GENETIC, MORPHOLOGICAL AND ECOLOGICAL RELATIONSHIPS AMONG HUDSON VALLEY POPULATIONS OF THE CLAM SHRIMP, *Caenestheriella gynecia*

A Final Report of the Tibor T. Polgar Fellowship Program

Jonelle Orridge

Polgar Fellow

Program in Ecology, Evolutionary Biology, and Behavior
City University of New York
Graduate School and University Center
New York, New York 10016

Research Advisors:

John Waldman
Department of Biology
CUNY- Queens College
Flushing, NY 11364

Robert Schmidt
Bard College at Simon's Rock
Great Barrington, MA 01230

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ABSTRACT

This project was designed to better understand the clam shrimp species, *Caenestheriella gyneca*, by comparing its ecological, morphological, and genetic characteristics from pools in three localities: the Hackensack Meadowlands of New Jersey; Saugerties, New York; and Pittsfield, Western Massachusetts (the first two occurring in the Hudson River Watershed). Little is known about the ecology of *C. gynecia. Caenestheriella gynecia* was first discovered in 1939 in a single pool in Oxford, Ohio. Schmidt and Kiviat reported four new localities of *C. gynecia* in New York and New Jersey, three within the Hudson Valley of New York and one in northeastern New Jersey. *Caenestheriella gynecia* may have originated from a very small founder population due to unusual dispersal vectors from its natural range to the west. Egg samples and hatched individuals were obtained from study sites. Specimens were raised in the lab to estimate several growth and survivorship traits. In the field, puddle habitats were observed between the months of May and August where water quality parameters (i.e. dissolved oxygen, temperature, conductivity and pH, and nutrient composition) were recorded. Genetic comparisons across the study sites were made using nuclear DNA sequencing and random amplified polymorphic DNA (RAPD). The investigations outlined in this proposal should provide a substantial extension of fundamental knowledge of this species. There is a wide variation among water quality values within and among sampling sites. Puddles found in the Meadowlands are warmer, deeper and have a higher salinity than the other two sites. Morphologically, all populations possess meristics within the range of those discovered by Mattox in 1950.

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INTRODUCTION

Clam shrimp are small freshwater organisms belonging to the Branchiopoda (Crustacea). There are about 800 species of Branchiopoda found worldwide, mostly inhabiting fresh or brackish temporary pools (Follo and Fautin 2001). Clam shrimp belong to the order Conchostraca. Conchostracans are frequently referred to as clam shrimp because of their similarities to bivalved mollusks. All conchostracans possess a bivalved carapace with a dorsal hinge controlled by a strong adductor muscle. Besides being filter feeders, these organisms can tear apart their food and will scavenge organisms in their environment (Martin and Boyce 2004). Clam shrimp are eaten by amphibians and other predators including notonectid hemipterans (backswimmers), Mallards and other ducks; shore birds like Killdeer, and Great Blue Heron, Great Egret and other wading birds. The protein from clam shrimp can provide important nutrition for migrating birds which visit vernal pools to gather nutrients they need to grow new feathers, migrate and lay their eggs. In Michoacan, Mexico, clam shrimp have been collected and used commercially as dry pet food (Martinez – Pantoja et al. 2002).

This project focused on *Caenestheriella gynecia* (suborder Spinicaudata; Fig. 1). *Caenestheriella gynecia* Mattox 1950 is a poorly known representative of the clam shrimp family Cyzicidae (Smith and Gola 2001). Little is known about the ecology of *C. gynecia*. *Caenestheriella gynecia* was first discovered in 1939 in a single pool in Oxford, Ohio. Schmidt and Kiviat (in press) reported four new localities of *C. gynecia* in New York and New Jersey, three within the Hudson Valley of New York and one in northeastern New Jersey.

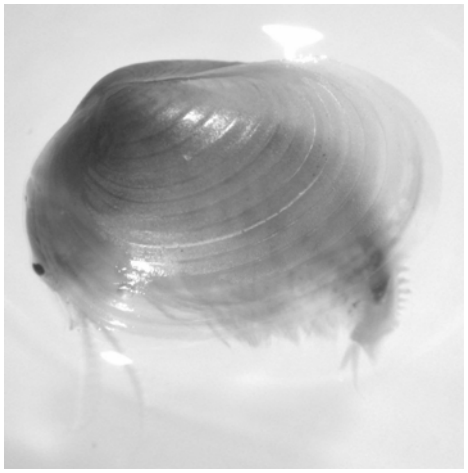

Fig. 1 Study organism – *Caenestheriella gynecia.* Lateral view of living organism, head to left. Specimen collected from Meadowlands, NJ.

The majority of clam shrimp are sexually dimorphic. The first two pairs of thoracic appendages in males have differentiated into claw-like claspers which they use to grab the shells of females and then hang on at right angles to the female's long axis (Martin and Boyce 2004). There are studies that show that "females" are actually functional hermaphrodites possessing both well-developed ovarian and testicular tissues (Sassaman and Weeks 1993). Contrary to the norm for clam shrimp, *Caenestheriella gynecia* is a parthenogenetic (asexual) species with no record of males. It is possible that males are uncommon and have yet to be discovered. Eggs are present in females between June and October and can remain viable without hatching for nearly 8 years. Individual specimens can live up to 6 months (Mattox and Velardo 1950). Egg viability is affected by temperature and time required for development and hatching varies with changes in temperature as was shown by Mattox and Velardo in 1950. Water persistence is necessary in a pool for a given amount of time in order for the animals to survive.

As a parthenogenetic species, the genetic variation of *C. gynecia* should be low. Parthenogenesis is a form of asexual reproduction in which females produce eggs that develop without fertilization. Another factor which would contribute to low variation is that the eastern populations of *C. gynecia* may have originated from a very small founder

population due to a dispersal event from its natural range to the west (Schmidt and Kiviat, in press).

Caenestheriella gynecia is so little known that only four scientific papers have been published about it, but it appears to have ecological significance as a food source for migratory birds. It also seems to be expanding its distribution, most likely through the unusual vector of all terrain vehicles (ATVs). Its habitat is found in open, muddy puddles on manmade dirt roads. Although this environment is odd for a clam shrimp, it is a common habitat in the U.S. and it seems likely that it will continue to expand its range to other similar locations. Wherever it occurs, either as a native or non-native, it contributes a unique set of life history characteristics that are intriguing, but still poorly defined. The investigations outlined in this report should provide a substantial extension of fundamental knowledge of this species. Moreover, as we gain a better understanding of *C. gynecia* habitat, behavior and distribution, this information can be used to create pools that support the species in the face of pool loss and insure the persistence of *C. gynecia.*

The goal of this project was to better understand the biology of *C. gynecia* by comparing its ecological, morphological, and genetic characteristics from pools in three localities: the Hackensack Meadowlands of New Jersey; Saugerties, New York; and Western Massachusetts (the first two occurring in the Hudson River Watershed). The first objective of the project was to determine the morphological and ecological relationships among populations of *C. gynecia*. The second objective was to determine the genetic relationships between *C. gynecia* found in vernal pools in the Meadowlands and the forms found in New York and Massachusetts. The third objective of the project was to

assess the differences among pools, found in the Meadowlands, which render them habitable or inhabitable to *C. gynecia.*

METHODS

Study sites

Hackensack Meadowlands

The Hackensack Meadowlands are composed of approximately 8,300 hectares of wetlands, uplands, and developed areas in the Hackensack River watershed of northeastern New Jersey (Kiviat and MacDonald 2004). *Caenestheriella gynecia* appears to occur only in puddles on the gas pipeline road in 1.07 km section of the Empire Tract and this is its only known locality in New Jersey. The actual study site was 10 rain puddles on the dirt surface of this road (Fig. 2 and 3). This road is regularly used by all terrain vehicles (ATVs) and other sport vehicles and these activities may help create and maintain the puddle habitats. The road is elevated around 1.5 m above the surrounding tidal marsh.

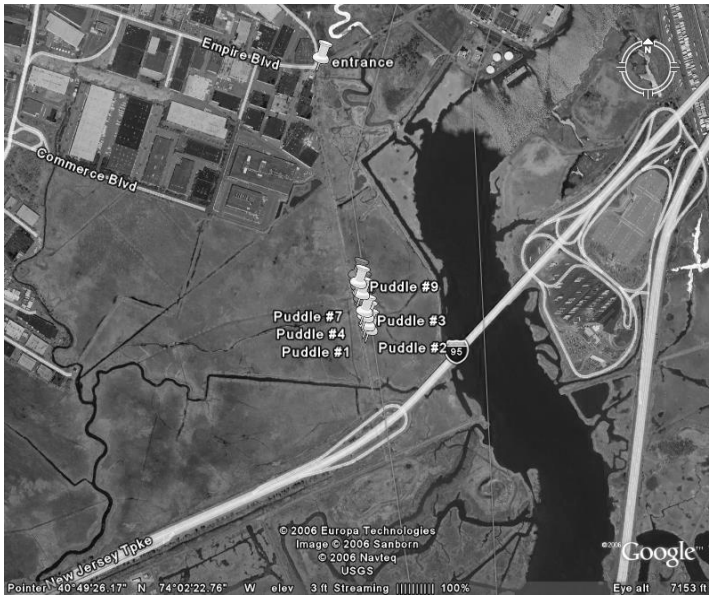

Fig. 2 Aerial map showing puddle sites located between Empire Blvd and I-95, Meadowlands, Bergen County, NJ. *Google maps.*

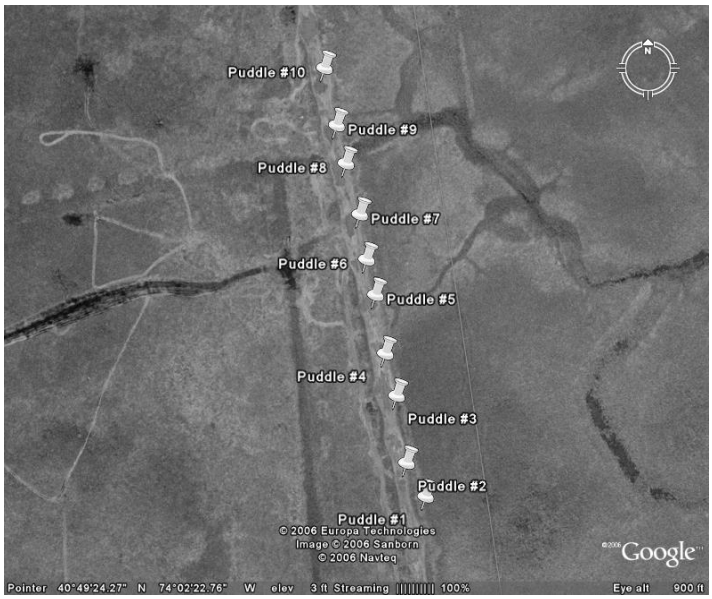

Fig. 3 Aerial map of puddle distribution on the gas pipeline road, Empire Tract, Bergen County, NJ. *Google Maps.*

Saugerties, New York

In July 2007, populations of *C. gynecia* were discovered by Erik Kiviat (Hudsonia Limited) in puddles on a dirt road in Bristol Beach State Park, Saugerties, New York (Fig. 4). The park is one of the 10 sub-units of the northern Ulster Scenic Area of State Significance found in the Hudson River Valley. In 1997, 58 acres of Hudson River shorefront was added to the 53 acres of undeveloped park. The park consists of riverfront, meadows, woodland, marsh and tidal flat habitats. In addition, the park is interspersed with several ATV trails.

Fig 4. Topographic map showing location of Bristol Beach State Park. Saugerties, NY. www.topozone.com

Lenox and Pittsfield, Massachusetts

Puddles located in Lenox, MA occur at the edge of a large wetland in the Housatonic River floodplain along an abandoned dirt road (Fig. 5). The areas surrounding the puddles provide a dense canopy of pine, maple, elm and other trees, which shades them. Like the Meadowlands site, the dirt road is visited and the puddles are probably maintained by off-road vehicles. Puddles located in Pittsfield, MA contain soils that are a mixture of tunbridge (loamy, well-drained soils that formed in Wisconsin-age glacial till) and muck and are slightly acidic to slightly alkaline (Fig. 6). Compared to the Lenox site, the pH of the water ranges from moderately to very slightly acidic. Direct sunlight is largely prohibited by a canopy of pine, hemlock, birch and oak trees (Smith and Gola 2001).

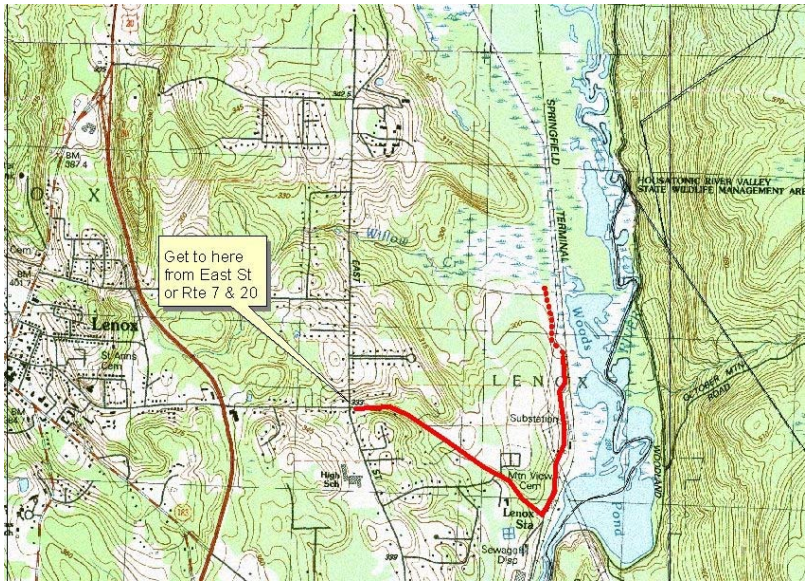

Fig 5. Topographic map of Lenox, MA site.
www.topozone.com

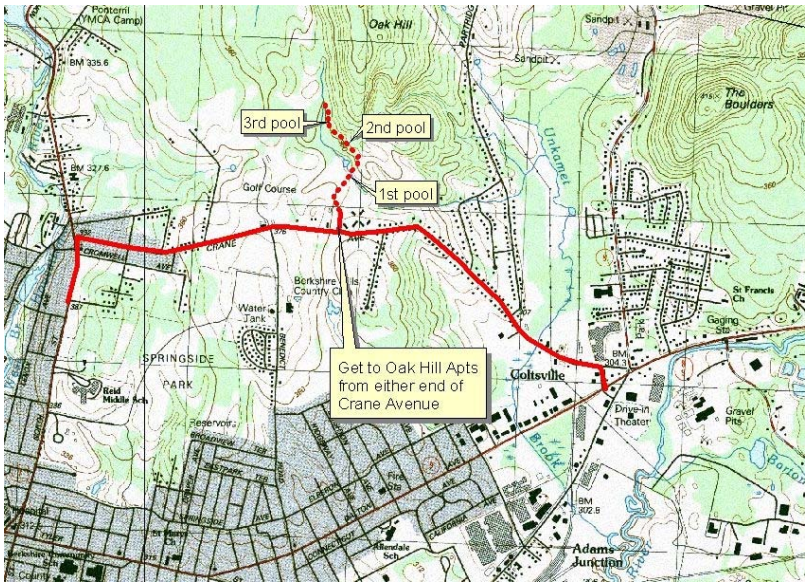

Fig. 6. Topographic map of Pittsfield, MA site.
www.topozone.com

Ecology

In the field, at the Meadowlands site, puddles were selected every 35m from a transect through the study site and marked for observation between the months of May and October 2008. Water quality parameters (dissolved oxygen, temperature, salinity and pH) were recorded weekly using an YSI 5562 Multiprobe Meter. Puddle depth was measured using a metric ruler. Nutrient composition (calcium, chloride and silica) of the

puddle was determined using a LaMotte 5917-01 water pollution kit. Three puddles were tested in July 2008 at the Pittsfield site and one at the Saugerties site.

Egg samples were obtained from dormant cysts in soil collected from these study sites (Zucker et al. 1997; Weeks and Zucker 1999; Marcus and Weeks 1997). Hatched individuals were collected via dipnetting. Water from the puddles was placed in plastic vials in which the specimens were stored. Specimens were raised in the lab to estimate growth and fecundity and to determine whether there is a correlation between age banding in their shells. Aquaria were kept at a temperature of 29 ± 2°C and under constant lighting using sunlight-simulating fluorescent bulbs. Crushed Tetramin© algal pellets were the only food source directly provided (algae and bacteria that colonize the tanks may have been consumed). Hatching time was recorded from the date/time of hydration while age was recorded as the time since hatching.

Soil samples were removed from the site, air dried and rehydrated in a 10L tank to collect larvae for observation. Daily size measurements were made using a Mitutoyo dial caliper. Daily growth increments were calculated for each shrimp by subtracting its size at day X from its size at day X + 1. Two measurements were attained, a dorsal measurement (measured between the two ends of the elliptical shell) and a lateral measurement (measured from the umbone of the carapace to the end of the shell, see Figure 7). Age at maturity was determined as the day at which eggs in the brood chamber were first noted. Longevity was determined by the length of time (in days) each individual survived and the carapace was preserved for ring counts at death. Molts were counted and collected/removed daily.

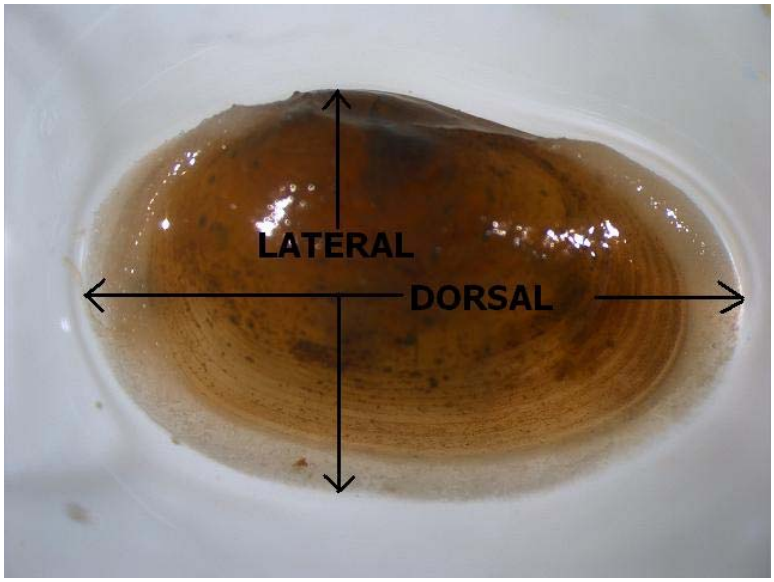

Fig. 7
Diagram of growth measurements

Population-Level Relationships

Populations examined included those in the Meadowlands, populations in the Hudson Valley, and Pittsfield and Lenox, Massachusetts. Population-level relationships were achieved by combining two sensitive molecular approaches: the random amplified polymorphic DNA (RAPD) and mitochondrial DNA sequencing. RAPD is a PCR-based method that can be performed without previous knowledge of DNA sequences of the species under study (Kautenburger 2006; Williams et al. 1990; Richardson et al. 1995). It uses arbitrary primers to detect changes in the DNA sequence at sites in the genome which anneal to the primer.

DNA extraction

Individuals were dissected and its digestive tract was removed. Genomic DNA was extracted from the remaining animal section using 250 µL aliquots of proteinase K extraction buffer. DNA was extracted with phenol chloroform followed by ethanol precipitation (Martinez et al. 2006).

Nuclear DNA sequencing

COI, 12S, 16S and 18S was examined in *Caenestheriella gynecia* and other species belonging to order Spinicaudata using the primers developed by deWaard et al. (2006), Carvalho et al. (2004), and Duff et al. (2004).

PCR conditions

The 22 µL PCR reactions contained 1µL of DNA template, 5 µL of 10x PCR buffer, 5 µL of each primer at 5µM (0.5 µM final) , 3 mM of $MgCl_2$, 2 mM of each dNTP, and 1 unit of *taq* DNA polymerase (Table 1).

Table 1. Primer sequences and PCR conditions

Primers	COI	12S	16S	18S
Forward	5'-CTGGTATAGT GGGAACTGCT – 3'	5'- TCCCTTTATTA GGGAGAGCG-3'	5'-TGAACGGCTA AACGAGAAAA-3'	5'-TTAAGCCAT GCATGTCTA AG-3'
Reverse	5'- AGGGTCAAAA AAAGAGGTGT-3'	5'- GTTAGACGAA GGACCCAAAT -3' or 5'-GTTAGAGAAG GACCCAAATA-3'	5'-AGGTCGAACA GACCTTTTGT-3'	5'-CAACTACG AG CTTTTTAAC C-3'
PCR cycling	1.5 min at 94°C, followed by 35 cycles of 45s at 93°C, 1 min at 50° C and 1 min at 72° C, followed by 1 cycle of 5 min at 72° C.	40 cycles of 94°C, followed by 30s denaturing, 40° C/45s annealing, and 72°C/45s extensions	1.5 min at 94°C, followed by 35 cycles of 45s at 93° C, 1 min at 50 C and 1 min at 72° C, followed by 1 cycle of 5 min at 72° C.	1 cycle at 94° C, 35 cycles of 30s at 93° C, 30s at 50° C, and 3 min at 72° C, followed by 1 cycle of 5 min at 72° C

PCR purification

One unit of Shrimp Alkalane Phosphotase (SAP) and 3 units of Exonuclease I was added to the PCR product. PCR products were diluted to 100 µl with Tris/EDTA and another100 µl of phenol was added. After vortexing, it was placed in a centrifuge (Eppendorf Centrifuge 5417R) for 10 minutes at 10,000 rpm at 22˚C. The aqueous top layer was placed in a new tube with 100 µl of phenol chloroform/isoamyl mix. It was again placed in the centrifuge for 10 min. One µl of glycogen was added to the aqueous top layer. Ten to twenty µl of sodium acetate was then added, depending on the final volume retrieved. Three hundred µl of 100% ethanol was added and the mixture was kept at -40˚C for 5 minutes. After incubation, the mixture was spun at 10,000 rpm for 15 minutes at 4˚C. The ethanol was poured off and 300 µl of 70% ethanol was added. The mixture was spun in the centrifuge again for 5 minutes. Thirty µl of distilled water was added and PCR fragments will be sent to Microgen in Korea to be sequenced or the solution will be stored at 37˚C until ready to be used.

RAPD analysis

PCR reactions were performed in a final volume of 25 µl, containing 25 pmol RAPD primer (see Table 2), 50 – 100 ng template DNA and a standard quantity of Ready To Go RAPD Analysis mixture using the kit from Amersham Pharmacia Biotech, Inc. #27-9502-01. The mixture was denatured for 5 minutes at 95°C followed by 45 cycles of 1 minute denaturation at 95°C, 1 minute annealing at 36°C and 2 minutes extension at 72°C. The amplified product was resolved by electrophoresis on 1.5% agarose gel in 1x

TAE buffer for 3 hours at 150 volts. The gel was stained with ethidium bromide and immediately photographed under UV light. Sizes were estimated by comparison with a 100 bp ladder.

RAPD bands were scored as present/absent and only well-resolved bands were considered. Selection of primers were based on reproducibility of the RAPD profiles and its consistency of producing polymorphic bands for DNA concentration ($1 - 5$ ng μl^{-1}) Williams et al. 1990).

Table 2. RAPD analysis primers

RAPD analysis primers	Primer sequence
Primer 1	5' – d[GGTGCGGGAA] – 3'
Primer 2	5' – d[GTTTCGCTCC] – 3'
Primer 3	5' – d[GTAGACCCGT] – 3'
Primer 4	5' – d[AAGAGCCCGT] – 3'
Primer 5	5' – d[AACGCGCAAC] – 3'
Primer 6	5' – d[CCCGTCAGCA] – 3'

RESULTS

Ecology

Table 3. Water chemistry of pools containing *Caenestheriella gynecia* in three sites in Pittsfield, MA, one site in Saugerties, NY and the main study site in the New Jersey Meadowlands. New Jersey values are the average of the 10 puddles at this site. All measurements were taken around midday. (DO: Dissolved oxygen saturation.)

Location	Temperature ºC	DO %	DO Mg/L	Salinity ppm	pH
Pittsfield 1	23.01	26.0	2.22	0.02	7.25
Pittsfield 2	24.32	6.8	0.53	0.01	5.58
Pittsfield 3	21.39	17.5	1.4	0.04	5.95
Saugerties	26.36	91.9	7.4	0.08	6.99
New Jersey	28.91	86.8	6.85	0.28	6.88

Water temperature in Pittsfield averaged 23˚C (Table 3). The water temperatures were higher in both Saugerties (26.4°C) and New Jersey (28.9°C). Dissolved oxygen saturation ranged from 6.8 – 26% in Pittsfield. However, the other sites had higher dissolved oxygen values – Saugerties at 91.9% and New Jersey at 86.8%.

The Meadowlands has higher temperatures than Saugerties and Pittsfield sites with an average minimum temperature of 70˚F. Figure 8 shows the minimum, average and maximum temperatures among the three sites for the month of July.

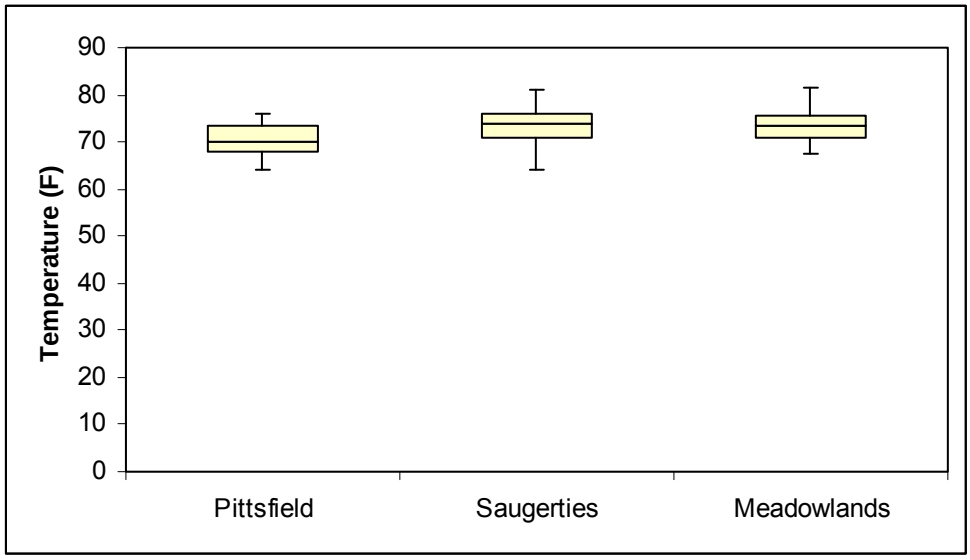

Figure 8. Temperature comparisons for the month of July among the three sampling sites. Data retrieved from Teterboro Airport at www.wunderground.com

Pittsfield, MA received almost five times (4.96x) the amount of precipitation (4.81 in.) than the Meadowlands site (0.97 in.) and almost three times (2.75x) the amount of precipitation than the Saugerties site (1.75 in.) Data was collected from Teterboro Airport at www.wunderground.com in July 2008.

Puddles (n=10) found in the New Jersey Meadowlands are 2.26x deeper (10.4 cm) than puddles found in Pittsfield (4.6 cm) and 1.79x deeper than puddles found in Saugerties, NY (5.83 cm) (see Fig. 9).

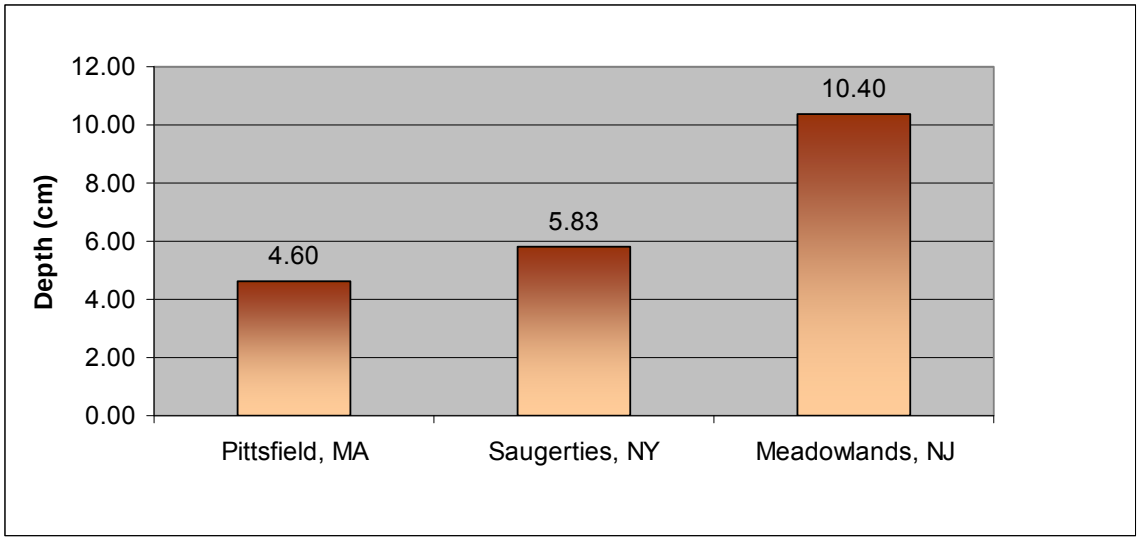

Fig. 9 Depth comparisons among the different sampling sites, Pittsfield, Saugerties and the Meadowlands.

Life history

Table 4. Life history comparisons among New Jersey and Pittsfield, MA populations of *Caenestheriella gynecia.*(D = dorsal length; L = lateral length)

	Daily Growth (D) (mm/day)	Daily Growth (L) (mm/day)	Molts (days/molt)	Age at maturity (days)	Daily egg count (eggs/day)	Long-evity (days)	Ring count (rings/day)
NJ (n=267)	0.037	0.040	2.0	14.2	11.30	25.43	0.396
MA (n=31)	-0.004	-0.007	1.3	0	0.00	35.67	0.180

New Jersey populations of *C. gynecia* had an average daily dorsal growth of 0.037 mm/day, an average lateral growth of 0.040 mm/day, and produced 0.40 rings/day. From the day of collection, individuals lived on average 25.43 days within the laboratory setting. Individuals molted every two days, developed eggs around 14.2 days and produced 11.30 eggs/day.

Massachusetts populations of *C. gynecia* had an average daily dorsal growth of -0.004 mm/day, an average lateral growth of -0.007 mm/day, and produced 0.18 carapace

rings/day. From the day of collection, individuals lived an average 35.67 days within the lab. Individuals molted every 1.3 days. None of the MA individuals reached maturity.

Morphology

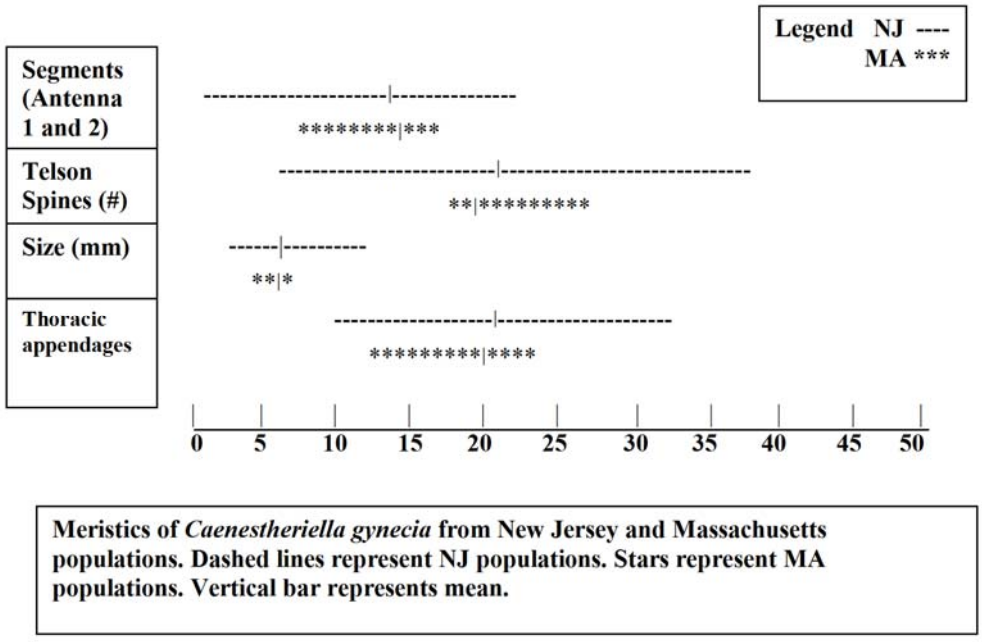

Fig. 10 Summary of meristics among *Caenestheriella gynecia* populations. New Jersey (n = 267); Pittsfield, MA (n=31)

Morphological measurements varied for populations of *C. gynecia* found in New Jersey and Massachusetts (Fig. 10). Individuals found in New Jersey had an antennal segment range between 1 and 23 ($\bar{x}$ = 13), while individuals from Massachusetts had an antennal segment range between 7 and 18 ($\bar{x}$ = 14). Individuals found in New Jersey possessed 6 to 38 ($\bar{x}$ = 19) spinal pairs (telson), while individuals from Massachusetts possessed 17 to 26 ($\bar{x}$ = 20) spinal pairs. Individuals from New Jersey ranged in size from 2.12 mm to 11.54 mm ($\bar{x}$ = 6.6 mm) total carapace length. Individuals from Massachusetts ranged in size from 4.86 mm to 8.12 mm ($\bar{x}$ = 6.6 mm). Number of thoracic appendages ranged from 13 to 24 pairs ($\bar{x}$ = 20) in the New Jersey population,

while it varied from 10 to 33 pairs ($\bar{x}$ = 20) in the Massachusetts population (Figure 11). All individuals examined were females as no specimens possessed any male claspers.

Figure. 11
Thoracic appendages of *Caenestheriella gynecia*.

We were unable to find live individuals to dissect for morphological comparisons on the sampling trip to Saugerties, NY. However, we successfully collected larvae from the rehydrated soil and are in the process of monitoring the life history traits and use them for genetic analysis.

Genetics

This portion of the project is still underway due to the unsuccessful collection of organisms from the Lenox and Saugerties site. A total of 100 individuals were collected from NJ and 15 from Pittsfield, MA. Soil was collected from all sites and taken back to the lab to dry and rehydrate to obtain larvae usable for genetic comparisons. To date, we have successfully collected 13 larvae from New York and 15 from Lenox, MA. These were the two sites that were dried out and no live individuals were found.

DISCUSSION

Ecological trends

There is a wide variation among values for the water quality parameters within and among sampling sites (Table 1). When looking at the water chemistry data for the three pools in Pittsfield, MA, it is evident that there is a range of values even though the pools are in close proximity to each other (within 2 - 4 minute walking distance). Variations in water temperature may arise from the amount of shade provided by the surrounding canopy. Some pools were completely shaded while others were exposed to pockets of sunlight. This also holds true for pools monitored at the main sampling site in the Meadowlands of New Jersey. Difference in water temperature among puddles can be as large as 20°C in a day. Pools here also share the same characteristics of the Pittsfield site in that some puddles receive direct sunlight at all times of the day and others are shaded by cottonwood, silver maple or honey locust trees. Variations in dissolved oxygen values may also result from the presence of dense algal mats that were found at both of these sites.

Salinity

Freshwater invertebrates inhabit discrete sites that are typically surrounded by inhospitable terrestrial landscape (Bilton et al. 2001). Freshwater is defined as water with less than 0.5 parts per thousand dissolved salts. Salinity values are consistent with freshwater aquatic systems in the Pittsfield and Saugerties (0.03 ppm and 0.08 ppm respectively). However, salinity is significantly higher in the Meadowlands (0.28 ppm) where salinity has reached to brackish levels (0.95 ppm). This is due to the fact that the dirt road in which these pools are located is surrounded by tidal marsh. After heavy

rainfall and extremely high tides, water from the marsh overflows onto the road and mixes with the water in the pools.

pH

When Smith and Gola (2001) sampled the Pittsfield site in 2001, they found that pools located there had a pH ranging from moderately to slightly acidic (6.2 – 7.6) with an average pH of 6.65. During my 2008 sampling of the Pittsfield site, pH values ranged from moderately acidic to nearly neutral (5.58 – 7.25). Pools found in the New Jersey Meadowlands, on average had a pH of 6.88 although values have dipped to as low as 4.34 and as high as 9.66. This is in part due to the increased truck presence within the area. They have recently started to repair the levees on the Hackensack River and are repeatedly transporting and dumping tidal soil back and forth between puddles. It has been shown that soil that is more saline is known to have a lower pH and puddles with high amounts of plant and animal detritus have a high pH (Al-Busaidi and Cookson 2003, Yee and Juliano 2006).

Depth

Comparing the maximum depths of all sampling sites, the New Jersey Meadowlands were 2.26x deeper than puddles found in Pittsfield and 1.79x deeper than puddles found in Saugerties, NY. All sites are frequented by recreational traffic of ATVs and SUVS. The major difference is that the Meadowlands site are subjected to not only the 650 lb. all terrain vehicles, but also 30,000 lb dump trucks and plow trucks whose immense tires kick up a lot of dirt and mud while traveling along the road.

Air temperature and precipitation

The Meadowlands are considerably warmer than the other two sites, as shown in Figures 8 and 9. However, the Meadowlands is also the site which received the least amount precipitation. This might correlate with the fact that we were only able to find 1 clam shrimp among the 10 puddles in the Meadowlands for the month of July. Higher temperatures combined with little rainfall does not leave enough water necessary for *C. gynecia* to complete its life cycle. Factors that affect pond duration constrain the length of life and time available for reproduction. Clam shrimp that live in smaller ponds, with low average rainfall, experience a shorter total time available for development than those found in larger ponds, with higher rainfall (Marcus and Weeks 1997).

Conversely, the Pittsfield site received the most rainfall and "cooler" temperatures out of the three sites. Of the three puddles sampled, all contained clam shrimp. Although cooler, pools in Pittsfield are inundated longer, which increases the time period in which branchiopods can complete their reproductive life cycle (Pike 2005).

These results indicate that even though pools are in close range, they can serve as micro-habitats that may result in differences in clam shrimp densities among pools. Modest changes in climate affects small pools which provide marginal reproductively suitable habitats with the potential to shift from favorable to unfavorable conditions in short periods of time (Pike 2005).

Life History

The life history measures reported herein are as a result from collection of individuals at an unknown stage in life. Exact ages at collection were undetermined and longevity and age at maturity are denoted as days since collection in the field.

Nonetheless, we feel that these data can be used for uncovering the relationship between certain life history traits in these organisms.

The last published study to look at life history traits for *C. gynecia* was by Emberton (1980). However, this study only looked at the absence/presence of eggs within the carapace and the maximum carapace length of individuals. Mattox and Velardo (1950) found ovigerous C. *gynecia* as small as 7 mm and laboratory cultures of *C. gynecia* to have a life cycle as brief as 23 days, growing from <1 to 11 mm in that time. Within my study, an individual completed its life cycle (egg to egg bearing) in 24 days which is consistent with their results. However, this individual grew to a maximum length of 5.91 mm. It must be noted that only one of 20 larva reached maturity under laboratory conditions.

When the project first began, it was observed that when daily dorsal measurements were taken alone, clam shrimp would increase and then decrease in size. This puzzlement led to the recording of and distinction between the two carapace measurements as dorsal and lateral. It was observed that at some instances, if the dorsal length decreased, the lateral length increased leading to the negative values in Table 4. More studies on the development of this species, from egg to maturity, must be done to establish its typical colonist life history traits in determining best conservation practices of the species. *Eulimnadia texana*, for instance, is optimized for life in short-lived water bodies displaying high initial growth, early reproduction, and then early senescence and death (Weeks et al. 1997).

Morphology

A total of 304 individuals from New Jersey and 31 individuals from Massachusetts were dissected. As shown in Figure 10, there is a wide range in variation of the different meristic measurements between the two populations of *C. gynecia.* These measurements included number of antennal segments, number of dorsal telson spines, carapace size, and the number of thoracic appendages.

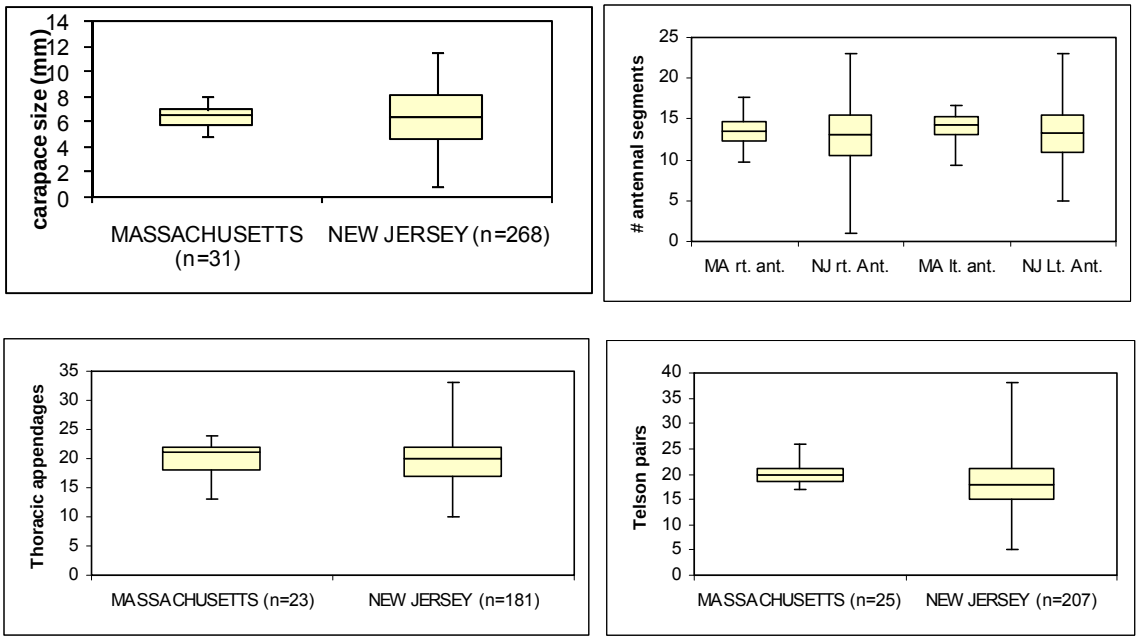

Fig. 12 Comparison of meristics among Massachusetts and New Jersey populations of *Caenestheriella gynecia.* rt. ant: Right antenna segment. lt. ant: Left antenna segment.

There has been speculation as to whether *C. gynecia* has been wrongly placed within *Caenestheriella* and perhaps should be included within *Cyzicus*. This hypothesis was based solely on morphological comparisons and the fact that there have been no males recorded for *C. gynecia* (Smith and Gola 2001). Although wide in range, the mean values of each meristic feature are actually close if not the same between the NJ and MA populations (Fig. 12). All values are within those described by Smith and Gola of the original population discovered by Mattox in 1950 and also their sample population (n

=186) in Massachusetts (2001), see Figure 13. Individuals (n = 11) collected by Schmidt and Kiviat also had measurements which fell into the same range as those that I examined (2005).

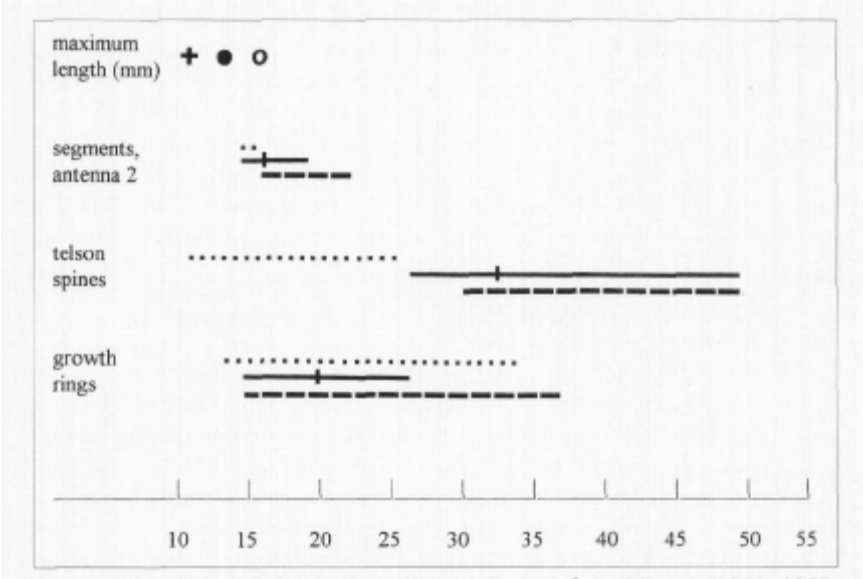

Fig. 13 - Figure 6. excerpted from Smith and Gola, (2001). Dotted lines, Ohio *C. gynecia* population; dashed lines, *Cyzicus species*; solid lines, Massachusetts populations.

Genetics

Although we were unsuccessful in collecting individuals from New York during this study, preliminary RAPD analysis of the New Jersey and the Pittsfield populations of *C. gynecia* were performed in 2007, (Figure 14). The DNA of the individual from Massachusetts (lane 6) shows a marked difference from individuals collected in NJ. It also shows that there are intra- and inter-puddle differences in DNA as well and there is a strong possibility that *C. gynecia* is not a true clonal species. The next step is to sequence the DNA fragments to see if there are more than one clone per puddle and if the New Jersey population is geographically centered.

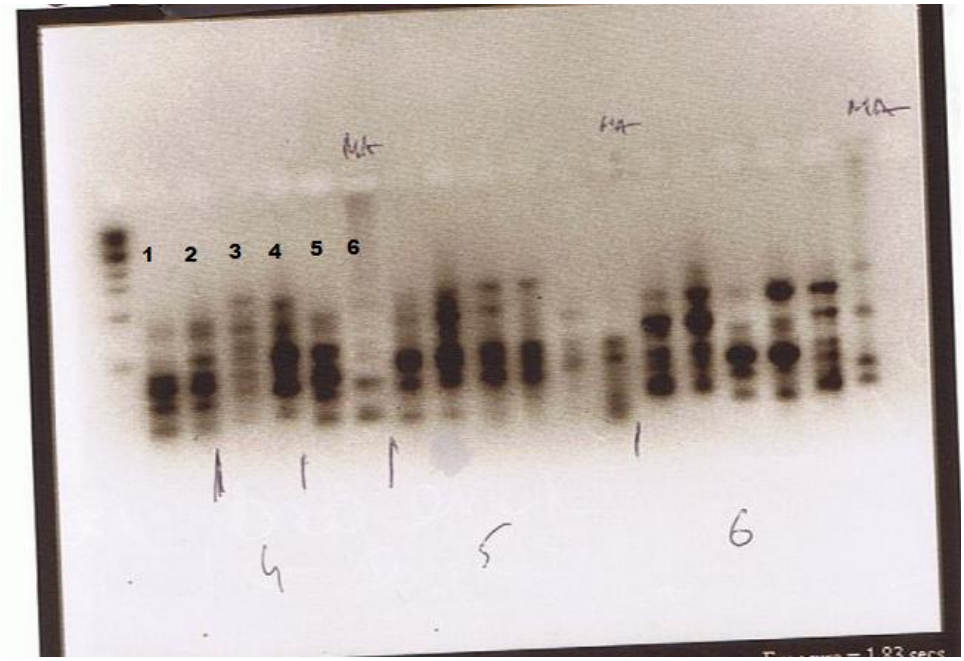

Figure 14. Image of RAPD analysis of New Jersey individuals (lanes 1-5) and an individual from Pittsfield, MA (lane 6). Lanes 1 and 2 are individuals from puddle #2; lanes 3 and 4 are individuals from puddle #5; Lane 5 is an individual from puddle #7.

Hopefully, as we gain a better understanding of *C. gynecia* biology and ecology, the information gathered from this project will assist in preserving the vernal pool habitat of *C. gynecia* as well. Clam shrimp are designated as an "At Risk" invertebrate species. Vernal pools serve as habitat and food sources to a variety of wildlife such as birds, amphibians and invertebrates, many of which are state-listed rare species. Invertebrates are vital to the vernal pool ecosystem as they function as both predator and prey.

Vernal pools formed in the Meadowlands may support *C. gynecia* because of the reduced predation or competition that would have been present in larger vernal pools. From what is known, *C. gynecia* occurs only in the pools formed on the gas pipeline road in a 1.07 km section and is the only known locality for this species in NJ.

Despite having a passive dispersal, migration among puddles is possible during periods of increased precipitation. However, during periods of drought, connectivity is

decreased. Populations will be lost if inter-puddle movement is prevented and there is a reduced likelihood of re-colonization events following population extinction. Hopefully, the genetic comparisons of the different populations will also lend a hand in determining the dispersal of the species from its first known location in Ohio by showing divergence times between populations.

There are many proposals to construct mega-malls, housing, and hiking trails to "renovate" the Empire Tract area. Providing awareness of the inhabitants of the area should help preserve and/or create and maintain the vernal pools that support *C. gynecia* in its new localities.

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