FOUNDATION FOR ECOLOGICAL RESEARCH IN THE NORTHEAST
P.O. Box 962
Upton, NY 11973

Phone: (631) 344-5810
Fax: (631) 344-5812
www.fern-li.org

Foundation for Ecological Research in the Northeast

10 August, 2007

PROJECT REPORT

Assessing Benthic Macroinvertebrate Sampling Procedures for the Development of the Freshwater Wetland Health Monitoring Protocols of Long Island's Central Pine Barrens.[1]

Sarah Miloski
SUNY College at Brockport

Foundation for Ecological Research in the Northeast
Brookhaven National Laboratory
Upton, NY

[1]. Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Science Undergraduate Laboratory Internship

Abstract

While wetlands, among the most productive ecosystems in the world, are often called the nurseries of life, little is known about the current health status of Long Island's freshwater wetlands. Such vital systems should be monitored over a period of time to determine the health of the wetlands. However, before gathering data in the field, it is necessary to assess and choose methods that will obtain the most representative results. Appropriately designed protocols will achieve the goals of establishing baseline data of the current wetland health and provide land managers with the data they require to make management decisions to optimize the health of the wetlands under their supervision. Monitoring methods need to be consistent, informative, efficient and replicable in order to be comparable to future data. Benthic macroinvertebrates are crucial indicators of wetland health, since the number and type of species present yield significant information regarding water quality. Due to their limited migration patterns, these organisms allow researchers to determine the sustainability of a wetland. In this research, appropriate procedures for sampling these organisms were reviewed and assessed using protocols developed by other states, such as Ohio and Florida. These protocols were then adjusted to accommodate the unique conditions of the wetlands of Long Island's Central Pine Barrens. To test the protocols, invertebrates were acquired using a d-frame dip net to jab and sweep various targeted wetland habitats. Invertebrates were then randomly chosen from an observation tray and identified in the field, or preserved for laboratory identification using a dissecting microscope. Several protocols called for a sampling total of 100 organisms. This task, however, consumed time that could have been allotted to other aspects of the protocol. Therefore, the benthic macroinvertebrates encountered were noted as present, thus providing a list of organisms that existed in the wetland at a given time. When this list is compared to data collected during the revisit of a site, the absence of a formerly present organism provides information about the current state of the wetland and how it has changed. Despite a low amount of diversity while sampling, there was a plethora of adult Odonates in the wetland. This occurrence would support the existence of a substantial supply of microorganisms, such as algae and periphyton. It was concluded that simply monitoring benthic macroinvertebrates in the water of the wetlands may not be an informative way of monitoring the aquatic organisms. Therefore, for the freshwater wetlands protocol of Long Island's Central Pine Barrens, further analysis should delve into a smaller scale of aquatic biota assemblages, such as periphyton and algae.

Introduction

Picture a healthy wetland. Do you see the sun gleaming off the clear water, home to various kinds of salamanders, frogs, invertebrates, and fish? Imagine diverse vegetation surrounding the wetland sheltering birds, amphibians and reptiles from the harshness of the sun. Now envision the same wetland after degradation. Fish kills, silence from a lack of bird and amphibian calls, and barren banks come to mind. By monitoring a wetland over a period of time, collected data can delineate changes in the wetland that occur during degradation to make a pristine wetland become tainted and victim to low diversity.

The Long Island Central Pine Barren region, an area of over 100,000 acres, boasts more than 400 protected wetlands, yet very little research has been done to determine their current health status. These wetlands are home to various state threatened and endangered species of plants, fish, and wildlife. Such vital and sensitive systems should be individually monitored over a period of time to determine the overall health of the wetlands and to have baseline data for future comparison. Appropriately designed protocols will achieve the goals of establishing baseline data of the current wetland health and provide land managers with the data they require to make management decisions to optimize the health of the wetlands under their control. Methods need to be consistent, informative, efficient and replicable in order to be comparable to future data.

The Foundation for Ecological Research in the Northeast (FERN), a not-for-profit organization, is currently fostering a step- by-step monitoring protocol specifically designed for the freshwater wetlands of the Central Pine Barrens of Long Island. This project rectifies the lack of baseline data regarding the current state of the freshwater wetlands in the Central Pine Barrens. The data attained by using the protocols for monitoring will be utilized to compare the health of the wetland to future biomonitoring data [1]. In turn this information will allow land managers to make informed decisions to preserve, protect and restore the wetlands under their supervision.

An essential aspect of wetland systems is benthic macroinvertebrate assemblages. Defined by the Environmental Protection Agency (EPA) as "animals without backbones, living in or on sediments or other substrates, of a size large enough to be seen by the unaided eye, and which can be retained by a U.S. Standard No. 30 sieve" and "of the bottom of a waterbody," these organisms are crucial indicators of wetland conditions [2]. Since many benthic macroinvertebrates have limited migration patterns and specific levels of tolerance to pollution, they are valuable in assessing site-specific impacts. Also, this group of organisms is composed of species that represent an extensive range of trophic levels, serving as a food source to other wetland organisms, such as amphibians and fish. So any changes in populations can be detected through monitoring and possible effects of alterations can be foreseen.

Not only is the sampling of these organisms crucial, it is advantageous. Most states have data on existing macroinvertebrate populations within the state, so identification is simplified to a certain extent of species. One should question an identified species that is not on the list. The New York State Department of Environmental Conservation has data regarding the macroinvertebrates found in New York from its Stream Biomonitoring Unit. Another large advantage of using these organisms as a biological indicator is that sampling is relatively simple and has minimal detrimental effect on the resident biota [3].

Before heading out into the wetlands of the Central Pine Barrens, parameters should be followed regarding the sampling of benthic macroinvertebrates. Protocols developed by other states were reviewed and adapted to suit the wetlands in this region. Since benthic macroinvertebrates had to be sampled in addition to monitoring other aspects of the wetland, such as water quality and vegetation, time in the field was a constraining factor and had to be taken into consideration when the protocols were prepared. With this limitation in mind, the sampling of benthic macroinvertebrates had to be informative, replicable, efficient and representative of the wetland as a whole. The resulting data provided a list on what assemblages of benthic macroinvertebrates were present at that given time. When this list is compared to data collected during the revisit of a site, the absence of a formerly present organism provides information about the current state of the wetland and how it has changed. This information will aid land owners in making management decisions and show the results of management practices tried after the baseline data was collected.

Materials and Methods

Assessing benthic macroinvertebrate protocols involved the review of protocols developed by other states and made available by the EPA [4-8]. The methods used in these protocols were altered to accommodate the specific requirements of wetlands within the Central Pine Barrens by evaluating them in the field and assessing how favorable the methods were to the goals of the protocol.

To sample for invertebrates, one meter sweeps were taken using a d-frame dip net with US 30 mesh in various substrates, including open water, vegetation, soft substrate and submerged macrophyte. Sweeps taken in each habitat were counted and recorded on the field data form, along with the time expenditure and the total number of organisms collected (Appendix A). After sweeping in a habitat, contents were emptied in a 20cm x 30cm gridded pan of 5cm squares. Debris was rinsed and organisms attached to the debris were taken off. While noting the time expended, it was attempted to count 100 specimens by randomly choosing grids and removing the organisms in the chosen grid with forceps. The number of grids picked for each habitat type also had to be noted on the data form (Appendix A). Specimens were identified to Order in the field to assess biodiversity and presence was recorded. After counting, organisms were released. The methods were discussed among the sampling crew in terms of adjustments that were necessary to make this section of the protocol simple and time efficient, while achieving the most useful and accurate data. Also, ways of creating a user-friendly data sheet to optimize field data collection had to be discussed.

Results

The EPA case studies were reviewed and summarized based on the methods utilized regarding benthic macroinvertebrate monitoring. Below, Table 1 depicts relevant case studies by state, their sampling window, frequency, and methods.

Table 1. EPA case studies for macroinvertebrate sampling.

State	Time of Year Sampled	Number of Visits Per Pond	Method
Florida	n/a	1	20 sweeps per wetland. Number of sweeps proportional to percent of total wetland
Michigan	Various	Many	D-frame dip nets -late July-August, and 2-3 wks after snow melt, during high-water, and just before wetlands dry up, if perm. in midsummer and fall
Minnesota	June- early July	1	D-frame dip nets; bottle trap activity trap (funnel trap)
Montana	April-September	1	D-nets
Ohio	Early, middle and late Spring	3	Funnel Traps, Dip Nets, Hester-Dendy Artificial Sampler
Vermont	April, May, June	2	Funnel Traps, D-Nets, Qualitative Search

As shown in table 1 above, each wetland monitoring program varied depending on state, needs, location, purpose, etc. To apply these methods and alter them to the wetlands of interest for the Long Island protocol, an outing was necessary to survey the benthic macroinvertebrates at hand. Table 2 and 3 below are from sampling a Coastal Plain Pond on 20 July 2007.

Table 2. Macroinvertebrates sampled in an open water habitat.

Habitat: Open water		**Sweeps: 5**	
Common Name	**Class**	**Order**	**Total**
Aquatic worm	Oligochaetae		1
Midge larvae	Insecta	Diptera	11
Mosquito larvae	Insecta	Diptera	1
Water boatman	Insecta	Hemiptera	1
Total Individuals Collected: 14			

Table 3. Macroinvertebrates sampled in a vegetated edge habitat.

Habitat: Vegetated Edge		**Sweeps: 5**	
Common Name	**Class**	**Order**	**Total**
Aquatic worm	Oligochaetae		35
Blackfly larvae	Insecta	Diptera	1
Mayfly nymph	Insecta	Ephemeroptera	4
Midge larvae	Insecta	Diptera	10
Mosquito larvae	Insecta	Diptera	24
Total Individuals Collected: 74			

As demonstrated above in Table 2 and Table 3, there were a considerably greater number of organisms in the more vegetated habitat, with essentially the same kinds of organisms as compared to the open water. The vegetated edge sample contained 2 mayfly nymphs (Order: Ephemeroptera) that the open water sample lacked.

Discussion and Conclusion

When developing the wetland health monitoring protocols EPA case studies from Ohio, Florida, Vermont, were reviewed in terms of benthic macroinvertebrate methods. These case studies had varying techniques that were either useful or unnecessary in terms of the Central Pine Barrens wetlands of interest. The researchers involved with each case study all seemed to share the idea that permanent plots were not a thorough representation of the ever-changing wetland, and therefore invertebrates sampled in a permanent plot would not be conducive to the goal of having data that could be easily compared to future data. Wetlands are heavily affected by seasonal change. Removal of the tree leaves surrounding ponds should increase light availability, due to reduced canopy cover, and decrease coarse particulate organic matter influx. The water hydrology of the wetland changes with seasons, due to, for example, flooding, atmospheric conditions and snow melt. With these seasonal variations, the macroinvertebrate population is expected to change [3]. Even over a 24-hour period, nocturnal macroinvertebrates could be sampled using traps. Sampling in the day alone will not accurately assess the benthic macroinvertebrate population.

In the Ohio case, qualitative samples were collected using dip nets in a location within a site that comprised of diverse habitat that was expected to sustain a variety of benthic macroinvertebrates. A minimum of 30 minutes was spent on collecting and sampling continued until the field crew determined that further sampling effort would not produce new taxa. To capture the nocturnal organisms, the Ohio researchers used and checked funnel traps and Hester-Dendy artificial samplers in early, middle, and late spring. They preserved each trap's contents separately from other traps so that location of macroinvertebrate populations would not be compromised [4]. This protocol was useful when creating the Long Island protocol in that there was no delineated way on how much to sample. It did not seem to consume an excessive amount of time if there was repetition of taxa, so precious field time could be used for other sections of the protocol.

In the Florida case study researchers used a d-frame dip net. Each wetland had a sample of 20 sweeps that were divided among different habitats based on percentage of said habitat in that wetland. All organisms collected were placed in a gridded tray and 100 specimens were chosen randomly and placed in jars for identification [5]. This method was great in limiting biased data, but the organisms were all placed together from the different habitats (such as open water, woody debris, submerged macrophyte, and vegetated banks) that were sampled. Although this method gave an overall picture of what lived in the wetland, this compilation of habitat samples seemed to lose location of the populations, which could be beneficial data.

The wetlands monitoring case study of Vermont sampled macroinvertebrates using three different methods: funnel traps to sample the actively swimming invertebrates, a d-frame dip net to sample benthic invertebrates in the leaf litter and muck, and a qualitative search for any taxa that might have been missed with the previous two methods. Funnel traps were made of window screen and designed to function like minnow traps. The traps were placed approximately 10m apart and were left in place for approximately 24 hours. When the traps were emptied macroinvertebrates were collected and preserved. The contents of each trap were stored separately. The sampling window was April-June [6]. Vermont seemed to thoroughly sample macroinvertebrates by capturing the various kinds of organisms in the wetland and keeping the contents apart for the sake of the data. The 3 month sampling period may not have captured the macroinvertebrates present during other seasons.

The Michigan case study seemed to remedy this lack of macroinvertebrate representation during other times of the year by having researchers make multiple visits to the wetland per year. Macroinvertebrate samples were collected with standard d-frame dip nets containing a 0.5-mm mesh. All major plant community zones were sampled at each site, including an emergent zone and a shallow, wet meadow zone. Samples were placed in white enamel pans, and 150 invertebrates were collected by focusing on small areas of the pan and removing all of the specimens. Special consideration was made to ensure that smaller organisms were not missed, as there is a bias towards larger, more mobile individuals using this technique. Plant detritus was left in the pan and sorted through for a few additional minutes to ensure that sessile species were included in the sample. Three replicate samples were collected within each plant community zone in order to obtain a measure of variance associated with sampling. Dip net samples were collected from late July through August. Samples taken from ice-out through mid-July generally contained less diversity and a greater proportion of early instars of aquatic insects, making identification very difficult. The July-August time period also corresponded to the time when plant communities, characteristic of these wetland systems, achieve maximum annual biomass production [7]. This case study brought up a great point regarding the difficulty in identifying non-mature specimens. More dichotomous keys tend to be available for mature specimens and the July-August time frame is ideal for sampling. The Minnesota case study kept this issue in mind when determining the time frame of sampling for macroinvertebrates using a dip-net and activity traps during June and early July to ensure the acquired organisms will be at the maximum level of maturity [8].

With the dynamics of wetland systems in mind, combined with the aforementioned case studies, benthic macroinvertebrates sampling should be part of the protocols for every season in the freshwater wetlands of the Long Island Central Pine Barrens. However, collection methods for each season will differ. Hester-Dendy activity traps will be deployed late spring and collected mid-summer. Summer is the season of high plant productivity and limited light availability, so during this time period, it is recommended that a d-frame dip net be used to jab and sweep vegetation. Each season will have leaf litter bags implemented at the beginning, and checked the start of the following season. This would give way to an idea of what macroinvertebrates were present in that season without physically sampling every day. For future monitoring, the leaf litter bags should be deployed and retrieved the same period of time as the last monitoring for the best accuracy and comparability. The same goes for d-frame dip net sampling. Environmental conditions may affect what organisms are active within the water when sampling is taken place, and therefore the sample may be compromised due to poor conditions. So, for future monitoring, the sampling dates should be close and the weather conditions should be analogous to build a more comparable collection of data.

Another way of keeping data collection consistent and comparable is informative field data sheets. Upon assessing the appropriate sampling methods, field data forms were discussed in terms of adaptability to wetland macroinvertebrate presence. Appendix A includes a draft of the data form that will be used for the benthic macroinvertebrate sampling portion of the protocol. As displayed in the form, the amount of sweeps, grids picked, time and total organisms will be recorded for each habitat type. This data shows the amount of effort put into that specific habitat to achieve the total organisms collected. This portion of the form will be useful in wetlands with a lower abundance of benthic macroinvertebrates. It will transcend the amount of time spent just to collect a minimal amount of organisms.

The form also includes a table that divides the organisms into Order or Class in each habitat type. This portion of the form includes a check box for presence along with tallies in each substrate type to record the number of organisms of that classification within each habitat type. This kind of data is important to biomonitoring since benthic macroinvertebrates have a specific tolerance to pollution. The presence of a low tolerance organism, such as a Mayfly nymph (Order: Ephemeroptera) can indicate good water quality. An abundance of high tolerance organisms, such as Leeches (Subclass: Hirudinea) and Blackfly larvae (Order: Diptera) can indicate poor water quality. Over time, through biomonitoring, any changes in the populations of these organisms can indicate a change in the health of the wetland. With increased perturbation, the taxa richness of those organisms considered to be sensitive to perturbation is expected to decrease. EPT tests can be done to assess water quality since it investigates the totals of Ephemeroptera, Plecoptera and Trichoptera. Organisms in these Orders tend to be more sensitive to pollution. Dominant taxa can also be calculated from the collected data. This calculation measures the dominance of the single most abundant taxon. With increased perturbation, the dominant taxa are predicted to increase [9].

It order to sample the organisms, equipment has to be effective and efficient. After testing out the methods, equipment will have to be changed for this section of the protocol. A mesh screen to sieve the contents of the d-frame dip net would have quickened the process by easing the sorting of debris, which consumed 4 minutes after a single sweep just to see what type of organisms were attained. A screen may enable more organisms to be seen, and therefore not go unaccounted for. Also, a considerable amount of water flea-like organisms was observed. To identify this kind of invertebrate in the field seemed impossible, due to their minute size. An eyedropper to collect these specimens would enable the preservation and identification in an effort to better display the invertebrate assemblages present.

All of the case studies saved identification for a later time. After investigating the protocols in the field, laboratory identification was decided to be the most effective way to achieve accurate data. Order, seldom Family, was easy to observe without a microscope, but getting down to the taxon of Genus or Species would require an instrument such as a dissecting microscope. Additionally, dichotomous keys, teesing needles to manipulate the specimen and Petri-dish filled with distilled water are all necessary to correctly identify an organism. Also, since time is a constraining factor, organisms could be placed in a labeled vial of 95% ethyl alcohol (ETOH). This allows laboratory work to be saved for a day of inclement weather during which field research would not occur.

Interesting enough, all ponds visited had a large population of adult dragonflies (Suborder: Anisoptera) and adult damselflies (Suborder: Zygoptera). For a population of this size to exist, an adequate supply of algae and periphyton should also be present. Keeping this observation in mind, there is a need to extend the wetland invertebrate monitoring protocols to monitor algae and periphyton presence. Although there does not seem to be a great deal of diversity on the benthic macroinvertebrate scale, there can be significant biodiversity on a smaller scale. If unmonitored, these assemblages could potentially have an undetected impact on the wetland [10].

In conclusion, utilizing other protocols enabled the development of a protocol specifically designed for the wetland monitoring of Long Island's Central Pine Barrens. Field assessments allowed for the necessary adjustments to make monitoring the benthic macroinvertebrate population informative, efficient, replicable and comparable to future data. This assessment also forced the monitoring protocols to include a section that delves into smaller scaled organisms, such as algae and periphyton to achieve a better idea of the aquatic fauna within the wetland.

Acknowledgments
This research was conducted in association with FERN and Brookhaven National Laboratory using a grant generously provided by the Central Pine Barrens Joint Planning and Policy Commission. I would like to thank my mentor, Ariana Breisch and my advisor, Timothy Green, for all of their aid, guidance, and passion regarding this project throughout the course of the summer. I also thank Melanie Theisen, our Office Manager, for her assistance with our research. I would especially like to express my gratitude to the U.S. Department of Energy, Office of Science, and the SULI program for allowing me the opportunity to participate in this exceptional internship program. Lastly, I would like to give my gratitude to my fellow FERN intern, Emily Efstration for her constant support throughout the summer.

References

[1] The Foundation for Ecological Research in the Northeast. Aug 2007. <http://www.fern-li.org/>

[2] Glossary for the Rapid Bioassessment Protocols Module Aug 2007 <http://www.epa.gov/watertrain/rbp/glossary.html>

[3] Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Jun 2007. <http://www.epa.gov/owow/monitoring/rbp/>

[4] Sampling Methods and Analysis: Macroinvertebrates and Amphibians, Mar 2006. < http://www.epa.gov/owow/wetlands/bawwg/case/oh1macro.html >

[5] Center for Wetlands, University of Florida, Mar 2000. < http://www.epa.gov/owow/wetlands/bawwg/case/fl1.html>

[6] Vermont Wetlands Bioassessment Program, Mar 2006. <http://www.epa.gov/owow/wetlands/bawwg/case/vt.html>

[7] Great Lakes Coastal Wetland Bioassessments (Michigan BAWWG Case Study), Mar 2006. <http://www.epa.gov/owow/wetlands/bawwg/case/mi.html>

[8] Minnesota Pollution Control Agency Wetland Bioassessment Program, Mar 2000. <http://www.epa.gov/owow/wetlands/bawwg/case/mn1.html>

[9] Classification of Macroinvertebrates, Dec 2006. <http://www.epa.gov/bioindicators/html/invertclass.html>

[10] EPA Methods for Evaluating Wetland Conditions, Mar 2002. <http://www.epa.gov/waterscience/criteria/wetlands/>

APPENDIX A- Sample Data Forms

FRESHWATER WETLANDS HEALTH MONITORING Draft 7
DATE: WETLAND NUMBER:

6. Algae, Macroinvertebrates, and Invertebrates

Algae

Qualitative Listing of Aquatic Biota	(KEY: A = Absent/Not Observed; R = Rare; C = Common)						
	A	R	C		A	R	C
Periphyton	☐	☐	☐	Slimes	☐	☐	☐
Filamentous Algae	☐	☐	☐	Macroinvertebrates	☐	☐	☐

Benthic Macroinvertebrate Sample Collection

Habitat Type	Sweeps (#)	Grids Picked (#)	Time Expenditure	Total Organisms (#)
Vegetated Edges				
Submerged Macrophytes				
Woody Debris/Snags				
Unvegetated Soft Soil[1]				

[1]Sand and other fine sediments

Macroinvertebrates

(Key: **V** – Vegetated Edges, **S** – Submerged Macrophytes, **W** – Woody Debris, **U** – Unvegetated Soft Soil)

Organism	Name	Present	V (#)	S (#)	W (#)	U (#)
Oligochaeta		☐				
		☐				
Hirudinea		☐				
		☐				
Isopoda		☐				
		☐				
Amphipoda		☐				
		☐				
Decapoda		☐				
		☐				
Ephemeroptera		☐				
		☐				
		☐				
		☐				
Plecoptera		☐				
		☐				
		☐				
		☐				
Trichoptera		☐				
		☐				
		☐				

Data Entry: Page Entered: ☐ by ______ Page Verified: ☐ by _____

FRESHWATER WETLANDS HEALTH MONITORING Draft 7
DATE: WETLAND NUMBER:

Organism	Name	Present	V (#)	S (#)	W (#)	U (#)
Hemiptera		☐				
		☐				
		☐				
Megaloptera		☐				
		☐				
		☐				
		☐				
Coleoptera		☐				
		☐				
Diptera		☐				
		☐				
Gastropoda		☐				
		☐				
Pelecypoda		☐				
		☐				
		☐				
Odonota		☐				
		☐				
		☐				
		☐				

(Key: **V** – Vegetated Edges, **S** – Submerged Macrophytes, **W** – Woody Debris, **U** – Unvegetated Soft Soil)

Invertebrates -- INSECTS

	Common Name	Observed	Photo Number
1	Comet Darner	☐	
2	Little Bluet	☐	
3	Mantled Baskettail	☐	
4	Needham's Skimmer	☐	
5	New England Bluet	☐	
6	Pine Barrens Bluet	☐	
7	Rambur's Forktail	☐	
8	Scarlet Bluet	☐	
9	Southern Sprite	☐	
10	Yellow-tailed Skimmer	☐	

NOTES (Use #s to reference item.)

Data Entry: Page Entered: ☐ by ______ Page Verified: ☐ by _____