-direction is given by right-hand rule. The origin of the primed coordinate system is defined by the intersection of $r_1$ and the shear layer and has principle axes aligned with the primary system. The principle axes of the double primed system are also aligned with the primary system and the origin is fixed to the retarded source position.

The derivation given by Amiet (1978) for the general case depicted in Fig. 6 is too detailed for inclusion here but demonstrated the independence and generality of the relations chosen for the correction algorithm. They are summarized in equations (8) and (9).

\[
\frac{x_c}{\sigma_c} - M = \beta^2 \frac{x_m'}{r_m'} \\
\frac{y_c}{\sigma_c} = \frac{y_m'}{r_m'}
\]  

Equation 8

\[
\beta^2 = 1 - M^2 \\
\sigma_c = \sqrt{x_c^2 + \beta^2(y_c^2 + z_c^2)}
\]  

Equation 9

The variables $\beta^2$ and $\sigma_c$ are temporary variables employed by Amiet given by

The origin of the primed system $(x'_0, y'_0, z'_0)$ is constrained to lie along $r_c$ coincident with the shear layer. It is important to note that the source, observer, corrected observer, and $(x'_0, y'_0, z'_0)$ all lie in the plane that is normal to the shear layer and passes through the source and observer as seen in Fig. 7.
The illustration is an extension of the various coordinate systems and three-dimensional geometry previously developed in Fig. 6. The z-directions are reversed from that prior description but all other axes and ray paths are otherwise defined as before. Fig. 7 depicts a specific geometry that will be used formulate the 3D correction algorithm but is shown here to emphasize the concept that the point at which the refracted ray crosses the shear layer does not independently define the refracted plane. The plane is instead uniquely formed by the source, observer, and free vector normal to the shear layer. The point of intersection is then determined by knowledge of the corrected observer position. This is again a result of the boundary conditions requiring equal trace velocities on either side of the vortex sheet. Stated another way, if the refracted ray intersected the shear layer at an independent point that created a plane with the source and observer which was not perpendicular to the vortex sheet, the trace velocities on either side would not be equal and the boundary conditions would be violated. These constraints mean that original measured ray and both segments of the corrected ray lie in the same plane and a simple construction of similar triangles gives the following relationships.

\[
y'_0 = \frac{y_c}{x_c} x'_0 \\
z'_0 = \frac{z_c}{x_c} x'_0
\] (12)

Since the origin of the primed coordinate system is constrained by definition to lie on the shear layer, the position of \(z'_0\) is also independently defined by the relation in equation (14).

\[
z'_0 = h
\] (14)

Additionally, since \(r'_m\) is determined by the known observer position in x-y-z and the origin of the primed system, its magnitude can be used to impose a length on \(r_c\) and obtain a sixth independent equation.

\[
r'_m = \sqrt{x'_c^2 + y'_c^2 + z'_c^2}
\] (15)

The system of six equations can be solved numerically to locate the corrected observer position and the point of intersection between the refracted ray and shear layer. The geometry is completed by finding the retarded source position which is given by Amiet and is related to the corrected observer position as follows.

\[
y''_0 = y_c \\
z''_0 = z_c
\] (17)

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**Beamforming Application.** The generalized relationships developed above were used to formulate a shear layer correction algorithm specific to the typical anechoic wind tunnel experimental setup. In this configuration, the LAMDA is positioned beneath, and parallel to, the test section where the vertical distance between all microphones and the shear layer is a fixed parameter. A global coordinate system is defined with the origin at the center of the array, the positive P-direction aligned with flow from inlet to diffuser, the positive R-direction perpendicular to the array directed toward the ceiling, and positive Q-direction completing the right-hand orthogonal coordinate system. The designations were chosen for clarity when working with the traditional x-y-z systems used to construct acoustic ray geometry.

The refracted ray path clearly alters the phase information upon which beamforming relies to steer the array to a point in space. Uncorrected beamforming on acoustic signals passing through a shear layer would therefore steer each microphone to a different point in space; none of which would coincide with the desired location. The timing is influenced by both propagation distance and the effect of convection on the speed of propagation. For beamforming application then, the correction algorithm should give the difference in propagation time between \(r_m\) and the refracted ray defined in Fig. 6 as the sum of \(r_1\) and \(r_2\). Transmission time in the case of \(r_m\) is simply the propagation distance divided by the speed of sound.

\[
t_m = \frac{r_m}{c}
\] (18)

The propagation speed of \(r_1\) (\(c'\)) is altered by jet convection (positive x-direction by definition). A unit vector in the direction of propagation (\(\hat{e}_x\)) is introduced to facilitate the vector addition of the speed of sound in the direction that direction and the convection term (\(\vec{U}\)).

\[
c' = c \cdot \hat{e}_x + \vec{U}
\] (19)

The transmission time of the refracted is then found by equation (20).

\[
t' = \frac{r_1}{c'} + \frac{r_2}{c}
\] (20)
The shear layer correction for the large array is a multi-step process beginning with a known Mach number (M), distance between the array and bottom of the inlet (shear layer), microphone coordinates and beamforming spatial coordinates in the P-Q-R global system defined in Fig. 7. For every combination of microphone and source position, an $r_m$ vector is formed. Equations (8) through (15) are solved simultaneously for the ray plane geometry. Then, with equations (16) and (17) providing $r_r$, the difference in propagation time is calculated. Though not strictly necessary for the beamforming correction, points of interest are easily translated to the global system for visualization.

**Results**

Intermediate results were obtained during development of algorithms addressing each stage of the correction process. All of the algorithms were implemented as functions in MATLAB.

**General Correction vs Established Special Cases.** The first correction was developed using the angle relations from equations (1), (4), and (7) for the specialized coplanar case. This was partly motivated by the availability of tabulated results from both Amiet (1978) and Soderman & Allen (Mueller, 2002) as well the need for quick implementation with data from individual microphone measurements. A similar reference for three-dimensional corrections was not available so the general correction was initially tested against the verified two-dimensional correction. The following tables were generated by using both algorithms and reproduce the referenced 2D results. Table 2 matches a table of results given by Soderman & Allen where the Mach number and the ratio between the source to observer distance and source to shear layer are used to find all angles given measured angles ranging from 40 to 140 degrees.

Table 3 is similar tabulation of results given Amiet (1978) again using constant Mach number and distance ratio to find three unknown angles. The table is shown with columns in the order presented by Amiet who employed an algorithm with 0 as the independent variable. The present investigation is concerned with obtaining refraction geometry with the measured angle known so the table was reproduced using the calculated value from Amiet to find the remaining parameters.

**Simulated Refraction Geometry.** The basic correction algorithms were incorporated into functions designed to provide a visual interpretation of the results. This was especially important for the 3D case to assess whether the results were at least physically meaningful in the absence of any other reference. The visual depiction also proved useful in the 2D case for a sense of actual scale relative to the schematic representations in Fig. 3 and Fig. 4. Fig. 7 represents the first test case from Table X and is a good illustration of how significant the refraction phenomena can be for a real physical case. On a one-meter

<table>
<thead>
<tr>
<th>$0m$</th>
<th>$0'$</th>
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<tr>
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<td>47</td>
<td>62</td>
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<td>53</td>
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<td>43</td>
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<td>60</td>
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<td>77</td>
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</table>

Table 2: Tabulated results from Soderman & Allen replicated using 2D and 3D corrections. Input parameters are $0m$, $M = 0.3$, and $Rm/Rt = 4$ (ref. Figure 4)

<table>
<thead>
<tr>
<th>$0m$</th>
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<table>
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<td>90.0</td>
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<td>105.3</td>
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<td>124.4</td>
<td>89.1</td>
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<tr>
<td>170</td>
<td>161.8</td>
<td>102.0</td>
<td>131.3</td>
</tr>
</tbody>
</table>

Table 4: Tabulated results from Amiet replicated using 2D and 3D corrections. Input parameters are $0m$, $M = 0.5$, and $h/r = 0.15$
scale, for example, the figure suggests a 30 centimeter displacement between actual and retarded source positions. The line styles in the simulated plot are consistent with the ray designations in Fig. 5, Fig. 6, and Fig. 7, while the blue horizontal line indicates the position of the shear layer.

The 3D plots were critical to revealing flaws in early implementations of the solution routine that allowed non-physical solutions to the given constraints. Fig. 9 illustrates the correct solution where the verified 2D behavior occurs in ray planes perpendicular to the shear layer connecting the source and the observer. The observer positions are actual microphone locations on the array and only the refracted path is displayed for clarity.

**Uncorrected LAMDA Measurements.** Two data sets from the large array were selected for initial evaluation of the 3D correction algorithm in its intended application.
Figure 8: Scale plot of the first row of results in Table 3 for an arbitrary length scale where the shear layer is depicted by the horizontal blue line.

Figure 9: Refracted ray paths from a source in the 1st quadrant of PQ to actual microphone coordinates on the large array. Additional input parameters are $d = 0.25$ m and $M = 0.2$. 
These were cases where the source location exhibited a clear shift from its actual measured position when measured by the array in the presence of flow. The only differentiating parameter between the data sets was source frequency of the speaker suspended in the flow field. Fig. 10 and Fig. 11 show an uncorrected acoustic map for the 5 kHz and 8 kHz tests respectively. The measured position in quiescent condition is located at the origin of the coordinate system shown in the plots.

The results were obtained prior to the definition of the coordinate systems used in the development of the correction algorithm. They differ in only the definition of the P and Q global axes which have negative sense with respect to the description in Fig. 7. The important distinction is in the flow direction which is in the negative x-direction as labeled in both figures.

**Corrected Results.** In its early developmental form, the shear layer correction code solves the nonlinear system of equations using a built-in MATLAB optimization scheme. It was employed for expedition and, at approximately one correction per second, was adequate for calculating the simulated results previously presented. In a beamforming application however, the time required to find 63 separate solutions for each of the 10,000 grid locations in concurrence with the beamforming algorithm proved excessive. This limitation was temporarily addressed in the form of a MATLAB script written by Tarik Yardibi which constructed the entire corrected beamforming map in a single matrix. The matrix is valid for a given Mach number and array position so only one implementation was required.

The correction algorithm was readily modified for coordinate system consistency with the available data sets and used to construct the corrected beamforming matrix. The following figures show the adjusted maps and indicate a shift toward the origin in both cases.

**Conclusions**

Simulated results demonstrated the successful execution of an algorithm for theoretical shear layer corrections in three dimensions. Two dimensional correction results from available references were reproduced exactly while three dimensional solutions displayed the behavior predicted in the derivation. There is substantial benefit to be gained in the practical application of the algorithm with further refinement. The built-in MATLAB optimizer used in the correction algorithm will be replaced with a more efficient solution specific to the correction’s nonlinear system of equations. This, along with general streamlining of the code to eliminate unnecessary calculations, will be pursued to improve calculation time.

A final conclusion regarding the suitability of the theoretical correction for the UF anechoic wind tunnel cannot be supported by the limited data set evaluated to date. In both cases examined, the shear layer correction shifted the source position in the appropriate direction but failed to completely restore the original source position. These results need to be augmented with data from a broad range of test conditions where Mach number, source position, and source type are varied and judged against an experimental control in the form of an equivalent zero-velocity source image.

Before proceeding with a more extensive testing regime, the amplitude component of the shear layer correction will be addressed. The shear layer correction itself is separable by frequency and amplitude and is, in theory, frequency-independent. This is not the case for the beamforming algorithm which depends on phase differences and is thus sensitive to variations in amplitude and frequency. It is therefore worthwhile to develop this component of the correction before judging its overall efficacy. Poor results after the implementation of these measures would justify a more extensive investigation of the shear layer refraction in the UF anechoic wind tunnel by allocating facility time to an experiment that would emulate the original research conducted by Schlinker and Amiet (1980).

**Literature Cited**


Figure 10: Large array beamforming results with a 5 kHz source and 0.091 test section Mach number

Figure 11: Large array beamforming results with an 8 kHz source and 0.091 test section Mach number
Figure 12: Large array beamforming results incorporating shear layer correction with a 5 kHz source and 0.091 test section Mach number

Figure 13: Large array beamforming results incorporating shear layer correction with an 8 kHz source and 0.091 test section Mach number
LabView Program for Thermal Conduction Measurements

Erica Douglas  College of Liberal Arts and Sciences, University of Florida

Introduction

Some antiferromagnetic geometric shapes have unique and complex behaviors. In the case of a triangular lattice, once two of the three spins on the lattice are anti-aligned in order to appease the antiferromagnetic (AF) behavior of the material, the third spin is unable to point in a direction which will be opposite to the other two spins while minimizing the energy states. Hence, the symmetry of the triangular lattice is not compatible with AF interactions. This situation is known as triangular geometric frustration.\(^1\) The consequence of the geometric frustration on the cooperative behavior can affect the ground state selection which is established by interactions such as the “next-nearest-neighbor.”\(^2\) Geometric frustration can also affect quantum fluctuations and usually results in various degenerate ground states.\(^1,2\)

Geometrically frustrated antiferromagnets are studied in order to determine what happens when the triangular lattice prevents the spin configuration from becoming simple and ordered. There are very few materials, however, which exhibit this behavior.\(^3\) Cs\(_2\)CuBr\(_4\) is an example of a crystal which displays the triangular lattice, AF interactions, and it has a spin S = \(1/2\). Magnetocaloric-effect and specific-heat measurements were performed on a sample of Cs\(_2\)CuBr\(_4\) in order to determine the phases, such as up-up-down (uud) of the spins. The focus of the research was on that of the specific-heat measurements. Specific-heat data uncovers the “dramatic enhancement of the magnon gap.”\(^3\) This, in turn, gives way to the presence of the Dzyaloshinskii-Moriya interaction, though only weak.

There are several factors that can affect specific-heat measurements. One of these is a fluctuation in the thermal conductance of the weak links in the calorimeter used for taking specific-heat measurements. In order to verify that the thermal conductance is consistent over time and magnetic fields, an addition to the LabView program used to acquire data was written.

Background

In order to understand essential features of geometrically frustrated magnetism, one can look at an isotropic Heisenberg model consisting of classical spins interacting via the nearest neighbor exchange described by the Hamiltonian for a layered triangular lattice:

\[
H = -\sum_{i<j} J_{ij} S_i \cdot S_j
\]

where S is a unit length, classical three-component vector and \(J_{ij}\) is the strength of the exchange interaction between spins at sites \(i\) and \(j\). This can be simplified by looking at a simple set of Hamiltonians that describe only antiferromagnetic interactions of nearest neighbors:

\[
H = J \sum_{i,j} S_i S_j
\]

\(J\), with a positive exchange energy, will favor antiparallel alignment of the spins S. This is labeled by the site indices of \(i\) and \(j\). The sum is then taken over the nearest-neighbor bonds and the Heisenberg vector spins S are again a three component vector where \(S = (S_x, S_y, S_z)\) of spin length \(|S|\) which is again fixed. Each spin has two degrees of freedom. Since the length of the spin is fixed, it is “free to take any value on the surface of a sphere.”\(^1\) These two degrees of freedom can be thought of as longitude and latitude for this case. The total number of degrees of freedom in the ground state \((F)\) can be estimated to be \(D - K = F\), where \(D\) is the total number of degrees of freedom of the spins and \(K\) is the total number of constraints that have to be met in order for the system to be in a ground state. This, however, may not always be the case since the constraints due to the equations may not be independent or they are mutually exclusive. Typically, when the total spin sums to zero, the state is in its ground state. When the magnetization plateau is present in Cs\(_2\)CuBr\(_4\), it signifies that the material is in the ground state. This ground state can be present as a “spin liquid, a collection of spin multimers, or an ordered state that is collinear with the magnetic field.”\(^3\)

With respect to Equation 1, \(J_{ij} < 0\), when the spins of the system are interacting antiferromagnetically in a triangular lattice. In the case of magnetic Cu\(^{2+}\), ions are located on the CuBr\(_2^2-\) tetrahedra and form the triangular lattice in the bc lane. In the \(b\) direction, the Cu\(^{2+}\) nearest-neighbor exchange, \(J_{ij}\), is larger than \(J_{ij}\) in the other “principal directions” in the bc plane.\(^4\) According to Tsujii et al., the ratio of \(J_{ij}/J_1\) = 0.74 for Cs\(_2\)CuBr\(_4\).\(^3\) Maximum geometric frustration occurs at \(J_{ij}/J_1 = 1\), which indicates that Cs\(_2\)CuBr\(_4\) is very close to this maximum frustration. In comparison to Cs\(_2\)CuCl\(_4\), another triangular frustrated antiferromagnet with \(S = 1/2\), the ratio of \(J_{ij}/J_1\) ranged from 0.34 to 0.37.\(^3\)
In zero-field, the Heisenberg model of the nearest-neighbor in zero-field exhibits 120° spin order. An extremely important finding in the crystal is that of a magnetization plateau. It is in this region that the magnetization of the material is constant despite the fact that magnetic field is increasing around it. In order for the magnetization plateau to exist, the spin system must satisfy the plateau condition of \( n(S - m) = \) integer, where \( n \) is the period of the spin state, \( S \) is the magnitude of the spin, and \( m \) is magnetization per site in the unit of \( g\mu_B \). Essentially, this effect arises from the gap in energy of the “low-lying magnetic excitations” and is a result of the continuous rotational symmetry of the Hamiltonian.

In order to determine the heat capacity of a sample, heat is applied to the sample in the form of

\[
C = \frac{\Delta Q}{\Delta T},
\]

where \( C \) is the specific-heat, \( \Delta Q \) is the change in heat applied to the sample, and \( \Delta T \) is the change in temperature of the sample. Thermal relaxation is used in order to determine the specific-heat. Once the system is in a steady state with current flowing through the system, as shown in Fig. 2, the power is then turned off. This, in turn, leads to a decay in temperature (voltage) to \( T_0(0) \) within a time constant of

\[
C = \kappa (RC),
\]

where \( \kappa \) is the thermal conductance. When the thermal conductance of a sample is low, the specific-heat of it may not be accurately measured with this method. This is due to a temperature gradient that becomes present in the sample.

The internal relaxation time, denoted by \( \tau_2 \), accounts for this effect by

\[
\tau_2 = \frac{C}{\kappa_{\text{int}}},
\]

where \( \kappa_{\text{int}} \) is the thermal conductance of the sample.

**Apparatus**

A relaxation calorimeter was used to gather both specific heat and magneto-caloric effect data. A dilution refrigerator was used in order to cool the calorimeter and the sample. Compared to the typical quasi-adiabatic calorimeters, this method allows the experimenters to be in the milli-Kelvin range. Fig. 3 is a schematic of the calorimeter.
The silver block, as shown in Fig. 3, is used as a thermal reservoir. This is made entirely of silver in order to prevent the time constant from becoming too long. Silver was used because of its very small nuclear heat capacity. In order to monitor the temperature of the silver block, a 220 Ω Speer carbon resistor that was ground to a thickness of 0.5 mm. Another important feature of the calorimeter is the brass casing that encloses it. The brass prevents thermal radiation and heating due to eddy-currents. It also thermally isolates the liquid helium in the mixing chamber from the calorimeter. The sample is placed on a platform made of sapphire. This design of the calorimeter allows the experimenters to use samples that are small, on the order of a few milligrams. This is essential since it is often difficult to obtain bulk crystals. The platform heater is constructed from a thin evaporated layer of a 7% Ti-Cr alloy. The platform is actually supported by the electrical leads for the thermometer and the heater. These leads also provide a weak thermal link between the platform and the silver ring.

In order to calculate the heat capacity of a sample that is placed on the sapphire platform, the temperature difference is measured, ΔT, between the silver ring and the weak thermal link of the platform. Once the Ti-Cr heater is turned off, the relaxation time is measured by the use of a lock-in amplifier as well as the null detector of a Wheatstone bridge. In order to actually obtain the heat capacity of the sample, the thermal conductance of the weak links are measured and a non-linear least-squares fit is performed on the relaxation data.

Method

Once measurements at varying magnetic fields are taken and the calorimeter is calibrated, the specific-heat measurements should be consistent. However, it is quite possible that the thermal conductance of the weak links between the silver thermal reservoir and the sapphire platform where the sample is placed fluctuates over time. This may be due to a buildup of material on the weak links. As a change in the thermal conductance can ultimately effect where the phase transitions appear, it is extremely important to make sure that the calorimeter is calibrated at all times. The addition, the LabView program was designed in such a way that a separate file is written and appended for the platform voltage, independent of the master file. Previously, voltages were read directly from the voltmeter and manually written down. These values were then recorded into a computer file, a process open to user error. Thus, this program is intended to reduce user error and allow analysis on the thermal conductance to be carried out independent of the master file.

As can be seen in Fig. 5, a For loop was designed with five iterations. Within this For loop, the Keithley sub-VI is used to read the voltage of the platform. This sub-VI reads in the platform heater GPIB address, the platform heater current in micro-amps as a local read variable, and the platform heat current GPIB address. This in turn allows the sub-VI to determine the resistance in ohms as well as the voltage of the platform. The voltage is read five times and averaged in order to obtain more accurate data.
Figure 6: User required inputs for GPIB addresses and data file names

Figure 7: First sequence of True case

Figure 8: Second sequence of true case

Conclusion

In summary, triangular geometrically frustrated antiferromagnets can provide insightful evidence into the phases as well as enhancing the magnon gap. \( \text{Cs}_2\text{CuBr}_4 \) is an exceptional material to study since it is very close to the maximum geometric frustration. By determining the specific-heat, the experimenters can also study effects from the gap in energies of low-lying magnetic excitation. The heat capacity of the sample is determined by applying heat to the sample and measuring the temperature difference the heat causes. This, however, depends largely on the temperature difference between the silver block of the calorimeter and the weak thermal links that are connected to the sapphire platform and the sample. Since it is possible that the thermal conductance of the weak links can change over time, it was the purpose of the experiment to design a program that would read the voltage of the platform and write the data to a file. This additional program will eliminate user error due to transcription of data and also allow future researchers to periodically verify the thermal conductance of the weak links. This in turn should allow for more accurate data analysis of the phase transitions for \( \text{Cs}_2\text{CuBr}_4 \) and other materials to be studied in the future.

Acknowledgments

I would like to thank Dr. Takano for the guidance and helpful discussions, as well as Younghak Kim for his assistance. This work was supported by the Center for Condensed Matter Sciences at the University of Florida Physics Department.

References

Economic Determinants of Faculty Union Membership at Florida State University

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This study explores why associate professors, professors, and eminent scholars at Florida State University belonged to the United Faculty of Florida in 2006. It is hypothesized that union members gain some economic benefits in membership. Faculty members within the lowest paid departments, those producing the fewest amounts of publications, those earning the lowest salaries, and those closest to retirement were expected to be more likely to join the union. The 347 faculty members eligible for tenure were examined in the study. A regression analysis of the data was performed with variables describing information about these faculty members. Data on each faculty member’s productivity, represented by each faculty’s salary for 2006, the number of books he or she published, the number of articles that he or she published, and the year that he or she acquired a PhD, were compiled. Each faculty member’s department affiliation was also noted. This data functioned as the independent variables in the four regressions performed. The dependent variable described faculty membership in the union (yes/no). Consistent with the hypothesis, the analyses showed that the most experienced faculty members were the least likely to join the union. Also as predicted, members of higher paid departments and those earning the highest salaries were less likely to join the union. The book and article variables proved to be unrelated to union membership. The study concluded that some levels of productivity, experience, and department affiliation can be common among faculty who joined the union and among faculty who did not join the union.

Introduction

Unions are formed when employees decide working conditions, wages, or benefits can be improved when they negotiate as a group rather than as individuals. However, not every employee is a union member. Some employees decide not to join a union even though the union continues to bargain on their behalf for wages, benefits, and working conditions. Examining similarities and differences between union members and non-members can expose the benefits and costs of union membership.

Florida State University (FSU) faculty was the group chosen to study trends in union membership. Information on faculty members’ department affiliations, salaries, numbers of articles and books published, and years of experience is publicly available and was gathered to create profiles of faculty members. The information gathered may explain why a faculty member did or did not join the union.

The United Faculty of Florida (UFF) is the state-wide bargaining unit for higher education faculty and professionals. A chapter of the UFF bargains for FSU faculty and is known as the UFF-FSU. A large function of UFF-FSU activity is to achieve across-the-board monetary raises for all faculty members during contract negotiations. These raises are universal and are not based upon individual faculty performance. An across-the-board-raise is in contrast to a merit-based raise, which is determined by individual faculty member performance. Through across-the-board monetary raises, faculty in lower-paying departments gain an increase in average salary relative to that of faculty in higher-paying departments. Additionally, faculty members who publish fewer books and articles and those who earn lower salaries are better represented by a union. In 2006, members of UFF-FSU paid 1% of their bi-weekly salaries in dues. Therefore, it can be assumed that union members received some benefits in exchange for these dues.

Through contact with union President Jack Fiorito, I was able to obtain data on each faculty member’s union membership status. Names were not disclosed, and information about each faculty member was placed into intervals in the final data set to protect privacy.

Materials and Methods

The sample contained the 347 associate professors, professors, and eminent scholars employed at Florida State University who were eligible for tenure in 2006. Instruc-
tors, assistant professors and other faculty members who had not achieved enough success in publications or other work to become eligible for tenure were not included in the sample. Faculty members eligible for tenure were assumed to be the most likely to join the union. The faculty members studied were employed in 23 departments. All departments employed 10 or more associate professors, professors, or eminent scholars. Every faculty member examined had published at least one article or book. The books and articles published in these departments could be easily compared. Departments in which faculty published forms of art or media were not included in the sample. The name, rank, salary, and department affiliation of each faculty member in 2006 were gathered from the UFF-FSU salary archive.

Statistical Analysis

Dependent Variable. The dependent variable was a faculty member’s UFF-FSU membership status. The variable took a value of 1 if the faculty member was a member of the union in 2006 and a 0 if the faculty member was not a member of the union.

Independent Variables. Average Department Salary was the average salary of all the faculty members within their respective departments within the sample in 2006. The data were taken from the UFF-FSU salary archive. In departments earning a lower average salary, faculty members may have felt that they would benefit from across-the-board raises and collective bargaining power. These department members had more incentives to support the union, and union members may have encouraged their fellow department members to join as well. It is hypothesized that Average Department Salary will have a negative effect on UFF-FSU membership status.

The Salary, Articles Published, and Books Published variables measured individual motivations in union membership. The 2 salary variables and 4 publication variables were used as 2 different measurements of productivity for the entire career of the faculty member. The salary variables were used in Regressions 1 and 3 and the Books and Articles Published Variables are used in Regressions 2 and 4. For each characteristic the faculty member was segmented into a top, middle, and bottom third division of his or her department within the sample. Two dummy variables were used to describe to which division a faculty member belonged. One variable indicated that the member fell within the top segment of his or her department within the sample and the other indicated that the member fell within the middle segment. This method was used to control for differences in salary, article publications, and book publications between departments.

Salary Variables. The salary of a faculty member could be an indicator of productivity and value to the University. Florida State may have used the promise of a higher salary to lure a faculty member away from the private sector or another university. Salary could also be used as a measure of productivity and the desire of the University to retain that faculty member. Salary data for each faculty member in 2006 was obtained from the UFF-FSU Salary Archive.

The Top Third Salary variable took a value of 1 if the faculty member’s salary was in the top third segment of the salaries in his or her department within the sample and took a value of 0 otherwise. A faculty member that received a higher salary may have been more valued by the University and had less to gain from an across-the-board monetary raise. It is hypothesized that the Top Third salary variable will have a negative effect on UFF-FSU membership status.

The Middle Third Salary variable took a value of 1 if the faculty member’s salary was in the middle third segment of salaries within his or her department within the sample. It took a value of 0 otherwise. These faculty members might have been highly productive but had less experience. They may have been more valued by the University and benefited more from merit-based raises. It was hypothesized that this variable will have a negative effect on UFF-FSU membership status.

Articles Published Variables. The productivity of faculty members was partly measured by the number of articles they published. Articles were examined because it was the most consistent measure of productivity among all departments studied. The number of articles published was gathered directly from faculty resumes. If the resume for a faculty member was not available, the number of articles was taken from the Web of Science Database. The articles counted were authored or co-authored journal publications by the faculty member through 2006.

The Top Third Articles Published variable took a value of 1 if the number of articles published by the faculty member was in the top third segment of his or her department within the sample and took a value of 0 otherwise. A faculty member who produced more articles would have been better compensated by a merit-based raise. They would have had little incentive to support an organization which advocated across-the-board raises. It

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3 Departments include: Accounting, Biological Science, Chemistry & Biochemistry, Communication, Computer Science, Criminology & Criminal Justice, Economics, Education Psychology & Learning Systems, English, Finance, History, Information Department, Management, Marketing, Mathematics, Mechanical Engineering, Meteorology, Oceanography, Physics, Political Science, Psychology, Public Administration, and Sociology

4 The archive can be found on the UFF-FSU Website: www.uff-fsu.org/saldata.html

5 This is an international database containing millions of articles from the years 1900 to 2007 published in academic journals. The Database is maintained by Thomas Scientific™. The website can be accessed at: http://isiwebofknowledge.com/
was hypothesized that this variable would have a negative effect on UFF-FSU membership status.

The Middle Third Articles Published variable took a value of 1 if the number of articles published by the faculty member was in the middle third segment of the department within the sample and a value of 0 otherwise. The faculty members who published a number amount of articles may have been more productive in an effort to move up in rank. These faculty members would have gained less from an across-the-board-raise. It was hypothesized that this variable would have a negative effect on UFF-FSU membership status.

**Books Published Variables.** Significant time and effort are required for a faculty member to publish a book. Therefore, books published could be considered an indicator of productivity. In some departments faculty published as many books as articles. To control for these effects, a measure of the number of books published by a faculty member through 2006 was used. This number was taken directly from the faculty member’s resume. If a resume was not available, the quantity of books was gathered from Amazon.com. Only full books authored or co-authored by the faculty member were counted.

If the amount of books a faculty member published was within the top third segment of his or her department within the sample, the Top Third Books Published variable took a value of 1. Otherwise, the variable took a value of 0. Faculty members publishing the most books in their departments were conducting a great amount of research and could have been considered highly productive. These faculty members had less incentive to support an organization lobbying for higher across-the-board raises because they would have benefited more from merit-based raises. This variable was hypothesized to have a negative effect on UFF-FSU membership status.

The Middle Third Books Published variable took a value of 1 if the number of books published by the faculty member was within the middle third segment of the department within the sample and a value of 0 otherwise. Members in this group may have been productive but had less experience. They would have gained more from merit-based raises. This variable was expected to have a negative effect on UFF-FSU membership status.

**Experience Variables.** Experience could be an indicator of a faculty member’s productivity. The difference between the year the faculty member acquired a PhD and the year 2006 was used as a proxy for experience. The year that the PhD was acquired was taken from the department website or the faculty member’s resume. Thirty-nine faculty members did not have data available from either location. For these members the average difference between the year the PhD was acquired and the year of first publication was calculated among all other department members within the sample. This average difference was then subtracted from the year of the faculty member’s first publication. This difference was used as the proxy for experience. Experience was expected to play a greater role in the decision to join or not join the union and greatly influence faculty members who had the most experience. For this reason, the top two segments of experience were included in the four regressions.

The 30+ Years variable took a value of 1 if the faculty member had a difference of more than 30 years between acquiring a PhD and 2006 and a value of 0 otherwise. Faculty members with more than 30 years of experience could have been less productive because they were close to retirement. These members would have benefited more from across-the-board-raises. This variable was hypothesized to have a positive effect on UFF-FSU membership status.

The 15-29 Years variable took a value of 1 if the faculty member had a difference of 15-29 years between acquiring a PhD and 2006 and a value of 0 otherwise. Faculty members with this amount of experience were further from retirement and were likely to still be productive. These members would have gained less from an across-the-board raise. The variable was expected to have a negative effect on UFF-FSU membership status.

**Results**

A total of 4 regressions were run. The Average Department Salary, 30+ Years, and 15-30 Years Variables were used in all four regressions. The salary variables were used as an alternate productivity measurement to the Articles and Books Published Variables. The salary variables were used in Regressions 1 and 3 while the Articles and Books Published Variables were used in Regressions 2 and 4. Regressions 1 and 2 contained the entire sample of 347 professors. Regressions 3 and 4 examined a subset of the sample. The 16 departments studied in the subset contained at least 1 union member. In no departments were 100% of the members also union members. The subset of 16 departments is studied because the costs of joining the union in the excluded 6 departments may have been greater due to departmental influences. These regressions were run to remove the effects of the 6 departments that contained no UFF members. Decisions to join or not join the union would not have been due to individual motivations. Variations in union membership

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6 The highest degree attainment for three faculty members within the English Department was a Masters. For these faculty members, the year they acquired their Masters was used in place of the year a PhD was acquired.

7 In the sub-set the Departments studied included: Biological Science, Communication, Computer Science, Criminology & Criminal Justice, Education Psychology & Learning System, English, History, Management, Marketing, Mathematics, Meteorology, Oceanography, Physics, Psychology, Public Administration, and Sociology
would have been non-existent. A total of 265 faculty members were studied in Regressions 3 and 4.

**Average Department Salary.** The coefficient of Average Department Salary proved to be negative and statistically significant at the 95% level in Regressions 1 and 2. The coefficient was negative but not statistically significant in Regressions 3 and 4. The results were consistent with the hypothesis that faculty members belonging to departments in which members earned a lower average salary felt that they would benefit more from across-the-board monetary raises. Members of lower-paying departments could have been more likely to join and support the activities of a collective bargaining unit. The impacts were calculated by multiplying the coefficients by the standard deviation of the variable located in the Table 1 of Appendix 1. The variable’s impact was -0.068875577 in Regression 1 and -0.069849868 in Regression 2. The impacts represent the effect of the variable on UFF-FSU membership status. A faculty member employed in a department with a higher average salary was 6.8-6.9% less likely to join the union. The impact of both coefficients was very low suggesting that, overall, average department salary was not a huge determinant for department members in the decision to join the union. When the departments containing no union members were removed from the sample, the coefficient became statistically insignificant.

**Salary Variables.** The Top Third Salary coefficient was negative and marginally significant at the 90% level in Regression 1. The coefficient in Regression 3 was negative but not significant. The signs of the coefficients were consistent with the hypothesis. Faculty members who received the highest salaries in their departments would have been less likely to join the union because they would have benefited more from merit based raises than across-the-board raises. Since the variable was dichotomous and indicated if the faculty member belonged in the category, the impact is the value of the coefficient. The variable had an impact of -0.06896 in Regression 1. The impact was the probability that a faculty member within this category would join the union. A faculty member earning in the top third of salaries within his or her department was 6.8% less likely to join the union. The low impact in Regression 1 suggested that salary for top-third earners in the department was not a huge determinant in the decision to join or not join the UFF-FSU.

The coefficient of the Middle Third salary variable was negative and insignificant in both Regressions 1 and 3.

Only one salary variable was marginally significant in both regressions. This might suggest that in 2006 the decision to join the union was determined little by how much salary a faculty member earned.

**Articles Published Variables.** The Top Third Articles coefficient was positive and insignificant in Regressions 2 and 4. The signs of the coefficients contradicted the predicted negative sign. It was hypothesized that faculty producing the most articles in the department would have been highly productive and therefore less likely to join a union advocating across-the-board raises.

The Middle Third Articles coefficient was negative and insignificant in Regressions 2 and 4.

**Books Published Variables.** The Top Third Books coefficients were positive and insignificant in Regressions 2 and 4. The coefficient of the Middle Third Books Variable was negative and insignificant in Regressions 2 and 4.

The mean values for the Top and Middle Third Books Variables were not in the same range as the Articles or salary variables. Segmentation of faculty members into three categories should have resulted in mean values that were within the range of 0.3 to 0.36 for the Salary, Articles, and Books Variables. The mean values for the Books Variables were between 0.083018868 and 0.219020173. The unusual range was present because the sample included departments in which few or no members published books. The Book Variables’ mean values were skewed because members of these departments could not be separated into three separate categories. A sample eliminating departments which faculty members could not be properly segmented did not yield enough data points to run a viable regression.

**Experience Variables.** The coefficients for the 30+ Years Variable were negative in all four regressions. The coefficient was statistically insignificant in Regression 1. The coefficients were statistically significant at the 95% level in Regressions 2, 3, and 4. The sign of the coefficients were inconsistent with the hypothesis that faculty members closest to retirement would join the union because they were declining in productivity and would gain more from across-the-board monetary raises. The signs suggested that faculty members closer to retirement were less likely to join the UFF-FSU. This may be because the union negotiates contracts for future years. Faculty members very close to retirement would have had less incentive to financially support an organization that negotiated for future benefits that they would not receive. Since the variable is dichotomous, describing if a faculty member has a characteristic or not, the coefficient value is also the impact. The variable had an impact of -0.127855993 in Regression 2, -0.161880839 in Regression 3, and -0.2122975 in Regression 4. The impacts measured the effect of belonging to this category on a faculty member’s decision to join the union. Faculty members with more than 30 years of experience were 12.7-21% less likely to join the union than faculty with 0-30 years of experience. The impact was noticeably large in Regression 4, which examined a sample containing only departments with at least 1 union member. The impact of the variable in Regression 4 was highest of any variable in any regression run. The results suggested that in departments in which at least 1 union member, the faculty with more than 30 years of experience were the least likely to join the UFF-FSU.
The 15-30 Years coefficient was negative in all four regressions. The coefficient was not significant in Regression 1. It was marginally significant at the 90% level in Regression 2. In Regression 3 the coefficient was marginally significant at the 90% level. In Regression 4 the coefficient was statistically significant at the 95% level. The signs of the coefficients were consistent with hypothesis that faculty members in the middle range of experience were productive and would have benefited more from merit-based raises. The variable was dichotomous, indicating if a faculty member was within the category represented by the variables. Therefore, the values of coefficients were also the values of the impacts of the variable on union affiliation. The variable had impacts of -0.070785351 in Regression 2, -0.104089579 in Regression 3, and -0.128149172 in Regression 4. The impacts measured the effect of belonging to this category on a faculty member’s decision to join the union. Faculty members in Regression 2 with 15-30 years of experience were only 7% less likely to join the UFF-FSU. This suggests that in the entire sample of faculty, a middle-range of experience was not a strong determinant of union affiliation. In Regressions 3 and 4, a faculty member with 15-30 Years of experience was 10.4-12.8% less likely to join the union. This suggests that experience played a larger role among the measured variables in the decision of a faculty member who had a mid-range of experience within his or her department to join or not join the union. The impact increased from 10.4% to 12.8% when a subset of departments with at least one union member was examined, magnifying the effect.

Conclusion

This study has yielded interesting findings. The variables having the greatest impact on a faculty member’s decision to join UFF-FSU were the experience variables. There was also evidence that members of higher-paid departments and those receiving the highest salaries in their departments were less likely to join the union. The findings for the individual publication variables were not conclusive. Alternate variables such affiliation to other union members, political ideals, and personal views may have better measured a faculty member’s individual motivation in joining or not joining the union. However, these variables were difficult if not impossible to measure. A larger sample measuring more indicators of productivity such as conference presentations, class level taught, and experience outside the teaching field may have provided better results. However, these variables were outside the scope of the time and confidentiality constraints of the project. The findings in this study suggest that members of higher-paid departments, earning the highest salaries in their departments, and the faculty that had the most experience had lower probabilities of joining the UFF-FSU.
### Appendix 1

#### Table 1. Summary of Statistics

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<th>Regression</th>
<th>Variable</th>
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<th>Standard Deviation</th>
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The Relationship between Periplasmic Nitrate Reductase and the Length of Diauxic Lag in Denitrifying Bacteria Switching from Oxic to Anoxic Conditions

Kyle Fischer et al.*

College of Engineering, University of Florida

The efficiency of biological denitrification processes in wastewater treatment is adversely affected by the length of diauxic lag when denitrifying bacteria are switched from oxic to anoxic conditions. Periplasmic nitrate reductase (Nap) is hypothesized to shorten diauxic lag. A Nap-containing bacterium and a Nap-deficient mutant of the same strain were grown and the lengths of their diauxic lags were compared to test this hypothesis. Nap-containing (wild type) Paracoccus pantotrophus displayed a significantly shorter diauxic lag than the Nap-deficient mutant bacteria. This result supports the hypothesis that the presence of Nap in bacteria decreases the length of diauxic lag. Enrichment of Nap-containing bacteria in wastewater treatment facilities could increase treatment rates by reducing diauxic lags.

Introduction

Nitrogen removal is an integral part of wastewater treatment due to the detrimental environmental and health effects associated with excess nitrogen. In an effort to remove nitrogen efficiently and economically, biological removal processes have been employed in many wastewater treatment facilities (Lisbon et al., 2002). These processes involve cycling bacteria between oxic (aerated) and anoxic (unaeated with nitrate present) conditions in order to first oxidize ammonia to nitrate with nitrifying bacteria and then reduce nitrate to dinitrogen with denitrifying bacteria (WEF, 2006).

Under aerobic conditions, synthesis of membrane-bound denitrifying enzymes is repressed, so the intracellular levels of these enzymes drop due to decay and production of new biomass. When denitrifying bacteria are switched from oxic to anoxic conditions, they must re-synthesize these enzymes, which causes a diauxic lag that can last several hours (Liu et al., 1998). Casasús et al. (2007) observed that bacteria which have both membrane-bound nitrate reductase (Nar) and periplasmic nitrate reductase (Nap) display shorter diauxic lags than bacteria that contain only Nar. They hypothesized that Nap is responsible for shortening diauxic lag.

The goal of this research was to test the hypothesis that Nap plays a role in shortening diauxic lag. This was accomplished by characterizing both the Nap-containing Paracoccus pantotrophus and a Nap-deficient mutant of the same organism in terms of diauxic lag. The specific objectives of the research were:

- Develop software for estimating the length of diauxic lag from growth measurements.
- Develop software for estimating the activity of Nap and Nar based on measurements of reduced benzyl viologen concentration as a function of time in nitrate reductase activity assays.
- Apply growth measurements for characterization of the mutant and wild-type P. pantotrophus.

Materials and Methods

All chemicals were obtained from Fisher Scientific, except as noted. Tris buffer contained 7.36 g/L Tris (hydroxymethyl)aminomethane hydrochloride (BP153) and 0.47 g/L Tris Base (Promega H5133).

Freeze-dried P. pantotrophus was revived as follows. Four 250 mL Erlenmeyer flasks were filled with 125 mL of 20 g/L Luria-Bertani (LB) (Difco 240230) solution, topped with foam stoppers and aluminum foil, and autoclaved for 17 minutes. The flasks were then cooled to room temperature in a water bath. After cooling, the flasks were inoculated with freeze-dried P. pantotrophus and placed in a shaker at 37º C for one day.

The revived bacteria were colonized on agar plates for future use. Plates were made from an autoclaved 40 g/L solution of LB Agar (Difco 244510) and were held for 24 hours at room temperature and then stored at 4º C. Bacteria from the flasks were streaked onto plates. The plates were inverted and placed into an incubator at 37º C for 24 hours. The plates were then wrapped with Parafilm and stored for up to 2 weeks at 4º C.

The bacteria were pre-cultured before each growth experiment. A 250 mL Erlenmeyer flask containing 125 mL of LB solution supplemented with 2.88 g/L of potassium nitrate was inoculated with a bacterial colony from 1 of the plates and placed in a shaker at 37º C for 18–24 hours.

To initiate the growth experiment, the absorbance of the pre-culture was measured with a Thermo-Spectronic Genesys 10 UV at a wavelength of 550 nm. A sufficient
volume of pre-culture was added to 3 flasks containing 125 ml of the supplemented LB solution to give an initial absorbance of 0.08.

The initial absorbance of each flask was measured, and the flasks were incubated in a 200 rpm shaker at 37º C. Absorbance was measured periodically. Samples for the enzyme assay were taken after the bacteria entered the exponential growth phase. Each culture flask was then purged with nitrogen gas for 4 minutes and placed on a shaker in an anaerobic hood (Coy Laboratory Type ‘A’) at 37º C. Absorbance was measured periodically using a spectrophotometer in the anaerobic hood. A second set of samples was taken for analysis of enzyme activity after the bacteria resumed exponential growth. *P. pantotrophus* with the *nap* gene deleted were grown in the same manner.

The enzyme assay procedure was adapted from Hamilton et al. (2005) and Casasús et al. (2007). A sample of sufficient volume to give 55 mg dry weight of bacteria was taken from each flask and centrifuged for 10 min at 13,000 x g and 4ºC in an Eppendorf 5810R refrigerated centrifuge. The pellet was resuspended in 30 mL of Tris buffer and centrifuged again. A volume of 2.0 mL Tris buffer was added to the final pellet.

Within the anaerobic hood, two borosilicate cuvettes were filled with 3-5 3 mm glass beads and 4 mL of a 0.12 g/L solution of benzyl viologen (1,1′-Dibenzyl-4,4′-bipyridinium dichloride; Sigma B8133) in Tris buffer (pH 6.2), followed by addition of 200 μL biomass in Tris buffer. The benzyl viologen is colorless in the oxidized state (*BV*2+) and is reduced to a deep-blue colored cation radical (*BV*) by adding 25 μL of a basified (0.4 g/L NaOH) solution of 4.35 g/L sodium hydrosulfite. The hydrosulfite reduces *BV*2+ to a deep-blue colored cation radical (*BV*) (Kamogawa et al., 1980). One of the two cuvettes was amended with 25 μL of a 1.76 g/L sodium azide solution to inhibit Nar activity and both cuvettes were filled (leaving no headspace) with 0.12 g/L benzyl viologen in Tris buffer solution, topped with rubber stoppers, and crimp-sealed with aluminum caps.

The two cuvettes were placed in separate identical spectrophotometers and absorbance was measured every 15 seconds. After 3 minutes, 35 μL of 101.1 g/L KNO3 was injected through each rubber stopper with a tuberculin syringe and the cuvettes were inverted to facilitate mixing and initiate the enzymatic reaction. At the end of the assay the cuvettes were unsealed, partially emptied, recapped, and shaken to decolorize the benzyl viologen. A final absorbance measurement was then taken to estimate the biomass concentration. This process was repeated for each biomass sample.

**Results**

*Software for Estimating Diauxic Lag*. Figure 1 depicts a typical growth curve and diauxic lag calculation for *P. pantotrophus*. The natural logarithm of absorbance is plotted (blue data points connected by a solid blue line) as a function of time. A period of exponential growth under anoxic conditions (to the left of the vertical dashed line) is followed by diauxic lag after conditions are switched to anoxic. Eventually, exponential growth is resumed under anoxic conditions. The lag begins at the switch point (vertical dashed line at 4.25 hours) and ends when the horizontal brown dashed line extending from the switch point intersects the green solid line fit to the secondary exponential portion of the absorbance plot (vertical dotted-dashed line at 4.55 hours). The 0.3-hour period between these two vertical lines is the diauxic lag.

A template that automates the calculation of diauxic lag length was implemented in Excel 2007 in order to provide consistent and rapid data analysis (templates can be downloaded from http://www.ees.ufl.edu/homepp/koopman/downloads/). The program is divided among seven worksheets. The function of each worksheet is described below. Cells with blue text show where user data should be entered and cells with black text represent calculated or static values that should not be changed. Worksheets that are named in all caps are reserved for calculations.

The first worksheet (*Data Input*) is where information about the experiment and the experimental data are entered. Absorbance measurements for up to three replicates and the corresponding dates and times are entered on this worksheet. The time and date of the anoxic switch, preculture absorbance, and parameters for desired experimental flasks can also be entered. The worksheet calculates the time (in hours) from the start of the experiment (first absorbance measurement) as well as the mean and standard deviations for each set of replicate absorbance measurements. A rough estimate for the amount of preculture needed to inoculate a growth experiment is calculated based on preculture absorbance, experimental flask size, and desired starting absorbance.

![Figure 1: Typical diauxic lag calculation for *P. pantotrophus*.](image-url)
The second worksheet (Growth Curve) plots the mean of the replicate absorbance measurements as a function of time. Error bars on the graph display standard deviations. The vertical dashed line depicts the time of the switch from oxic to anoxic conditions. The period to the left of the vertical dashed line is the oxic phase and the period to the right is the anoxic phase.

The third worksheet (Diauxic Lag Calc) allows the user to graphically adjust the fit to the linear portion of the anoxic phase growth plot, which is necessary for calculation of the length of diauxic lag. (The details of the diauxic lag calculation are described in the fourth worksheet.) The third worksheet is split by default into two planes. The top plane displays a graph to assist the user in visually fitting the data. Information about the fit, based upon a least-squares linear regression, and the calculated length of the lag are also displayed. The absorbance data set that was entered in the first worksheet is displayed, along with an interpolated data point corresponding to the time of the anoxic switch.

The user chooses which data to fit by placing an “x” in the row corresponding to the data point of choice in the data selection column. Selected data points are utilized in a linear regression calculation that is coded on the LINEAR REGRESSION worksheet. The results of the linear regression of the growth curve are utilized to plot the fitted line. The fit and calculated diauxic lag are updated as different points are selected.

The fourth worksheet (Diauxic Lag Graph In) shows the result of data selection in the third worksheet. The natural logarithm of biomass absorbance is plotted as a function of time (solid, blue line). The length of the lag is calculated by extending a line (solid green line) fitted to the linear portion of this plot in the anoxic phase (after the bacteria have resumed exponential growth) to a horizontal (dashed) line extending from the biomass absorbance at the time of the switch. The length of time between this intersection and the time of the switch gives the lag (Swinnen et al., 2004).

Software for Estimating Activity of Nar and Nap before and after Diauxic Lag. Figure 2 depicts a typical measurement of the enzymatic reduction of nitrate. Absorbance is plotted as a function of time. The slope in terms of change in absorbance per second is converted to nanomoles BV oxidized per second per unit of mg biomass by multiplying the slope by the inverse of the molar extinction coefficient of reduced BV and dividing by the biomass concentration in dry weight. Bacterial density in dry weight is determined by converting an absorbance measurement to dry weight, using a factor appropriate for the culture and the instrument variables. The default is a ratio of 360 mg dry weight per liter per unit absorbance (Ohmomo et al., 1988).

Utilization of the molar extinction coefficient to convert absorbance to species concentration is based upon the Beer-Lambert Law. An experimentally determined molar extinction coefficient for the chemical species in question (εBV) was found from extensive review of scientific literature. Spencer and Guest (1973) reported this coefficient to be 7.78 x 10^6 M^-1 cm^-1 at 550 nm, which is the wavelength used in the assay procedure. This value has been widely cited in subsequent studies (Proctor et al., 2000, Tian et al., 2005, Bastian et al., 1991).

In a given worksheet, the slope of the initial linear portion of the plot after the injection of nitrate is determined for each assay (both with and without azide) by placing an “x” in the column labeled "N" for each row containing a desired datum. The selected data points are employed in a linear regression that is coded on separate
worksheets. The results of the linear regression are reflected in the graph. The column titled “B” has the same functionality for determining the slope of the baseline. To assist the user in determining linear portions of the data, a tolerance measure flags points that meet the criterion for linearity. When the second derivative (a measure of curvature) falls below 0.0001 (by default) an asterisk is displayed. The threshold for linearity can be varied.

**Application of Software in Determining Diauxic Lag and Enzyme Activities.** Figure 3 displays the growth curve of the wild type (Nap containing) *P. pantotrophus*. Exponential growth is quickly resumed after a switch to anoxic conditions. The calculated lag can be seen as 0.3 hours.

Figure 4 displays the growth curve of the Nap-deficient mutant. After a switch to anoxic conditions there is a long period of little to no growth before exponential growth resumes. The diauxic lag was calculated to be 15.4 hours.

Figure 5 displays the growth curves for the Nap-containing wild type and Nap-deficient mutant side by side. The vast difference in the length of diauxic lag is readily apparent when the two growth curves are plotted on the same time scale.

**Discussion**

The nap-deficient mutant displayed a significantly longer diauxic lag than the wild-type *P. pantotrophus*. The 15-hour difference in diauxic lags supports the hypothesis that Nap is responsible for shortening diauxic lag in this species. This result also agrees with the results of Casasús et al. (2007), which showed that denitrifying bacteria containing both Nar and Nap display shorter diauxic lags than bacteria that contain only Nar.

The software templates were created to increase the speed and consistency with which diauxic lag and enzyme assay calculations were made. Data gathered from absorbance measurements in both of these cases often contain unexplained outliers that hinder the attainment of a proper fit. These calculations also require the fitting of only a portion of the data which limits the use of a standard non-interactive template with pre-set calculations for the data entered. Hence, finding the proper fit is best accomplished with human judgment and interaction. This can be a slow and cumbersome process, however, when manually selecting which points and or ranges to use with standard Excel formulas. The templates provide a more fluid and interactive process that gives the user instant visual and statistical feedback to show how the fit changes with the selection of each data point. Recommendations are also provided to help pick out linear portions of the data for enzyme assay calculations. In this manner the user can quickly find a starting point and easily adjust the fit until it is satisfactory. The ability to quickly compare the effects of different data selection choices should help to improve the consistency and accuracy of the fit since many options can be attempted in the time necessary to try one selection with the use of standard formulas.

The results from this project will help to characterize the role of Nap in biological denitrification processes and improve mathematical models of biological nitrate reduction to dinitrogen, which could lead to better design and operation of nitrogen-removing wastewater treatment.
systems. The enrichment of Nap-containing denitrifying bacteria in wastewater treatment facilities could reduce the length of diauxic lag thereby increasing treatment rates and efficiency. More efficient wastewater treatment processes will gain in importance as growing global populations lead to an increase in the amount of wastewater produced.

**Literature Cited**


Ohnomo, S., Daengsubha, W., Yoshikawa, H., Yui, M., Nozaki, K., Nakajima, T., Nakamura, I. 1988. Screening of anaerobic bacteria with the ability to decolorize molasses melanoidin. *Agricultural and Biological Chemistry*. 52(10): 2429–2435


