Brookhaven National Laboratory

NATURAL RESOURCE MANAGAMENT PLAN

ANNUAL REPORT

CALENDAR YEAR 2007

1.0 Introduction

This document summarizes activities carried out under Brookhaven National Laboratory's (BNL) Natural Resource Management Plan (NRMP) during calendar year 2007. Previous year's reports may be obtained from BNL's Cultural and Natural Resource Manager. All activities carried out under the NRMP during CY2007 will be discussed and the report will facilitate development of summary information for the Site Environmental Report for 2007.

2.0 Comprehensive Natural Resource Management Plan

The Laboratory completed and issued the Comprehensive Natural Resource Management Plan in December 2003. The development of this plan was carried out over several years with the assistance of the Technical Advisory Group (TAG) that was established to provide input to the Natural Resource Program and the Upton Ecological and Research Reserve.

This report discusses work related to actions established within the NRMP and through subsequent annual reviews. Incremental changes from annual reviews will be incorporated annually with the completion of the required Annual Report. All incremental changes will be addressed during the 5-year re-write of the Plan scheduled to begin in late 2008.

3.0 Progress

3.1 Transition Wildlife Management Plan Actions into NRMP

This was completed in December 2003 with the publishing of the current NRMP.

3.2 Annual Summary Report

An annual summary report of activities under the NRMP was written for CY2006.

3.3 TAG Review of Annual Report

The CY2006 report on the NRMP was submitted to the TAG for informational purposes.

3.4 Adaptive Management Cycle

The current report is the fifth Annual Report in the Adaptive Management Cycle. It is not expected to result in a need for significant changes. As actions identified in the NRMP are implemented, monitored, and reported on in the future, the need for change will be identified.

3.5 Improve Decision making through use of Innovative Tools

The use of global positioning systems (GPS) and geographic information systems (GIS) are routinely utilized in the Natural Resource Management program. No significantly new data has been added in 2007.

3.6 Maintain and Improve Relationships with Stakeholders

BNL continues to maintain good relationships with all of its stakeholders. Through interactions with the Office of Education Program's – Open Space Stewardship initiative increased activities with stakeholders is taking place. More than 20 school districts, 4 towns, Suffolk County, and over two dozen teachers are participating. The Natural Resource Program at BNL plays a role in training teachers to carryout monitoring of open space throughout Suffolk County. This activity strengthens BNL's relationship with numerous stakeholders.

The FERN, in working with BNL, began the development of a set of freshwater wetland monitoring protocols in 2007. These protocols are being developed on behalf of the Central Pine Barrens Joint Planning and Policy Commission. In the development of these protocols BNL and FERN held a planning meeting with various stakeholders seeking input. The protocols are expected to be completed in the spring 2008.

FERN and the Natural Resource Program at BNL are closely allied to ensure sound operation of the Upton Reserve and to encourage the use of the Reserve, BNL and the Pine Barrrens for ecological research.

3.7 Peconic River Flow Monitoring

Peconic River flow is measured at several locations including above the outfall (HE), down river at the East Firebreak (HMn), and near the boundary of the Laboratory (HQ). In addition flows from the central wetlands are monitored before they enter the Peconic River station at the East Firebreak (HMs), and flows from the STP are measured prior to discharge into the Peconic River. Flow data is presented in Figure 1. The chart shows three peaks. The January and March 2007 peaks are likely due to snow fall. The April 2007 peak is associated with rainfall. The continued decline in flows after the April peak is indicative of small rainfall events and a generally drier year. Off-site flows stopped around mid-September. Although off-site flows stopped, the river on site never totally dried and standing water was present throughout the later months of 2007.

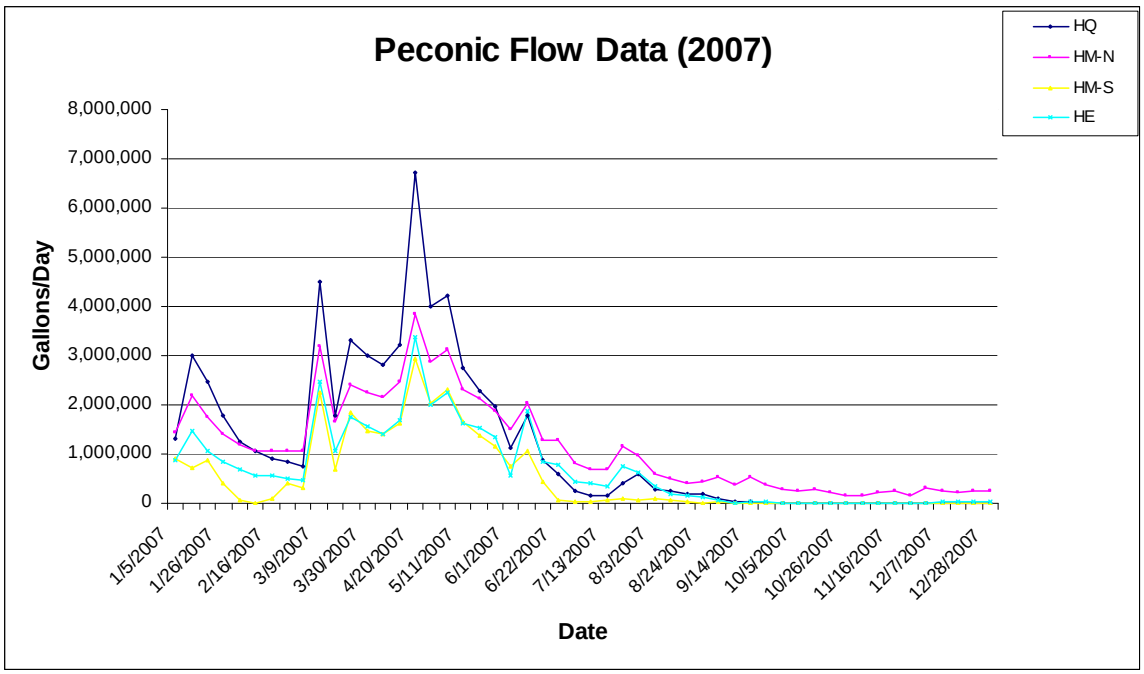

Figure 1. Peconic River flow data for 2007.

3.8 Water Quality Monitoring

Water quality is monitored as a requirement of BNL's State Pollutant Discharge and Elimination System (SPDES) permit. Water quality is measured at various outfalls including the STP discharge to the Peconic River and at several recharge basins that receive stormwater and/or once through cooling water. Results are reported to the NYSDEC on a monthly basis and summarized in the Site Environmental Report each year. The Site Environmental Report for the previous year is made available in October and may be viewed via the Internet at http://www.bnl.gov/esd/SER.asp. Sampling in 2007 did not indicate any concerns for threatened or endangered species within basins or the Peconic River.

3.9 Fish Sampling Peconic River

A population assessment of the onsite portion of the Peconic River was not completed in 2007 and was not necessary since numerous larger fish were captured during surveillance monitoring and sampling. Numerous schools of fry of various species were also noted during the spring months. Fish sampling on the Peconic River was initiated in April and continued through early June. The early season sampling allowed for more and larger fish to be obtained. Historically, late summer sampling resulted in fewer samples taken which was likely due to low dissolved oxygen content in the shallow slow moving waters. Sampling did not appear to affect the population within the onsite portions of the river as numerous smaller fish were seen during sampling events.

The health of the banded sunfish population in Zeke's pond was reassessed during the summer months in 2007. Two students from Southern New Orleans University worked

to assess the population. Their efforts resulted in a population estimate of around 4,000 fish. This is much lower than the estimate of roughly 10,000 fish in 2005, but may be in part due to the estimating technique or the fact that Zeke's pond nearly dried up in the drought of 2006.

3.10 Deer Management

While the need for deer population management continues to be an issue for BNL, there was little change in deer management in 2007, except for updated population estimates and the completion of an Issue and Decision Paper that was submitted for management review.

Discussions on various deer management issues are provided below.

3.10.1 Issue and Decision Paper on Deer Management

The Issue and Decision Paper on Deer Management was completed in November 2007 and the Laboratory Director was briefed on the paper. The paper was being reviewed for comment at the close of the year.

3.10.2 Environmental Assessment for Deer Management

No change in 2007

3.10.3 Implement Deer Management

No additional work has been done on this action in 2007.

3.10.4 Deer Population Estimation

Deer population estimates were conducted in both spring (prior to birth of fawns) and in the fall (after birth of fawns and while bucks had antlers). Figure 2 shows the population trend over the past several years. Population levels are considered to be above the ecosystems carrying capacity and the effects of over population on the ecosystem are still evident.

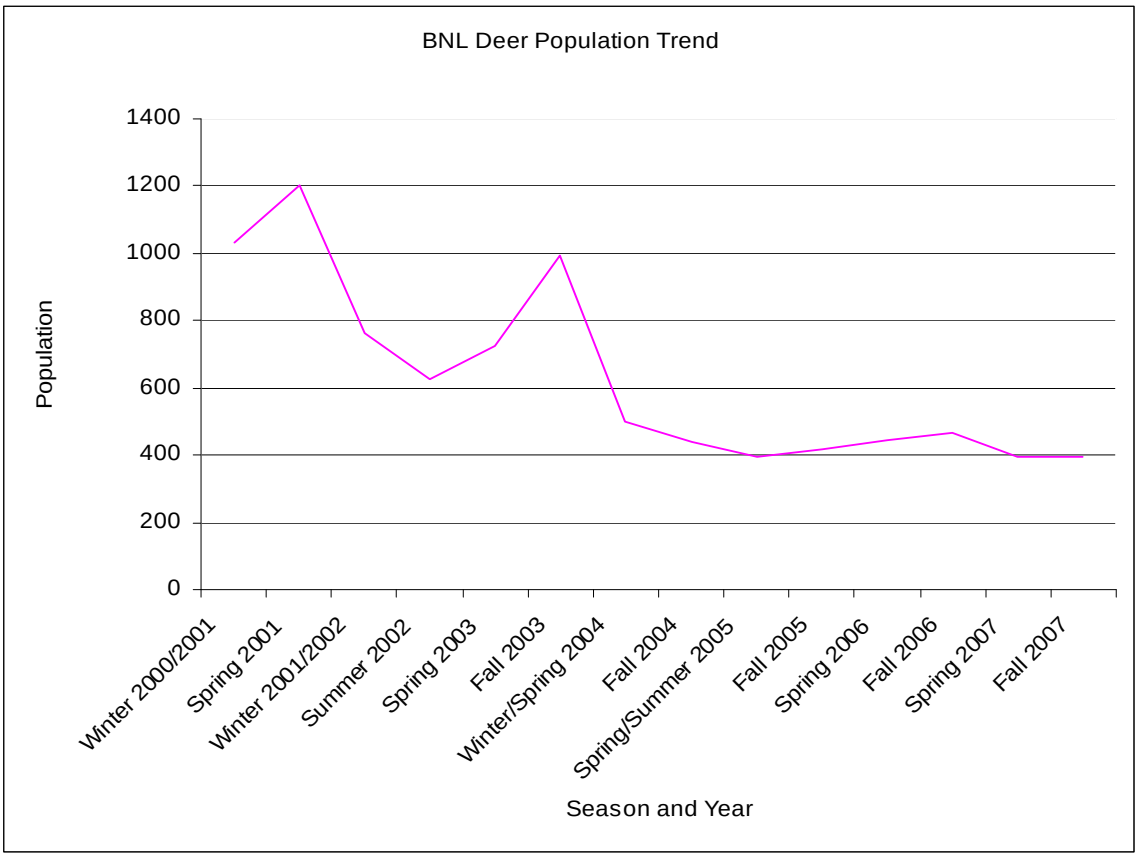

Figure 2. Trend in population estimates of white-tailed deer between 2001 and 2007.

3.11 Special Status Species

BNL is home to a number of plants and animals that are considered special status species including the New York State endangered tiger salamander and Persius duskywing, and the state threatened banded sunfish, swamp darter, frosted elfin butterfly and northern harrier (Table 1). Endangered and threatened plants include the crested fringed orchid, stargrass, and stiff goldenrod. There is also a relatively long list of species of special concern, and rare or vulnerable plants. Under the NRMP the Lab is working to identify areas that may be suitable habitat for species on this list.

In addition to the list in Table 1, species like the wild turkey and Canada goose are also of interest due to their prominence and potential to interact with humans. Information on these species is maintained simply to be aware of potential issues that may arise.

3.11.1 Maintain Special Status Species List

Table 1 is the most recent update of the special status species list. A threatened damselfly species, the pine barrens bluet (*Ennalagma recurvatum*) was placed on the list in 2005 after it was confirmed to exist onsite. Table 1 contains all species identified onsite since the mid-1980s. The sharp-shinned hawk and osprey were added in 2006 based on repeated identifications during routine bird surveys or other observations. The

sharp-shinned hawk may nest on-site and the osprey has been documented using cellular communication towers as roosts and construction of false nests.
Two "likely" species, the horned lark and vesper sparrow were taken off the list as they have not been documented on the BNL property in many years. Should they ever be identified onsite during bird surveys they will be added back to the list.

Table 1. New York State Threatened, Endangered, and Species of Special Concern.

Common Name	Scientific Name	State Status	BNL Status
Insects			
Frosted elfin	*Callophrys iris*	T	Likely
Mottled duskywing	*Erynnis martialis*	SC	Likely
Persius duskywing	*Erynnis persius persius*	E	Likely
Pine Barrens Bluet	*Enallagma recurvatum*	T	Confirmed
Fish			
Banded sunfish	*Enniacanthus obesus*	T	Confirmed
Swamp Darter	*Etheostoma fusiforme*	T	Confirmed
Amphibians			
Eastern tiger salamander	*Ambystoma tigrinum tigrinum*	E	Confirmed
Marbled salamander	*Ambystoma opacum*	SC	Confirmed
Eastern spadefoot toad	*Scaphiopus holbrookii*	SC	Confirmed
Reptiles			
Spotted turtle	*Clemmys guttata*	SC	Confirmed
Eastern box turtle	*Terrapene carolina*	SC	Confirmed
Worm snake	*Carphophis amoenus*	SC	Confirmed
Eastern hognose snake	*Heterodon platyrhinos*	SC	Confirmed
Birds (nesting, transient, or potentially present)			
Whip-poor-will	*Caprimulgus vociferus*	SC	Likely
Grasshopper sparrow	*Ammodramus savannarum*	SC	Confirmed
Northern harrier	*Circus cyaneus*	T	Confirmed
Cooper's hawk	*Accipiter cooperii*	SC	Confirmed
Osprey	*Pandion haliaetus*	SC	Confirmed
Shap-shinned Hawk	*Accipiter striatus*	SC	Confirmed
Plants			
Stargrass	*Aletris farinosa*	T	Confirmed
Butterfly weed	*Asclepias tuberosa*	V	Confirmed
Spotted wintergreen	*Chimaphila maculata*	V	Confirmed
Flowering dogwood	*Cornus florida*	V	Confirmed
Pink lady's slipper	*Cypripedium acaule*	V	Confirmed
Winterberry	*Ilex verticillata*	V	Confirmed
Sheep laurel	*Kalmia angustifolia*	V	Confirmed
Narrow-leafed bush clover	*Lespedeza augustifolia*	R	Confirmed
Ground pine	*Lycopodium obscurum*	V	Confirmed
Bayberry	*Myrica pensylvanica*	V	Confirmed
Cinnamon fern	*Osmunda cinnamomera*	V	Confirmed
Clayton's fern	*Osmunda claytoniana*	V	Confirmed
Royal fern	*Osmunda regalis*	V	Confirmed
Crested fringed orchid	*Plantathera cristata*	E	Likely
Swamp azalea	*Rhododendron viscosum*	V	Confirmed
Long-beaked bald-rush	*Rhynchospora scirpoides*	R	Confirmed
Stiff goldenrod	*Solidago rigida*	T	Confirmed
New York fern	*Thelypteris novaboracensis*	V	Confirmed
Marsh fern	*Thelypteris palustris*	V	Confirmed
Virginia chain-fern	*Woodwardia virginica*	V	Confirmed

Notes: * information based on 6 NYCRR Part 182, 6 NYCRR Part 193, and BNL survey data.

No federally listed threatened or endangered species are known to occur at BNL.

E = endangered, T = threatened, SC = species of special concern, R = rare, V = exploitably vulnerable

3.11.2 Identify Habitats of Special Status Species

When special status species are identified as being present on the BNL site, their habitats are also identified, if possible. If applicable, surveys for the correct habitat take place with surveys for the species in question and information concerning presence or absence of the species is recorded and maintained in BNL's GIS. Currently surveys for four species take place at least annually, these are the tiger salamander, banded sunfish, swamp darter, frosted elfin, and pine barrens bluet.

3.11.3 Tiger Salamander

The eastern tiger salamander, a New York endangered species, is locally abundant on the BNL site. This species has been documented using at least 22 of the 27 ponds or pond systems on site. During the development of the NRMP pond designations were modified to lessen the confusion between confirmed (TS) and unconfirmed (ts) habitat.

3.11.3.1 Tiger Salamander Annual Egg Mass Surveys

Annual egg mass surveys were conducted between the end of December and mid-April. A total of 11 ponds out of the 22 confirmed ponds were visited with egg masses confirmed at TS-10, TS-13a, TS-A7 and TS-W6b.

3.11.3.2 Tiger Salamander Larval Surveys

Larval surveys are conducted at ponds that had positive egg mass identification during the spring breeding season, all ponds with egg masses were also confirmed to have larvae. Fourteen larvae were found dead along the shoreline of TS-10. No chemical or disease could be confirmed as causing the mortality. However, pH was higher than typically measured in the pond.

3.11.3.3 New Pond at RHIC

No additional actions took place with this pond in 2007

3.11.3.4 Cover Board Surveys on one TS Pond

Cover board surveys were discontinued in 2004, but several boards were left around both TS-7 and TS-10 to provide shelter for emerging metamorphs. Remaining cover boards are occasionally checked when other work is being conducted around various ponds. They are simply used as alternative habitat for various reptile and amphibian species. Drift fence surveys of TS-6, TS-7, TS-A7, and TS-13a continued in 2007.

3.11.3.5 TS-A7 Restoration of Meadow Marsh

Restoration was completed in 2003. This pond is currently being monitored as part of a long-term study of tiger salamanders being conducted by the State University of New York at Binghamton to compare its use by tiger salamanders to typical use of coastal plain ponds by salamanders. Drift fencing was installed in 2004 and egg mass, larval,

and metamorph surveys have been routinely conducted. Several metamorphic and adult tiger salamanders have been captured, tagged using radio transmitters and followed to determine the extent of their migration. This work continued through 2007 and will continue into the summer of 2008 to gain a better understanding of tiger salamander biology.

3.11.3.6 TS-W6b Pond Remediation ER Program

The TS-W6B Pond is located on the northwest edge of the Former Hazardous Waste Management Facility (FHWMF). The clean up and restoration of the wetland was completed in September 2005 and was conducted under a wetlands equivalency permit issued by the NYSDEC. Tiger salamander egg mass surveys were completed in 2007 with egg masses documented. Larval surveys in June 2007 resulted in approximately two dozen confirmed larvae present in the pond.

3.11.4 Banded Sunfish

The banded sunfish (*Enneacanthus obesus*) is a New York threatened species that inhabits backwater areas of the Peconic River and Zeke's Pond. Two interns working under the Science Undergraduate Laboratory Internship program worked to document the population in Zeke's pond. The estimate of the population was between 3,000 and 4,000 fish. Documentation also linked the fish to emergent and submergent vegetation (lily pads and bladderwort). A poster of the research results is attached.

3.11.4.1 Peconic River Flow Monitoring HMn

As mentioned above in section 3.7 Peconic River flows are recorded at numerous locations including at HMn. Flow is important for the survival of the banded sunfish in the Peconic River system.

3.11.4.2 OU V Peconic River Remediation Program

Post cleanup monitoring of the Peconic River continued in 2007. Sediment, fish, and water column samples were obtained. Sediment was analyzed for radiological, PCB, and mercury content; fish were analyzed for mercury content; and water was analyzed for mercury, methyl mercury, and total suspended solids. Results of all sampling is published annually in the Peconic River Monitoring report and summarized in the Site Environmental Report.

3.11.5 Frosted Elfin

The frosted elfin (*Callophrys iridis*) is a small orange-brown butterfly that is dependent on wild lupine. Historically, the frosted elfin was found along the south boundary and LIRR right of way at the south east corner of the Lab. This area is typified by soil disturbance that enhances habitat for wild lupine that in turn provides habitat for the butterfly.

3.11.5.1 Confirm Presence/Absence of Frosted Elfin

Surveys of the primary area of lupine occurring on site showed little or no increase in plant production over 2006 surveys.

3.11.5.2 Establish Monitoring Protocols for Frosted Elfin

No additional information on this has been forthcoming. At this point only surveys of host plants appear to be effective.

3.11.5.3 Maintain and Enhance Habitat for Frosted Elfin

Wild lupine likes disturbed soil areas as is found along the south firebreak at the southeast corner of the Lab. Disturbance of the primary area of lupine has not yet resulted in additional plants establishing.

3.11.5.4 Habitat assessment for Lupine

Since lupine is not spreading due to disturbance practices, the Natural Resource Program must consider either purchasing and planting or attempt to collect and plant seeds to encourage the spread this host plant to more areas.

3.12 Habitat Enhancement other species

Several species of birds have been targeted for improvements in nesting habitat. These include the eastern blue bird, kestrel, and wood duck. As information is gained on other species of special interest, habitat improvement needs will be identified and implemented as necessary.

3.12.1 Bird nests/boxes

Nest boxes are important for many species of birds because of the lack of proper habitat. This is particularly true of birds that utilize cavities for nesting. The eastern bluebird is one of the better know birds for which nest boxes are important. BNL currently has 58 boxes distributed across the site in appropriate habitat (open fields near forested areas). House wrens, tree swallows, chickadees, and tufted titmouse also use the bluebird boxes (Table 2). The successful use of boxes declined in all species except use by house wrens. This is due in part to several boxes being removed because activities in the area would greatly disturb the boxes. The use of the nest boxes is evident as indicated in Figure 3. The percent use of nest boxes by bluebirds decrease to about 40% in 2007 while use by other species like tree swallows declined.

All nest boxes including bluebird, wood duck, and kestrel boxes continue to be monitored by volunteers several times each year. To date, four years of monitoring suggest very limited use of wood duck boxes near the biology fields and in the RHIC ring, and apparently no use of kestrel boxes is occurring.

Table 2 Results of Bluebird Nest Box Monitoring 2001 – 2007. Some boxes are used more than once per season.

Summary of Nesting Success							
Year	# of Boxes	Empty/other	Bluebird	House Wren	Tree Swallow	Chickadee	Tufted Titmouse
2001	37	12	19	6	1		
2002	46	13	19	6	6	2	
2003	46	14	21	4	4		2
2004	48	12	23	6	6	1	
2005	53	9	39	6	6	1	
2006	56	8	38	9	6	1	
2007	54	20	27	14	3	3	

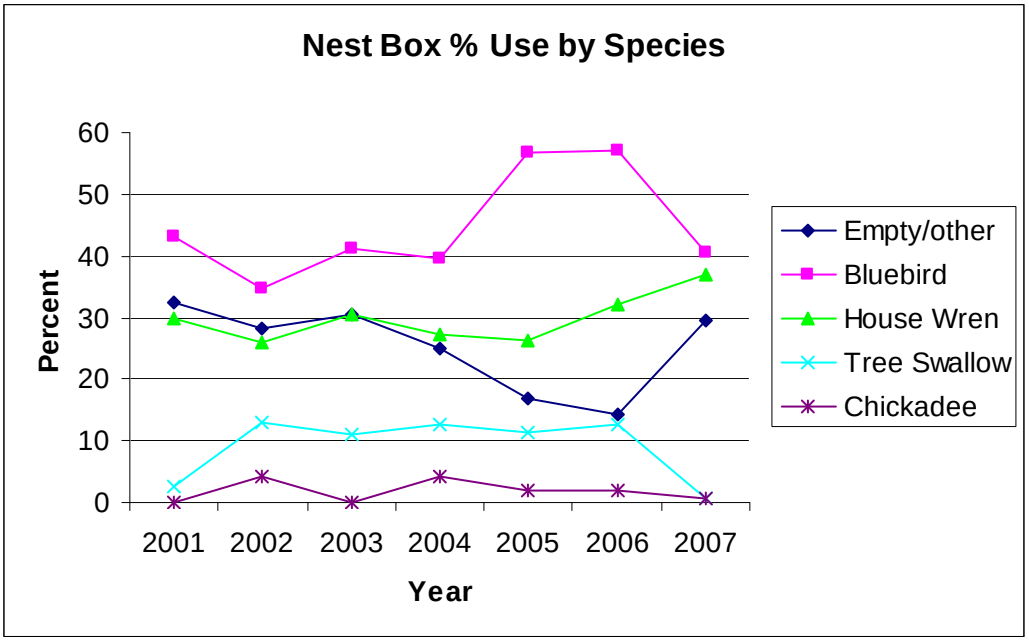

Figure 3. Bluebird nest box use based on a percentage of available boxes.

3.12.2 Surveys and Monitoring

Conducting surveys and routine monitoring allows BNL to identify, track, and trend population status for a number of species. New surveys for reptiles and amphibians, Odonata (damselflies and dragonflies), and incidental reporting of other species during routine activities results in a better understanding of which species are present. The following discussions will touch on the results of various surveys and monitoring in 2006.

3.12.3 Develop Survey Methodology to document all Biota on BNL

A full set of monitoring and survey protocols are still needed. Contracts for a research data base and forest health monitoring protocols were completed in 2005. The forest health monitoring protocols have been implemented across BNL. Additional details are

discussed below. Working with FERN, development of protocols for freshwater wetlands was started in the fall of 2006 and was submitted as drafts to the Central Pine Barrens Joint Planning and Policy Commission in November 2007 for review.

A three year survey of BNL plants was initiated in 2007. Dr. Richard Stalter from St. John's University began random collection of plant species. The intent is to collect from spring through late fall each year in order to document as many species as possible from all habitats across the Laboratory. This work is to supplement the work completed in the 1994-95 biological surveys of BNL.

3.12.3.1 Reptiles and Amphibians

Interns began marking all eastern box turtles (*Tarrapene carolina*) found by notching their carapace and releasing them. The practice started in 2003 and continued in 2007. A database of marked turtles was started. As reported in last year's annual report, turtles are routinely inspected and recaptures documented.

In 2005 several box turtles were found with upper respiratory infections with three documented as having an amphibian ranavirus (iridovirus) isolated from their tissues. Based on this information, a study of the box turtles was initiated. Several turtles were captured in the TS-7 area, radio transmitters attached and then followed during the summers of 2006 and 2007. A poster of the results from 2007 is attached.

3.12.3.2 Monitor Canada Goose & Wild Turkey Populations

The Canada goose (*Branta canadensis*) population on site was estimated to be 120 birds prior to spring 2007. Due to results from previous bird bandings by NYSDEC limited nest management was initiated in 2007. Even with limited nest management, bird banding in late June resulted in an additional 37 goslings being banded. This accounted for most of the production in 2007 bringing the total resident population to 157 birds.

3.12.3.3 Turkey Sighting Reports to NYSDEC

The NYSDEC did not request assistance in monitoring of wild turkey populations in 2007. Rough estimates of the turkey population continue to place the number around 300 birds.

3.12.3.4 Song Bird Surveys

Songbird surveys have been carried out since May 2000. Monitoring involves recording ambient weather conditions at the beginning and end of each of the six routes, and counting the number of individuals of each species heard or seen during a five minute period at each point on the route. Points are spaced approximately 300 meters (Fig. 4) apart to prevent overlap of counts from point to point. Monitoring is carried out monthly from April through September each year.

The current results of monitoring are provided in Table 4 below. In 2007, 69 species of birds were detected. Routes next to wetlands (Peconic River, Biology Fields, and Z-path

routes) continue to have the highest number of species detected. This is likely due to higher biodiversity in these habitats that support a greater variety of nesting sites and foraging opportunities. Results along the Z-Path route are also beginning to indicate high number of species, likely due to the variability of habitats along this route. The Z-Path route goes through the most diverse habitats, ranging from pine forest, to wetlands, to mixed forest.

BNL will continue monitoring in 2008.

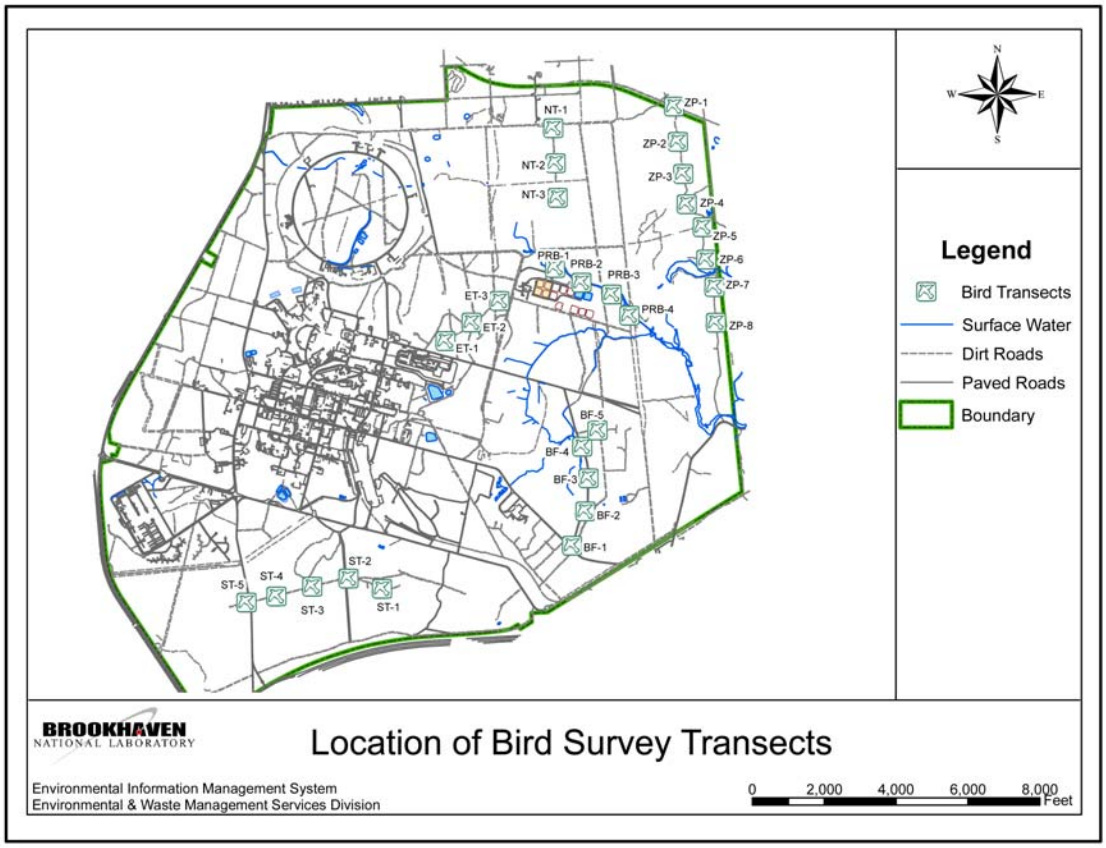

Figure 4. Songbird survey routes.

Table 3. Results of Bird Surveys

Bird Survey Results 2000 - 2007									
Year	# of Species Identified Yearly	# New Species Identified	Total # of Species ID'd	# of Species Biology Fields	# of Species East Trenches	# of Species North Transect	# of Species Peconic River	# of Species South Transect	# of Species Z-Path
2000	70		70	50	31	23	48	32	
2001	73	23	93	53	32	34	45	39	
2002	73	6	99	45	29	30	43	29	47
2003	79	4	103	49	27	31	47	33	44
2004	68	2	105	45	24	33	44	28	41
2005	67	3	108	49	26	32	43	26	43
2006	70	2	110	58	29	33	42	25	37
2007	69	1	111	51	23	34	43	23	46

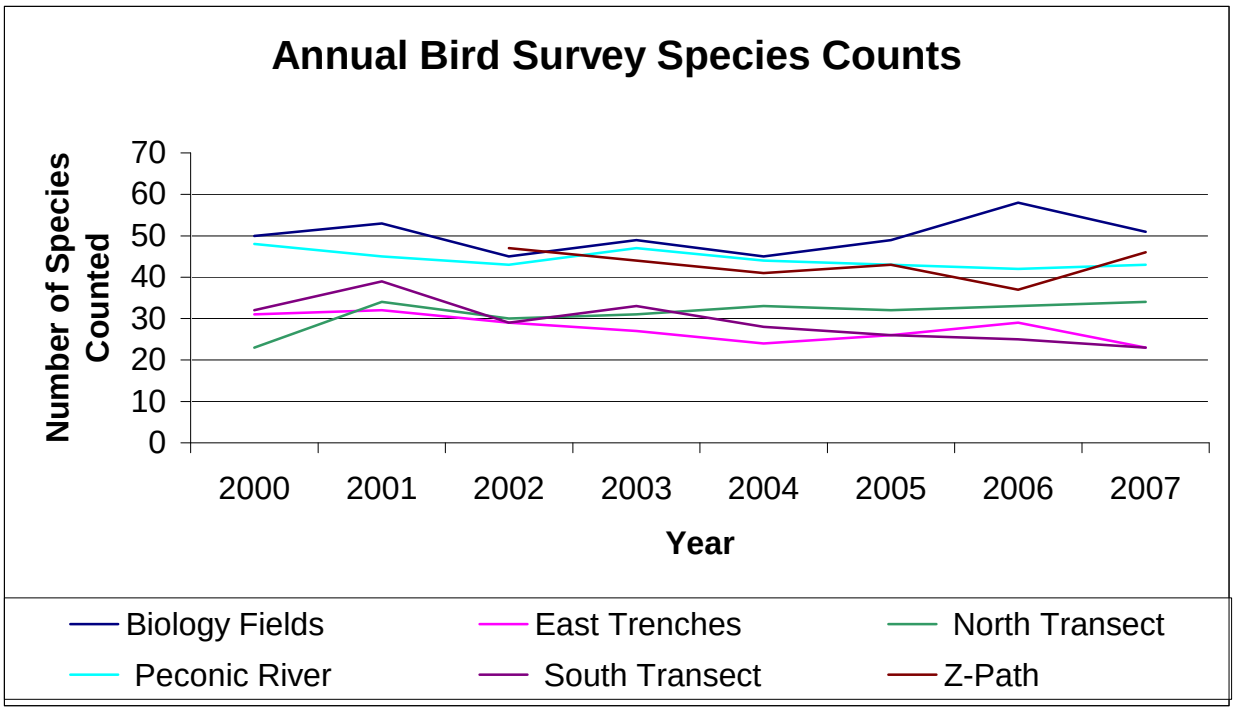

Figure 5. Trends in species counts of songbirds per transect from 2000 – 2007.

Table 4. Data concerning routinely documented bird species

		Year - Number							
Common Name	Scientific Name	2000	2001	2002	2003	2004	2005	2006	2007
Acadian Flycatcher	*Empidonax virescens*		4						
American Crow	*Corvus brachyrhynchos*	71	74	87	121	49	29	43	41
American Kestrel	*Falco sparverius*				1			1	
American Redstart	*Setophaga ruticilla*	1	2						
American Robin	*Turdus migratorius*	207	120	492	231	176	178	278	128
Baltimore Oriole	*Icterus galbula*	6	41	39	53	53	35	61	41
Barn Swallow	*Hirundo rustica*		2	2	5	1	2	2	1
Belted Kingfisher	*Ceryle torquata*			1	1	3			
Bewick's Wren	*Thryomanes bewickii*		1						
Black-and-White Warbler	*Mniotilta varia*	11	10	11	12	1	9	10	1
Black-billed Cuckoo	*Coccyzus erythropthalmus*	10	14	9	3		3	2	3
Black-capped Chickadee	*Poecile atricapillus*	84	114	122	111	173	135	186	168
Blackpole	*Dendroica striata*		1	2					
Black-throated Green Warbler	*Dendroica virens*	2	1						
Blue Jay	*Cyannocitta cristata*	123	216	319	288	253	199	230	180
Blue-Grey Gnatcatcher	*Polioptila caerulea*		5	6	3	3	4	2	4
Blue-winged Warbler	*Vermivora pinus*	1		3	3	1	1	4	4
Brown Creeper	*Certhia americana*	13	1		3	3	1	1	1
Brown Thrasher	*Toxostoma rufum*	9	6	1	7	1		2	1
Brown-headed Cowbird	*Molothrus ater*	9	34	98	81	84	78	69	71
Canada Goose	*Branta canadensis*	28	82	46	216	103	93	85	17
Carolina Wren	*Thryothorus ludovicianus*	1	1	7	1	9	5	10	9
Cedar Waxwing	*Bombycilla cedrorum*	39	2	22	2	1	8	46	44
Chestnut-sided Warbler	*Dendroica pensylvanica*	1							
Chimney Swift	*Chaetura pelagica*			1	1				

Common Name	Scientific Name	Year - Number							
		2000	2001	2002	2003	2004	2005	2006	2007
Chipping Sparrow	*Spizella passerina*	124	130	195	182	237	197	249	148
Common Grackle	*Quiscalus quiscula*	40	55	64	90	153	89	556	127
Common Peafowl	*Pavo cristatus*					1		1	
Common Yellowthroat	*Geothlypis trichas*	11	10	20	15	11	16	13	15
Cooper's Hawk	*Accipiter cooperii*		1	4	2	1			3
Dark-eyed Junco	*Junco hyemalis*		2		1	1		2	
Double-crested Cormorant	*Phalacrocorax auritus*		3	70	42		15		
Downy Woodpecker	*Picoides pubescens*	7	17	30	24	35	26	30	13
Eastern Bluebird	*Sialia sialis*	1	2	7	3	3	3	5	2
Eastern Kingbird	*Tyranus tyranus*	2	1	4	8	3	3	5	7
Eastern Phoebe	*Sayornis phoebe*	3	10	9	2	10	3	12	3
Eastern Screech Owl	*Otus asio*				1				
Eastern Towhee	*Pipilo erythrophthalmus*	151	216	252	211	219	220	213	179
Eastern Wood Peewee	*Contopus virens*	68	51	67	59	70	52	56	67
European Starlings	*Sturnus vulgaris*	32	21		18	7		1	11
Field Sparrow	*Spizella pusilla*		1		8	7	4	5	7
Fox Sparrow	*Passerella iliaca*							3	
Fish Crow	*Corvus ossifragus*		3	1	2	2			2
Glossy Ibis	*Plegadis falcinellus*				8				1
Golden Eagle	*Aquila chrysaetos*	3						6	
Golden-crowned Kinglet	*Regulus satrapa*	1				2			
Goldfinch	*Carduelis tristis*	54	35	49	70	82	47	87	74
Grasshopper Sparrow	*Ammodramus savannarum*	2				1			
Great Black-backed Gull	*Larus marinus*					1			
Great Blue Heron	*Ardea herodias*				1	1	1	1	2
Great Crested Flycathcer	*Myiarchus crinitus*			17	14	25	12	22	33
Great Egret	*Ardea alba*						1		
Great Horned Owl	*Bubo virginianus*		1				1		2
Green Heron	*Butorides virescens*				2				1
Grey Catbird	*Dumetella carolinensis*	57	65	68	62	49	47	59	45
Hairy Woodpecker	*Picoides villosus*		3	3	2	2	4		5
Hermit Thrush	*Catharus guttatus*		5	4	1	4	10	4	1
Herring Gull	*Larus argentatus*	2		1	24	6	5	3	1
Horned Lark	*Eremophila alpestris*	2							
House Finch	*Carpodacus mexicanus*		1		2		1	1	
House Wren	*Troglodytes aedon*	14	4	7	11	3	3	9	8
Indigo Bunting	*Passerina cyanea*		5	11	15	21	8	12	12
Killdeer	*Charadrius vociferus*	1				1	2		2
Magnolia Warbler	*Dendroica magnolia*	2							
Mallard Duck	*Anas platyrhyncos*	2	7	3	2	1	6	1	
Merlin	*Falco columbarius*						2		
Mourning Dove	*Zenaida macroura*	55	41	78	39	46	27	16	33
Nashville Warbler	*Vermivora ruficapilla*	6							
Northern Bobwhite	*Colinus virginianus*	31		1	1				
Northern Cardinal	*Cardinalis cardinalis*	15	13	7	16	8	14	17	14
Northern Flicker	*Colaptes auratus*	31	21	38	20	27	21	42	12
Northern Mockingbird	*Mimus polyglottos*	6	13	13	9	6	7	8	11
Northern Parula	*Parula americana*	1			2		5		
Northern Rough-winged Swallow	*Steligdopteryx serripennis*	8				2			
Orchard Oriole	*Icterus spurius*					1			2

Common Name	Scientific Name	Year - Number							
		2000	2001	2002	2003	2004	2005	2006	2007
Osprey	*Pandion haliaetus*								2
Ovenbird	*Seiurus aurocapillus*	19	71	86	58	65	56	89	62
Palm Warbler	*Dendroica palmarum*		1	3	1				4
Pine Warbler	*Dendroica pinus*	5	23	54	25	81	57	91	57
Plain Pigeon	*Columbus livia*		1				5	4	
Prairie Warbler	*Dendroica discolor*	3					1		
Purple Martin	*Progne subis*				6				
Red-bellied Woodpecker	*Melanerpes carolinus*	12	8	4	7	15	3	4	4
Red-breasted Nuthatch	*Sitta canadensis*	5	19	13	11	25	43	38	49
Red-eyed Vireo	*Vireo olivaceus*	24	31	15	20	28	19	32	20
Red-tailed Hawk	*Buteo jamaicensis*	3	2	2	6	6	5	5	5
Red-winged Blackbird	*Agelaius phoeniceus*	8	12	45	16	34	25	18	9
Ring-billed Gull	*Larus delawarensis*	4					4		
Rose-breasted Grossbeak	*Pheucticus ludovicianus*	2			2			1	
Ruby-crowned Kinglet	*Regulus calendula*	2		1	1			7	1
Ruby-throated Hummingbird	*Archilochus colubris*			1	1		1	1	3
Savannah Sparrow	*Passerculus sandwichensis*							3	
Scarlet Tanager	*Piranga olivacea*	3	8	7	15	11	13	25	18
Sharp-shinned Hawk	*Accipiter striatus*	1		1	1	2		1	
Song Sparrow	*Melospiza melodia*				2		1		
Summer Tanager	*Piranga rubra*						1		
Tree Swallow	*Tachycineta bicolor*	6	3	8	9	17	3	10	
Tufted Titmouse	*Baeolophus bicolor*	34	19	29	32	25	26	17	20
Veery	*Catharus fuscescens*	3	1	6	3	3			7
White-breasted Nuthatch	*Sitta carolinensis*	5	3	3	3	3	9	8	4
White-eyed Vireo	*Vireo griseus*	1							
White-throated Sparrow	*Zonotrichia albicollis*	12	1	1					4
Wild Turkey	*Meleagris gallopavo*	15	3	7	8	9	36	38	15
Wood Duck	*Aix sponsa*				7	3	4	6	2
Wood Thrush	*Hylocichla mustelina*	43	16	10	10	12	10	20	18
Worm-eating Warbler	*Helmitheros vermivorus*							1	
Yellow Warbler	*Dendroica petechia*	1		1	1	1			6
Yellow-bellied Sapsucker	*Sphyrapicus varius*	1						1	
Yellow-billed Cuckoo	*Coccyzus americanus*	13	8	20	26	5	6	18	8
Yellow-rumped Warbler	*Dendroica coronata*		3		12			7	1
Yellow-throated Warbler	*Dendroica dominica*		1						

3.12.3.5 Odonate Surveys

Surveys of Odonata (dragonflies and damselflies) continued in 2007. No new species were added to the list for BNL. There were no rare damselflies identified in 2007. Table 5 presents a compiled list of all species found at BNL from 2003 through 2007.

In 2006 a pilot study to determine whether Odonate populations can be estimated using mark recapture techniques was conducted. This study was continued in 2007 and was expanded to look at multiple ponds and the potential for migration between ponds. A copy of the paper relating to the research is attached to this report.

The results of the 2006 study and conclusions was presented at the Northeast Fish and Wildlife Administrators conference in April, 2007.

Table 5. Dragonfly and damselfly species identified during surveys at BNL.

DRAGONFLIES			
Aeshnidae	***Scientific Name***	***Libellulidae (cont.)***	***Scientific Name***
Shadow Darner	*Aeshna umbrosa*	Slaty Skimmer	*Libellula incesta*
Common Green Darner	*Anax junius*	Widow Skimmer	*Lebellula luctuosa*
Comet Darner	*Anax longipes*	Twelve-spotted Skimmer	*Libellula pulchella*
Swamp Darner	*Epeaeschna heros*	Painted Skimmer	*Libellula semifasciata*
Harlequin Darner	*Gophaeschna furcillata*	Band-winged Meadowhawk	*Sympetrum semicinctum*
Gomphidae		Black Saddlebags	*Tramea lacerata*
Unicorn Clubtail	*Arigomphus villosipes*	Blue Dasher	*Pachydiplax longipennis*
Ashy Clubtail	*Gomphus lividus*	Carolina Saddlebags	*Tramea carolina*
Corduliidae		Cherry-faced Meadowhawk	*Sympetrum internum*
Common Baskettail	*Epitheca cynosura*	Common Whitetail	*Libellula lydia*
Williamson's Emerald	*Somatochlora williamsoni*	Eastern Amberwing	*Perithemis tenera*
Libellulidae		Great Blue Skimmer	*Libellula vibrans*
Bar Winged Skimmer	*Libellula axilena*	Black Setwing	*Dythemis nigrescens*
Calico Pennant	*Celithemis elisa*	Spot-winged Glider	*Pantala hymenaea*
Halloween Pennant	*Celithemis eponina*	Wandering Glider	*Pantala flavescens*
Martha's Pennant	*Celithemis martha*	White-faced Meadowhawk	*Sympetrum obtrusum*
Eastern Pondhawk	*Erythemis simplicicollis*	Double-ringed Pennant	*Celithemis verna*
Dot-tailed Whiteface	*Leuchorrhinia intacta*	Ruby Meadowhawk	*Sympetrum rubicundulum*
Spangled Skimmer	*Libellula cyanea*	Frosted Whiteface	*Leucorrhinia frigida*
Blue Corporal	*Libellula deplanata*	Elfin Skimmer	*Nannothemis bella*
DAMSELFLIES			
Calopterygidae	***Scientific Name***	***Coenagrionidae (cont.)***	***Scientific Name***
Ebony Jewelwing	*Calopteryx maculata*	Azure Bluet	*Enallagma aspersum*
Lestidae		Familiar Bluet	*Enallagma civile*
Spotted Spreadwing	*Lestes congener*	Northern Bluet	*Enallagma cyathigerum*
Common Spreadwing	*Lestes disjunctus*	Atlantic Bluet	*Enallagma doubledayi*
Amber-winged Spreadwing	*Lestes eurinus*	Big Bluet	*Enallagma durum*
Sweetflag Spreadwing	*Lestes forcipatus*	Skimming Bluet	*Enallagma geminatum*
Elegant Spreadwing	*Lestes inaequalis*	Pine Barrens Bluet	*Enallagma recurvatum*
Slender Spreadwing	*Lestes rectangularis*	Citrine Forktail	*Ischnura hastata*
Lyre-tipped Spreadwing	*Lestes unguiculatus*	Fragile Forktail	*Ischnura posita*
Swamp Spreadwing	*Lestes vigilax*	Rambur's Forktail	*Ischnura ramburii*
Coenagrionidae		Eastern Forktail	*Ischnura verticalis*
Marsh Bluet	*Enallagma ebrium*	Sphagnum Sprite	*Nehalennia gracilis*
Variable Dancer	*Argia fumipennis*		

3.12.4 Population Management

There are currently four species on site whose populations either do or may require management in the near future. These are the white-tailed deer (discussed above), Canada geese, wild turkey, and feral cats.

3.12.4.1 Manage Canada Goose Population

As mentioned above, the Canada goose population is currently estimated at 157 birds living year round on the BNL site. Numerous requests for management of the geese were received in 2007 mainly dealing with presence of goose droppings on sidewalks and walkways. In several instances geese were causing safety issues due to their defensive posturing to protect their nests. Due to the location of the nesting geese, the nests were destroyed, under permit, forcing the geese to move to another location.

Because of the continued nuisance situations, the Natural Resource Program obtained a permit under new U.S. Fish & Wildlife Service (FWS) regulations established late in 2006 to manage nesting geese. These regulations allow nest management to occur based on registering with the FWS and reporting data back to them. A total of 10 nests were either destroyed or the eggs oiled as the opportunity presented itself or in response to complaints; aggressive nest management was not practiced in 2007. However, because the population continued to increase, as mentioned above, a more aggressive approach to nest management will be implemented in 2008.

3.12.4.2 Manage Wild Turkey Population

The wild turkey population seems to be stabilizing around 300 - 350 birds. In 2007 only a few instances of nuisance situations, birds pecking at paint on cars, or blocking access to buildings, were reported. In general the turkeys have not cause significant nuisance situations, but if they do, NYSDEC is willing to issue permits for capture and transport of nuisance animals to other locations onsite. Issues with feeding turkeys and other animals seem to have diminished in most cases. When feeding is documented the issue is dealt with on a case by case basis.

3.12.4.3 Feral Animals

The existing feral cat colonies continue to be managed by an ad-hoc group of employees. There has been no significant change in the number of cats present.

3.12.4.3.1 Establish BNL Policy on feral animals

A general agreement on feral cats was established with the ad hoc cat managers. This agreement basically provides for the continued care of existing cats and does not allow any additional cats to be added to the colonies regardless of whether they are introduced or wander into a colony. This policy ensures that the colony size will be reduced over time.

3.12.4.3.2 Protocols for monitoring and managing feral cats

Data collected by simple ad hoc monitoring of cat colonies by care givers is routinely submitted to the Natural Resource Manager for review.

3.13 Vegetation Management

BNL met its commitment to the EPA Region 2 Performance Track program with 42 acres of land either restored or place into fire rotation. An additional 15 acres were scheduled for prescribed fire in November 2007. However, weather conditions were outside of the prescription and therefore no fire occurred.

3.13.1 Native Vegetation

No additional plantings occurred in 2007

3.13.1.1 Establish Protocol for Use of Native Vegetation

BNL now routinely uses native vegetation in its landscaping efforts. Where possible native vegetation is specified during the planning and design stages of projects

3.13.1.2 Use Native Vegetation on Restoration and new Construction Projects

This is routinely implanted.

3.13.1.3 RHIC Revegetation

Pitch pine seedlings planted in 2001 and 2005 are beginning to grow more rapidly, slowly returning the area to typical Pine Barrens habitats.

3.13.1.4 Establish Policy and procedure for cutting trees

An informal process is used in which the Assistant Laboratory Director for Facilities and Operations makes a decision based on input from Plant Engineering and the Natural Resource Manager. This informal process appears to be efficient as it has become a routine procedure and no additional formalization is deemed necessary.

3.13.2 Invasive Species

BNL continued to participate in the Long Island Weed Management Area efforts in 2007. BNL working with the Central Pine Barrens Commission and The Nature Conservancy had several wetlands onsite surveyed for the presence or absence of invasive plants. Of the six wetlands surveyed, none had any invasive plants present.

3.13.2.1 Identify and Monitor Distribution of Invasive Species

Nearly all areas of BNL have been surveyed for invasive species and documented in the GIS.

3.13.2.2 Establish Volunteer "Weed Watchers" group

No further action has occurred on this since determining that the group is not needed.

3.13.2.3 Removal or Control of Invasive Plants

Towards the end of 2007 three areas were being developed for implementation as "Weed Free Areas." These include the southern portion of the Laboratory south of Princeton Ave.; the area east of the east firebreak; and the area north of the north firebreak.

3.13.2.4 Identify Funding Sources

At the end of 2007, the Central Pine Barrens Joint Planning and Policy Commission was preparing a work plan for utilizing a Student Conservation Association work crew to complete invasive plant removal on public lands. At that time BNL was evaluating the feasibility of participating in this work plan.

3.14 Ecosystem Monitoring & Management

Through efforts of student interns, several wetlands on BNL and within the Pine Barrens were sampled for water quality parameters. The results of these surveys were reported in posters at the end of the summer (attached) and data provide to The Nature Conservancy for further analysis.

3.14.1 Wetland Health Monitoring

As mentioned above water quality analysis of several wetlands was completed in 2007. Additionally, two students working with FERN developed freshwater wetland monitoring protocols as part of a larger project to develop protocols to be used across the Central Pine Barrens.

3.14.1.1 Determine Functionality of BNL Central Wetlands

No activity on this item took place in 2007.

3.14.1.2 Maintain or improve wetland functions

This action cannot be undertaken until wetland health monitoring and a determination on functionality is completed. Once the previous two actions are completed then plans for management of the wetlands can be made.

3.14.2 Forest Health Monitoring

No additional work was completed on this. The total data set for the Pine Barrens Forest Health Monitoring is being analyzed and a final report is forthcoming.

3.14.2.1 Develop Criteria

No additional work was completed on this in 2007.

3.14.2.2 Establish Forest Health Monitoring locations

No additional plots were established under the Forest Health Monitoring.

3.15 Security

Security issues associated with illegal ATV use continued in 2007.

3.15.1 Illegal Use of ATVs

Illegal ATV use continues to be a problem. The Central Pine Barrens Protected Lands Council began planning mitigative actions to attempt to reduce ATV traffic in the Sarnoff Preserve in Riverhead. The effectiveness of these efforts appears to be promising at reducing illegal ATV traffic. Should the project prove successful, BNL will evaluate implementing a similar program.

3.15.2 Other Trespass issues

No new issues have been identified in 2007.

3.16 Pesticide Use

No new work occurred in 2007. BNL continues to use Integrated Pest Management for determining pesticide use.

3.16.1 SBMS Subject Area

An SBMS subject area is likely not needed.

3.16.2 Use in Natural Resource Management

In the future, the use of pesticides, primarily herbicides, will be necessary for control of invasive plants. Protocols for use and approvals must be developed when determined necessary. The BNL site maintenance team was asked to obtain the necessary certifications on their Applicator's licenses in order to apply herbicides in wetlands to control phragmites.

3.17 Wildland Fire Management

BNL is working under an approved Wildland Fire Management Plan. This plan is due for review beginning late in 2008.

3.17.1 Implement Wildland Fire Management Plan

The Wildand Fire Management Plan continues to be implemented.

3.17.2 Implement Use of Prescribed Fire

An updated prescribed fire plan was developed for a 60 acre block of oak forest located in the northeast portion of the Laboratory. A fire was planned for the week of the annual Fire Academy. However, due to weather conditions not meeting the prescription the fire was not carried out.

A draft Memorandum of Understanding between BNL/DOE, NYSDEC, and The Nature Conservancy was developed and submitted for review. It had not been approved by the end of 2007.

3.18 Integration of Cultural Resources

Cultural Resource Management issues are now routinely incorporated in natural resource planning.

3.18.1 Identify Cultural Resources and Develop GIS layers

Cultural resource map layers are routinely maintained within the GIS.

3.19 GIS and GPS

The Natural Resource Management program has integrated GIS and GPS into much of its management. No additional changes were made to this action in 2007.

3.19.1 Develop Natural Resource data layers for GIS

The GIS has been used to map home range information for all species that are being tracked with radio telemetry equipment. This effort is routinely implemented as new work is identified and carried out.

3.19.2 Plan Trails and paths that limit impact

No new actions were taken on this in 2007.

3.19.3 Fill data gaps concerning flora and fauna

Filling data gaps is documented throughout this annual report in earlier sections concerning endangered, threatened, and species of special concern, reptile and amphibian studies, and Odonate studies as examples.

The presence of red and gray fox on BNL has resulted in initial investigation into their habits and habitats. In both 2006 and 2007 the natural resource management program used remotely deployed cameras to document the presence of both species on site. Non-

invasive genetic identification of both species and their distribution has been accomplished using fecal material. Because both cameras and genetic techniques suggest some level of overlap of these two species, a radio collar survey was formalized late in 2007 for implementation in 2008. This three year study is intended to gather data on the amount of overlap by these two species, and provide a better indication of their habitat needs.

3.20 Education Programs

In 2007, the Natural Resource Management program and the Foundation for Ecological Research in the Northeast hosted nineteen individuals that included a Faculty and Student Team (1 Professors and 2 -students), five Academies Creating Teacher Scientists (ACTS) interns, nine undergraduate research interns, and one high school intern, all working on various projects. These interns completed work on salamanders, radio telemetry work on eastern box turtles, inventory of Odonate species, island wide surveys for southern leopard frog, genetic surveys for red and gray fox, water chemistry of coastal plain ponds, tiger beetle population estimation, determining the presence of burying beetles, and development of freshwater wetland protocols.

Each intern was responsible for their own research as well as assisting each other in the collection of data. Results of the research were presented in a poster session sponsored by the Office of Education Programs, and the research was also presented at a poster session at the Pine Barrens Research Forum. Copies of all posters are attached to this report.

Many students and BNL staff participated in the BNL Science Museum's Summer Camp program. Each week, camp participants met on Thursday at the Weaver Rd. pond to learn about the environment. Each intern also presented their research to the campers. These lessons introduced students in grades 4 –6 to the various research topics, and gave the student interns an opportunity to learn teaching skills.

3.21 Research

Research carried out in 2007 through funding from FERN included the microbial study of the Gamma Forest soils and soils associated with Forest Health plots, and an island wide search for the southern leopard frog. As mentioned above in the plant inventory section, a three year study to identify as many plant species at BNL was initiated.

Additionally, Jonathan Mawdsley from the Heinz Center conducted a survey for tiger beetles. During the survey a total of five species of tiger beetles were identified including: *Cicindela formosa generosa, C. repanda, C. scutellaris rugifrons, C. sexguttata,* and *C. tranquebarica*. All are considered common. No rare species were identified.

3.21.1 Identify, attract, and support ecological research to BNL

A researcher from SUNY Binghamton continued working on tiger salamander research in 2007. As mentioned above, FERN funded research looking at the microbial make-up of Gamma Forest soils and forest health plots.

The FERN continues to work on identifying outside sources for funding research in the Pine Barrens.

3.22 NRMP Plan Update

Since the NRMP was completed in December 2003 the update for the plan has been scheduled to begin in fiscal year 2009. Appendix C of the NRMP has been updated to reflect progress made in 2007. Appendix C is attached.

This report once completed will be provided to the TAG for their information.

APPENDIX C

NATURAL RESOURCE MANAGEMENT PLAN – ACTION ITEMS

Action Item	Site ID	Action	Planned Date	Action Taken
1	*Site-wide	Transition WMP Action into NRMP	December 2003	Complete
2	Site-wide	Annual Summary Report	Annual by April 30	Ongoing
3	Site-wide*	TAG Review of Annual Report	Annual by May	Ongoing
4	Site-wide*	Adapt Management based on new information	As Required	4th annual report 4/26/07, ongoing
5	Site-wide*	Improve decision making through use of innovative tools	As Necessary	Implemented 2003, ongoing
6	Site-wide*	Maintain and Improve relationships with stakeholders	Continual	Ongoing
Peconic River/Basins				
7	Peconic River Station HMn	Monitoring for flow: water quality	Monthly sampling SPDES Program	Ongoing
8	Fish Sampling Peconic River	Fish sampling with NYSDEC/Cold Spring Harbor: population assessment of banded sunfish and swamp darter	Annual Spring/Summer	Ongoing
9	TS-7	Monitoring for water quality	Monthly sampling SPDES Program	Ongoing
Deer Management				
10	*Site-wide	Issue and Discussion Paper on deer management by Natural Resource Manager	Fall 2007	Draft submitted Dec. 2007
11	*Site-wide	Environmental Assessment under NEPA for deer management		On hold
12	*Site-wide	Implement Deer Management		Decision to be made based on I&D paper
13	Site-wide	Deer population estimation	Nov-Jan May-June	Ongoing. Routine estimates made twice a year, new protocol developed in 2004
Special Status Species				
14	*Site-wide	Maintain Special-status species list	Annual Review	Ongoing
15	*Site-wide	Identify habitats of special-status species	Continual	Ongoing
Tiger Salamander				
16	Site-wide	TS annual egg mass surveys at breeding ponds	Feb-April 2003	Ongoing
17	Site-wide	TS Larval Survey	Annual June-July	Ongoing
18	Education	Provide educational material or opportunities to BNL staff and public on environmental issues	Continual	Ongoing
19	*RHIC	New pond being added at RHIC	Summer 2004	completed
20	Tiger salamander	Set up cover boards around one breeding site (as a test case)	Summer	Summer 2001 & 2002, completed, drift fences installed
21	TS-A7	Lining of pool ER program	Aug 2003	Completed
22	TS-W6b	Pond Remediation ER program	2004-2005	Completed

APPENDIX C

NATURAL RESOURCE MANAGEMENT PLAN - ACTION ITEMS

(continued)

Action Item	Site ID	Action	Planned Date	Action Taken
Banded Sunfish				
23	OU V	Peconic River Remediation Program	Spring 2004	Completed, tracking success of restoration
Frosted Elfin				
24	*Habitat Specific	Confirm presence/absence of Frosted Elfin	May-June Annually	Ongoing
25	*Habitat Specific	Establish standard monitoring protocols for the Frosted Elfin		
26	*Species Specific	Maintain and Enhance habitat for the Frosted Elfin	Continual	Ongoing
27	*Site-wide	Habitat assessment for lupine	Spring 2004	Ongoing
Habitat Enhancement/ other species				
28	Site-wide	Bird nests/boxes	Ongoing	Routine monitoring and maintenance of bluebird, kestrel, wood duck nest boxes
29	*Site-wide	Develop survey methodology to document all biota on BNL	2004	Contract through Upton Reserve
30	Site-wide	Monitor Canada Goose and Wild Turkey populations	Ongoing	Ongoing
31	Site-wide	Turkey sighting reports to NYSDEC	Ongoing	Reports sent annually in September or upon request
32	Site-wide	Song bird surveys	April – Sept.	Continuing
33	*Site-wide	Odonata Surveys	Summers	Initiated 2003, ongoing
34	*Site-wide	Reptiles and amphibian Surveys	Ongoing	Reptiles & Amphibians started 2003
Population Management				
35	*Site-wide	Manage Canada Goose population	As necessary	Implemented April 2007
36	*Site-wide	Manage Wild Turkey population	As necessary	Not needed, yet
37	*Site-wide	Establish BNL policy on feral animals	General policy implemented	
38	*Site-wide	Establish monitoring and management protocols for feral animals	Fall 2003	Initiated, ad hoc group providing monitoring information
Vegetation Management				
39	*Site-wide	Establish protocol for use of native vegetation		Routinely done w/out protocol
40	*Site-wide	Use native vegetation on restorations and new construction landscaping	As necessary and applicable	Initiated 2003, ongoing
41	RHIC Revegetation	Implement Revegetation	Ongoing	Grasses planted 2002 and 2003, Completed 2005
42	*Site-wide	Establish policy and procedure for cutting trees		Informal Procedure appears adequate.
Invasive Species				
43	*Site-wide	Identify and monitor distribution of invasive species.	Ongoing	Mapping started Summer 2003, completed 2005

APPENDIX C

NATURAL RESOURCE MANAGEMENT PLAN - ACTION ITEMS

(continued)

Action Item	Site ID	Action	Planned Date	Action Taken
44	*Site-wide	Establish volunteer "Weed Watchers" group	Ongoing	Group formed May 2003, disbanded 2005
45	*Site-wide	Removal or control of invasive plants where possible.	As necessary	Planning started
46	*Site-wide	Identify funding for removal or control of invasive plants where possible.	As necessary	Central Pine Barrens initiating work plan.
Ecosystem Monitoring and Management				
47	*Site-wide	Develop criteria to monitor wetland health	2007	Started in 2007
48	*Site-wide	Determine functionality of BNL Central wetlands	2007-2009	
49	*Site-wide	Maintain or improve wetland functions		
50	*Site-wide	Develop criteria to monitor forest health	Fall 2004	Completed 2005
51	*Site-wide	Establish forest health monitoring locations	Summer 2005	Initiated 2005, continued 2006, completed
Security				
52	*Site-wide	Coordinate with Security to reduce illegal use of ATVs	Continual	Ongoing
53	*Site-wide	Other trespass Issues	Continual	Ongoing
Pesticide Use				
54	*Site-wide	Determine need for a SBMS subject area on pesticides	As necessary	Not currently necessary
55	*Site-wide	Pesticide use for natural resource management	As identified	Requested site maintenance to gain certification.
Wildland Fire Management				
56	*Site-wide	Implement Fire Management Plan	Sept. 2003	Plan Approved September 2003
57	*Site-wide	Implement use of prescribed fire and mechanical fuel reduction	March 2003	1st Fire November 2004 CY2006 Approved. CY2007 plan approved
Cultural Resource Management				
58	*Site-wide	Identify cultural resources and develop into GIS layers	Ongoing	LEED Area Identified 2005
GIS and GPS				
59	*Site-wide	Develop natural resource data layers of GIS	Ongoing	
60	*Site-wide	Plan trails and paths that limit impact on the environment while introducing employees to forest diversity.		
61	*Site-wide	Fill data gaps concerning all flora and fauna, including the following: terrestrial and aquatic invertebrates, Lepidoptera, wild flowers, and grasses.	Ongoing	

APPENDIX C

NATURAL RESOURCE MANAGEMENT PLAN - ACTION ITEMS

(continued)

Action Item	Site ID	Action	Planned Date	Action Taken
62	Site-wide	Education Programs	Ongoing	Utilize Office of Education Programs Interns, etc. 18 interns 2005, 21 interns 2006, 19 interns 2007
	Research			
63	Site-wide	Cooperate with Upton Reserve, support and conduct research as needed	Ongoing	Assisting Upton Reserve in coordinating research programs, Transitioned to FERN
64	*Site-wide	Identify, attract, and support ecological research at BNL	Ongoing	Coordinating with FERN
65	Site-wide	NRMP Plan Update	Every 5 years Next update 2008	

Notes: * New initiative

ER – Environmental Restoration
GIS – Geographical Information System
NEPA – National Environmental Policy Act
NYSDEC - New York State Department of Environmental Conservation
NRMP – Natural Resource Management Plan
OU V – Operable Unit V
RHIC - Relativistic Heavy Ion Collider
TS – Tiger Salamander

ATTACHEMENTS

STUDENT INTERN POSTERS

Population Assessment of the New York State Threatened *Enneacanthus obesus* (Banded Sunfish) Conducted in Zeke's Pond and the Peconic River.

Carmen Maldonado, Tyra Bunch, and *Timothy M. Green, Southern University at New Orleans, New Orleans, LA 70126, *Brookhaven National Laboratory, Upton, NY 11973

Abstract

Enneacanthus obesus (Banded sunfish), the smallest species of sunfish inhabiting rivers, lakes, and ponds along the Atlantic coast, has been declared a threatened species in the state of New York. Approximately 200 sunfish were relocated to Zeke's Pond in 2004 during the remediation of the Peconic River, which runs through Brookhaven's property. However, in 2005 a drought nearly eliminated the relocated sunfish population. A population assessment was conducted in the Peconic River, and Zeke's pond, which is found on the eastern most point of Brookhaven's grounds. To capture and assess a sampling of the sunfish population, a seine net, a dip net, a bucket, a measuring tape, a pen, and an all weather writing tablet were utilized. The first step was to complete a survey of the aquatic vegetation by calculating the amount of vegetation in the immediate area that was to be seined. The sunfish were collected from the seine net, stored in the bucket, counted, measured, and then returned safely back to the water. No sunfish were found in the Peconic River. An area of approximately 25785.5 ft.^{2} was covered in Zeke's Pond during a series of thirteen visits resulting in a total of eighty seines. Final fish counts yielded 369 sunfish, sixty-six catfish, and thirteen pumpkinseeds. The estimated total population is 4,027, which is 4% of the previous study's count of 95,900. Further studies are necessary to document the life cycle and population trends of the *Enneacanthus obesus*.

Fig. 1 Zeke's Pond

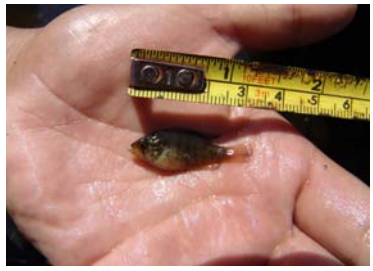

Fig.3 Juvenile Banded sunfish

Fig.2 Adult Banded sunfish

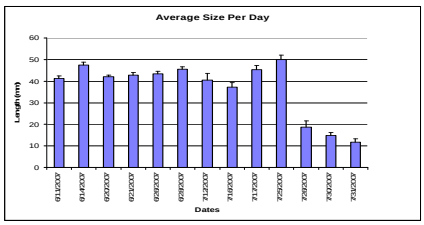

Table 1 Average Sizes of Fish Per Day

Materials and Methods

To capture and assess a sampling of the sunfish population, the materials required included a seine net, a dip net, a bucket, a measuring tape, a pencil, and an all weather writing booklet. Once in the water the preliminary procedure consisted of completing an Aquatic Vegetation Survey (AVS) by calculating the amount of Submerged Aquatic Vegetation (SAV) in the area that was to be seined. The aquatic vegetation survey was conducted by visually observing the quantities and densities of vegetation present in the area to be seined. Upon observation a rubric was utilized to calculate the amount of vegetation present in each seining site.

Once the AVS was completed for each approximate area of 8' X 20', seining was done in the assessed area. To seine an area, the seine net had to be dragged along the floor of the pond and pulled rapidly from the water; the sunfish were collected from the seine net by sifting through the SAV that was captured in the net. Next, the fish were counted and stored in the bucket until the seine net was completely emptied. Then the fish were measured individually, and their size was recorded for future reference. Finally the fish were returned safely back to the water. Once the assessment of the fish was completed, the next seining process would begin where the last process ended immediately following the AVS.

Results

An area of approximately 25785.5 ft.^{2} was covered in Zeke's Pond during a series of thirteen visits resulting in a total of eighty seines. Final fish counts yielded 369 sunfish, sixty-six catfish, and thirteen pumpkinseeds

Fig.4 Peconic River

Introduction

Enneacanthus obesus is a fresh water fish of the order Perciformes and the family Centrarchidae; their common name is banded sunfish. The word Ennea, meaning nine, and canthus, meaning rim of wheel or edge, represents the 9 "bands" circling the little fish's body.

Averaging about 40 to 70mm in length ,other distinguishing characteristics of the banded sunfish include an upturned mouth, rounded pectoral and tail fins, and an olive green body covered with purple, green and gold iridescent specks. *E. obesus* are carnivorous fish; they eat crustaceans, mollusks, other small aquatic or living life forms, and insect larvae.However, the most ecologically significant part of their diet is mosquito larvae, which helps control the mosquito population.The banded sunfish inhabit rivers, lakes, and ponds along the Atlantic Coast; however, *E. obesus* is a threatened species in the state of New York.As a threatened genus, it has become important to reestablish the banded sunfish and prevent them from moving to the endangered species list. Therefore, Brookhaven National Laboratory in conjunction with the Department of Energy launched plans to prevent the sunfish from becoming endangered.The Peconic River, which runs through the grounds of Brookhaven National Laboratory, was home for the sunfish however, in 2004, lab personnel began remediation on the river to remove harmful chemicals that had spilled into the river over the past decades of scientific research and discovery. During the initial steps of the remediation process, scientists and students at the lab began removing banded sunfish and relocating them into other parts of the Peconic River and Zeke's Pond, which is on the eastern most point of the laboratory's grounds.As a result of those initial steps in the remediation process, Zeke's Pond received approximately 200 banded sunfish. In 2005 a drought nearly eliminated the relocated sunfish population.Six sunfish were rescued from the practically waterless pond.Once the pond water had returned to adequate levels, the surviving sunfish were re-released into the pond to flourish .

Discussion/Conclusion

The estimated total population is 4,027, which is 4% of the previous study's count of 95,900. The sizes of the fish also contrasted between initial and final runs. In runs one through sixty-seven the sizes of the fish caught ranged from 17mm to 60mm. However, in runs 68 through 80 the sizes of the fish caught ranged from 12mm to 57mm. Most of the fish caught in the final runs were very small in length in comparison to the fish caught initially as a result of the fish spawning during the week of July 1, 2007. Schools of fry could be observed during that time. Due to this fact, seining had to be suspended for a week to allow the fry to grow. Further studies are necessary to document the life cycle and population trends of the *Enneacanthus obesus*.

Acknowledgements

We would like to thank the Department of Energy, National Science Foundation (NSF), Brookhaven National Laboratory, Science Undergraduate Laboratory Internship (SULI) and the Office of Educational Programs (OEP) for facilitating us with this internship. Our gratitude goes out to Dr. Timothy Green, our team leader, and our professor and mentor Dr. Murty Kambhampati for their guidance. We sincerely appreciate the associates and staff of OEP and the Environmental Services Division of Brookhaven National Laboratory for their support and assistance. Lastly, we would like to thank Southern University at New Orleans for this opportunity.

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The Use Of Mark-Recapture To Estimate Odonate Populations At Vernal Pools At Brookhaven National Laboratory

Dianna Rodriguez

Office of Science, Science Undergraduate Laboratory Internship (SULI)

SUNY Old Westbury

Brookhaven National Laboratory

Brookhaven, New York

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Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Science Undergraduate Laboratory Internship under the direction of Timothy Green in the Environmental Sciences department at Brookhaven National Laboratory.

Participant:_____
Signature

Research Advisor:_____
Signature

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Abstract

The Use Of Mark-Recapture To Estimate Odonate Populations At Vernal Pools At Brookhaven National Laboratory. DIANNA RODRIGUEZ (SUNY Old Westbury, Old Westbury NY), TIMOTHY GREEN (Brookhaven National Laboratory, Upton NY)

Dragonflies are insects of the order Odonata, suborder Anisoptera. In the state of New York there are 60 known, documented species of odonates of the 3000 species known worldwide. Odonates are important because they play a role in maintaining the delicate ecosystems of vernal pools and other bodies of water such as marshes, streams, and wetlands. Monitoring and tracking odonates can present much difficulty due to their numerous population, migration, extraordinary flight speed, and relatively short life span. The use of a tracking system is needed in order to keep accounts of odinate species populations that are being monitored and observed. With the use of a very simple form of the mark-recapture method, odonates are caught using nets and numbers are drawn on their wings then released. The study was conducted for ten weeks; the first three weeks was spent observing all odonate species at four ponds. However, due to a very low population, one of the ponds was eliminated from the study leaving only the remaining three ponds to be studied for the entire ten-week period. As well as employing the mark-recapture method, surveys were conducted at each of the three ponds once a week at the same time each week. These surveys provide an account of all species visibly present and their apparent abundance. From the use of mark-recapture, 525 dragonflies were captured and marked with 18 dragonflies recaptured at least once. The study was actually conducted for eight and a half weeks with the remaining week and a half used to generate the population estimates and attempt to verify their validity. Once all data was collected the program Noremark was used to make population estimates. Two population estimates

were generated for each pond, the program including a variable to account for emigration and immigration. The program estimated about 629dragonflies inhabiting pond 7, 2,700 dragonflies inhabiting 9 O'clock pond, and inconclusive results for Meadow Marsh. The surveys have shown that there are at least ten common species amongst all three ponds, and the most abundant species at the ponds have changed in the past eight and a half weeks. It has also been noted that several species, such as the Widow Skimmer (*Libellula luctuosa*) and the Calico Pennant (*Celithemis elisa*), have a greater presence at the ponds in comparison to previous studies and observations of the ponds. It has also been observed that a species, the Band-Winged Meadowhawk (*Sympetrum semicinctum*), which has not been previously documented as part of Brookhaven National Labs Odonate population, has gained a significant abundance during weeks five through eight. These studies are indicators of pond health and speciation, and have thus far shown that the ponds at Brookhaven Lab are clean and habitable because of the great abundance of these sensitive insects. This research is part of an ongoing project that was started in 2003 to observe the Odonate populations of the Brookhaven National Laboratory campus and will be continued until an accurate account of species is created. Future studies may include the effects of hydroperiod on Odonate populations and abundance.

Introduction

Odonates are predacious flying insects that inhabit bodies of water such as vernal pools, ponds, lakes, and streams. Within the order Odonata there are two sub-orders, Anisoptera (dragonflies), and Zygoptera (Damselflies). Odonates are physically characterized by a head with 2 compound eyes and three small "simple" eyes, a thorax with six bristly legs and two pairs of membranous wings, and a long brightly colored abdomen consisting of 10 segments. Since 2003 research has been conducted at Brookhaven National Laboratory (BNL) to identify the species inhabiting the ponds and Peconic River onsite. Currently there are approximately 36 identified species of dragonflies onsite at BNL. The purpose of this project is to try to identify new species, and attain population estimates of the species inhabiting the ponds at Brookhaven National Labs. Since Odonates play a role in maintaining the delicate ecosystem of vernal pools and other bodies of water such as marshes, streams, and wetlands it is desirable to be able to estimate the health of a population within a given area. Tracking and monitoring Odonates can be extremely difficult due to their relatively short lifespan, numerous populations, and extraordinary flight speed. To observe and monitor Odonates, the use of a tracking system is needed to keep accounts of individual species populations. In 2006, a study was conducted to see if the use of mark-recapture could be successfully employed on dragonflies to attain population estimates. Proven applicable to dragonflies, the marking system was refined and employed once again for this study. This study was conducted to observe all odonate species and their populations at three ponds on the BNL campus.

Methods and Material

A 15-inch diameter net was used to catch the specimens while in flight or perched on vegetation. Waders were worn to wade through ponds and flooded areas around the ponds. A magnetic board was used to hold down individual dragonflies by pinning down their wings with a magnet. Non-water-soluble markers were used to mark the dragonfly's right hind wing with distinguishing marks in a color to denote the pond they were found inhabiting. Once a dragonfly was caught with the net it was removed, by hand, by pinching its wings together. The marks were either drawn on it while in the hand, or while they were held down on the magnetic board. Once marked, the dragonfly was promptly released. Each additional recapture was noted but no additional marks were drawn onto the wings. The marking system employed involves a base color that represents the pond in which the dragonfly was found inhabiting, and numbers were used as the marks. The same numbers are used for different species and both sexes within a species. The numbers distinguish one individual dragonfly of a species from another as well as provide the amount of a species of dragonfly captured. The colors of the marks are pond indicators, so all the odonates of one specific pond have the same color marking. This can be useful in migratory species; we will be able to see if a dragonfly migrates to another pond. Once a dragonfly is caught and marked it is immediately released for future recapture. The color magenta was used solely for pond 7. Blue was used solely for the pond called Meadow Marsh. And green was used for the pond called 9 O'clock pond. Therefore, every dragonfly caught at pond 7 would have a magenta number on their right hindwing, at Meadow Marsh they would have a blue number on their hindwing, and at the 9 O'clock pond they would have a green number on their right

hindwing. The first individual of each sex of all species captured would get a number one on their hind wing. The second would get the number two and so on. Once all the data on marked individuals was recorded, the program called NOREMARK was used to calculate population estimates with variable's for Odonates that were marked and unmarked, and those that immigrate and emigrate.

Results

During the summer of 2007, a total of 3 ponds were visited on site at BNL. The Ponds visited were pond 7, Meadow Marsh and the 9 o'clock pond. Over the four years of odonate research at BNL, 35 species have been found out of 56 recorded in Suffolk County, which includes the Band-winged Meadowhawk (Sympetrum semicinctum) that was found for the first time this year at BNL. Through the use of the program NOREMARK, estimates were generated for the odonate populations of the three ponds Meadow Marsh, 9 O'clock Pond, and Pond 7. For pond 7, the population estimates the program generated were 517 and 732, which average to about 629. For 9 O'clock Pond the estimates the program generated were 3,050 and 2,348 which average to about 2,700 dragonflies inhabiting the area. For Meadow Marsh the results were inconclusive. The program generated averages of 274,102, and 372,067. Both of which were completely implausible and must be revisited. The surveys have shown that there are at least ten common species amongst all three ponds, and the most abundant species at the ponds have changed in the past eight and a half weeks. At two ponds, pond 7 and 9 O'clock pond, the most abundant species was the Common Whitetail (*Libellula lydia*) during the first five weeks. Now, however, at pond 7 there are two more species with nearly the

same abundance as the Common Whitetail, the Black Saddlebag (*Tramea lacerata*) and the Widow Skimmer (*Libellula luctuosa*). At 9 O'clock Pond, the most abundant dragonflies are now the Calico Pennants (*Celithemis elisa*), and the Carolina Saddlebags (*Tramea carolina*); the Common Whitetails no longer have much of a presence at this pond. At the third pond, called Meadow Marsh, the most abundant dragonfly is still the Spangled Skimmer (*Libellula cyanea*). It has also been noted that several species, such as the Widow Skimmer (*Libellula luctuosa*) and the Calico Pennant (*Celithemis elisa*), have a greater presence at the ponds in comparison to previous studies and observations of the ponds. It has also been observed that a species, the Band-Winged Meadowhawk (*Sympetrum semicinctum*), which has not been previously documented as part of Brookhaven National Labs Odonate population, has gained a significant abundance during weeks five through eight.

Discussion

The estimates computed for Pond 7 were 517 and 732, which give an average of 629 dragonflies. The averaged estimate of 629 dragonflies appears to be low. Though there have been no previous studies on total dragonfly a population at pond 7, a study was conducted in 2006 estimating the population of the Cherry-faced Meadowhawks (*Sympetrum internum*). Those estimates yielded populations between 300 and 600. In comparison to the estimates for total dragonfly population, it would support the idea of a larger average population. Such a low estimated average for total dragonflies captured may be due to the low rate of recapture. For the study on the Cherry-faced Meadowhawks, about 150 were captured with 33 recaptured at least once. For the total

population study, 178 were captured with only 14 recaptured. Also, the Cherry-faced Meadowhawk study was conducted over four weeks, compared to total dragonfly population, which was conducted over eight and a half weeks. So the factor of time may also play a role in the estimates because the program, NOREMARK, generates the estimates using the number of pond visits in the equation. It must also be noted that during most visits to Pond 7, at maximum, only about one third of the dragonflies seen were actually capture; on some occasions less were capture, and even less often were more than one third of the dragonflies seen captured. Further studies of the pond would probably yield more accurate results. The estimates computed for 9 O'clock Pond were 3,050 and 2,348 which give an average of 2,700 dragonflies. Unfortunately, this was the first time estimates have been generated for dragonfly population at the 9 O'clock pond so there is no data to directly make comparisons with. However, using the estimates from pond 7 as an indirect comparison, an estimated population average of 2,700 dragonflies appears to be a plausible. Though again, this estimate may also be lower than the actual average. Comparing pond size alone, 9 O'clock pond is at minimum three times the size of pond 7, and has a visually larger population of dragonflies. There were also sixty more dragonflies captured at 9 O'clock pond than at pond 7; 9 O'clock pond also had fewer recaptures, only 4. It must also be noted that during most visits to 9 O'clock pond, at maximum, maybe one fifth of the dragonflies seen were captured due to the largeness of the pond. As for Meadow Marsh, the program NOREMARK generated estimates of 274,102 and 372,067. These estimates are completely implausible and inaccurate. Judging by pond size alone, there should be an estimate similar, if not slightly smaller, than pond 7. Visually, fewer dragonflies were present at Meadow Marsh than at either

pond 7 or 9 O'clock pond. These estimates may have been generated so inaccurately by the program because of fewer visitations to the ponds, fewer dragonflies captured, 111, or because, unlike the other two ponds, there were no recaptures of any of the marked dragonflies. This was due to the lack of accessibility to the ponds edges; most of the ponds perimeter is blocked by the aquatic plant known as Cattail (*Typha latifolia*), which made it difficult to successfully swing a net and capture a dragonfly. There have been no previous studies of dragonfly populations at Meadow Marsh to make any further data comparisons with. Further studies of the pond would probably yield more accurate results.

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

Sarah Miloski, SULI intern, SUNY College at Brockport, Brockport, NY 14420

Timothy Green, PhD, Program Advisor, Department of Environmental Sciences, Brookhaven National Laboratory, Upton, NY 11973

Ariana Breisch, MS, Mentor, Foundation for Ecological Research in the Northeast, Upton, NY 11973

ABSTRACT

While wetlands, among the most productive ecosystems in the world, are often called nurseries of life, little is known about the current health status of Long Island's freshwater wetlands. Such vital systems should be individually monitored over a period of time to determine the overall 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 regarding to optimize the health of the wetlands under their control. 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 adjusted to accommodate the wetlands of Long Island's Central Pine Barrens. 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.

Dr. Timothy Green and Sarah Miloski observing benthic macroinvertebrates.

INTRODUCTION

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.

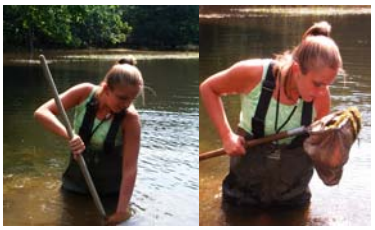
The Foundation for Ecological Research in the Northeast (FERN), a not-for-profit organization, is currently fostering a step by step monitoring protocol specifically designed for the freshwater wetlands of the Central Pine Barrens of Long Island. This project rectifies the lack of baseline data regarding 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].

An essential aspect of wetland systems are benthic macroinvertebrate assemblages. Since many benthic macroinvertebrates have limited migration patterns and a certain level 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. Since benthic macroinvertebrates are to be sampled in addition to the monitoring of other aspects of the wetland, such as water quality and vegetation, field time 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 as a rapid bioassessment, one meter sweeps were taken using a d-frame dip net with US 30mm mesh in various substrates, including open water, vegetation, soft substrate and submerged macrophyte. Sweeps taken in each habitat were counted and recorded on data forms. 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. 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.



Sarah Miloski sweeping macroinvertebrates with a dipnet and then observing the collected content.

Emily Efstration and Sarah Miloski viewing collected organisms in a gridded tray.

RESULTS

Table 1 depicts relevant case studies by state, their sampling window, frequency, and methods. Each wetland monitoring program varied by state depending on needs, location, purposes, 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. As demonstrated below 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.

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

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			

DISCUSSION AND CONCLUSION

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. 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 with out 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 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 similar to build a more comparable collection of data.

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. Therefore, 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 [9].

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 time 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.

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Testing Vegetation Sampling Methods of Central Pine Barren Freshwater Wetlands in Preparation of the Wetland Protocol

Emily B. Efstration, SULI intern, University of Delaware, Newark, DE 19717

Timothy Green, PhD, program mentor, Department of Environmental Sciences, Brookhaven National Laboratory, Upton, NY 11973

Ariana Breisch, MS, Advisor, Foundation for Ecological Research in the Northeast, Upton, NY 11973

ABSTRACT

The current health of the freshwater wetlands of the Long Island Central Pine Barrens is unknown. In order to determine the health of the wetlands, a protocol must be established to determine a baseline. The baseline will then aid in monitoring future wetland conditions. Several bioassemblages of the wetland community will be examined because each element has an affect on the overall health of the wetland. Vegetation is an element that plays a major role in determining the health of the wetlands. It is the primary source of energy flow in the wetland ecosystem and forms the foundation of the wetland food chain. No other life forms are able to exist without the presence of vegetation. Plants, both dead and alive, form a structural habitat for many species to live and thrive in. Not only does vegetation affect taxonomic groups, but it also has a major impact on the wetland's water and soil quality. Therefore, vegetation is very important for the survival of the entire wetland community and must be closely monitored. By reading bioassessment case studies of Florida, Michigan, Minnesota, North Dakota, Oregon, Wisconsin and Maryland, different methods for analyzing wetland vegetation were collected and examined. Information on how to carry out various analytical techniques of vegetation was gathered and organized. The techniques that best suited our purpose, along with the necessary equipment, were taken into the Pine Barren Wetlands to be tested. Many different methods for analyzing the wetland vegetation was carried out in and around the wetland ponds of Long Island. The procedures that were the most practical and informative for the wetlands being assessed were noted. Many methods that were tested did not apply to the Pine Barren wetlands being examined because many of the case studies established permanent plots. Since the wetlands being studied will be on public lands, permanent plots were not a viable option. Upon investigating different methods of vegetative analysis, it was found that the case studies were very helpful, but many of the procedures were altered in order to accommodate the ponds being studied. Further investigation must be conducted in order to determine the precise vegetative methods that will be used to examine plants of the freshwater wetlands in the Long Island Pine Barrens.

INTRODUCTION

The current state of the freshwater wetlands of the Long Island Pine Barrens is unknown because there is not a wetlands protocol established in the state of New York to address this issue. The health of the freshwater wetlands is critical because it has an affect on the health of the aquifer of Long Island. This aquifer provides most of Suffolk County Long Island with drinking water [1]. Therefore, unhealthy freshwater wetlands could possibly have a bad affect on the quality of Long Island's drinking water.

Another factor that must be considered is that fact that Long Island is home to many threatened and endangered species. All animals rely on water as a source of nutrients and some of these endangered animals, such as the Tiger Salamander, live in and near the water. Fish as well as many amphibians and reptiles rely on water to reproduce and raise their offspring. An unhealthy freshwater wetland could not only affect the human population, but it could be devastating to threatened or endangered native species.

An organization that has realized this problem and is putting an effort forth to construct a freshwater wetlands protocol is the Foundation of Ecological Research in the Northeast (FERN). One of the main purposes of FERN is to establish a step-by-step freshwater wetland protocol in order to monitor the health of the wetlands on Long Island [1]. This protocol will be composed of many parts, all of which have an affect on the overall health of the wetland. Some of the wetland assessment will be based strictly on observations, but there will be aspects that yield empirical data. Analyzing water quality, soil quality and macroinvertebrates will yield quantitative data while the vegetation, reptile, amphibian, bird and mammal sections will consist of observations.

There are seven different freshwater wetland community types on Long Island that will be studied. These wetland types are the Coastal Plain Ponds/Pondshores, Coastal Plain Poor Fen, Highbush Blueberry/Bog Thicket, Pine Barrens Shrub Swamp, Coastal Plain Atlantic White Cedar Swamp, Emergent Marsh and Red Maple-Black Gum Swamp.

MATERIALS AND METHODS

The first step was to take photographs of the wetland using a waterproof camera and a compass. Photo points were set up and panoramic shots of the wetland were taken from that location. The points were marked by GPS to make them easy to find again. Panoramic pictures were taken from spots that best represented the overall wetland community. The file number of the photograph was noted for future observation and comparison. The bearing at which the picture was taken was also observed.

The next step was to note emergent vegetation, estimated surface area and the dominant species on a data entry form. Vegetation included both aquatic and terrestrial species.

A thorough sketch of the wetland was then drawn. Distinct features of the wetland and surrounding area, different vegetative communities, photograph points and water/soil sample points were noted on the sketch.

Finally, other information that was gathered when researching vegetation in the area was a comprehensive species list, cover estimate of each species, cover class, relative cover of each plant species, relative density of the species, stems per unit area, basal area, importance values, standing biomass, DBH of living plants, dead plants and shrubs, length and state of downed logs, abundance of a species and dominant species present in the wetland. The DBH of the plants was determined by using DBH measuring tape and the length of the downed logs was measured with a tape measure. Most other information was determined by estimations done by two or more crewmembers. All information collected was noted on data sheets.

Emily Efstration studying a red maple tree

RESULTS [2]

State	Plot Size/ Description	Comprehensive Species List	Cover estimate	Cover Class	Relative Cover	Relative Density	Stems per Unit Area
Ohio	transects	X	X	X	X	X	X
Oregon	quadrats						
Minnesota	releve plots	X		X			
Michigan	2 transects per bird census plot	X					
Maryland	transects	X					
Florida	4 cardinal transects	X					

State	Basal Area	Standing Biomass	DBH	Downed Logs	Abundance	Dominance
Ohio	X	X				
Oregon						X
Minnesota						
Michigan			living, dead, shrub	length, state of decomposition		
Maryland					X	X
Florida					X	X

DISCUSSION AND CONCLUSION

Although many procedures and methods were tested out in the field, it was found that most of the procedures were hard to conduct in all seven Long Island Central Pine Barren freshwater wetland community types. Line transects, quadrats and permanent plots were all tested in the wetlands, but none of the methods worked well in all communities. A major concern was the fact that a large percentage of the freshwater wetlands is made up of ponds, many of which are too big and deep to cross in waders. Since multiple species of vegetation are in and on the water, the vegetation in the water must be analyzed in some manner. If a permanent plot were established on the shores of the wetland community, an overall representation of the wetland would not be met because some species of vegetation could be present outside of the plot [3].

The idea of setting up a series of quadrats was also discussed, but the idea was dismissed because it would not represent the wetland community as a whole.

Certain plant species are hard to identify when they are not flowering while others are quite easy. Since it was decided that an overall observational analysis of the wetland community type was going to be performed, vegetative analysis would be conducted seasonally. An estimated percent cover of each species present in the wetland would be determined by multiple crewmembers.

Another concern was the issue of time. Multiple components of the wetlands are going to be analyzed including the water, soil, macroinvertebrates, amphibians, reptiles, birds, mammals, the area around the wetland and also the vegetation. To analyze all aspects of a wetland will be very time consuming so it was decided that an overall estimation of the vegetation would be ideal. This form of vegetative analysis was not decided before testing out other analytical methods in the field, which were presented in different state case studies.

Panoramic pictures of the wetland location will be taken each time it is visited. The location and bearings of where the pictures were taken will be recorded. This is so that the pictures can be retaken from the same angles and at the same point in the wetland.

The sketch of the wetland is one of the most important factors when visiting the wetland. The sketch will document where roads and paths are, where the photo points and water sample points are located, any defining features of the wetland as well as other characteristics that must be documented.

Emily Efstration and Sarah Miloski identifying vegetation

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SULI

Comparative Ecological Study: Long Island Pine Barren Ponds, NY

LaTonya Stemley, Ha'Wanna St. Cyr, Murty S. Kambhampati, and Timothy Green* Southern University at New Orleans, New Orleans, LA 70126, *Brookhaven National Laboratory, Upton, NY 11973

Abstract

The purpose of this research was to collect scientific ecological data on water and sediments from the Long Island Pine Barren Ponds including the ponds on Brookhaven National Laboratory (BNL) site and to compare results between the on-site (Zone-I) and off-site (Zone-II) ponds. The specific objectives were to: (a) analyze samples for physico-chemical factors; (b) compile and analyze data statistically; and (c) to identify the interrelationships between abiotic factors in ponds of two zones. We have collected 99 surface water and sediment samples (<15cm deep at 50 - 200m intervals, depending on size of each pond) randomly from 5 experimental sites (Groups 1-5). Experimental sites were plotted using eXplorist 200 Global Positioning System (GPS) and ArcInfo Geographic Information Systems (GIS). Field data were obtained on dissolved oxygen (DO), temperature, pH, turbidity, and conductivity using Yellow Spring Instruments, Inc. (YSI) probe. Water samples were analyzed using Hach DR890 colorimeter. Filtered and acidified water samples (pH<2) were used to estimate metal content using Directly Coupled Plasma Spectrometer (DCP). Sediment samples were air dried, sieved, and saved for elemental analysis using DCP. Macro and micronutrients were analyzed using LaMotte Soil Test Kits. Samples were also dried in an oven at 65ºC for 36-48 hr to obtain moisture. Majority of the water and sediments were acidic and nutrient poor. Soil texture is mostly either sand or silt. Moisture content varied between 20.98±10.35 to 50.02±6.13% in NRP and BNL samples, respectively. Sediment ANOVA results indicated positive and negative significances (P<0.05 and P<0.01) between elements, aluminum (Al), iron (Fe), lead (Pb), and chromium (Cr). In conclusion, the Long Island Pine Barren pond water and sediments are acidic and nutrient poor. Sediments have higher concentrations of metals (Al and Fe), in general.

Introduction

High acidity in the Long Island Pine Barren ponds is one of the major concerns to the Natural Resource Management group of Environmental Science at BNL and the Suffolk County Chapter of The Nature Conservancy, for several ecological reasons, such as: quality of water and sediments; role of acid rain and deposition; effect of salting roads during the winter months and its impact on the quality of water and sediments and consequently on biota including some rare and endangered species, to name a few. Water and sediment quality in wetlands of northeastern regions of the USA has been documented [1]. Available literature on ecological studies of the Long Island Pine Barren complex wetlands is very scanty and very minimal [2 & 3]. We have investigated a total of 33 ponds across the Suffolk County and collected data on a wide range of limnological factors, including heavy metals in water and sediments, to compare with previous research findings. Sediment in pond waters is an important ecological factor and plays a critical role on biotic organisms and the water quality. Phosphorus plays a critical role in water quality and plant growth in fresh water bodies [4, 5, 6, & 7].

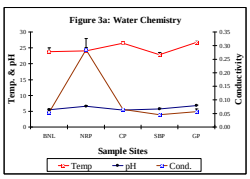
No peer-reviewed literature published in scientific journals is available on the environmental health issues, such as water and sediment chemistry and its impact on biota, of the Long Island Pine Barren Ponds. Hence, the purpose of this research was to collect scientific ecological assessment data on water and sediments from the on- and off-sites of BNL and to establish a database for future studies, and management and conservation of natural resources on Long Island. The specific objectives were to: (a) analyze samples for physico-chemical factors; (b) compile and analyze data statistically; and (c) identify the interrelationships between abiotic factors. Our hypothesis is that the Long Island Pine Barren pond water and sediments would be acidic, nutrient poor, and free of contaminants. There would be no significant difference in mean values (<0.05 and 0.01) of physico-chemical factors between and within the groups. We have investigated a total of thirty three ponds in two major sections of the Long Island Pine Barrens (LIPB): on-site zone (BNL: P1-10 & P21-24) and off-site zone (Near Road Ponds-NRP: P11-13; Calverton Ponds-CP: P14-16 & P25-28; Sears Bellow Ponds-SBP: P17-20; and Greenbelt Ponds-GP: P29-33) as shown in Figure 1. The experimental sites are located between 18.679729-18.727803 E and 45.27536-45.40748 N.

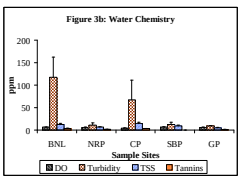
Materials and Methods

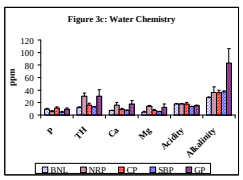
Three surface water and sediment samples (not more than 15 cm deep) at 50-200 m intervals (based on pond area and accessibility) were collected from each pond, over a period of 10 weeks. Sediment samples from each pond were thoroughly homogenized and saved a representative sample in 250 mL Nalgene bottles for physical and chemical analyses. The sampling sites were plotted, as shown in Figure 1, using eXplorist 200 Global Positioning System (GPS) and ArcInfo Geographic Information Systems (GIS). Water samples were kept on ice for chemical analysis (within 24 hrs.). Field data on DO, temperature, conductivity, pH and turbidity in water were obtained using the YSI (Yellow Spring Instrument Inc.) probe. HACH-DR 890 (the colorimeter) was used to test total tannin, phosphorus, and suspended solids. The Digital Titration-16900 was used for testing total hardness and alkalinity. Approximately 2-5 mL 1:1 nitric acid:DI water was added to each sample (100 mL filtered water using Whatman 40 filter paper), and preserved the samples for trace metal analysis. Sediment samples were air-dried and sieved through 2mm sieve to remove organic matter such as roots. Air dried samples were used to measure macro and micro nutrients using LaMotte Soil Test Kits (pH, K, P). Percentage of moisture was obtained by drying samples in an oven at 65º C for 36 to 48 h. Air-dried sediment samples were used to determine soil texture using Fisher Scientific Standard Testing Sieves (45 - 2000µm). Soil samples (5g each) were digested using 100mL Kjeldahl flasks, following EPA 3050B method. Samples were digested with concentrated 10mL nitric acid (HNO_3) and 10mL hydrochloric acid (HCl) and were allowed to soak overnight. Samples were then digested on hot plates (not more than 95ºC) for 3-4 h and let the samples to cool overnight and filtered using Whatman 541 filter paper. Digestion extracts were diluted with deionized distilled water and made the final volume to 100 mL using volumetric flasks, labeled, and saved in 125 mL Nalgene bottles for Direct Coupled Plasma (DCP) spectrometer analysis to estimate Al, Pb, Mo, Cr, Mg, K, Fe, Ca, and Mn.

Results

Water chemistry: Water was acidic (5.31±0.35 to 6.84±0.13 at CP and GP, respectively) and low in DO as shown in Figure 3b (4.12±0.89 to 6.92±0.55 ppm at CP and SBP, respectively). Alkalinity ranged from 27.71±1.60 ppm in BNL ponds to 82.93±23.50 ppm in GP (Figure 3c). Maximum conductivity (0.29±0.04µs/cm³) was recorded in NRP samples. Metal content in water samples is summarized in Table 2. One-way ANOVA results indicated mean differences between groups (df = 4) and within groups (df = 28) and two-tailed Pearson correlations indicated significant relationships between various physico-chemical factors at P<0.05 and P<0.01, as shown in Tables 4a & 4b.







Sediment Chemistry

The sediments were acidic (6.16±0.17 to 7.00±0.35 in NRP and GP samples, respectively) and nutrient poor. Moisture content varied from 20.98±10.35 to 50.02±6.13% in NRP and BNL samples, respectively. One-way ANOVA results confirmed positive and negative significant (P<0.05 and P<0.01) relationships between elements, aluminum (Al), iron (Fe), lead (Pb), and chromium (Cr). Two-tailed Pearson correlation data are summarized in Tables 5a & 5b. Among all the variables studied in sediments, Mg and Fe had highest positive significant relationship (0.922**; P<0.001). Most of the sediments have higher concentrations of Al and Fe. Results are summarized in Figure 4b. Trace metal concentrations are very low in all sites we studied.

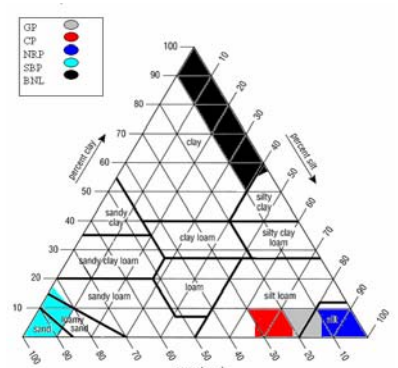
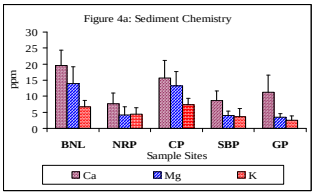


Figure 2: Long Island Pine Barren Ponds: Soil Texture



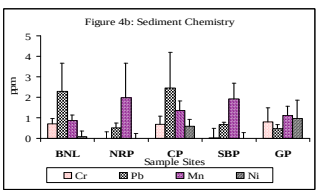


Figure 1. Sample Sites

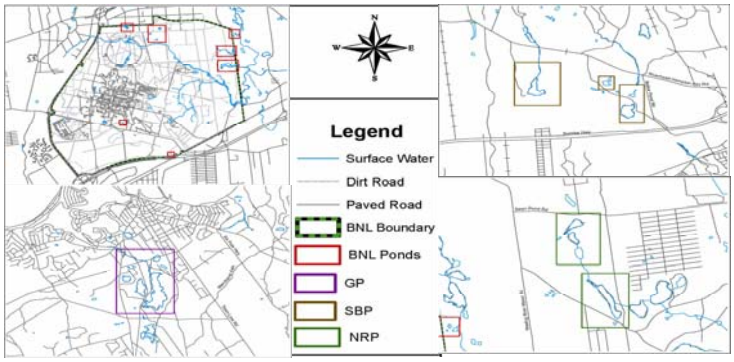


Table 2: DCP Data on Water Samples

Table 1. DCP-DL	
Elements	DL
Ca	0.044
Mg	0.002
Fe	0.028
Mn	0.014
Ni	0.036
Al	0.05
K	0.24
Cr	0.022
Pb	0.097

Site		Ca	Mg	Fe	Mn	Ni	Al	K	Cr	Pb
BNL	Mean	1.78	0.88	0.52	0.05	0.01	0.10	0.93	0.01	0.07
n = 14	SE	0.44	0.19	0.12	0.01	0.01	0.01	0.21	0.01	0.02
NPR	Mean	5.55	2.53	1.30	0.15	-0.01	0.05	2.03	0.00	0.17
n = 3	SE	1.64	0.42	0.54	0.05	0.00	0.01	0.47	0.01	0.16
CP	Mean	2.05	1.04	1.10	0.04	0.00	0.08	1.09	0.01	0.10
n = 7	SE	0.50	0.18	0.26	0.01	0.00	0.03	0.41	0.01	0.06
SBP	Mean	1.17	0.85	1.98	0.05	0.02	0.09	0.57	0.03	0.07
n = 4	SE	0.27	0.11	0.96	0.01	0.01	0.03	0.19	0.01	0.06
GP	Mean	5.46	2.65	0.37	0.04	0.08	0.08	1.63	0.07	0.26
n = 5	SE	2.08	0.90	0.12	0.01	0.07	0.06	0.69	0.06	0.22

Statistical Analysis

Mean, variance, standard deviation, standard error, Pearson two-tailed correlations, and one-way ANOVA were applied to measure significance levels between and within groups of sample sites (Zones 1 & 2) using SPSS 10.0 version.

Table 4a. Pearson Correlations Water Data(*P<0.05; n=33)

Temp	Tan	-0.425
Temp	Mn	-0.346
pH	Cond	0.356
pH	Turb.	-0.388
Cond.	Alka.	0.433
Turb.	TSS	0.383
Turb.	Mg	0.375
Acid.	Al	-0.37
Alka	K	0.436
Ca	Al	-0.394
Ca	K	0.435

Table 4b. Pearson Correlations Water Data(*P<0.01; n=33)

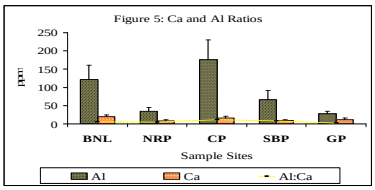
DO	P	-0.463
Temp.	TSS	-0.5
pH	Tan.	-0.442
pH	Acid.	0.617
pH	Alka	0.522
pH	Ca	0.642
pH	Fe	0.45
Cond.	Acid.	0.487
Cond.	Ca	0.494
Cond.	Mn	0.643
Turb.	Tan.	0.451
TSS	Tan.	0.78
TSS	Mg	0.545
TSS	Al	0.486
Tan.	Mg	0.679
Tan.	Al	0.456
Acid.	Ca	0.951
Acid.	Fe	0.824
Acid.	K	0.454
Alka.	Ca	0.813
Alka.	Fe	0.827
Ca	Fe	0.739
Mg	Al	0.484
Fe	K	0.451
Ni	Al	0.537
Ni	Cr	0.985
Al	Cr	0.483

Table 5b. Pearson Correlations Sediment Data(*P<0.01; n=33)

Ca	Mg	0.602
Ca	K	0.469
Ca	Cr	0.568
Ca	Fe	0.598
Ca	Ni	0.483
Mg	K	0.828
Mg	Cr	0.543
Mg	Fe	0.922
Mg	Al	0.892
K	Cr	0.498
K	Fe	0.843
K	Mn	0.48
K	Al	0.876
Cr	Fe	0.516
Cr	Mn	0.483
Cr	Ni	0.781
Cr	Al	0.463
Fe	Mn	0.55
Fe	Al	0.82

Table 5a. Pearson Correlations Sediment Data(*P<0.05; n=33)

Ca	Pb	0.415
Ca	Mn	0.431
Ca	Al	0.42
Mg	Mn	0.405
Mn	Ni	0.369
Mn	Al	0.393



Discussion and Conclusion

Historically a majority of the Long Island Pine Barren Ponds are shallow with acidic waters and sediments with low concentrations of essential nutrients such as nitrogen and phosphorus. Research results have indicated high acidic sediments along with excess amounts of Al and acidic waters in the Long Island Pine Barren Ponds. Borg (1987) made similar observations that surface water in North America has become acidic due to acid compounds and metals [7]. Ramachandran *et al* (1997) reported that carbon dioxide concentrations are higher in the summer, which can lead to the cause of the water being very acidic. They have also observed that the suspended solid concentrations were higher in the summer when compared to autumn [8]. Experimental results indicated that all our study sites have low DO, and excessive amounts of tannins and suspended solids in acidic waters and sediments. Studies have shown, however, when the pH is higher the nitrogen levels decline or were at a minimal [9]. Singer and Stumm (1970) suggested that there are multiple factors, including biological and chemical factors, affecting the pH of water [10]. Kemp (1970) reported that waters of low pH have higher alkalinity and total hardness and water of high permanent hardness should be corrosive [11]. Salzman et al. (2001) opined that the chemical behavior of water can be affected by the oxygen's direct influence by plants [12]. Our results indicated NRP samples have high conductivity which could be attributed to salting during winter seasons and their consequent runoff into road side ponds. We found that higher concentrations of Al in acidic soils. This particular feature has influence on Ca concentrations as reflected in Figure 5 and Tables 4a and 5a.

In conclusion, experimental results were in partial agreement with our hypothesis (nutrient poor, low DO, and high turbidity). However, we reject null hypothesis, since our hypothesis was proven wrong regarding contaminants and mean differences among the groups of data sets. We have also observed that water and sediments of ponds in different locations of the LI Pine Barrens have higher concentrations of metals (Al and Fe).

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Long Island Pine Barren Ponds: Water Quality

Shakera Pinder (Tallahassee Community College, Tallahassee, FL 32304), Murty S. Kambhampati (Southern University at New Orleans, New Orleans, LA 70126), Timothy Green (Brookhaven National Laboratory, Upton, NY 11973)

Abstract

Ponds in the Pine Barren complex at Brookhaven National Laboratory (BNL), Near Road Ponds (NRP), Calverton Ponds (CP), Sears Bellows County Park Ponds (SBP), and ponds of the Long Pond Greenbelt (GP) of Suffolk County, NY were studied. A Magellan eXplorist 200 Global Positioning System (GPS) was used to mark each pond. A YSI 650 MDS Probe was used to measure the real-time data on temperature, pH, dissolved oxygen (DO), conductivity, and turbidity of the water. This study is specifically focused on the alkalinity, the acidity, and the buffering capabilities of ponds within the Pine Barrens. We have collected three random surface water samples directly into 500 mL Nolegn bottles and one random water sample at two feet deep, from each pond of a total of 33 ponds located on and around BNL's site and of Suffolk County, NY, using a Plano Horizontal Polycarbonate Water Sampler. Water samples were analyzed using Hach Digital Titrator and TitraVer Solutions and were tested for acidity, alkalinity, calcium (Ca) and magnesium (Mg) hardness, and total hardness. The water temperature of each pond was greatly affected because some ponds were shaded by surrounding forest trees, while other ponds were directly exposed to the Sun. One of the goals of this project was to obtain the results of the physico-chemical analyses of water samples and focus on the most pressing water quality of pond problems in Long Island, NY. This study also gives a look at the variations of the pH levels of each pond and how they are affected by the atmospheric acid deposition. The results of this research show that there is no correlation between near-road ponds and off-road ponds. Results of this study will assist ecologists on how to manage the habitats of wildlife in the Long Island Pine Barren ponds.

Introduction

Due to native acid conditions in Pine Barren ecosystems, ecologists are concerned about increased acid deposition from atmospheric sources, impacts on the local ponds, and effects on wildlife and local habitat. Acidity of water will have a greater impact on water quality and sediments. Eventually acidity due to natural and anthropogenic causes will have drastic effects on biota including some rare and endangered species of these pristine natural habitats, long Island Pine Barren aquatic ecosystems. Water and sediment quality in wetlands of northeastern regions of the USA has been documented [1]. Several investigative reports were published on impact of acid rain and other man-made causes on loss of equilibrium in buffering mechanisms in wetlands of North America and Canada [2, 3, 4]. We have investigated a total of 33 ponds across the Suffolk County and collected data on limnological factors such as pH, acidity, alkalinity, calcium and magnesium hardness, total hardness and aluminum in water samples. Historical data of DOE at Brookhaven National Laboratory (BNL) indicated the presence of organic and approximately 14 inorganic contaminants (methyl mercury, copper-Cu, mercury-Hg, lead-Pb, silver-Ag, and iron-Fe) in the sediments of the PR complex, due to the laboratory practices during the 1940's through the 1980's [5, 6].

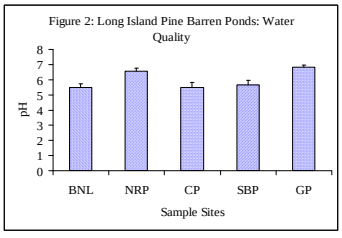
No peer-reviewed literature in the recent past, published in scientific journals, is available on limnological issues on the Long Island Pine Barren Ponds. Hence, the purpose of this research was to collect scientific ecological data on water from both on- and off-site experimental areas of BNL and to establish a database for future studies and management of natural resources on Long Island. The specific objectives were to: (a) analyze samples for physico-chemical factors; (b) compile and analyze data statistically; and (c) identify the interrelationships between abiotic factors such as pH and Ca, Mg, and Al. Our hypothesis is that the Long Island Pine Barren pond waters would be acidic, nutrient poor, and free of contaminants. There would be no significant difference in means (<0.05 and 0.01) of of physico-chemical factors between and within the groups. We have investigated a total of 33 ponds in two major sections of the Long Island Pine Barrens (LIPB): on-site zone (BNL: P1-10&P21-24) and off-site zone (Near Road Ponds-NRP: P11-13; Calverton Ponds-CP: P14-16&P25-28; Sears Bellow Ponds-SBP: P17-20; and Greenbelt Ponds-GP: P29-33) as shown in Figure 1. The experimental sites are located between 18.679729-18.727803 E and 45.27536-45.40748 N.

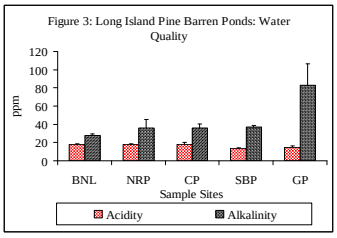
Materials and Methods

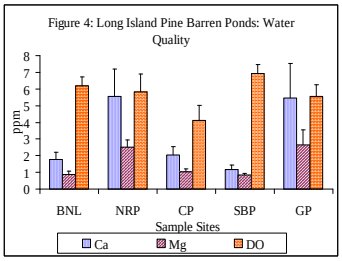
Three random surface water samples and one random water sample at 2' deep, using a Plano Horizontal Polycarbonate Water Sampler were collected and directly placed into 500 mL Nolgen polyethylene bottles. Acidity, alkalinity, calcium hardness, magnesium hardness, and total hardness in water were determined using Hach's Digital Titrator and TitraVer Solutions. Real-time field data on temperature, pH, dissolved oxygen (DO), conductivity, and turbidity were collected using a portable YSI 650 MDS Probe. Samples were stored on ice and analyzed immediately (<24 hrs.) for Ca and Mg hardness and total hardness, acidity, and alkalinity. Samples were acidified to pH <2 with 1:1 HNO_3 and preserved in 125 mL Nolgen polyethylene bottles for further analysis of aluminum concentrations using Direct Coupled Plasma (DCP) spectrometer following EPA 3050B method.

Results

Results on water quality are summarized in Figures 2 to 4. Among all groups studied, GP study sites have higher pH values, close to neutral (6.8±0.13) and the minimum mean pH was recorded at BNL sites (5.5±0.25). Alkalinity readings varied between 27.9±1.69 to 82.9±23.5 ppm at BNL and GP, respectively. Calverton Pond sites have minimum amount of DO compared to Sears Bellow Pond site samples (4.1±0.89 vs. 6.9±0.55 ppm). NRP site samples have maximum concentrations of Ca (5.55±1.64 ppm) and the lowest mean acidity was recorded at SBP sites (13.7±0.69 ppm). Among various physico-chemical factors analyzed using one-way ANOVA, mean differences between groups (df = 4) for DO, acidity, and Al were significant (P<0.05). Two-tailed Pearson correlations indicated significant relationships between various physico-chemical factors at P<0.05, as shown in Table 2. There is no significant difference in data between surface water samples and 2' deep water samples.







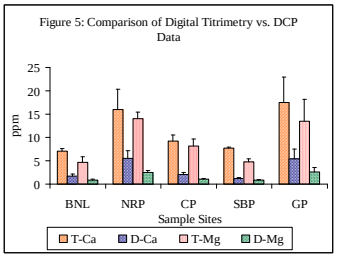
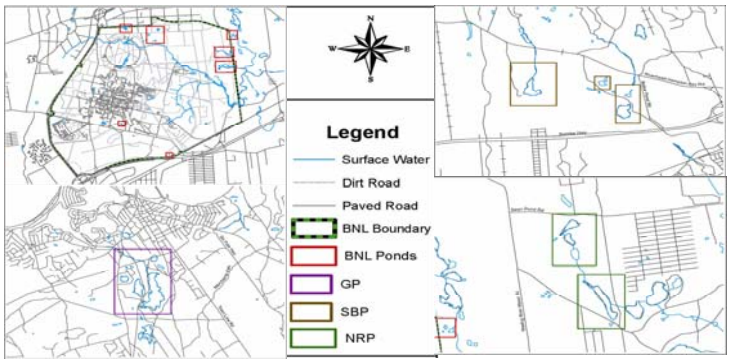


Figure 1. Experimental Sites



Statistical Analysis

Mean, variance, standard deviation, standard error, Pearson two-tailed correlations, and one-way ANOVA were applied to measure difference in means and their significance levels between groups (BNL-n = 14; NRP-n= 3; CP-n =7; SBP-n = 4; and GP-n = 5) using SPSS 10.0 version. Outputs of statistical analyses are summarized in Tables 1 & 2.

Table 1. One-way ANOVA (p <0.05*) (*Equal mean variances Assumed)

		SS	F	Sign.
Ca	BG	86	4.058	0.01
	WG	149		
Mg	BG	17	4.764	0.005
	WG	25		
Alkalinity	BG	11475	6.241	0.001
	WG	12871		
Acidity	BG	95	2.028*	0.118
	WG	328		
Al	BG	0	0.354*	0.839
	WG	0		
pH	BG	9	3.621	0.017
	WG	18		
DO	BG	27	1.861*	0.145
	WG	101		

Table 2. Pearson Correlations (*P<0.05; **P<0.01) Sediments

Ca	Mg	0.960**
Ca	Alkalinity	0.799**
Ca	Al	-0.367
Ca	pH	0.669**
Mg	Alkalinity	0.812**
Mg	Al	-0.438
Mg	pH	0.680**
Alkalinity	pH	0.481**
Acidity	Al	0.474**
pH	Al	-0.35

Discussion and Conclusion

BNL has a long history on inorganic and organic contaminants in sediments (1940s-1980s) and is listed as one of the US Environmental Protection Agency's (EPA) National Priorities List. It is necessary to quantify the extent of risks of these contaminants to BNL's aquatic sites and to its biota (plants, animals, microbes). In the current research project, we attempted to investigate several ponds (on-site and off-site) to identify the interrelationships between pH, Al, Ca and Mg hardness to understand the quality of water in test sites. Our results indicated that Ca, Borg (1987) made similar observations that surface water in North America has become acidic due to acid compounds and metals [7]. Stow (2001) reported that symptoms of excessive eutrophication are algal blooms, low dissolved oxygen, fish kills and outbreaks of toxic microorganisms in the Neuse River, North Carolina [8]. Ramachandran *et al* (1997) reported that carbon dioxide concentrations are higher in the summer, which can lead to the cause of the water being very acidic. They have also observed that the suspended solid concentrations were higher in the summer when compared to autumn [9]. Experimental results indicated that all our study sites have low DO without any visible fish, with a few encounters of frogs, and excessive amounts of tannins and suspended solids in acidic waters. Low pH can have a negative impact on metabolic processes of biota, biodiversity, excess amount of toxic metal accumulation in sediments, which in turn have bioaccumulation of metals in biotic tissues. Kessel-Taylor (1985) has proposed wetland-sensitivity rating to classify wetlands [10]. Based on his classification, most of the ponds that we studied currently fall under category # 4 in which we found low pH, low alkalinity, nutrient poor waters ultimately have an impact on buffering capacities. High acidic soils facilitate the mobility of elements such as Al, Ca, Mg and may enhance leaching activities. This may eventually cause ground water and surface water pollution during rains and snowmelt [4]. Kulp (2007) reported that a pH of 5 occurs approximately when the acid-neutralizing capacity of the lake equals zero and hence the lake is considered "acid"[11]. Most of the current study sites fall under this category. We observed an inverse interrelationship between DO (4.12±0.89 ppm) and total suspended solids (14.67±2.49 ppm) in sample sites of CP. This finding is in direct agreement with report published by Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers, 1979 [12].

In conclusion, experimental results were in partial agreement with our hypothesis (nutrient poor, low DO and pH). However, we reject null hypothesis, since our hypothesis was proven wrong regarding contaminants (such as Al) and mean differences among the groups of data sets.

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Non-Invasive Indexing of Red and Gray Fox Populations at Brookhaven National Laboratory

Patrick Mallin[1] and Jennifer Higbie[2]

[1]College of William and Mary, Williamsburg, VA 23186

[2]Brookhaven National Laboratory, Upton, NY 11973

Gray fox – field camera – Brookhaven National Laboratory

Red fox (photo – Jennifer Higbie) – Brookhaven National Laboratory

ABSTRACT

The red fox (*Vulpes vulpes*) and the gray fox (*Urocyon cinereoargenteus*) have sympatrically inhabited the greater Long Island area over the last several hundred years. In recent years, speculation has grown regarding the population size of each species. While the red fox has historically been known to adapt well to ecological disturbances, including those of an anthropogenic nature, and is largely considered to have a thriving population in the Long Island area, recent studies of the last thirty years suggest the gray fox populations have struggled with such anthropogenic disturbances of the last century. A previous Brookhaven National Laboratory (BNL) study in 2006 confirmed the presence of gray fox on BNL property using non-invasive fecal DNA analysis via mitochondria DNA markers and automated camera documentation. This project further studied the extent of the gray fox presence at BNL for the 2007 season by using the non-invasive techniques of fecal DNA extraction and automated field cameras. Gray fox presence was confirmed through both methods over the course of the study. While apparently much less common than the red fox, the gray fox species appears to be present and established at BNL and, presumably, in similar habitats throughout the Long Island area.

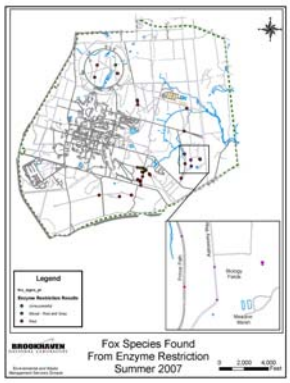

Figure 1: Scat samples with successful enzyme restriction from summer 2007 collection – labeled by species (refer to legend)

INTRODUCTION

Throughout many parts of North America, the gray fox (*Urocyon cinereoargenteus*) and the red fox (*Vulpes vulpes*) coexist sympatrically. While in many parts of the country, gray fox populations have grown in the past 100 years due to abandonment of farmland and subsequent woodlands growth [1], it is likely that gray fox populations of the Long Island area have followed the opposite trend [2]. Data on fox populations in the area is scarce, and most of the available information is outdated and may not account for recent changes in habitat due to anthropogenic disturbances [2].

Gray foxes typically prefer a habitat of mixed hardwood/pine with fairly dense undergrowth [3]. While the diet of the red fox is comprised of mostly small mammals and insects with a mix of some plant material, the gray fox is considered more omnivorous, with over half of its diet (with some seasonal variation) coming from plant materials such as berries [4]. With a balanced omnivorous diet, it seems to follow that gray fox species would tend to reside in dense forest habitats with high availability of both small game and vegetation. Anthropogenic disturbances affecting these preferred habitats of gray foxes would create a somewhat transient lifestyle in which the species would likely have a much lower survival rate [5]. In disturbed habitats, red fox tend to have a much higher survival rate than gray and have been found increasingly more often in suburban and urban settings over the last century [6].

A study at BNL in the summer of 2006 examined the presence of the gray fox species on laboratory property [7] by using techniques and strategies largely based on a 1997 Smithsonian Institution zoological study [8]. Based on these and other studies, this project relied on several key points: a) DNA can be successfully extracted from fecal samples due to the shedding of epithelial cells from the digestive tract b) these fecal samples can be effectively preserved to undergo DNA extraction c) samples can easily be obtained by walking forest paths and roads, as canids tend to follow these established routes [9].

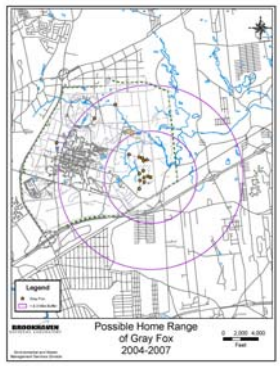

Figure 2: Buffer around known activity of gray foxes gathered from 2004-2007 -- smaller circle represents 1 mile radius range, larger circle represents 2 mile radius range. Foxes typically maintain a home range with a radius of about 2 miles.

MATERIALS AND METHODS

The Laboratory was first divided into walking transects from a vegetation map to be walked regularly. The GPS location was recorded for each sample prior to collection using a Thales handheld GPS/GIS device. The scat samples were collected in resealable bags, preserved with silica gel in the ratio of 4g of silica gel to 1g of sample and then stored in a freezer in preparation for DNA extraction.

For the mitochondrial DNA (mtDNA) extraction, the protocol from the Qiagen QIAamp DNA Stool Mini Kit was followed. After DNA extraction, a portion of the DNA was then run through a Polymerase Chain Reaction (PCR) following standard protocols of a Taq PCR kit. A portion of each PCR product was run on a 0.8% agarose gel to test the success of the PCR. Successful PCR products then underwent enzyme restriction using AluI and HinfI enzymes following standard protocol. Enzyme restriction products were then run on a 2% agarose gel to determine species of sample.

In addition to scat samples, a digital field camera was used to supplement the results of the scat species identification. Camera sites were chosen based on a variety of factors, including: area of scat collection success, reported sighting locations, and likely habitats.

RESULTS

Overall, 51 scat samples were collected from the field. All of these samples plus one additional sample from 2006 underwent DNA extraction and PCR. Of the 52 samples, 40 had a successful PCR (77% success rate). Of the 40 successful PCR products run through enzyme restriction, 28 returned a positive red fox result, 9 samples returned positive bands for both red and gray fox, and 3 samples returned no result. In the case of the double species positive results, the result could not be classified as exclusively gray fox or red fox. A result with two positive readings was therefore classified as a "mixed" positive sample for this project. In these "mixed" results, intensities of the bands varied, but distinct bands were observed for each species. These 9 mixed samples underwent an additional PCR and enzyme restriction and were run through an acrylamide gel to confirm the initial results. From the reading of the enzyme restriction products on the acrylamide gel, 8 samples came back exclusively gray positive, with one sample yielding no result.

In approximately four weeks of use, the Reconyx automated field camera also returned positive results for the gray fox. In one camera location, the camera captured the gray fox on film on at least five distinct occasions. Additionally, in one set of pictures during a brief time period, two gray foxes appeared together in one picture. Furthermore, in the same camera location, a gray fox and a red fox appeared within seconds of one another.

Two gray foxes (at night) – field camera– Brookhaven National Laboratory

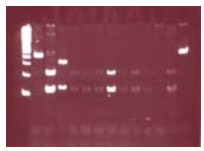

Figure 3: 7.5 % acrylamide gel – enzyme restriction products -- Lane 1: ladder – Lane 2: red fox positive control – Lane 3: gray fox positive control – Lane 4: domestic dog positive control – Lanes 5-13: scat samples – Lane 14: control (no enzyme)

DISCUSSION AND CONCLUSION

While foxes are known to be transient in their juvenile life stages as they establish their territory and disperse from their natal dens, it appears that the gray foxes identified through this study are permanent residents of the study site. Since two adult gray foxes were captured on camera in the same photo, it is very unlikely that both foxes were transient individuals just passing through the area; instead, it is more likely that these individuals have an established home range that encompasses a part or parts of BNL.

Additionally, it appears that red and gray fox populations have direct habitat overlap with one another. With appearances by both species with such a small temporal gap on the camera, it is clear that the species have some level of habitat overlap, albeit to an unknown degree. Moreover, the scat samples that returned mixed species positive results were obtained in an isolated geographic area of approximately 0.25 square miles. In the first round of laboratory testing, the only positive gray fox results were coupled with a red fox positive result was well. This mixed positive result was possibly the result of territorial marking by one or both of the species upon the other's feces. It would therefore be unclear which species produced the actual fecal sample, but the mixed positive result would confirm the species presence of the gray fox nonetheless. It is also possible that some degree of contamination between samples occurred. To test the validity of the first set of results for these mixed samples, a second round of tests was run. The second run returned results of exclusively gray for 8 of 9 of these previously "mixed" samples (with the other sample returning no result). Although the second round of testing showed the exclusive gray fox species identification result, it is unclear whether the first round of testing actually showed trace red fox DNA within the sample or if it was simply a result of contamination. To make this matter more clear, further tests on the sample would have to be run to retest for this trace red positive result.

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Office of Science U.S. Department of Energy

SULI

BROOKHAVEN NATIONAL LABORATORY

Spatial distribution of Iridovirus in the Eastern box turtle population at Brookhaven National Laboratory: Implications for transmittance based on home range size

SARAH SNYDER[1] and VALORIE TITUS [2,3]

[1] Unity College, Unity, ME 04988
[2] Brookhaven National Lab, Upton, NY 11973
[3] Binghamton University, Binghamton NY, 13903

There are currently four recognized genera of the icosohedrally symmetric iridoviruses that infect both invertebrates (*Iridovirus* and *Chlorirdovirus*) and poikilothermic vertebrates (*Lymphocystivirus* and *Ranavirus*). Ranaviruses have only been documented in a relatively few number of reptiles when compared to the number of viruses that have been documented in amphibians and fish. Relecent detection of ranaviruses in five species of chelonians, including a virus outbreak in a population of Eastern box turtles (*Terrapene carolina carolina*) at Brookhaven National Laboratory, is especially alarming. This discovery poses a threat to box turtles in surrounding areas since the species is listed as special concern in the state of New York. This is a continuing study to ascertain the current distribution of infected turtles at Brookhaven National Laboratory. Turtles were sampled during 2006 and 2007 using systematic transect searching. Cloacal and oral samples were collected from each turtle encountered and DNA was isolated from swabs using DNeasy kit protocols. PCR was used to amplify virus DNA and products were subsequently run on 0.8% agarose gels to determine the presence or absence of Ranavirus. Ranavirus was detected in a liver tissue sample and oral swab obtained from one turtle collected during the summer of 2006 which exhibited advanced symptoms of viral infection including an aural abscess which later died. These results preliminarily suggest that swab sampling and PCR testing may not be adequate methods for detecting ranavirus in pre-symptomatic turtles, yielding falsely negative results from turtles sampled during the early stages of infection. To further explore the potential transmission of the Ranavirus within the box turtle population, determining individual home range size specific to turtles at the study site was necessary. Radiotransmitters were attached to 5 box turtles inhabiting the area of Ranavirus discovery and their daily movements were recorded for two summers. Geographic Information Systems was used to digitally map turtle movements and estimate home range size by creating minimum convex polygons. Home ranges of individual turtles are not significantly different from one another, varying between 1.8 ha and 8.2 ha, which is comparable to home range sizes found in other studies. Home ranges also grossly overlap which suggests favorable conditions for virus spread, depending on encounter rates and mode of transmission.

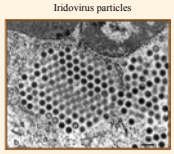
Introduction

Iridovirus

Characteristics of Family Iridoviridae

- Icosahedral symmetry
- Large, diameters 125 to 300 nm
- Linear double-stranded DNA 140 to 303 kilobase pairs

Iridovirus particles



Box turtle found with aural abscess

http://www.cdc.gov/ncidod/EID/vol5no6/daszakG4.htm

Four Genera

- *Iridovirus* and *Chlorirdovirus*- invertebrates
- *Lymphocystivirus*- freshwater and marine fishes
- *Ranavirus*- fish, reptiles, and amphibians
- Majority of reptile ranaviruses observed in chelonians

Iridovirus in box turtles

- Multiple observations of iridovirus infections in Eastern box turtles (*Terrepene carolina carolina*) [1,2]
- Two found at Brookhaven National Laboratory on 2 August 2005 with ocular discharge and swelling, aural abscesses, and yellow caseous plaques
- Histopathology, PCR, and virus isolation confirmed a ranavirus infection [1]
- Species is listed as Special Concern in New York

Box turtle home range

- Habitat quality, structure, diversity, and individual preference all account for variation in size and spatial distribution of home ranges [3]
- Home range estimations vary from 1 to 9.77 ha [3,4]
- GIS can be an effective tool in investigating disease spread within populations through digitally mapping the non-infected and infected turtle distribution, home range area, and home range overlap [5]
- Radiotelemetry useful to assess habitat use and movement patterns over a long time span

Materials and Methods

- Cloacal and oral samples were collected from turtles encountered on the Laboratory property from chance encounter and through systematic transect searching at site where *Ranavirus* was discovered.

- DNA was extracted from swabs using the Buccal Swab Spin Protocol for the DNeasy kit (Quiagen, Valencia, CA, USA). The *Ranavirus* major capsid protein was amplified using the sense primer (5'-GACTTGGCCACTTATCAC -3') and anti-sense primer (5'-GTCTCTGGAGAAGAAGAA-3') as previously described [1].
- Using a Taq PCR Kit (New England Biolabs), mixtures containing the extracted DNA, primers, distilled water, 10x buffer, dNTP, Mg, and Taq were amplified in a thermal cycler. PCR products were resolved in 0.8% agarose gels and bands were examined.

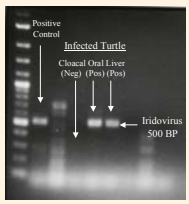
- Radiotransmitters were attached to 5 box turtles inhabiting the area of *Ranavirus* discovery.

- Turtles were tracked daily and their location was recorded using a GPS. Veg. data was collected.

- GIS was used to map GPS turtle location points and calculate home range area using minimum convex polygons (Hawths Analysis Tools).

Results- Iridovirus Testing

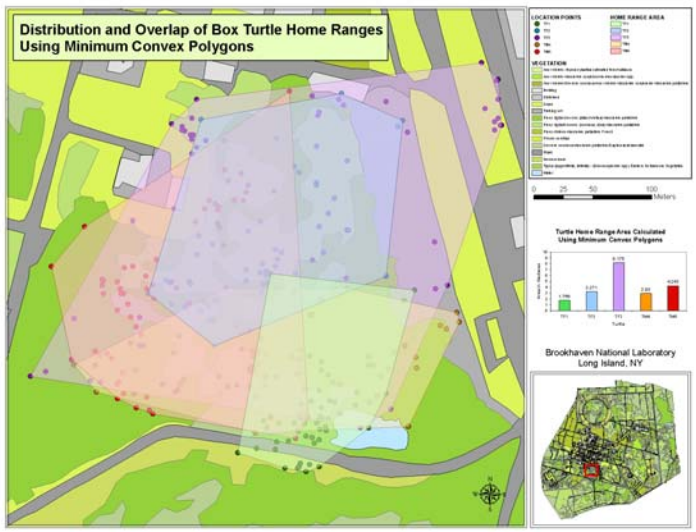
PCR Products Resolved on Agarose Gel



Infected Turtle

Results- Home Range Area

- Min. home range area= 1.756 ha (TF1)
- Max. home range area= 8.175 ha (TF3)
- Mean home range area= 4.08 ha
- No sig. diff. between home range area of individual turtles:
  - Chi^2 Value (5.147)< Crit. Value (9.488), df= 4, α= 0.05
- No sig. diff. between home range area of males and females:
  - T Stat (0.062)< Crit. Value (3.182), df=3, α= 0.05



Discussion and Conclusion

- Ranavirus is still present in the box turtle population at Brookhaven National Laboratory but was only detected in one turtle sampled during the summer of 2006 which exhibited advanced symptoms of viral infection including an aural abscess. This turtle was collected from a different site than the infected turtles found during the previous summer The turtle was admitted to a rehabilitation facility and later expired. Liver tissue and oral swab collected from the dead specimen yielded a positive result while the cloacal swab did not detect the presence of the virus.
- These results preliminarily suggest that swab sampling and PCR testing may not be adequate methods for detecting ranavirus in pre-symptomatic turtles. If this is true, infected turtles sampled may have gone undetected if they were in early stages of infection, yielding falsely negative results.
- Cloacal swabs have been unsuccessful for virus detection, however, oral swabs may be a useful noninvasive method of testing sick turtles for the disease.
- Home range area of the five radio tracked box turtles ranges from 1.756 ha to 8.175 ha and is consistent with the home range findings of similar studies [3].
- There is no significant difference between the home range area of individual turtles or between sexes, however, one female had a home range nearly twice as large as any other turtle.
- Although home range sizes are relatively small, they grossly overlap as is consistent with the literature which may encourage ranavirus transmission depending on encounter rates and mode or transmission [6].
- With only 3 confirmed virus infections, spatial mapping and disease modeling based on home range size is not a valuable management tool for controlling disease spread. Further refinement of virus detection techniques and more intensive sampling is needed to determine the extent to which ranavirus may impact the box turtle population.

Acknowledgements

- I would like to thank the Department of Energy and staff at Brookhaven National Laboratory's Office of Science for the opportunity to participate in the Student Undergraduate Laboratory Internship (SULI) Program. I would especially like to send my thanks to my exceptional mentor Valorie Titus, my enthusiastic volunteers Lauren Puccia and Shirin "Ragu" Jaggi who kept me sane, and Danny Kohut for his help in the lab.

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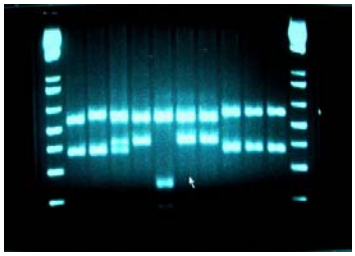
Genetic Techniques used in Detecting Wildlife Diseases



http://www.oceanexplorer.noaa.gov/explorations/03bio/logs/sept10/media/lasonolide3.html

SHIRIN JAGGI[1] and VALORIE TITUS [2,3]

[1] Hicksville High School, Hicksville, NY 11801

[2] Brookhaven National Lab, Upton, NY 11973

[3] Binghamton University, Binghamton, NY 13903

ABSTRACT

Iridovirdae, specifically genus Ranavirus, is responsible for the morbidity of frogs and turtles. Alongside, Chytridiomycota, a part of the fungi kingdom, is becoming the cause for the mortality of many amphibians including salamanders and frogs.[1] In order to prevent the spreading of the infection further, it is necessary to find methods to prevent the decline of animal populations. By examining the animal samples collected at Brookhaven National Laboratory, we wish to find genetic methods to detect the cause that is affecting such a large number of animal population so we can prevent the further spread to other animals. We hope to achieve this by using several techniques such as DNA extractions, Polymerase Chain Reaction (PCR), gel electrophoresis and others techniques that are widely used in the field of bioengineering. By finding methods to detect the virus, we hope to see the movement and spread of the virus and consequently find ways to prevent the virus from infecting other animals. As a result, we also wish to figure out how this virus even came to Long Island and how this can affect other animals in the area.

INTRODUCTION

Iridoviridae, a family of virus with only dsDNA is a genome of 150000-280000 nucleotides long.[2] Having a diameter of 120-300 nanometers and a icosohedral symmetry, the virus consists of five genera: Chloriridovirus, Iridovirus, Lymphocystivirus, Megalocytivirus, and Ranavirus.[3] The virus consists of three domains: an outer proteinaceous capsid, an intermediate lipid membrane, and a central core containing DNA-protein complexes. Usually, *Iridovirus* and *Chloriridoviurs* are known to infect vertebrates which are ectothermic. However this is not known for certain because *Iridovirus* has had recent findings in reptiles as well. *Ranavirus* in specific, can result in high morbidity in susceptible species, such as frogs and toads. [4]

Chytridiomycota, a phylum of the fungi group, are mostly saprobic (degrading chitin and keratin). There are approximately 1,000 chytrid species in 127 genera, which are then distributed among 5 orders. Some chytrid speices are known to kill amphibians in very large numbers. Chytridiomycosis is the disease that is known to fatally infect amphibians caused primarily by the chitrid- *Batrachochytrium dendrobatidis.* This infectious disease is known to have brought dramatic population decline of frogs in western North America, Central America, South America and Australia.[3]

MATERIALS AND METHODS

In order to carry out genetic techniques and procedures, it was first necessary to obtain animal samples. These samples were obtained by various methods. Eastern Box turtles were found in the woods at Brookhaven National Laboratory, near the area where some turtles were found to be affected with iridovirus. Cloacal and oral swabs were then taken to genetically test the presence of the virus. Two turtles that were found dead and were dissected in order for DNA to be extracted from their liver tissue. Furthermore, tiger salamanders were also tested by cutting part of their tail and tiger salamanders found dead were tested by cutting part of their tongues.

In order to carry out the procedures and test for the presence/absence of any disease, it was necessary to carry out the method of extractions. Extraction was a technique used after all the samples were collected. With this procedure we were able to extract the DNA from the cloacal and oral swabs and tissue samples of the turtles and salamanders. This was done by using a Dneasy kit (Quiagen, Valencia, CA, USA) that provided a protocol for extracting DNA from the various swabs collected and from the tissue samples that were obtained from the sick turtles and salamanders. After the conclusion of the process, DNA was run on an agarose gel just to confirm that the extraction was successful and the DNA was ready for the next step of Polymerase Chain Reaction (PCR).

PCR is used to amplify specific regions of a DNA strand in order to make enough copies that are sufficient to carry out testing. It is first carried out by making a 'master mix' that has the DNA template, which contains the fragment of DNA that needs to be amplified, primers that are complimentary to the DNA regions needed to be amplified, DNA polymerase used to synthesize a DNA copy, deoxynucleotide triphosphate (dNTP's) where the DNA polymerase makes new DNA, a buffer solution that provides a good chemical environment needed for maximum activity and stability of the reaction, and finally magnesium ions.[5] It was during this process that we were able to amplify the DNA to get it ready for the gel electrophoresis, which was to follow.

Gel electrophoresis is the separation of deoxyribonucleic acid, ribonucleic acid and protein using an electric charge.[6] A .8% agarose gel was used to separate the molecules and compare them with the control group. "Electrophoresis" refers to the electromotive force(EMF), which is used to push or pull molecules in a gel by applying an electric current. A blue dye was used to visualize the bands in the gel. A ladder was used during each process of gel electrophoresis.

Figure 1- collecting an oral sample from an Eastern Box Turtle

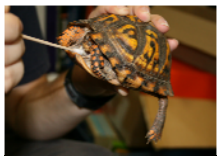


Figure 2- Sick Box Turtle found with an aural abscess

Figure 3- Dissecting a sick turtle to obtain the liver for DNA extraction.

RESULTS

After the completion of the extractions, performing PCR and running the samples on an agarose gel, it was seen that the sick turtle which exhibited physical characteristics of abnormalty in various regions of its neck, was positive for iridovirus. The positive was only observed in the liver and the oral samples. The other samples obtained from turtles seen from chance encounter and systematic transect searching, did not exhibit iridovirus. Also, the all the tiger salamanders were found negative for Chytrid fungus.

Figure 4- positive liver and oral sample in the sick turtle exhibiting the abscess as compared with the positive Irido sample in the second row,

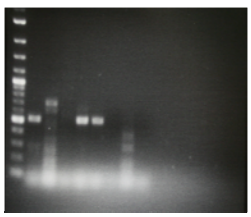


Figure 5- positive Chytrid sample

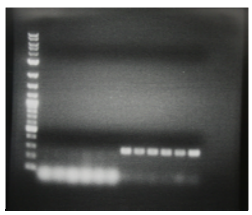
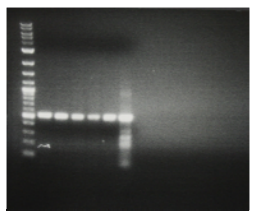


Figure 6- positive Irido sample



DISCUSSION AND CONCLUSION

According to the gel electrophoresis, there was clearly no iridovirus in the turtles from which cloacal and oral samples were taken. The dead sick turtle that had the aural abscess was positive for Iridovirus. As a result, it is possible to conclude that Iridovirus is positive in turtles that exhibit a physical abnormality as seen in the turtle that was tested. Also, after the DNA was extracted from the oral & cloacal swabs and the liver tissue, the extracted product was run on a gel to test for DNA. The procedure indicated that the swabs and the tissue contained DNA, however when the products were run on PCR and compared with a positive iridovirus sample, only the liver DNA and the oral DNA matched with the positive. To avoid false negatives in the PCR, a positive Iridovirus sample was run on a gel, and it confirmed that there was no iridovirus in the other turtle samples. As a result, it can be inferred that cloacal swabs may not be very effective in determining the presence of Iridovirus.

It was also evident that the sick tiger salamanders found at Brookhaven National Laboratory were not affected by Chytrid Fungus. To exclude the risk of PCR contamination, a Chytrid positive was also run on an agarose gel; the chytrid positive was clearly evident on the gel, confirming that the tiger salamanders did not have the Chytrid fungus.

Although the Chytrid Fungus was not responsible for the death of the several tiger salamanders found in Long Island, it makes one wonder what was the cause of the mortality of the tiger salamanders. Furthermore, how did Iridovirus even come to Long Island. It is necessary to investigate the origins of this virus on Long Island so we can possibly track the virus. If one turtle found on site is affected by Iridovirus, there is a possible risk for other species in the area to be infected as well. Perhaps, we can detect the virus using the spatial distribution of the virus and by using the home range area of the species. Since Brookhaven National Laboratory has a very high population of ticks, it is also necessary to investigate if the virus is transmitted through ticks within the species.

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BROOKHAVEN NATIONAL LABORATORY

Office of Science U.S. DEPARTMENT OF ENERGY

"Has the American burying beetle [*Nicrophorus americanus*] been extirpated from Brookhaven National Laboratory?"

Ann Ballester[1], Linda Dowd[2], Caroline Singler[3], Maria Metzger[4], and Tim Green PhD [5]

[1]Bellport High School, Brookhaven, NY 11719, [2] Riverhead High School, Riverhead, NY 11901, [3]Lincoln-Sudbury Regional High School, Sudbury, Ma 01776, [4]Southampton High School, Southampton, NY 11968, [5]Brookhaven National Laboratory, Upton, NY 11973

Introduction

The American burying beetle [*Nicrophorus americanus*] was placed on the endangered species list in August 1989. These species were formerly distributed throughout 35 states and 3 Canadian provinces in eastern North America from Nova Scotia to western Nebraska and from the upper peninsula of Michigan to Texas. The American burying beetle has disappeared from over 90% of its historic range.[1] In 1989 there were only two known populations, one on Block Island, Rhode Island and one in southeastern Oklahoma near Red Oak. Other populations have since been discovered in Oklahoma, Arkansas, Nebraska, and South Dakota.[2] Due to the proximity to Block Island, similar climate and weather patterns and geology, an attempt was made to identify a population of American burying beetles at Brookhaven National Laboratory, Long Island NY. [4,6]

Methods- Site ABB

- Locate existing traps (Fig. 2)
- Record GPS coordinates
- Bait traps with chicken [1,3]
- Create maps of GPS coordinates in GIS (Fig. 1)
- Check traps daily prior to 9 am., starting on the third day
- Re-bait traps when necessary
- Record data

Fig. 1

Fig. 2

Methods- Site BB

- Select trap locations
- Measure 20 meters between traps
- Flag locations
- Take GPS coordinates
- Create map in GIS with GPS coordinates (Fig. 3)
- Design and build new traps [1] (Fig. 4)
- Bait traps with chicken [1,3]
- Bury traps flush to ground level at flagged locations [1,5](Fig. 5)
- Check traps daily prior to 9 am., starting on the third day
- Record data

Fig. 3

Fig. 4

Fig. 5

Results

Site ABB no burying beetles were identified.

Site BB burying beetle data seen in Table 1.

Beetle Genus and species	Encounter #1	Encounter #2	Encounter #3	Encounter #4	Encounter #5
Nicrophorus *orbicollis and tomentosus*	0	0	11	30	2
Oiceoptoma *noveboracense*	0	0	0	4	1
Necrophila *americana*	0	3	18	21	7

Table 1

Nicrophorus orbicolis

Necrophila americana [7]

Nicrophorus americanus [8]

Nicrophorus tomentosus

Nicrophorus tomentosus

Discussion

Although no American burying beetles were encountered during the course of this study, the common burying beetle and other carrion beetles were found.

At site ABB no burying beetles were encountered. There were a number of items that could be altered to improve the likelihood of capturing burying beetles. One suggestion is to use a trap that would prevent access to other predators and another is the use of a greater mass of carrion as indicated by the literature.[1] On two separate occasions the bait was stolen from all of our traps, probably by raccoon or fox, necessitating a new trap design.

At site BB we constructed new traps to prevent the bait from being stolen; the carrion remained available for the beetles. Further modification of the traps to contain small holes for drainage of rainwater is recommended. This would allow for a greater survival rate and greater ease of collection of beetles.

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Acknowledgements

This research was conducted at Brookhaven National Laboratory as part of the DOE-ACTS program. Thank you to the U.S. Department of Energy for sponsoring a program designed to bring out the scientist in the science teacher and help teachers explore ways to bring "real world" science into the classroom. Thank you to Mel Morris of the BNL Office of Educational Programs for the opportunity to participate in the program and for his encouragement and moral support. Special thanks to Tim Green, our mentor at BNL, for suggesting this project and for his patience and guidance throughout the process. His expertise in environmental sciences and his commitment to making environmental science accessible to students of all ages inspired this group of teachers to do the same. Thank you, Jennifer Higbie, for patiently guiding us through the use of GPS and GIS technology to prepare maps of our study areas.

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Influence of Physical Factors on Occurrence and Distribution of Tiger Beetles at Brookhaven National Laboratory

Caroline Singler[1], Maria Metzger[2], Linda Dowd[3], Ann Ballester[4], and Timothy Green, PhD[5]

[1]Lincoln Sudbury Regional High School, Sudbury, MA 01776 [2]Southampton High School, Southampton, NY 11968 [3]Riverhead High School, Riverhead, NY 11901 Bellport High School, Brookhaven, NY 11719
[5]Department of Environmental Sciences, Brookhaven National Laboratory, Upton, NY 11973

Abstract

Tiger beetles (family Cicindelidae) are predatory insects with widespread geographic distribution that are considered to be important biodiversity indicators. Surficial geology, geography, and climate are important factors that influence the species diversity of tiger beetles in a region. Sandy substrates and the presence of open areas with sparse vegetation interspersed in undeveloped woodland areas make the property at Brookhaven National Laboratory (BNL) a favorable study area for several tiger beetle species that have historically been observed on Long Island, NY. This study, conducted in July 2007, was designed to identify and estimate populations of tiger beetles at BNL and to describe the physical factors of some of their preferred habitats on BNL property.

Introduction

Tiger beetles (Family Cicindelidae) are distinctive insects with recognizable physical characteristics and behaviors that make them relatively easy to identify in the field [1]. Cicindelids have widespread geographic distribution across a broad range of habitats with strong habitat preferences exhibited by individual species; therefore, tiger beetles are considered to be important indicators for biodiversity and conservation studies [2].

BNL is located in the western part of the Pine Barrens region of Suffolk County, Long Island, NY. BNL property lies between the Ronkonkoma moraine to the south and the Harbor Hill moraine to the north; the shallow subsurface is comprised of outwash -- sands and gravels deposited by glacial melt water as continental ice sheets receded at the end of the last stage of the Pleistocene glaciation [3]. Boring logs and down-hole geophysical logs from monitoring wells drilled throughout the site indicate that sand and gravel deposits ("upper Pleistocene deposits") are between 100 and 150 feet thick across BNL property [4]. Many tiger beetles species are known to inhabit a variety of sandy environments [1], a fact that makes BNL an excellent location to study tiger beetles.

Tiger beetles are ectothermic, relying on sunlight to maintain body temperatures needed for activity [5]. Furthermore, the tiger beetle life cycle is seasonally controlled – adults of some species are active in spring and fall, while others species are active in the summer [1]. Therefore, climate factors such as latitude, temperature, and precipitation patterns play an important role in determining the geographic ranges and specific habitats of different species. On a local scale, daily weather conditions such as wind, cloud cover and humidity influence the likelihood of tiger beetle encounters in the field.

Purpose

The primary purpose of this investigation was to identify tiger beetle species and estimate populations at BNL. A secondary objective was to characterize the physical factors of the study areas and assess the influence of substrate size and composition and weather conditions on tiger beetle occurrence and distribution.

Materials and Methods

Jonathan Mawdsley, an entomologist from the Heinz Center, visited BNL in May 2007. He conducted a preliminary survey of tiger beetle species and assessed potential tiger beetle habitats on site. Figure 1 shows the study areas of the investigation: pitfall traps were used at NF, NBA, NBB, TP and BB; insect nets were employed at NF, FB, FBB, and BL. Each site was visited several times a week during the period from 10 July through 31 July 2007. Time of day and general weather conditions were recorded for each site visit – when possible, sites were visited at approximately the same time each day. Beetles were identified and measured in the field, then marked with consecutive numbers and released near the area of first encounter. Subsequent sightings of marked beetles were recorded. Encounter histories for certain species at selected sites were summarized and entered into Program NOREMARK [6] to obtain population estimates.

Figure 1 Tiger Beetle Study Areas: North Firebreak (NF), New Burn (NBA and NBB), Firebreaks (FB and FBB), old burn area near Treatment Plant path (TP), Balloon Launch (BL), and Burying Beetle site (BB)

Substrate composition and texture were observed in the field. Shallow substrate samples were collected from four sites (NF, FB, FBB, and BL) and analyzed using standard grain size analysis techniques [7]. Preliminary weather data for July 2007 were obtained from the National Weather Service [8].

Figure 2 Tiger beetle capture locations, from left to right: NF, NB, FB, FBB, and BL (with close-up of substrate at BL)

Results

Table 1 summarizes the numbers and locations of tiger beetles encountered at BNL during July 2007. Five species, all from the genus *Cicindela*, were identified; Figure 3 shows photographs of four tiger beetles in the areas where they were captured and released. Tiger beetles were encountered at all sites surveyed except one (TP). *C. punctulata punctulata* was the most common tiger beetle found at BNL during this study. Individuals of the species were also observed in the developed areas of BNL on several occasions in addition to those encountered in the study areas.

Acknowledgements

This research was conducted at Brookhaven National Laboratory as part of the DOE-ACTS program. Thank you to the U.S. Department of Energy for sponsoring a program designed to bring out the scientist in the science teacher and help teachers explore ways to bring "real world" science into the classroom. Thank you to Mel Morris of the BNL Office of Educational Programs for the opportunity to participate in the program and for his encouragement and moral support. Special thanks to Tim Green, our mentor at BNL, for suggesting this project and for his patience and guidance throughout the process. His expertise in environmental sciences and his commitment to making environmental science accessible to students of all ages inspired this group of teachers to do the same. Thank you, Jennifer Higbie, for patiently guiding us through the use of GPS and GIS technology to prepare maps of our study areas. Finally, thank you to Jonathan Mawdsley of the Heinz Center for sharing his knowledge of tiger beetles and for his detailed responses to our email questions about tiger beetle identification.

Table 1 Tiger Beetle Species and Numbers Found at BNL

Species Name	Site Name*							
	BB	BL	FB	FBB	NBA	NBB	NF	TP
C. formosa generosa	0	2	1	2	0	0	1	0
C. punctulata punctulata	0	14	14	18	0	0	0	0
C. scutellaris rugifrons	0	0	1	4	0	0	1	0
C. sexguttata	2	0	3	1	1	2	1	0
C. tranquebarica tranquebarica	0	3	1	0	0	0	6	0
Total	2	19	20	25	1	2	9	0

* BB = Burying Beetle BL = Balloon Launch FB = Fire Break FBB = Fire Break B NBA/B = New Burn A/B NF = North Fire TP = Treatment Plant

Figure 3 Tiger Beetles at BNL, clockwise from top left: *C. tranquebarica* at BL; *C. punctulata* at FB; *C. scutellaris* at FBB; *C. sexguttata* at FB

Figure 4 shows results of the grain size analysis of substrate samples. The surface sediment in the study area is predominantly medium and coarse sand. Results are nearly identical for three sites (FB, FBB, and NF), where medium sand comprises the greatest percent by weight of samples analyzed (43.70%, 41.91%, and 38.70%, respectively). The sand at BL is somewhat coarser, with 40.17% by weight in the coarse sand range. Visual observations of surface sediment at the sites indicate that the sand is composed primarily of the mineral quartz. Surface deposits at all sites also contained small, well rounded pebbles of quartz and other rock fragments; these were removed from samples prior to sieving. Overall, the number and sizes of pebbles were greatest at BL and in low washout areas along FBB.

Figure 5 summarizes temperature and precipitation data for the region for July 2007[8]. Daytime temperatures were typically in the middle to upper 80ºF range during most of the investigation; days with lower than average temperature coincided with higher precipitation, including two rainfall events that interrupted field work on 18 July and 23 July 2007.

Figure 4 Frequency Curves for Grain Size Distribution of Substrate at Four Tiger Beetle Study Areas

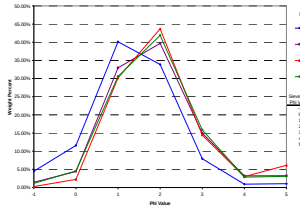
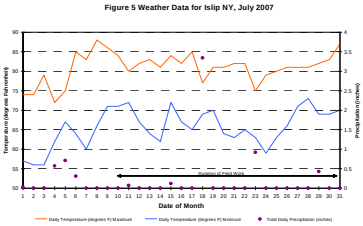


Figure 5 Weather Data for Islip NY, July 2007



Discussion

Tiger beetle findings at BNL were consistent with the life cycle and typical habitats of the species encountered [1, 9]. The assemblage of species at BNL is typical for the geology and climate of the site; the types of tiger beetles found in this study have often been observed together in similar settings [9].

C. punctulata punctulata is a summer species – adults emerge in early July and are active through the summer months. This species is found in a wide range of dry habitats, including rocky hillsides, dusty roads or trails, and sand pits, as well as sidewalks and parking lots[1]. The first noted observation of *C. punctulata* was on 13 July 2007 at site BL. All three study areas where *C. punctulata* were found are dry, sandy areas with little vegetation; other encounters at BNL occurred near buildings or paved roads. *C. punctulata* were most active in late morning and early afternoon, on warm, clear days with little or no wind.

The other species found at BNL are all spring/fall species [1]. Their small numbers are likely due to the fact that this study began near the end of the spring adult life cycle, so adults were sparsely distributed throughout the site. Each species was encountered in habitats where it would be expected – *C. tranquebarica* and *C. formosa* show a preference for open sandy areas like BL and NF; *C. scutellaris* occurs in open sandy areas with patchy vegetation that provides cover during escape flights, as at FB and FBB [1,9]. Exploration of these sites during the spring and fall will likely yield considerably higher counts of these species.

The small number of *C. sexguttata* and their locations are consistent with previous studies of the species – it is a woodland species with adults active primarily in spring; late in the season, adults tend to congregate in sunny patches in the forest in order to absorb solar radiation needed to maintain an acceptable body temperature [5]. When *C. sexguttata* was found in pitfall traps at NBA, NBA, and BB, it was always in traps located in sunny spots along the paths in wooded areas.

Conclusion

Brookhaven National Laboratory provides many favorable habitats for a variety of tiger beetle species common on Long Island. Large areas of the site are undeveloped and likely to remain that way. It is imperative to continue to provide and maintain tiger beetle habitats in suburban and urban areas [9, 10]. Habitat loss to human development is an often-cited cause of extirpation and possible extinction of certain tiger beetle species, and careful land management and conservation efforts are important for the preservation of these environmentally sensitive organisms [11,12]. Routine maintenance of firebreaks and dirt roads on the perimeter of BNL property ensures continued open habitat for species like *C. tranquebarica* and *C. formosa* and marginal species like *C. scutellaris*, while pine and oak woodlands provide habitat for *C. sexguttata*.

This investigation was not an exhaustive search of all potential tiger beetle habitats at BNL. Exploration of sites with similar surface geology and vegetation to BL, FB and FBB at seasonal intervals that coincide with emergence of adults of different species are encouraged to further delineate tiger beetle populations at BNL.

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BROOKHAVEN NATIONAL LABORATORY

Office of Science U.S. DEPARTMENT OF ENERGY

A Comparison of Efficacies of Pitfall Trapping to Netting of Tiger Beetles

Linda Dowd (1), Ann Ballester(2),Caroline Singler(3),Maria Metzger(4), and Tim Green(5)
1Riverhead High School, Riverhead, NY 11901, 2Bellport High School, Brookhaven, NY 11719, 3Lincoln-Sudbury Regional High School, Sudbury, Ma 01776, 4South Hampton High School, South Hampton, NY 11968, 5Brookhaven National Laboratory, Upton, NY 11973

ABSTRACT

As part of the DOE/ACTS program myself and three other teachers took part in a population study of tiger beetles at Brookhaven National Laboratory. Jonathan Mawdsley, PhD in entomology, had surveyed the laboratory property and identified five adult species of tiger beetles all of the genus Cincindela. Mawsdley's survey took place in the spring of 2007. We decided to survey and capture tiger beetles during the summer of 2007 with the hopes of determining what species are on site and active at this time as well as to determine population estimates at the particular sites. The team employed two different methods of capture, netting and pit fall trapping. As field work progressed it became apparent that the capture methods used had different efficacies. As a result of this learning we decided to examine trapping methods in more detail.

INTRODUCTION

Tiger beetles are an easily distinguishable group that is classified in the order Coleoptera and family Cinindelidae. Four genera occur in the North America: Omus, Amblycheila, Tetracha and Cincindela. Traits common to adult tiger beetles include long sickle shaped mandibles, teeth arrangement on the mandible, antennae width and segment number, position of antennae, and long, thin, running legs. Adult tiger beetles are similar in body shape, proportions and behavior. The head is generally larger than the thorax as to allow for the large eyes that help them in predation. Adults have transparent hind wings that are folded under the elytra, the front wings. The hind wings allow for flight and most species can fly for short distances at a low height. Characteristics used in identification of tiger beetles at the species level include the color of the elytra, the luster of the body, especially the abdomen and the pattern on the elytra referred as the maculation. Some maculations appear to be no more than a series of dots, some maybe absent and others entirely fused so to make the elytra entirely white. The normal maculation pattern includes an attractive band of light colored markings found at the front, middle and rear of the elytra. It is often helpful to look and see if the maculations are fully attached to a white line running along the outer edge of the elytra. (Pearson, Knisley/Kazile 2006)

The tiger beetles are so named for their predatory skills. Although they are fast sprinters they must be remain stationary in order to see their prey. Then and only then can they run down their prey and seize it with their mandibles. The tiger beetles need to chew the prey into a puree and nature has given them digestive juices from mandibular glands that help them in feeding as well as defense. (Pearson, Knisley/ Kazilek 2,006)

Tiger beetles are attractive insects that are often beautifully colored and marked and often times display a metallic sheen. Tiger beetles are found world wide in various habitats. Because of this tiger beetle collections by amateur hobbyists have been adding to the body of knowledge of this beetle for many generations. Tiger beetle studies date back to 1758 with a study from Linne. The vast wealth of tiger beetle information has allowed conservation biologists to use them as indicators of habitat health and biodiversity. Their distributions throughout time are well documented and are used in evaluating and authenticating historic declines as well as correlating some declines with long term environmental changes. Tiger beetle populations can be used as a bioindicator to make inferences about butterfly and bird populations. (Pearson, Cassola 2,005)

Collecting of adult tiger beetles can be attained by a variety of methods including netting, pit fall traps, nocturnal "sheeting" and sticky traps. The netting of tiger beetles involves a standard insect net, skill and patience. Movements by the collector must be slow or the tiger beetle will react suddenly. The net is to be slapped over the beetle and then can be removed from the net. Pitfall traps are containers that are buried flush to the ground. There are many variations and sizes that researchers have used but the concept is always the same: the insect inadvertently falls in the trap and can't go out. Sheeting of nocturnal insects involves luring insects at night with a light source to a sheet where they will be picked off by hand and sticky traps capture insects by causing them to adhere to vegetation or a plastic strips that have a sticky material applied. The team decided to employ pi fall trapping and then shortly later added netting to the sampling procedure.

METHOD

Five sites located at Brookhaven National Laboratory were used in the sampling of Tiger Beetles. The sites are: North Fire Break (NF), New Burn A and New Burn B (NBA, NBB), Treatment Plant (TP), Fire Break and Fire Break B (FB, FBB), Balloon Launch (BL). Traps were set at NF, NBA and NBB, TP and netting when tiger beetles were seen. FB and BL were sites for netting only. NF had twenty-four traps set at approximately twenty meters apart. NBA had thirteen traps at approximately twenty meters apart. NBB had fifteen traps at twenty meters apart. TP had nine traps twenty meters apart. GPS readings were taken at all trap and netting locations. Maps were created reflecting these areas. Traps were made by using twenty ounce water bottles. The tops of the water bottles were cut about 4-5 cm in length so that they resemble funnels and the caps removed and discarded. The top was inverted in to the body of the bottle and taped. Traps were planted flush to the ground and no bait was used. Traps were visited daily and the funnel was closed with a stone when left over the weekend.

RESULTS

TIGER BEETLE CAPTURES THROUGH TRAPS AND NETS

DATES OF SITE VISITS

SITES	7/10	7/11	7/12	7/13	7/16	7/17	7/19	7/20	7/24	7/25	7/26	7/30
NF	-	1N	2T	0	-	0	1N	4N	-	0	0	1T
NBA/NBB	0	0	1T	1T/1N	-	2T	0	-	-	0	0	1T
TP	0	0	0	0	0	0	0	0	0	0	0	0
FB/FBB	-	-	-	0	8N	11N	6N	2N	7N	9N	8N	7N
BL	-	1N	1N	1N	-	4N	3N	3N	-	7N	3N	2N
TOTALS	0N/0T	2N/0T	1N/3T	2N/1T	8N/0T	15N/2T	10N/0T	9N/0T	7N/0T	16N/0T	11N/0T	9N/2T

Legend:
- not visited
0 no captures
N net capture
T trap capture
NF: North Fire Break
NBA/NBB: New Burn A and New Burn B
TP: Treatment Plan
FB: Fire Break and Fire Break B
BL: Balloon Launch

RESULTS/DISCUSSION

Early in the research it was quickly determined that trapping was not an efficient means of capturing an adequate amount of tiger beetles to help the team estimate population. The collection period was twelve days over three weeks and in that time eight tiger beetles were trapped compared to the ninety that were netted. Although pitfall traps is a commonly employed method of invertebrate collection it is not without drawbacks in terms of its use in population estimates. Researcher G.G.E. Scudder warns that population studies using pitfall trapping for sampling needs to be correlated with independent measures. This sentiment is also echoed by researcher Kimberly Ogden for both pitfall and sweep netting. Pitfall trapping of course is only useful in surveying ground invertebrates and flying insects would need a different method of capture. Even though netting was a more successful method in beetle capture it is not without drawbacks. Nets require more on site time by the team and limits the range in which capture can occur where as traps allow for a practical survey of a large area without the constant supervision of the researcher. Considering all the obstacles in collecting insects for population studies it becomes important to give careful consideration to pitfall design. Further research suggests the following improvements to trap design:

1 – "Nesting of traps", the placement of traps of one into another is beneficial in two ways. The inner container is suspended to the outer and makes it easy to remove without disturbing the environment. (Scudder 2000) and it also increases capture number. Tiger beetles are very active and can sometimes escape a single funnel fitted trap. The nested trap will help capture rates by increasing the probability that an escaping tiger beetle will literally falling in the crack between the traps. (Young Entomologists Society)

2 – Trap layout, traps are more effective when planted in arrangement that will collect the most beetles in a specific area. Traps can be laid out in an x design with plastic lawn edging acting as barriers between the traps. This edging helps to catch beetles running in any direction. Traps placed near the edge of water and by boulders helps beetle capture rates. (Pearson/Knisley/Kazilek) Researcher Kimberly Ogden laid out 120 pitfall traps covering two different sites. Each set of sixty was set up in rows of five at one meter apart. (Ogden 2,004) A "trap circle" was a layout used by Scudder. The circle was ten meters in diameter with three to five traps placed on the circle perimeter. Scudder cites a similar design by van den Bergh that is suitable for any habitat. The traps are doubly nested and with three to five trap circles placed fifty to seventy five meters apart. (Scudder 2,000)

Future attempts by this researcher to trap tiger beetles will include closer placement of nested traps in a more limited site. More research into effective trapping so as to make reliable population estimates is needed.

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Using NOREMARK to Estimate Populations of Tiger Beetles (Coleoptera: Cicindelidae) at Brookhaven National Laboratory

Maria Metzger[1], Caroline Singler[2], Ann Ballester[3], Linda Dowd[4] and Timothy Green, PhD[5]

[1]Southampton High School, Southampton, NY 11968 [2]Lincoln-Sudbury Regional High School, Sudbury, MA 01776 [3]Bellport High School, Brookhaven, NY 11719

[4]Riverhead High School, Riverhead, NY 11901 [5]Department of Environmental Sciences, Brookhaven National Laboratory, Upton, NY 11973

Abstract

Tiger beetles (family Cicindelidae) are a group of insects which have been known to exist across the globe. More than 2600 species are described to date. It has been found that the family Cicindelidae is an appropriate indicator taxon for determining regional patterns of biodiversity therefore many conservation studies have utilized them as test organisms. The purpose of this study is to identify tiger beetle biodiversity at Brookhaven National Laboratory (BNL) and to estimate their populations using a mark recapture method.

Introduction

Tiger beetles (family Cicindelidae) are an intriguing group of insects which have been known to exist across the globe. More than 2600 species are described to date and have been found on every landmass with the exception of Antarctica, the Arctic north of 65º latitude, Tasmania and some isolated oceanic islands like Hawaii and the Maldives [1&2]. Inhabitable altitudes for tiger beetles are 3500 m above sea level to 220 m below sea level [1&2]. Favorite habitats, depending on the species, include sand dunes, ocean beaches and hardwood forest floors. Many tiger beetle species are restricted to one particular habitat [1]. As a result, they are among the most widely investigated families of insects in terms of their ecology and geographic distribution [1].

Most tiger beetles look very similar in body shape and behavior. They vary in size, color and elytra markings. Large, prominent compound eyes are set within a head that is wider than the pronotum and thorax [1&3]. On the head are antennae and large mandibles to grab and chew prey. Emerging from the thorax are segmented tarsi along with transparent flight wings which are hidden by hard protective elytra [1&3]. When approached, tiger beetles will remain motionless until they feel threatened. Once alarmed, they fly 5-10 m and tumble as they land [1&4]. Since they need to thermoregulate for activities, adults are usually diurnal [1&4].

Tiger beetles exhibit one of two types of life cycle patterns: spring-fall or summer. For spring-fall species, hibernating adults emerge in the spring, mature, mate, oviposit and die. The new brood emerges early fall, hibernate for the winter and emerge the following spring to repeat the cycle. The summer species emerge from the pupal stage in the early summer, mate, oviposit and die before the next winter. These species pass the winter in the larval, stage [1,3&5].

So, why study tiger beetles? It has been found that the family Cicindelidae is an appropriate indicator taxon for determining regional patterns of biodiversity because it has a stabilized taxonomy, individuals are easily observed and manipulated, the life history and biology is well understood, occurrences are global with a broad range of habitats while each species has a specific habitat, patterns of species richness are highly correlated with those of other vertebrate and invertebrate taxa and the taxon include species of potential economic importance [6&7]. When making policy decisions of national conservation efforts, governments focus on species richness or biodiversity [6,8]. Since tiger beetles meet the logistical and biological criteria to be used as a bioindicator taxon, many conservation studies have utilized them as test organisms [2,6,7&8].

Purpose

To identify species richness of tiger beetles at eight sites on Brookhaven National Laboratory (BNL) property and to estimate their populations using mark recapture methods.

Study Areas

Pitfall traps were buried at 4 sites (BB, NF, NB, TP) (Figure1) while netting occurred at 4 sites (NF, FB, FBB, BL) (Figures 1-3)

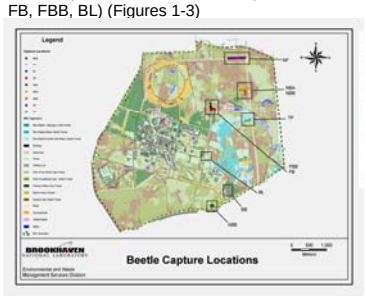

Figure 1

Figure 2

Figure 3

Materials and Methods

Eight sites were chosen for tiger beetle capture: New Burn A and B (NBA, NBB), North Fire Break (NF), Treatment Plant (TP), Balloon Launch (BL), Burying Beetle (BB), Fire Break (FB) and Fire Break B (FBB) [4]. GPS coordinates were taken at each site and maps were created using GIS software Arc View 9.0 (Figures 1-3). Pitfall traps were fashioned by inverting the funneled top of a water bottle into the bottom (Figure 4) [1&9]. They were buried every 20 m apart at NBA, NBB, NF and TP (Figure 5). Netting occurred at NF, BL, FB and FBB (Figure 6). All captured beetles were measured (length and width), sexed, marked on the elytra with a xylene paint pen and released (Figures 7-9) [1&9]. Trap checks and netting occurred daily. New and recaptured beetles were documented. Population estimates were computed using the program NOREMARK [10].

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Acknowledgements

This research was conducted at Brookhaven National Laboratory. I would like to thank the U.S. Department of Energy and Dr. Mel Morris, Director, the Office of Educational Programs at BNL for granting me this opportunity to participate in the DOE ACTS program and to work in such a prestigious lab. I thank my mentor Dr. Tim Green for his patience, knowledge and devotion to the field of environmental science. He is definitely an inspiration to minds of all ages. Thank you to Jennifer Higbie for assisting our group with GPS and the creation of GIS maps. I would last like to thank Johathan Mawdsley for his tiger beetle expertise and speedy replies to our emails. This was a wonderful learning experience, one in which the knowledge and skills gained can be brought back to the classroom.

Results

Table 1 indicates species richness at 8 sites on BNL property. Five different species were found within the genus *Cicindela*. Their total numbers captured for each site are given. Some individuals were recaptured either by netting or trapping. Table 2 shows the recaptured individuals and if immigration/ emigration took place from the marking site. Population estimates of 4 species were calculated using NOREMARK [10](Table 3). For statistical analysis purposes 4 different models were run (2 within a closed system and 2 within an open system) at a confidence interval of 95%. Estimates for each model were then be compared to each other for validity.

Table 1

Tiger Beetle Species and Numbers Found at BNL

Species Name	Site Name*							
	BB	BL	FB	FBB	NBA	NBB	NF	TP
C. formosa generosa	0	2	1	2	0	0	1	0
C. punctulata punctulata	0	14	14	18	0	0	0	0
C. scutellaris rugifrons	0	0	1	4	0	0	1	0
C. sexguttata	2	0	3	1	1	2	1	0
C. tranquebarica tranquebarica	0	3	1	0	0	0	6	0
Total	2	19	20	25	1	2	9	0

* BB = Burying Beetle BL = Balloon Launch FB = Fire Break FBB = Fire Break B NBA/B = New Burn A/B NF = North Fire TP = Treatment Plant

Table 2

Tiger Beetle Recaptures

Individual ID #	Species	# Times Recaptured	Site Marked	Site Recaptured
3	*C. sexguttata*	3	NBA-B	NBA-B
23	*C. tranquebarica*	1	BL	BL
25	*C. tranquebarica*	1	BL	BL
35	*C. formosa*	2	BL	BL
19	*C. punctulata*	1	BL	BL
6	*C. sexguttata*	1	FB	FB
7	*C. sexguttata*	2	FB	FB
9	*C. tranquebarica*	2	FB	FB
14	*C. formosa*	1	FB	FB
33	*C. scutellaris*	1	FB	FB
10	*C. punctulata*	1	FB	FB
11	*C. punctulata*	5	FB	FB
13	*C. punctulata*	1	FB	FB
51	*C. punctulata*	1	FBB	FBB
54	*C. punctulata*	1	FBB	FBB
50	*C. formosa*	1	FBB	FBB
41	*C. scutellaris*	1	FBB	FBB

Table 3

Total Population Estimates Using NOREMARK

Site	Model Types: JHE Closed Pop. Est. (95% CI)	JHE Closed MC Sim Est. (95% CI Length)	Immigration/Emigration Pop. Est. (95% CI)	Immigration/Emigration MC Sim Est. (95% CI Length)
Cicindela punctulata punctulata				
FB	31	31 ± 1.5	36	36 ± 1.2
	20 - 63	30.7	22.1-73.0	34.6
FBB	47	47 ± 4	70	70 ± 5
	21 - 249	67.4	20.4 - 382.5	110.6
BL	148	148 ± 26.5	178	178 ± 195.2
	39 - 2492	463.6	45.4 - 3022.3	676.8
Cicindela scutellaris rugifrons				
FB/FBB	11	11 ± 0.6	13	13 ± 1
	6 - 53	16.5	6.7 - 68.8	31.2
Cicindela sexguttata				
FB/FBB	10	10 ± 1.1	13	13 ± 113.7
	5 - 26	36.7	6.2 - 45.2	1162.3
Cicindela tranquebarica tranquebarica				
BL	15	15 ± 1.4	18	18 ± 130.5
	7 - 80	35.3	7.1 - 98.9	469.3

JHE = Joint Hypergeometric Estimator
MC Sim. Est. = Monte Carlo Simulation Estimator
Pop. Est. = Population Estimate
CI = Confidence Interval

Discussion

There were two purposes for this study. First, tiger beetle species richness was to be identified at different habitat sites on BNL property. Second, once these individuals were captured a population study based on mark and release techniques was to be employed. The results in Table 1 indicate that indeed there is biodiversity of tiger beetles at BNL. Five species were identified: *C. formosa generos, C. punctulata punctulata, C. scutellaris rugifrons, C. sexguttata and C. tranquebarica tranquebarica* and at least one of those species occurred at 7 sites. The most favorable habitats were BL, FB and FBB because they are open and have sandy substrate. One can see that out of the 78 individuals captured and released, 46 (58.9%) were *C. punctulata punctulata*. This species is a summer species while the other 4 are spring/fall species. Although we saw the other species, they were not prevalent.

Populations of tiger beetles were calculated using the program NOREMARK. The program could not estimate populations based on extremely small captures or no recaptures, therefore our estimations were limited to four species at their most prevalent sites (Table 3). One can see that *C. punctulata punctulata* has the largest population estimates at all 3 sites (BL=148, FB=31 and FBB=47) in a closed population and 178, 36, 70 respectively in an open population. Twelve out of the 78 marked individuals (15.3%) (Table 2) were recaptured at least once at their original location which leads us to believe tiger beetles tend not to immigrate or emigrate. BL numbers are larger because there were less recaptures whereas the most recaptures occurred at FB.

C. sexguttata, C. tranquebarica tranquebarica and C. scutellaris rugifrons populations were estimated in the teens (in both open and closed models) which validate their spring/fall life cycle. Although all models were set at a 95% confidence interval, one would need to visit each site at least 50 hours to reliably determine species numbers [6] and visit the sites during peak season of each species life cycle.

Conclusion

BNL has a diverse population of spring/fall and summer species of tiger beetles which may be used in conservation studies. These species are able to be captured, identified, marked, released and recaptured by way of pitfall traps or netting. Populations may be estimated from this mark and recapture technique.

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