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Project objectives from application:

- Identify the composition of zooplankton species in temporary ponds across a gradient of isolation in central Illinois
- Examine the hatching fraction of several zooplankton species across isolation and age gradients
- Examine temporal variability in community composition and life history traits

Project Description

Summary

Variation among individuals in phenotypic traits is the raw material for individuals to adapt to spatially and temporally variable conditions. Annual organisms face unique challenges in variable environments, because they must survive through temporal and spatial catastrophes. Prolonged dormancy is one strategy that allows these organisms to deal with variability and persist in metapopulations through time. Zooplankton employ prolonged dormancy to survive frequent catastrophic populations losses. However, little is known about differences among populations for prolonged dormancy investment. Currently, I am documenting species composition and cladoceran dormancy patterns across a range of environmental conditions through intensive field surveys of ponds, reciprocal transplants of diapausing eggs, and a regional survey of *Daphnia* hatching fractions. Results thus far suggest populations differ substantially in local limnological characteristics, population and ephippial densities and the fraction of newly produced ephippia emerging from diapause after one year (annual hatching fraction). Additionally, common garden and reciprocal transplant studies suggest that both genetic and environmental factors influence the annual hatching fraction of populations. This work suggests investment in prolonged dormancy may be a selectable trait contributing to adaptation to variable environments.

Introduction

Organisms are frequently exposed to conditions that challenge their abilities to survive and reproduce. Yet, populations persist, suggesting individuals find a way to survive or contribute offspring to future generations. Theory predicts population persistence in variable environments for short lived organisms can be accomplished through two primary mechanisms: prolonged dormancy during inhospitable conditions or dispersal of individuals to alternate habitat patches (Cohen 1966, Venable and Lawlor 1980, Levin et al. 1984, McPeek and Holt 1992, Ellner et al. 1998). Multi-year dormancy provides a temporal escape mechanism allowing individuals to survive through unpredictable, unfavorable conditions until conditions allow successful growth and reproduction. Dispersal is an alternate method by which populations may

cope with spatially variable conditions by providing opportunities for gene flow between populations, range expansion of species and persistence of populations. Theory suggests that there is an inherent tradeoff between optimal levels of dispersal and prolonged dormancy, and that optimal investment in each will change based upon population growth rates and the fitness costs associated with each strategy (Klinkhamer et al. 1987). *Daphnia*, a group of invertebrates common to lakes and ponds, employ these strategies to cope with annually shifting conditions. Dispersal in the *Daphnia* has been noted on many occasions and has been shown to be quite substantial (Bilton et al. 2001, Michels et al. 2001, Havel et al. 2002, Louette and DeMeester 2004, Figuerola et al. 2005). Previous work has suggested local dispersal may contribute to metapopulation dynamics in *Daphnia* (Louette and De Meester 2005, M. Allen, unpublished data). Concurrently, many populations of *Daphnia* engage in dormancy, as all sexual eggs are deposited in a desiccation-resistant ephippium. If viable eggs forgo hatching for one year, they are said have invested in prolonged dormancy. Thus, the "hatching fraction", or percent of viable eggs hatched after one year, provides a useful surrogate for examining variation in prolonged dormancy strategies. *Daphnia* employ this strategy, as individual ephippia have been harvested and hatched from over a century ago (Cáceres 1998). Thus, while prolonged dormancy exists, variation in this trait and its causes are relatively unknown.

The goals of this study are three-fold:

- To perform a broad survey of ponds in central Illinois that will identify the composition of zooplankton species in temporary ponds, and specifically find ponds that contain cladocerans producing dormant eggs
- To examine patterns of dormancy in these cladocera across a range of environmental conditions
- To access the influence of local environmental or genetic and maternal factors in determining the hatching fraction of *Daphnia* populations

Methods

I monitored 14 local ponds throughout their wet phase for biotic and abiotic variables. Additionally, 26 ponds were visited only one time (Figure 1). For the focal ponds, abiotic factors were measured using standard limnological procedures on a biweekly basis (pH, dissolved oxygen, temperature, pond size, etc.). Phytoplankton abundance was measured by chlorophyll a extraction using standard protocols. Zooplankton communities were sampled by standard dip sampling procedures. Cladocerans were identified to species, copepods to suborder and rotifers genus following Pennak (1989). Additionally, pond zooplankton composition was monitored throughout the spring to access the state of diapausing egg production of resident cladocerans.

To measure the hatching fraction of cladoceran species, I collected these eggs from the water column during peak production for use in dormancy investment estimates. The "hatching fraction," or the fraction of viable eggs unhatched after one season of environmental cues, was used as a surrogate for prolonged dormancy (Philippi 1993, Cáceres and Tessier 2003). Diapausing eggs were collected from *Daphnia obtusa, Daphnia pulex, Simocephalus vetulus* and *Ceriodaphnia reticulata.* Ephippia were brought back to the lab, dried and stored at room temperature for six months. I then placed the ephippia into 6-well culture trays, sealed with 120μm mesh to prevent egg escape but allow water exchange. Each tray contained 40 eggs (20 ephippia). In fall 2005, I placed sets of three replicate emergence containers from each pond back into the field. The trays were placed in an area of each basin approximately 0.5 m below the highest surface of the pond and allowed to overwinter. Trays were removed in May 2006. The

ephippia were retrieved from each tray, counted, and scored as hatched, unhatched and viable or unhatched and inviable. The hatching fraction for each pond was calculated as the number of hatched eggs divided by the total number of hatched and unhatched but viable eggs following Cáceres and Tessier (2003). *Daphnia* produced enough ephippia for these experiments in six ponds, *Simocephalus* three ponds and *Ceriodaphnia* three ponds. *Simocephalus* ephippia have not yet been scored. Ephippia from *Ceriodaphnia* were found to be unscorable.

Additionally, I sought to examine the extent of genetic and environmental control on prolonged dormancy in *Daphnia*. Using five ponds where I was able to collect a high number of eggs, I set up a series of common garden experiments. As the number of ephippia collected from each pond varied, I was not able to establish a full reciprocal transplant. Instead, I placed sets in the originating pond and in one or two common gardens (Figure 2). To test for a difference in hatching fractions among ponds, I used a one way ANOVA with Tukey's mean separation procedure. To explore genetic and environmental influences, I used a series of one and two way ANOVAs. All hatching fractions were arcsine-square root transformed to meet the assumptions of ANOVA.

Results

The studied ponds had diverse characteristics, with different hydroperiods, sizes, relative isolations and *Daphnia* population densities (Table 1). Additionally species composition varied dramatically (Table 2). While the presence of copepods and some cladocerans was nearly universal (e.g. *Daphnia* and Chydoridae), rotifier composition was quite mixed. Additionally, while *Daphnia* were present to some degree in nearly all ponds, density varied by nearly four orders of magnitude (Table 1).

Local hatching fractions for *Daphnia* varied substantially, ranging from 54 to 94%. Although variances for some populations were large, there were significant differences in hatching fraction among ponds ($F_{4,9} = 6.75$, $p = 0.009$) with Edge Pond significantly greater than all but Center Pond following Tukey's correction (Figure 3). There were no obvious correlations between the hatching fraction and environmental factors, although the sample size was very small ($n = 5$).

The common garden experiments suggested that both genetics and local environmental factors influenced the hatching fraction of *Daphnia* populations. However, there were not great differences among hatching fractions of some populations which reduced the ability to distinguish genetic or maternal from environmental influences. I used a series of 1 way ANOVA's to test for the environmental impacts on hatching fractions. Hatching fractions from Edge and Top pond eggs showed significant differences when placed in different ponds (Figure 4: $F_{2,5} = 7.2$, $p = 0.033$; $F_{1,4} = 16.15$, $p = 0.016$, respectively). Busey, Center and LTSP eggs showed no differences ($F_{1,2} = 13.71$, $p = 0.066$; $F_{2,4} = 0.91$, $p = 0.471$; $F_{1,4} = 2.49$, $p = 0.190$) either due to high intrapond variability or small differences among means (Figure 4A). I was able to test for genetic or maternal influences in Center, Edge and Top ponds which served as common gardens. There were only significant differences among hatching fractions in Center Pond (Figure 4B: $F_{3,7} = 39.92$, $p < 0.0001$). This pattern was primarily driven by the Top population which had a significantly lower hatching fraction than the other three populations in the common garden (Figure 4B). The two fully reciprocal transplants (Edge x Center; Center x Top) affirmed these results. Environmental, but not genetic or maternal, control was significant in the Edge x Center transplant (source: $F_{1,8} = 2.91$, $p = 0.1265$; host: $F_{1,8} = 21.16$, $p = 0.0018$), while genetic or maternal, but not environmental, control was significant in the Center x Top

transplant (source: $F_{1,8} = 18.96$, $p = 0.0024$; host: $F_{1,8} = 3.49$ $p = 0.0987$). The interactions terms were not significant. A fully reciprocal transplant among multiple ponds will improve the power of these tests.

Discussion

The hatching fraction was very high (54 to 94%), which contrasts markedly from a study conducted in lakes. Caceres and Tessier (2003) found hatching rates ranging from 6 to 50% in their studies of *Daphnia pulicaria*, a member of the *D. pulex* complex, from lakes in southern Michigan. While the design of the two experiments was similar, Caceres and Tessier (2003) placed their emergence traps at the bottom of their lakes. This deeper placement may have prevented the eggs from receiving as significant hatching cues as those experienced by the ephippia in the temporary ponds. Additionally, hatching cues may be different between the two species in the two environments. *D. pulicaria* ephippia, for example, is exposed rarely to dry conditions (as they sink to the bottom of the water column), while *D. pulex* ephippia likely experience those conditions every year.

The common garden experiment highlighted further contrasts with the *D. pulicaria* study. While both studies showed a strong influence of environmental controls on *Daphnia* hatching fraction, my study additionally found strong evidence for control by genetic or maternal factors. Caceres and Tessier (2003) only found genetic or maternal factors to be important when ephippia were hatched in the lab. Potential reasons for this difference include: 1) different species of *Daphnia* have different environmental or genetic responses to hatching, 2) temporary ponds have much less stable and predictable environments than lakes, and the environments may be so different that genetic or maternal influences cut through the noise, and 3) long-term selection pressure for hatching is very different between the two environments. Specifically, *Daphnia* must recolonize a temporary pond every year; while this may not always be the case for lake dwelling *Daphnia*.

These results are an important first step toward understanding the nature long-term dormancy in temporary pond populations. They demonstrate that the methods are quite sound, as within pond standard errors were often small. Ongoing work is assessing hatching in these ponds over two more years, such that interannual variability in hatching can be examined. Additionally, in spring 2006, I began a broad-scale study to explore regional dormancy patterns in *Daphnia* populations. The preliminary survey results show considerably more environmental variability among ponds than the six explored in the 2005 survey. Additionally, I was able to extract sufficient *Daphnia* ephippia to estimate hatching fractions for 23 of ponds. This survey will allow me to examine the effects of isolation and age with greater statistical power than possible from the 2005 survey data. I will use these estimates to distinguish among a number of hypotheses that may explain regional patterns of dormancy investment:

1. Patch isolation, or a lack of dispersal, may cause a decrease in the annual hatching fraction (Kalisz et al. 1997). Such a pattern would support the hypothesis that recent historical events, such as habitat fragmentation and destruction, are influencing the evolution of *Daphnia* dormancy.
2. The annual hatching fraction equals the frequency of catastrophic losses of the population (Cohen 1966).
3. Local environmental factors or adaptation may more strongly influence hatching rates than dispersal or annual catastrophes (e.g., different predator regimes, limnological variables)

Additionally, to better distinguish the third hypothesis, I will conduct a full reciprocal transplant experiment among four ponds with very diverse environmental conditions, using the 2005-2006 survey to guide planning. This experiment will provide more power for distinguishing any genetic by environmental components that may influence hatching.

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Table 1: General characteristics of focal ponds (2005). Dry down is the first sampling date that the pond was dry. Area is the maximum surface area measured by GIS (m^2). Density is the total *Daphnia* density on the date of highest ephippial abundance (individuals per liter). Ephippia is the ratio of the density of *Daphnia* ephippia in the water column to adult *Daphnia* on the day of maximum ephippial density. The sex ratio is the maximum male to female ratio (nm = no males). pH, DO (dissolved oxygen in mg/ml), and chla (chlorophyll a concentration in µg/l) are the average of all May sampling dates. Isolation is the number of independent ponds or lakes within 1 km of the source pond. (* = missing data)

Pond	Dry down	Area	Daphnia density	Ephippia	Sex ratio	pH	DO	chla	Isolation
LTSP	5/10/2005	130.3	281.11	0.001	0.325	7	12.4	*	3
Top	6/5/2005	291.2	20.12	0.028	0.431	7.6	3.47	6.24	6
Center	6/29/2005	386.3	6.74	0.171	0.013	7.55	6.81	3.3	1
BS	7/14/2005	2233.5	17.13	0.019	0.070	7.7	1.5	7.3	7
Busey	8/15/2005	12157.9	92.02	0.002	0.184	7.6	5.04	1.29	3
Edge	na	325.6	2.25	0.000	0.009	7.8	8.45	3.23	1
MFW	6/6/2005	1146	0.075	0.000	nm	6.95	1.15	37.61	4
Mittop	5/25/2005	154.7	0	0.000	nm	7.8	8.12	17.4	4
BN	8/15/2005	5006	*	*	*	7.9	2.975	6.1	7
ME	9/12/2005	16	*	*	*	8.45	11.95	13.82	2
MW	na	62.4	*	*	*	7.8	9.42	2.76	3
MC	7/14/2005	545	0.14	0	nm	7.65	5.3	5.06	7
Dump	na	267.1	0.19	0	nm	7.95	7.69	12.12	6
Fig8	6/29/2005	536.3	*	*	*	7.75	10.42	42.32	6

Table 2: Preliminary taxonomic identification for 10 studied ponds. '+' indicates presence in the pond during May 2005. Cladocerans have been identified to species, with the exception of the Chydoridae. Daphnia pulex/obtusa are currently undergoing diagnostic assessment. Rotifers were identified to genus. Copepods have been identified to the suborder. Four further ponds, VROMW, MFFig8, KSPMittop and KSPBN, have not yet been keyed out. Sample processing for the rest of the season is ongoing for all ponds.

Pond	Center	Edge	Busey	KSPBS	MFTop	LTSP	KSPMFW	MFMC	MFDump	VROME
Cladocerans										
Ceriodaphnia reticulata	+	+	+	+	+		+	+		
Daphnia pulex/obtusa	+	+	+	+	+	+	+	+	+	
Daphnia ephemeralis				+						
Moina micrura						+				+
Scapholeberis mucronata	+	+	+	+	+					+
Simocephalus vetulus	+	+	+	+	+		+	+	+	
Chydoridae	+	+	+	+	+	+	+	+		
Rotifers										
Bdelloidia			+		+	+	+	+	+	
Asplanchna							+			+
Brachionus	+	+		+	+			+	+	+
Cephalodella			+							
Keratella			+	+						
Lecane	+	+		+	+	+	+		+	
Lepadella			+		+			+		
Platyias				+	+					
Polyarthra				+			+			+
Trichocera			+	+			+			
Copepods										
Calanoid	+	+	+	+			+			
Cyclopoid	+	+	+	+	+		+	+	+	+
Harpactacoid		+	+	+	+			+		

Figure 1: Illinois map highlighting approximate locations of ponds studied in 2005. Inset in each star is the number of ponds in that area.

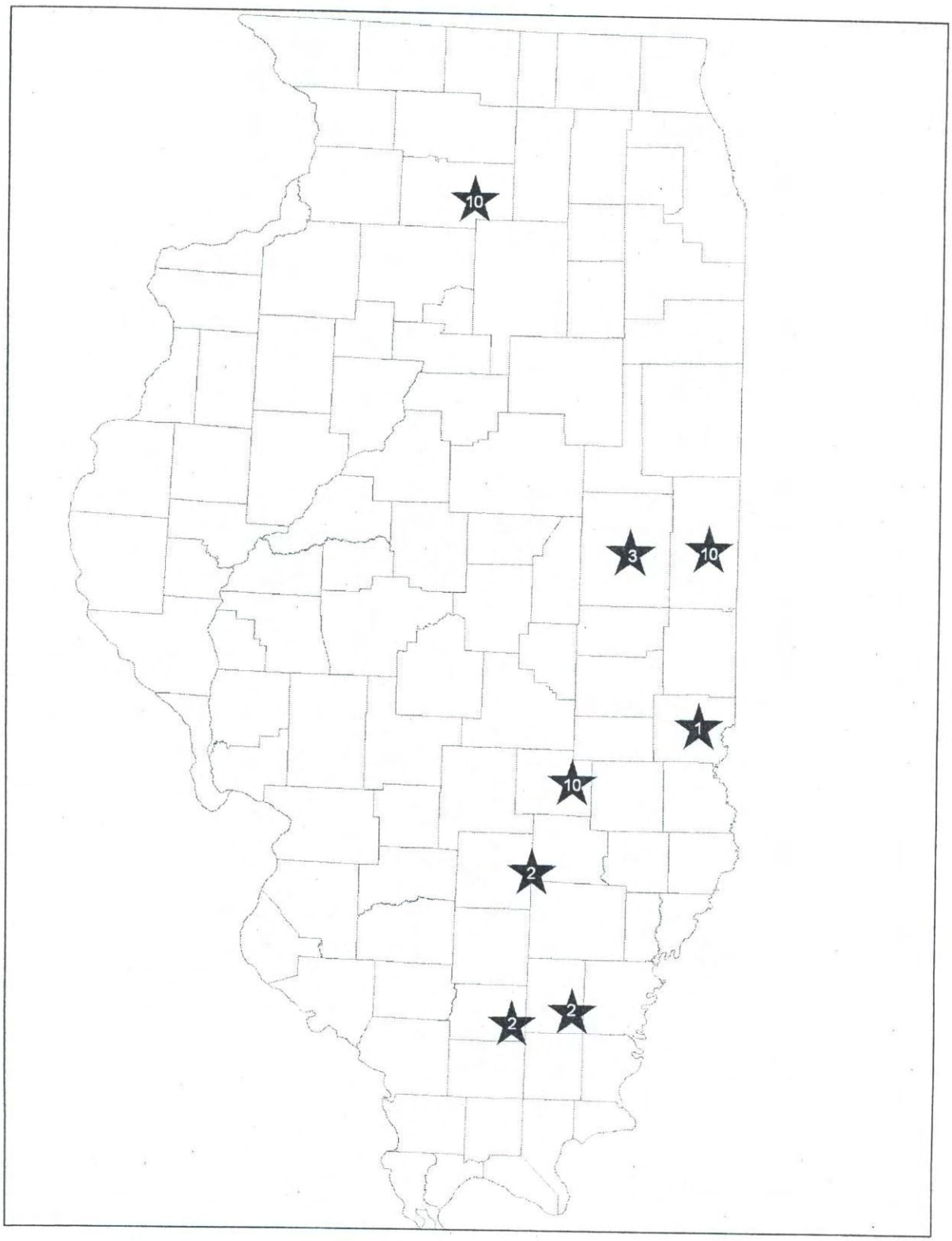


Figure 2: Hatching fraction setup for fall 2005-spring 2006. Each square represents a different pond. The dark squares, Center, Edge and Top, are common gardens. Green arrows represent hatching fractions obtained in each pond from eggs obtained in the pond. Maroon arrows represent transplants between ponds.

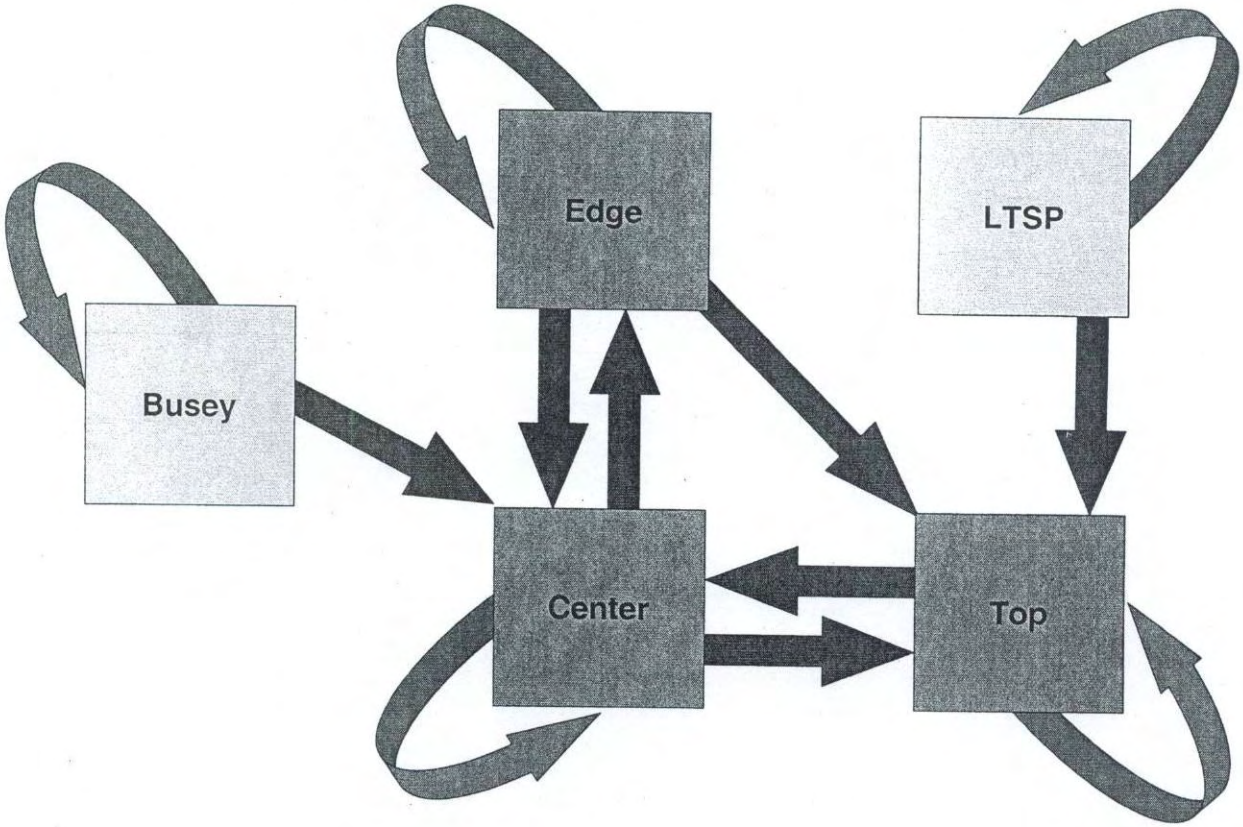


Figure 3: Hatching fractions from each pond. Populations differed significantly ($F_{4,9} = 6.75$, $p = 0.009$) with Edge pond greater than all but Center pond following Tukey's correction.

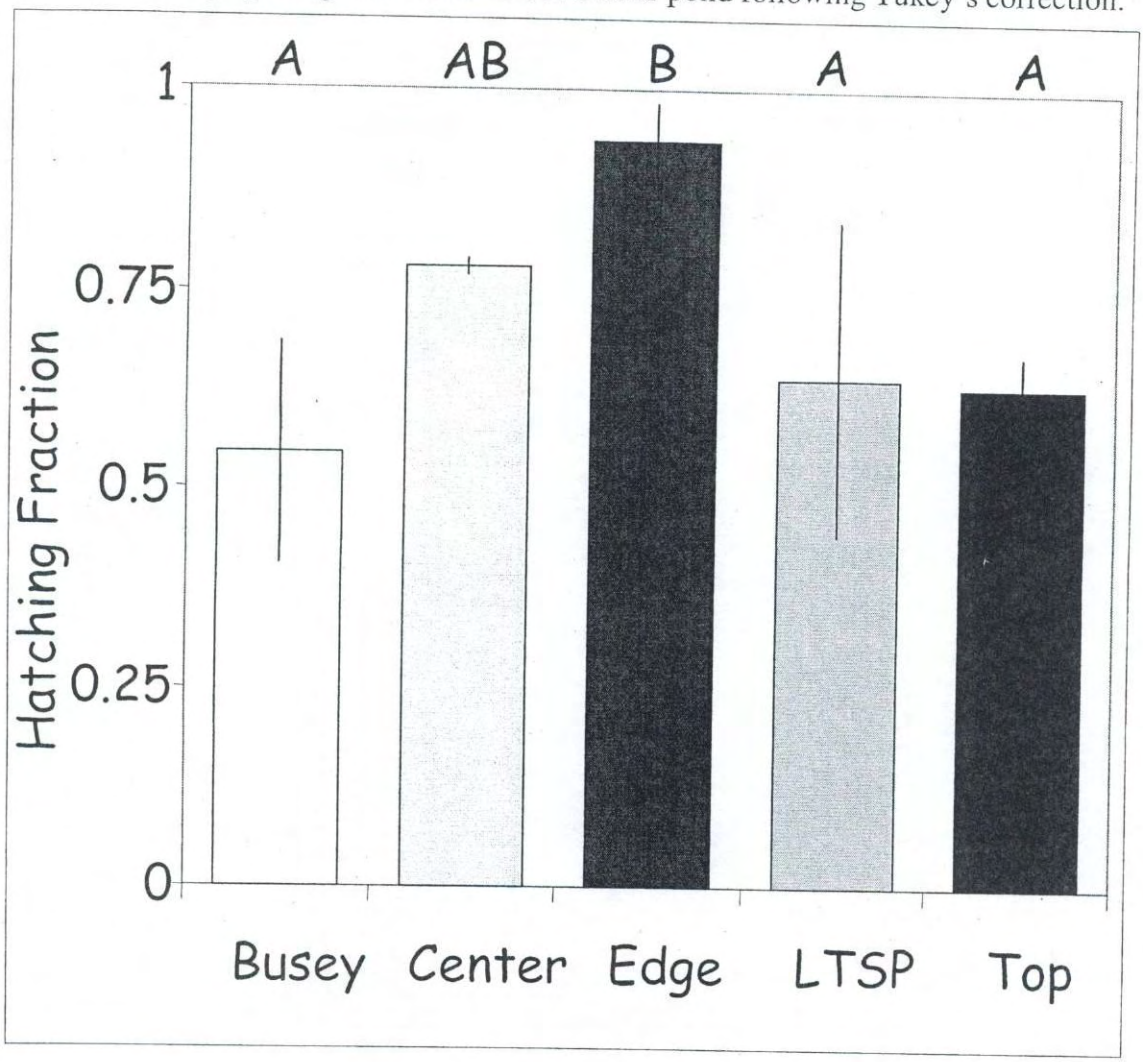
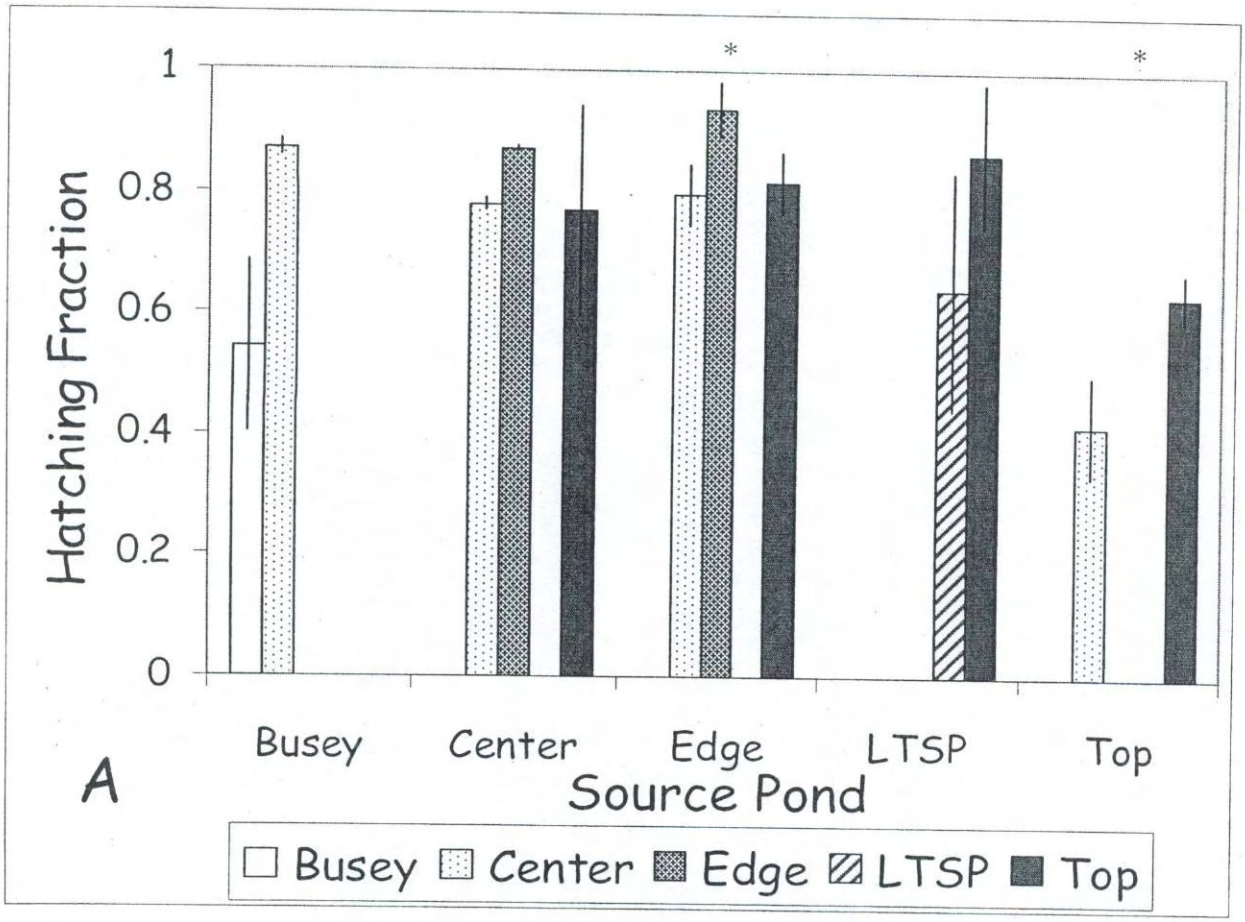
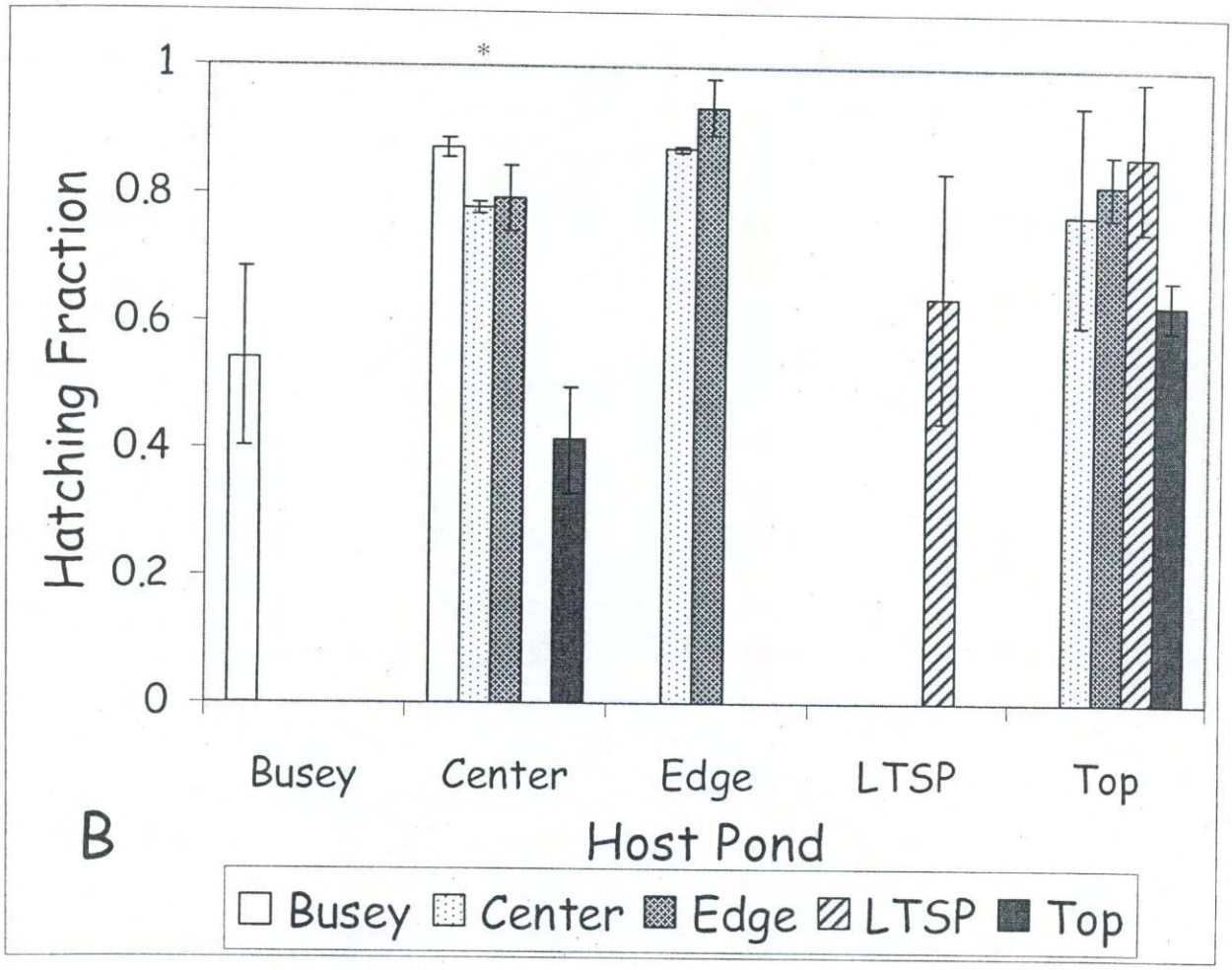


Figure 4: Hatching fraction for five ponds studied between fall 2005-spring 2006. Each bar represents an untransformed mean (±1SD). A) Means are grouped by the source pond from which the eggs were harvested. Differences within a group suggest the local environment influenced hatching fractions. B) Means are grouped by the host pond where the eggs were hatched. Differences within a group suggest genetic or maternal effects influenced hatching fractions. A '*' suggests there were significant differences within a group.




1
0.8
0.6
0.4
0.2
0
Hatching Fraction
*
Busey
Center
Edge
LTSP
Top
B
Host Pond
Busey
Center
Edge
LTSP
Top

Other Project Expenditures

Travel			
Project Mileage	2005 (0.37 per)	1100 miles	407.00
	2006 (0.41 per)	824 miles	337.84
	Covered by this grant	977 miles	-361.49
Materials/Other			
Microfibre filters – GFF			138.16
Garmin eTrex GPS unit			132.99
GPS adapter			15.98
Cable ties			3.20
Battery powered bubbler			15.98
Batteries for pH meter			18.55
Rite in the Rain notebooks			17.17
Ethanol		3 gal	20.40
Total other expenses			**745.78**