The Population Biology of the Spotted Salamander, *Ambystoma Maculatum,* Inhabiting Penn State Erie Wetlands

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P. Silver, M. A. Campbell, and J. K. Cooper
Penn State Erie, The Behrend College

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Pamela Silver, Michael Campbell, J. Kevin Cooper, Carley Gwin, and Jen Osmanski

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13. Abstract (Maximum 200 words)

Two wetland systems on the campus of Penn State Erie, The Behrend College, are under development pressure stemming from the construction of the East Side Access Highway and from expansion of the college. Both wetland systems are important breeding habitat for the spotted salamander, *Ambystoma maculatum*, which has extensive requirements for both wetland and terrestrial habitat during its life history. Though this species is prevalent in many vernal wetland systems in Pennsylvania, very little is known about how changing landscapes resulting from development, road construction, and wetland mitigation affect this species. The goals of this study were to: 1) describe the population biology of *A. maculatum* on the Penn State Erie campus, 2) use this information to assess the possible effects of campus development and the construction of the East Side Access Highway on the salamanders, and 3) recommend mitigation strategies that will minimize harmful effects of road construction on the salamanders. Data were collected between 1995 and 1999, and the results reported in this document address genetic variation and reproductive isolation of the populations, the temporal and spatial patterns of the reproductive migration, the abundance and distribution of the reproductive adults, and the importance of the upland habitat for the number and quality of migrating adults. From this study, we can conclude that the wetlands on the Behrend campus support a large, genetically-homogenous population of *A. maculatum*. However, sufficient variability is present for us to conclude that some degree of reproductive isolation exists between populations in different wetland complexes. The population is genetically distinct from others in the eastern United States. We also can conclude that the *A. maculatum* populations are dependent upon the area and quality of the upland habitat surrounding the breeding wetlands.

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EXECUTIVE SUMMARY

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The goals of this study were to: (1) describe the population biology of *A. maculatum* on the Penn State Erie campus, (2) use this information to assess the possible effects of campus development and the construction of the Eastside Access Highway on the salamanders, and (3) recommend mitigation strategies that will minimize harmful effects of road construction on the salamanders. Data were collected between 1995 and 1999; the results reported in this document address genetic variation and reproductive isolation of the populations, the temporal and spatial patterns of the reproductive migration, the abundance and distribution of the reproductive adults, and the importance of the upland habitat for the number and quality of migrating adults.

Chapter 2 describes *A. maculatum* and the two major wetland systems on the Penn State Erie campus subject to development pressure. The wetland complexes (WC1 and WC2) consist of two clusters of vernal forested wetlands separated by a distance of 1200 m. WC1 is located in relatively undisturbed mature forest, but WC2 has been and still is subject to extensive disturbance that will continue into the foreseeable future. Chapter 2 also describes the change in upland habitat resulting from the construction of the Behrend athletic fields surrounding WC2.

Chapter 3 examines the genetic differentiation between the two *A. maculatum* populations using sequence data from a 225 base pair section of the mitochondrial DNA. The two populations were found to be genetically homogeneous. However, minor differences between the populations are consistent enough to clearly distinguish individuals that breed in different wetland complexes, a pattern that is indicative of limited dispersal ability and reproductive isolation of interbreeding subpopulations of this species.

Chapter 4 examines the importance of climate and upland habitat on the timing and spatial pattern of the spring reproductive migration. Migration is episodic, occurring on rainy nights in early spring when the air temperature is between $0\,^{\circ}C$ and $12\,^{\circ}C$. Within wetland complexes, adult abundances are greatest where the wetlands adjoin intact mature forest. During breeding migrations, adults utilize wetlands with woody vegetation and debris rather than wetlands with submerged or emergent herbaceous vegetation. The movement of migrating adults into the wetlands is highly directional, rather than occurring along random paths. Adults enter the wetlands from upland locations characterized by mature forest, and they clearly favor certain entry points over others.

Chapter 5 describes the estimates of population sizes and the quality of migrating adults. The estimated size of the salamander population on the Penn State Erie campus is approximately 25,000 adults, but the majority of salamanders occur in WC1 (15,000 adults). Population levels fluctuated during the 4-year period of the study. Population size and the quality of adults remained the same throughout the study for salamanders in WC1. However, habitat changes around WC2 were associated with a marked decline in the number and quality (reproductive output) of the migrating adults.

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1. INTRODUCTION

Worldwide, many amphibian populations have declined (Jaeger 1980; Banks and Beebee 1987; Semb-Johansson 1989; Crump et al. 1992) or have gone extinct (Corn and Fogleman 1984; Pounds and Crump 1994) during the past 20 years. While climatic factors such as El Niño/ Southern oscillation have been shown to have a role in some population declines (Pounds and Crump 1994), the majority of studies have implicated anthropogenic impacts such as pollution (Blaustein et al., 1994), habitat degradation through draining and logging (Nunney and Campbell, 1993; Blaustein et al., 1994; Wyman, 1990), and roads (Fahrig et al., 1995) as primary reasons for the global population decline of amphibians.

The global concern regarding amphibian decline stems from the central ecological role that amphibian species play in wetlands and in surrounding upland habitats. In wetlands, amphibian larvae are significant predators (Duellman and Trueb 1986), herbivores (Morin et al. 1990), and prey (Duellman and Trueb 1986). In the upland habitat the adults are important both as predators and as prey (Porter 1972). Amphibians account for much of the vertebrate biodiversity in uplands and wetlands (Duellman and Trueb 1986). Thus, loss of amphibians is expected to have repercussions to the wetland and upland communities (Beebee 1996).

Despite the ecological importance and globally threatened status of amphibians, little resrach has been conducted to determine the connections between degradation of habitat and the population biology of amphibians (Blaustein et al. 1994). Few studies have been conducted for a length of time that allows differentiation of population fluctuations resulting from natural

population cycles from real declines in numbers resulting from the degradation of the habitat (Blaustein et al.1994). In addition, no long-term research has been published on the consequences of wetland mitigation for amphibian populations.

PROBLEM STATEMENT

In 1995, the Pennsylvania Department of Transportation (PennDOT) approached Penn State Erie concerning the construction of the East Side Access Highway and ca. 4 ha of mitigation wetlands on an undeveloped part of the campus. PennDOT asked the biology faculty at Penn State Erie to take advantage of the road construction to develop applied education and research opportunities for biology students.

Seven wetlands on the Penn State Erie campus were identified as having the potential to be affected either by the East Side Access Highway or by the expansion of Penn State Erie. These wetlands and the surrounding upland habitat support a rich amphibian assemblage. In common with many vernal wetlands and deciduous uplands in Pennsylvania, the dominant amphibian species here is the spotted salamander, *Ambystoma maculatum*. However, very little is known about the species and the effect of development on the biology of this species.

To quantify the population biology of *A. maculatum* on campus, we investigated the population genetics, demography, and seasonal use of upland and wetland habitat of this ecologically important species. This report is the culmination of the first four years of the study. The data generated by this study provide a baseline upon which we will assess the future impact of development on campus. The data can be used to guide the design and assessment of mitigation wetlands as habitat for *A. maculatum* populations. In addition, the results also

illustrate the importance of intact forested upland to the population biology of *A. maculatum*, as the upland habitat surrounding several of the wetlands was altered during the second year of this study. Because *A. maculatum* is present in many vernal wetland systems across Pennsylvania, the results from this study provide specific information for other PennDOT projects across Pennsylvania. Students from Penn State Erie and Mercyhurst College were actively involved in all aspects of this study (Appendix A).

2. *AMBYSTOMA MACULATUM* AND THE FORESTED WETLANDS OF PENN STATE ERIE

INTRODUCTION

Two major wetland systems are present on the Penn State Erie campus (see Figure 1). The two wetland systems are separated by a distance of approximately 1200 m. The area between wetland complexes 1 and 2 (WC1 and WC2) includes the Penn State Erie campus and several heavily trafficked roads. Both systems are threatened by development: the upland habitat surrounding WC2 was altered during the course of this study and the upland habitat surrounding WC1 is expected to be developed within the next five years. WC1 also will be affected directly by the construction of the East Side Access Highway.

Both WC1 and WC2 support a diverse assemblage of amphibians dominated, in particular, by the spotted salamander, *Ambystoma maculatum*. Other salamander species found in the wetlands include the red-spotted salamander *Notophthalmus viridescens viridescens*, the redback salamander *Plethodon cinereus*, the mountain dusky salamander *Desmognathus ochrophaeus*, and the mole salamander *Ambystoma jeffersonianum*. Only *A. maculatum* and *N. v. viridescens* are found in appreciable numbers on the Penn State Erie campus. Frog and toads using the wetlands include the wood frog *Rana sylvatica*, the spring peeper *Pseudocris crucifer crucifer*, the green frog *Rana clamitans melanota*, the chorus frog *Pseudocris feriarum feriarum*, and the American toad *Bufo americanus*.

THE NATURAL HISTORY OF *AMBYSTOMA MACULATUM*

Ambystoma maculatum is a member of the salamander family, Ambystomidae. *A. maculatum* is relatively large species with wet weights approaching 50 grams for females and 30 grams for males (Duellman and Trueb 1986). It has a maximum life span approaching 25 years (Flageole and LeClaire 1992). The biphasic life history of *A. maculatum* is typical of amphibians. *Ambystoma maculatum* spends much of its life in the terrestrial habitat surrounding the wetlands. Adults live in small burrows and crevices and forage on small invertebrates such as insects and worms. The home range of the adults is small (3.2 to 29.4 m^2) compared to that other amphibians and salamanders (Kleeberger and Werner 1983), but adults move long distances to reach breeding ponds (Shoop 1968). Many studies suggest that *A. maculatum* adults return to their natal ponds to breed (Stenhouse 1985; Whitford and Vinegar 1966). Males reproduce the spring after metamorphosis, but females do not reproduce until after two years in the upland habitat (Husting 1965). The reproductive migration of *A. maculatum* to wetlands begins in early spring. *A. maculatum* is often the first amphibian to initiate the spring reproductive migration because of its low temperature threshold of 0°C (Duellman and Trueb, 1986). Adults migrate synchronously in large masses. Once in the water, males deposit spermatophores on the bottom of the wetlands. Females then pick up the spermatophores and insert them into the cloaca, thereby fertilizing the eggs. Females deposit egg clutches approaching 134 eggs (Bishop 1941) on sticks and other structures in the water. After reproduction, adults emigrate back into the upland habitat. Eggs hatch 43 days after fertilization and immature adults emigrate from the wetland after approximately 87 days (Bishop 1941). Survival in the egg and larvae stages is low (22.2 percent and 71.2 percent, respectively), resulting in 15.8 percent reaching the adult stage

(Shoop 1974; Bishop 1941).

PENN STATE ERIE WETLANDS

Wetland complex 1 (WC1) is located between Station Road and Jordan Road on the top of the ridge on the north side of Jordan Run (Figure 1). The system has a perched water table and consists of a single large (880 m^2) wetland (Figure 2) and several smaller (< 10 m^2) wetlands (not illustrated). Only the larger wetland supports *A. maculatum*. This palustrine forested wetland has a perched water table, with a maximum depth of 1 m. The mature forest surrounding the system on all sides has been subjected to little human disturbance during the last 20 to 30 years. Species composition of the forest includes maples, tulip poplar, and oaks.

Wetland complex 2 (WC2) is located on the northeastern edge of the campus near the future location of the East Side Access Highway (Figure 1). The system has a perched water table and is composed of 6 wetlands, but only 4 (wetlands 3, 4, 6, and 7) support appreciable numbers of *A. maculatum* (Figure 3). Wetland 7 is 800 m^2, wetland 5 is 490 m^2, and wetland 4 is 1125 m^2. Wetland 4 and wetland 7 are palustrine forested wetlands, while wetland 6 is a palustrine emergent wetland. Wetlands 4, 6, and 7 are contiguous during periods of high water and become separated as they dry. Water drains from wetland 4 to wetland 6 to wetland 7. Wetland 3, a 1000- m^2 forested palustrine wetland, has long been used as a dump, and it is highly degraded. It is located at the base of a hill about 100 m from wetlands 4, 6, and 7 on property adjacent to the Penn State Erie campus. All four ponds are temporary, filling with snow melt and spring rains, and drying by late summer to early fall. The maximum water depth for each of the wetlands is approximately 0.75 m.

FIGURE 1. Map of the Penn State Erie Campus, Erie Pennsylvania. Wetland complex 1 (WC1) and Wetland complex 2 (WC2) are within the circled boundary.
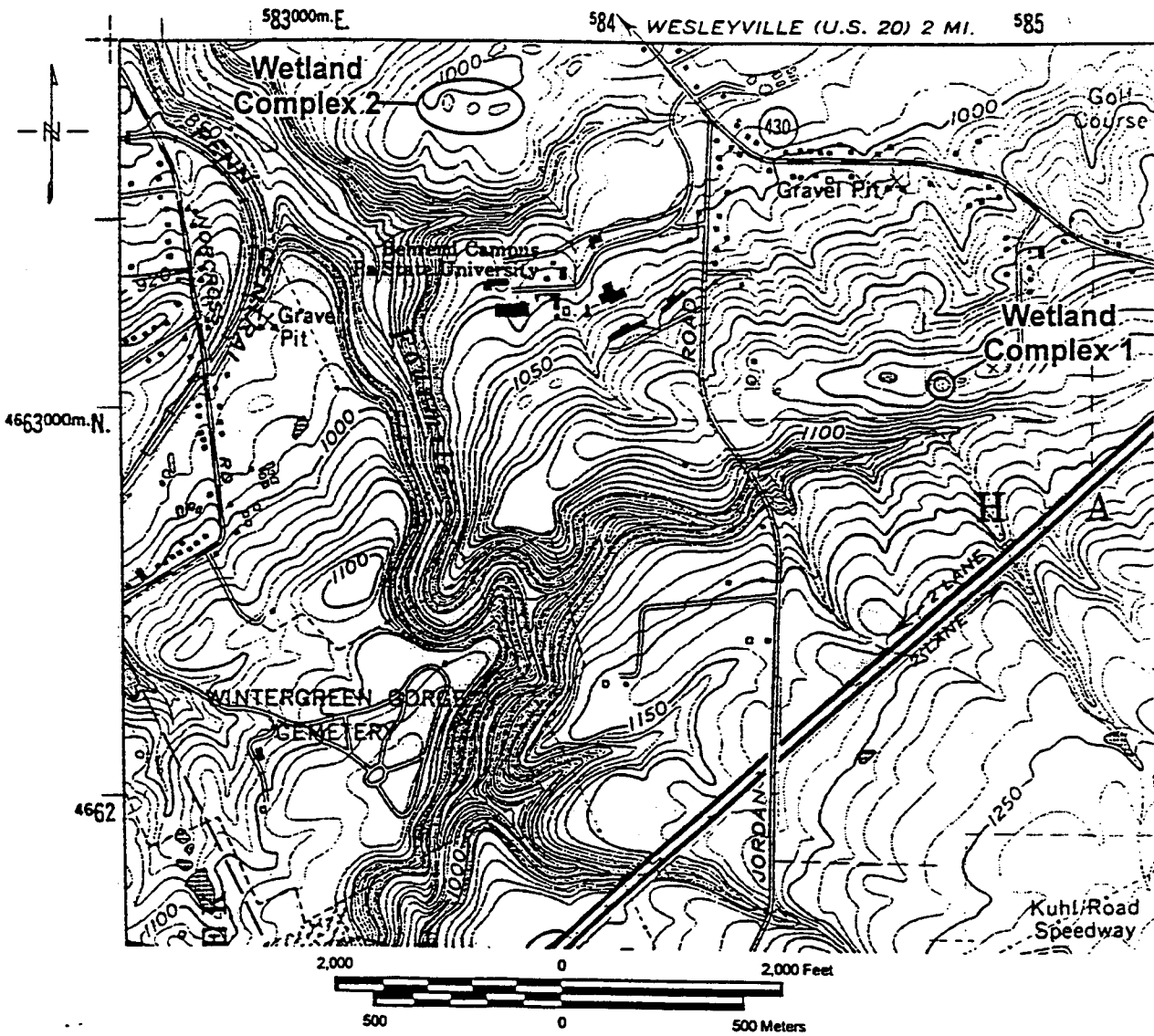
FIGURE 2. Map of Wetland Complex 1. Wetland fences are denoted by "F" and upland fences by "UF". The size of the wetland and position of fences are approximations based on survey distances.
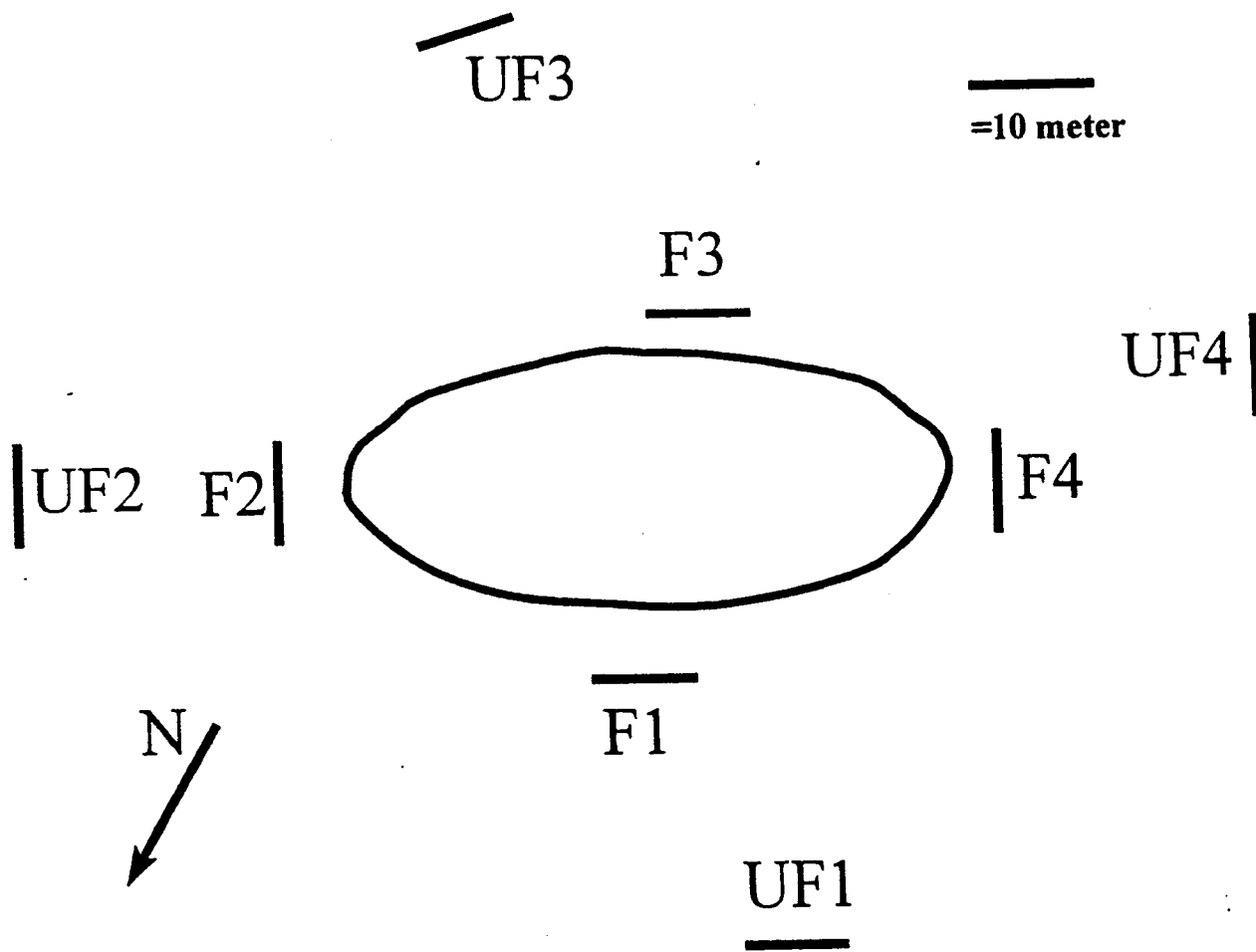
FIGURE 3. **Map of Wetland System 2.** Wetland fences are denoted by "F" and upland fences by "UF". The size of the wetland and position of fences are approximations based on survey distances. Stippled area reflects the current extent of the forested area surrounding the wetlands.

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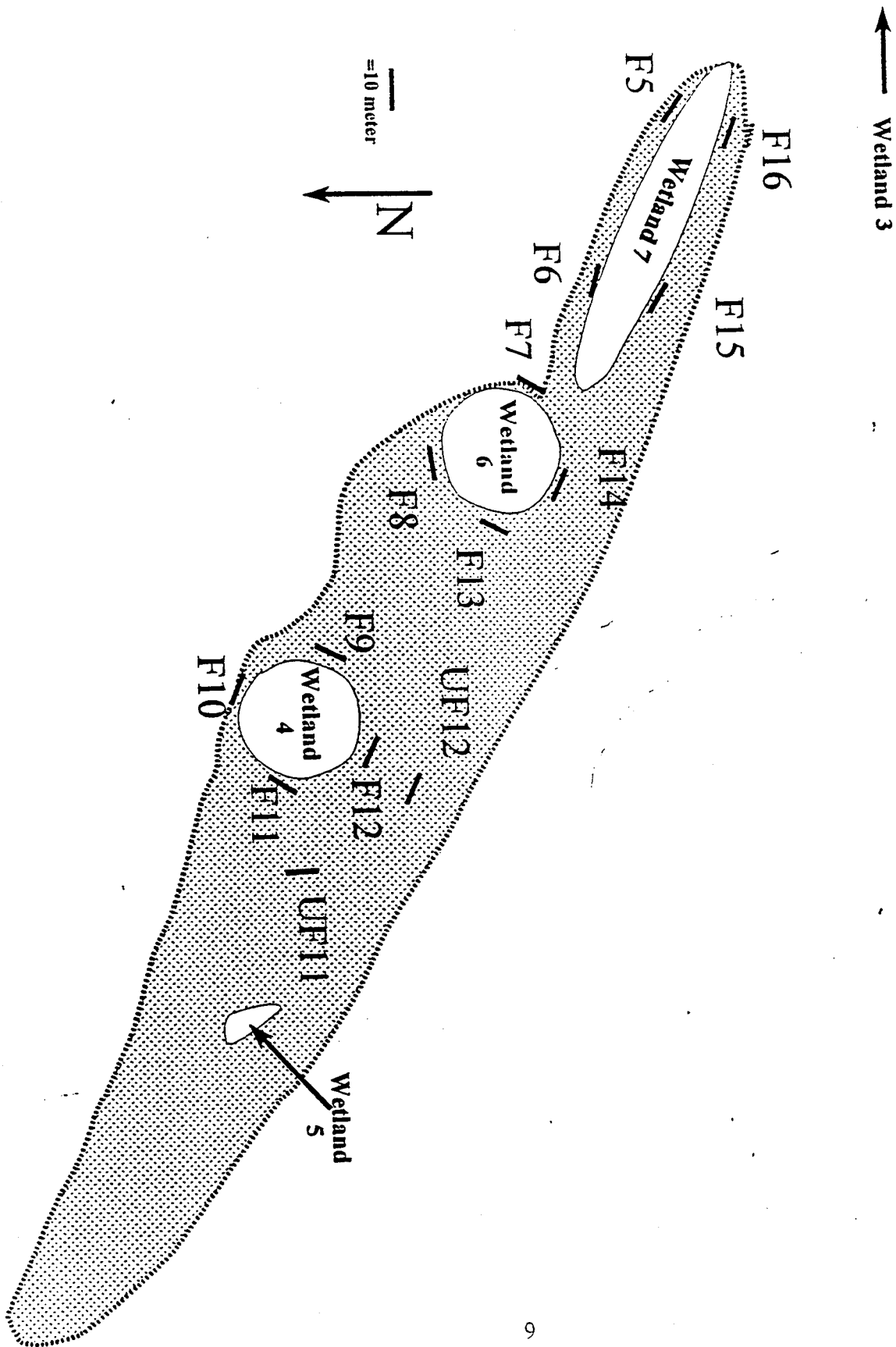
During the summer and fall of 1997, 40 acres of upland habitat surrounding WC2 were developed into athletic fields (Figure 3). Prior to this development, the upland area around wetland 7 was surrounded by a mix of old forest and abandoned vineyards (Table 1). The upland now consists of athletic field on one side and athletic field/old forest mix on the other side (Figure 3). Wetland 6 was surrounded on one side by old forest, on two sides by abandoned vineyard, and on the remaining side by wet meadow. The upland now consists of athletic field and wet meadow. Wetland 4 was surrounded by mature forest containing black cherry, maple and tulip poplar. The upland now consists of significantly reduced forest and, on the southwest corner, athletic fields less than 3 m from the wetland. During construction, a tile-drain system downhill from wetland 7 was removed, and the duration of the wet phase of the hydroperiod in all three wetlands was extended by several months.

Drift fences and pitfall traps for salamanders collection were established during the fall of 1995 along the edges of the wetland in both complexes (Figures 2 and 3). These fences were constructed of galvanized tin. Each fence was 8 m long and 0.5 m tall. Fences were established 1-3 m above the high water mark in spring. The bottom of each fence was dug into the soil to prevent animals from moving under it. Fences were supported with 1" x 2"x 3' wooden stakes located at each end and at 2 m intervals along the fence. Pitfall traps were dug at the ends and in the middle of both sides of the fences. Pitfalls were lined using 2 L plastic tubs set with the tops flush with the surface of the soil. In the fall of 1998, four additional fences were established 20 m into the upland from the original fences (Figures 2 and 3).

TABLE 1. Habitat description of WC1 and WC2.

Wetland	Fence	Before 1997	After 1997
7	5	old vineyard	athletic field
7	6	old vineyard	athletic field
7	15	old field	old field / athletic field
7	16	old field	old field / athletic field
6	7	old vineyard	athletic field
6	8	old vineyard	old vineyard / athletic field
6	13	meadow	meadow
6	14	meadow	meadow
4	9	wood	wood
4	10	wood	athletic field
4	11	wood	wood
4	12	meadow	meadow

3. POPULATION GENETICS OF *A. MACULATUM.*

INTRODUCTION

Assessing the genetic variation of populations is important because it provides a measure of the degree of isolation between the populations at a historical level, while also providing insight into present-day processes such genetic variation (Weir 1996). In the context of wetland mitigation, knowledge of such phenomena is important in determining whether active transplants of amphibians will be necessary to establish breeding populations in the new wetlands (as would be the case if the populations are genetically differentiated). Genetic information can also guide choices regarding which populations would be better candidates for transplantation (i.e., those with a higher degree of genetic variation).

Essentially no knowledge exists regarding small-scale population genetics of *A. maculatum*. For ambystomids, as well as for amphibians in general, much of the use of molecular genetics has been confined to determination of biogeographic patterns or phylogenies (Phillips 1994; McKnight and Shaffer 1997). However, the fidelity of *A. maculatum* to natal ponds (Stenhouse 1985; Whitford and Vinegar 1966) coupled with its limited home range implies a potential for *A. maculatum* populations to become genetically isolated. The large distance and the human-made barriers including major roads and buildings between the two wetland systems on the Penn State Erie campus makes the potential for reproductive isolation of the two populations high.

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To evaluate the genetic variability and the degree of isolation of the salamander populations, we utilized molecular tools. We analyzed mitochondrial DNA because this approach is considerably less time-consuming and expensive than other methods of molecular genetic assessment (e.g., microsatellites). A particularly variable segment of the mitochondrial DNA (mtDNA) located between the genes coding for Threonine and Proline transfer RNA has been shown to be effective in differentiating ambystomid populations over large geographic distances (McKnight and Shaffer 1997). We modified this segment to suit the Penn State Erie population of *A. maculatum*, and compared mitochondrial DNA sequences among individuals collected from different wetlands.

METHODS

Isolation of DNA from *A. maculatum*

The DNA utilized in this study was isolated from fertilized eggs collected during the breeding season in 1995 from each of the wetlands in the survey area. In 1996, 1997, 1998, and 1999, toes from individually identified adult salamanders were collected and preserved at -80°C. Each toe was stored in an individually labeled microcentrifuge tube.This repository of toes represents several hundred individuals collected from each year of the study from all wetland habitats; the DNA from toes will be analyzed at a future date.

DNA was isolated from eggs using 400μl of ice-cold extraction buffer (1.0 M NaCl, 50 mM Tris (pH 8.0), 5 mM EDTA, 2% hexadecyl ammonium bromide, 0.1% bovine serum albumin). The solution was cleared of debris by centrifugation at 12,000 X g for 10 minutes at

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4°C. The supernatant was extracted twice with 300μl of chloroform and the DNA was then collected by ethanol precipitation. Quantification of DNA was performed using a fluorometer.

Amplification of DNA

In the mitochondrial genome of the Ambystomidae there is an intergenic space region located between the tRNA threonine and tRNA proline (McKnight and Shaffer 1997). This spacer region is suspected to be noncoding and it demonstrates a significant level of variability between species within the ambystomids, as well as populations from geographically distant populations.To amplify this segment of the mitochondrial genome, we utilized polymerase chain reaction (PCR) in combination with primers that flank the intergenic region. Amplification of *A. maculatum* DNA using the THR and 007 primers resulted in a DNA fragment of about 500 base pairs (bp) in length. The size of this DNA amplification product is in agreement with the results of McKnight and Shaffer (1997) who demonstrated that the intergenic spacer in *A. maculatum* is about 400 bp in length. The resultant amplified segment mtDNA, however, was of poor quality. A new primer, SALLY SEQ (Appendix B), was created based on sequence information for 225 bp (Appendix C) within the intergenic spacer described above. It resulted in better yields of PCR product. A dye-terminator fluorescent cycle sequencing kit (Perkin Elmer Inc., Forest City, CA) was used for visualization. DNA sequence was then determined by the Penn State Nucleic Acid Facility.

Analysis of mtDNA sequences from *A. maculatum*

DNA sequences were aligned using the GCG program PILEUP (Oxford Molecular

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Group). The Penn State Erie populations of *A. maculatum* were compared to a control

population from Virginia. The pattern of genetic variation within each of the wetland populations

was assessed using the Jukes-Cantor and Maximum Likelihood Distance algorithm in the

computer package PHYLIP (Felsenstein 1993). Data were then plotted using the Neighbor

Joining Procedure in PHYLIP (Felsenstein 1993). Sequence data from the Virginia population

was included in this analysis to provide a benchmark for analyzing the Penn State Erie

population.

RESULTS

An alignment for the amplified 225 bp sequence is found in Appendix C. A shift in the

sequence at position 161 of the hypervariable region in the Virginia population to position 163

in the Penn State Erie population is indicative of divergence between the Virginia and

Pennsylvania populations. In general, sequences from the Pennsylvania salamanders were quite

similar, indicating a high degree of homogeneity within the PSU Erie population. However,

comparison of the PSU Erie sequences revealed a nucleotide deletion at position 7 that is unique

to the WC1 population. This deletion was not present in WC2 (wetlands 3, 5, and 7) nor was it

present in sequences from Virginia salamanders. This deletion can be used to identify the WC 1

salamanders, and its consistent presence in WC1 but not in WC2 strongly suggests that the

populations of the two wetland complexes are at least partially reproductively isolated.

FIGURE 4. Neighbor-joining tree of _A. maculatum_ individuals. The unrooted phenogram is based on Jukes-Cantor distance.
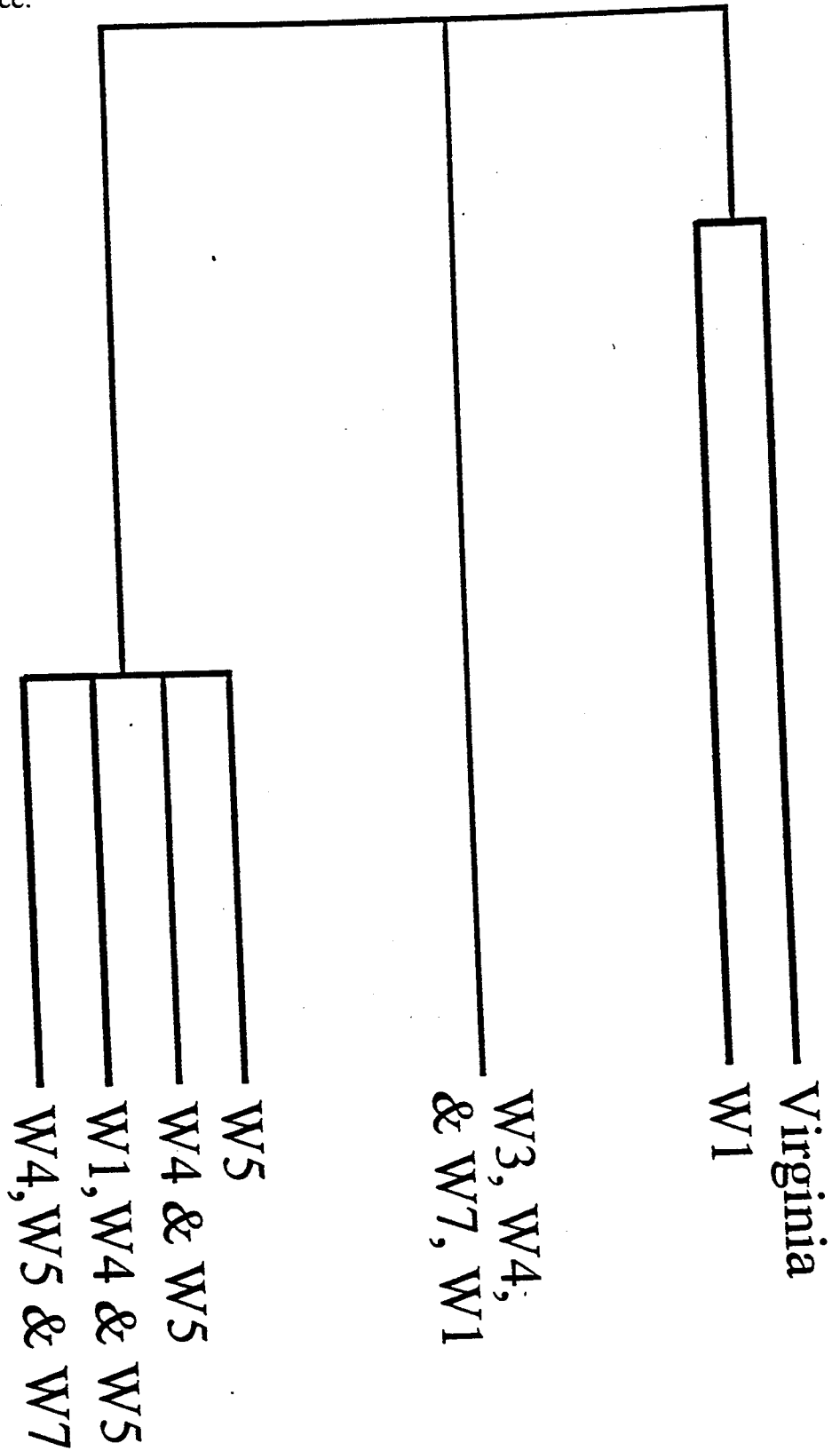
The DNADIST analysis further supports the notion that the populations of the two wetland complexes are reproductively isolated. The unrooted dendrogram (Figure 4) shows that the Virginia and two Penn State Erie populations occupy separate nodes. Overlap between the two Penn State Erie populations is indicated by the presence of individuals from each population in the limb dominated by the other population. However, no overlap was found between the Virginia population and the Penn State Erie population. The interpretation is that some gene flow (migration) occurs between the two wetlands, but that the gene flow is restricted, resulting in effectively isolated populations.The shape of the dendrogram also indicates that some gene flow occurs among the adjacent wetlands in WC2. Because these wetlands are essentially contiguous during the breeding season, it is surprising that there is not more mixing of the populations.

CONCLUSION

The salamanders in the Penn State Erie wetland complexes make up a single, relatively homogeneous population that can be subdivided into two genetically distinct populations on the basis of a single base pair change. Despite the contiguity of the wetlands in WC2, *A. maculatum* populations from each of the populations tend be more similar to one another than to the adjacent wetland population. This implies that gene flow does occur among the populations, but at a reduced rate, suggesting that the salamanders utilizing each wetland are best viewed as subpopulations.

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4. SEASONAL AND SPATIAL MIGRATION PATTERNS
IN *A. MACULATUM.*

INTRODUCTION

The reproductive migration of *A. maculatum* occurs during early spring and is dependent upon a combination of temperature and precipitation (Duellman and Trueb 1986). Movement occurs at night when the air temperature is 0°C or greater and precipitation occurs. Because the weather conditions during spring can vary daily from favorable to unfavorable conditions, the migration of *A. maculatum* is punctuated by episodes of mass migration during warm and wet conditions and little or no migration during colder and drier conditions. The migration period typically lasts until mid- to late April, when it ends regardless of air temperatures. Males often initiate migration at an earlier date than females (Beneski et al. 1986).

Several studies have demonstrated that *A. maculatum* migrates to breeding wetlands from upland habitat along specific corridors (Shoop 1965; Douglas and Monroe 1981; Stenhouse 1985; Beneski et al. 1986; Phillips and Sexton 1989). Adults are thought to locate migration corridors and wetlands by way of olfaction (Stenhouse 1985). The corridors are used year to year (Stenhouse 1985; Beneski et al. 1986), and the orientation behavior may expedite migration so that salamanders do not have to look for suitable wetlands for reproductive habitat (Phillips and Sexton 1989). Thus, disruption of migration corridors could reduce successful reproduction by preventing or delaying adult movement into breeding habitat. In general, adults return to their natal wetlands to reproduce. However, they are capable of colonizing new wetlands, and fidelity to natal wetlands probably is not absolute.

In this chapter we describe the spatial and temporal migration characteristics of breeding migrations of *A. maculatum* on the Penn State Erie campus over a four-year period.

METHODS

Migrating salamanders were captured using drift fences and pitfall traps as described in chapter 2. All pitfalls were checked each morning prior to the beginning of, and throughout the entire migration period, and the number of salamanders captured at each fence on each day was recorded. For each fence on each day, a minimum of 10 individuals was sexed, weighed to the nearest 0.1 g, measured from snout to vent to the nearest 1 mm, and toe clipped prior to release. Temperature and precipitation were recorded for each morning.

To help assess the distance salamanders migrate, we established upland fences during the fall of 1998 (see chapter 2, figures 2 and 3). Salamanders captured at the upland fence were handled in the same manner as described above. Toes were clipped in a manner so as to distinguish the adults captured at the upland fences from adults captured at the wetland fences.

RESULTS

Climate and Migration

As expected, yearly and seasonal patterns in the timing of migration and the number of migrants were significantly influenced by weather conditions. Timing of the breeding migration did not differ for salamanders inhabiting WC1 and WC2. Migration began much later during the cold and dry springs of 1996 and 1999 (migration began on April 12th and April 3rd respectively) than during the warmer springs of 1997 and 1998 (migration began on March 22nd and 19th

respectively). Despite the differences in starting dates, migration ended just before the last week in April in all four years. During any given year, migration was episodic as evidenced by the spikes in the number of salamanders captured each day. The number of captures was significantly higher in mornings when air temperature was above freezing and precipitation had fallen during the previous night (Figures 5, 6, 7, and 8).

The Spatial Pattern of Migration

Captures of migrating of *A. maculatum* were not evenly distributed among the fences (see Table 2 and Figure 9). In each wetland, captures at one fence accounted for greater than 50 percent of the total salamanders that moved into the wetland (Figure 9). In the case of wetlands in WC2, the "favored" fences were located near mature forest. It should be noted that fence 10, a "favored" fence previously located in mature forest, continued to be favored even after it became part of the sideline of a new athletic field. Non-random capture patterns also were observed in WC1 where the upland habitat appeared similar near all fences. Fence 3, the "favored" fence in WC1, was located near the outflow of the wetland where the soils were continuously saturated. It is probable that this fence was located directly in the migration corridor.

The number of captures at upland fences was approximately a third of the total number of salamanders intercepted in 1999 (Table 3). However, only eight of the salamanders marked at upland fences were recaptured at wetland fences.

FIGURE 5. Variation in migrating salamanders and temperature for 1996. The heavy line is the total number of salamanders captured from both wetland complexes. Hatched line is the temperature, in °C, at the time of surveying the pitfall traps (0700 - 0800 hours).
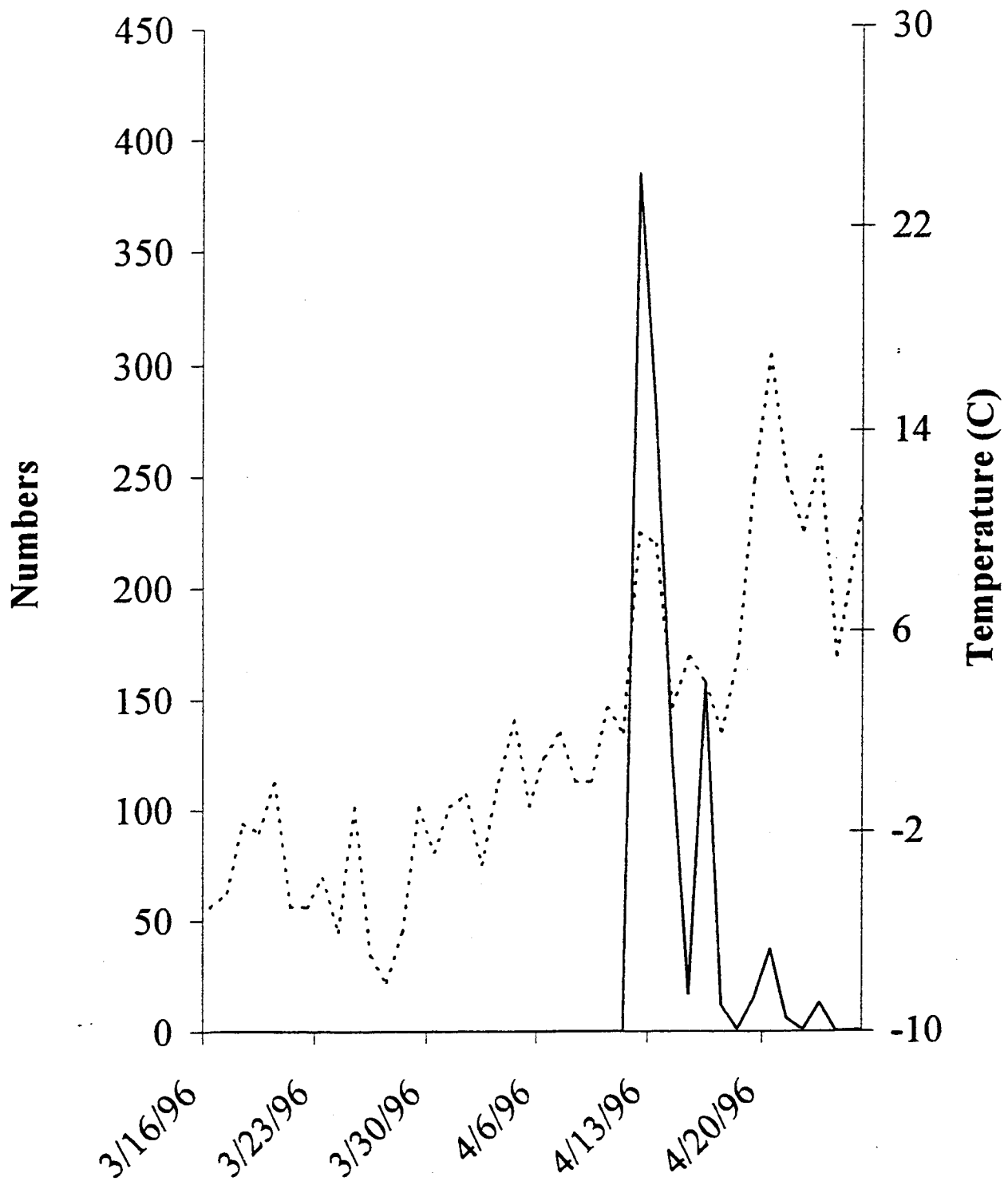
FIGURE 6. Variation in migrating salamanders and temperature for 1997. The heavy line is the total number of salamanders captured from both wetland complexes. Hatched line is the temperature, in °C, at the time of surveying the pitfall traps (0700 - 0800 hours).
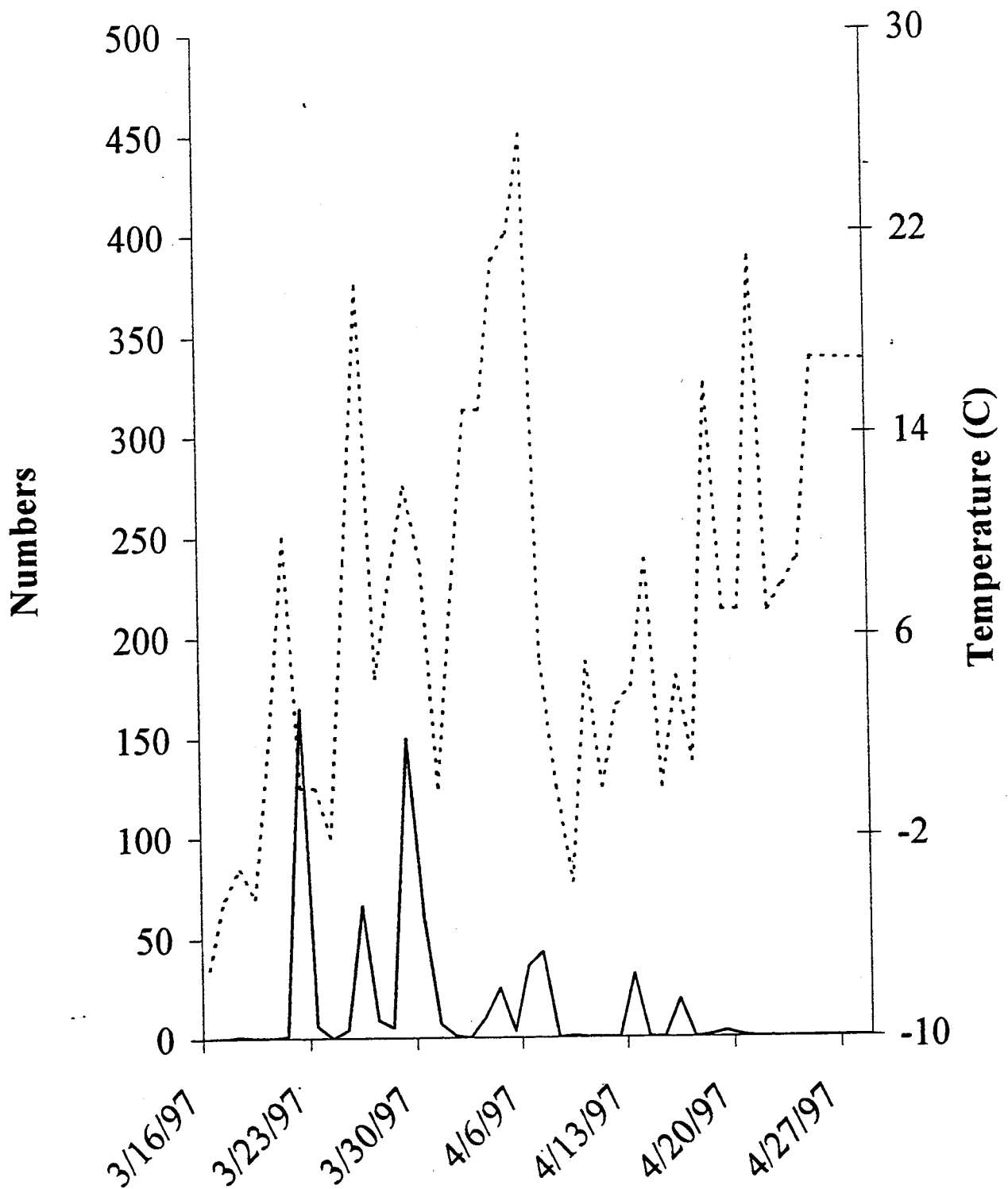
FIGURE 7. **Variation in migrating salamanders and temperature for 1998.** The heavy line is the total number of salamanders captured from both wetland complexes. Hatched line is the temperature, in °C, at the time of surveying the pitfall traps (0700 - 0800 hours).
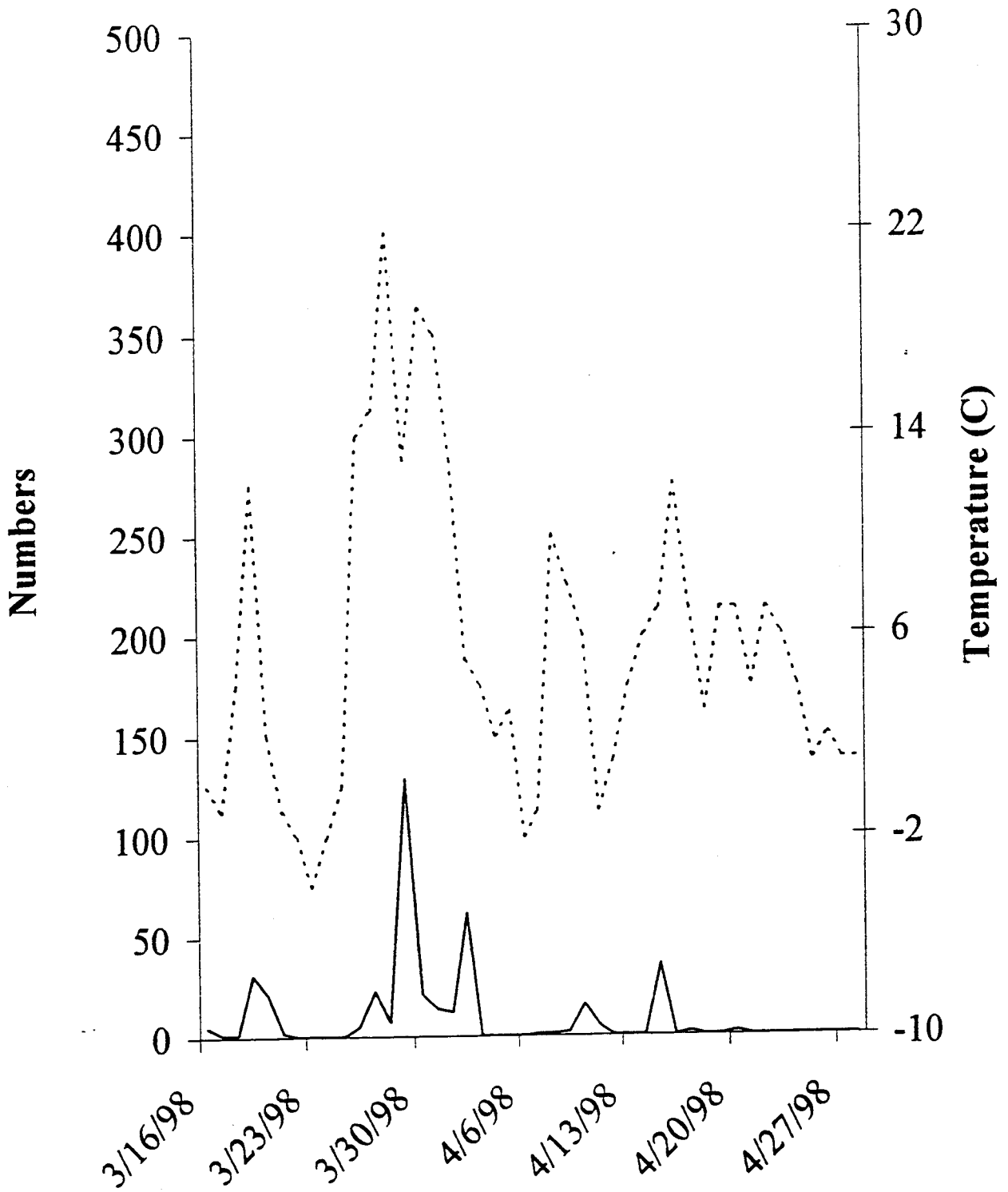
FIGURE 8. Variation in migrating salamanders and temperature for 1999. The heavy line is the total number of salamanders captured from both wetland complexes. Hatched line is the temperature, in °C, at the time of surveying the pitfall traps (0700 - 0800 hours).
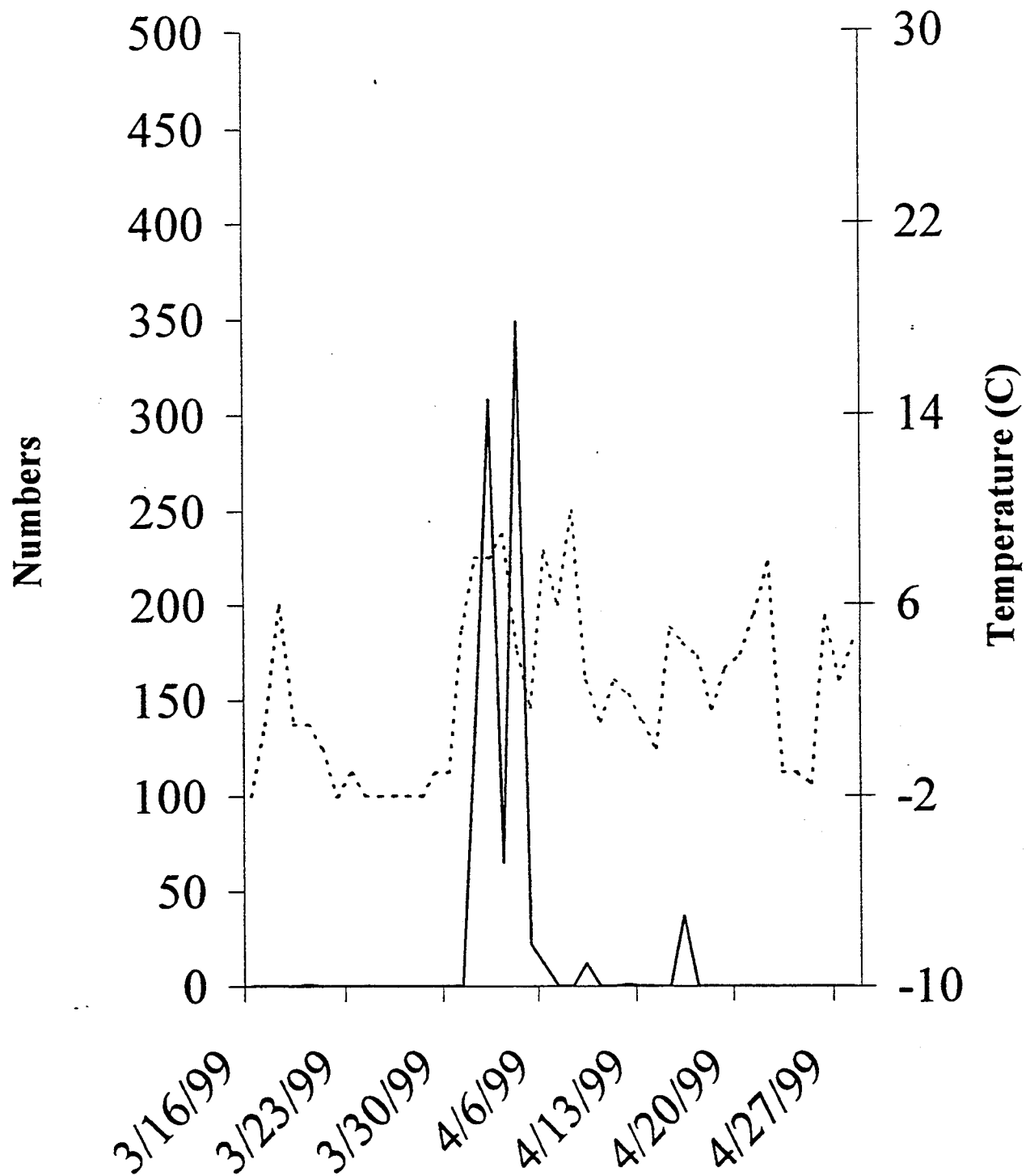
TABLE 2. Yearly salamander captures by fence.

	1996	1997	1998	1999	4 YEAR TOTAL	4 YEAR AVERAGE
F1	77	46	8	59	190	47.5
F2	106	135	59	110	410	102.5
F3	395	258	102	153	908	227
F4	35	60	44	65	204	51
F5	0	1	0	1	2	0.5
F6	0	0	0	1	1	0.25
F7	4	2	8	1	15	3.75
F8	10	2	3	11	26	6.5
F9	6	4	3	5	8	2
F10	77	63	39	29	208	52
F11	256	111	86	144	597	149.25
F12	59	42	20	42	163	40.75
F13	66	11	7	19	103	25.75
F14	5	8	4	13	30	7.5
F15	3	5	10	7	25	6.25
F16	6	4	6	7	23	5.75
Total	1105	752	399	809	3065	766.25

FIGURE 9. Percent of salamanders captured by fence. Each pie chart represents the percent contribution of fences to the total migrating salamander population over the 4 years study period to each wetland.

Wetland 1

(13.16%) — F4
(12.82%) — F1
(24.22%) — F2
F3
(49.80%)

Wetland 7

(3.89%) — F5
(1.95%) — F6
(44.86%) — F16
(49.30%) — F15

Wetland 6

(15.79%) — F14
(8.77%) F7
(15.80%) F8
F13
(59.64%)

Wetland 4

(16.18%) — F12
(1.82%) F9
(21.33%) — F10
F11
(60.67%)
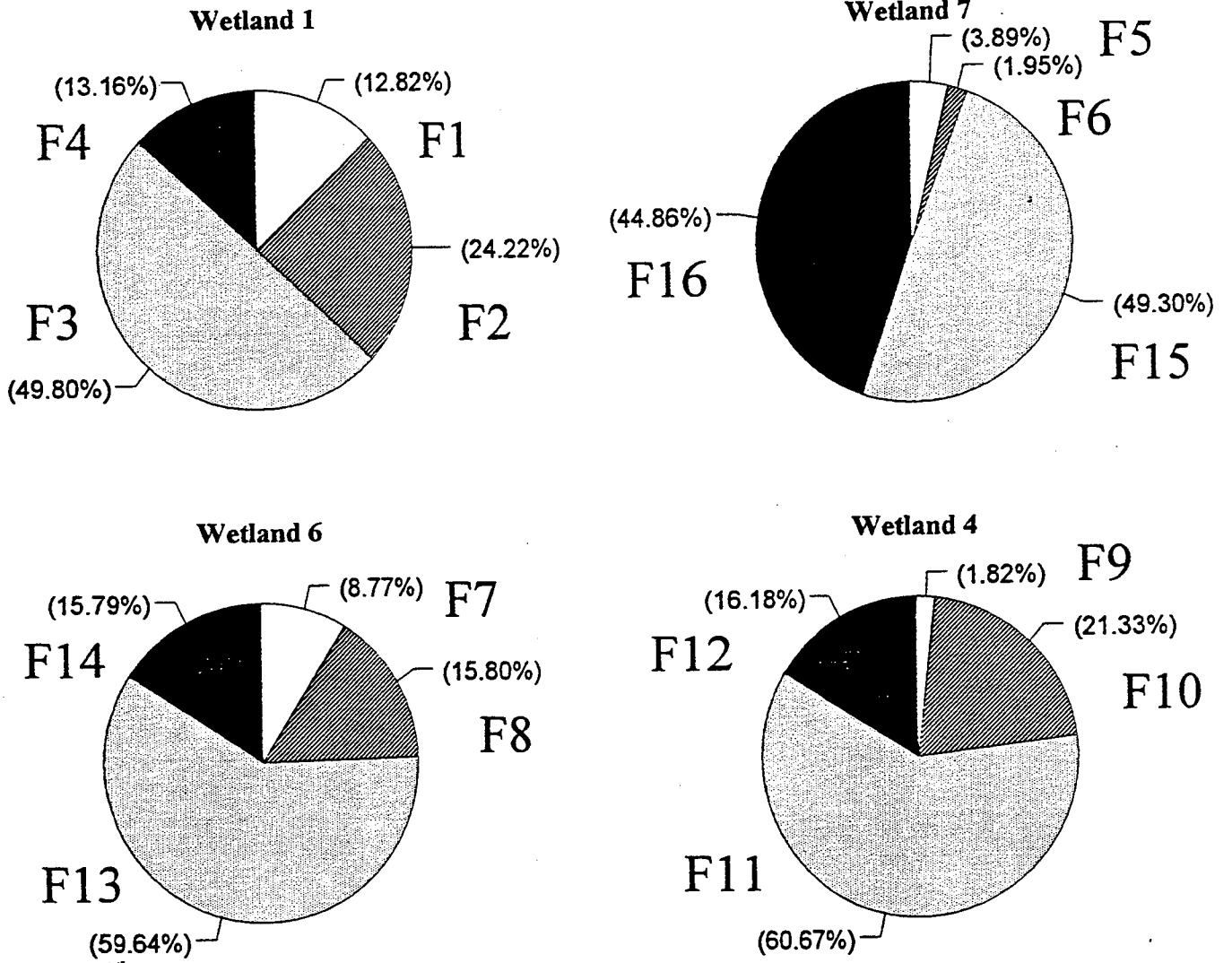
TABLE 3. The upland habitat migration.

Fence	Number Captured	% Contribution
F1	59	28.0
UF1	23	
F2	110	19.0
UF2	27	
F3	153	45.5
UF3	128	
F4	65	19.0
UF4	16	
F11	144	25.8
UF11	50	
F12	42	46.7
UF12	35	

CONCLUSION

The migration behaviors of the Penn State Erie populations of *A. maculatum* are similar to those described in the literature: migration is dependent upon favorable conditions including temperature and precipitation, and it does not originate randomly from the upland habitat. In general, migration does not begin until the third week in March and is completed by the end of April. During this interval, migration is dependent upon relatively warm and moist nights.

The majority of the adults migrated from specific regions in the upland area. The spatial pattern of captures was repeated every year. Fences favored in 1996 still were favored in 1999, in spite of changes in upland habitat quality. For each wetland system, one fence accounted for 50 percent of the total yearly migrating population to a wetland. Roughly 30 percent of the migrating population originated in habitat beyond the upland fence, a distance of greater than 20 m.

5. THE POPULATION BIOLOGY OF *A. MACULATUM* AND THE IMPACT OF UPLAND DEFORESTATION.

INTRODUCTION

Because the life span of *A. maculatum* approaches 20 years (Flageole and LeClaire 1992), long-term population studies, especially of populations threatened by development, are essential even though they may be difficult to conduct. Currently, the longest reported study of *A. maculatum* covered a span of five years (Husting 1965), during which the estimated population abundances fluctuated from year to year. In longer studies (12 years) of *Ambystoma* congeners, estimated population abundances also exhibited yearly fluctuations (Pechmann et al. 1991). It is not clear whether the fluctuations in the yearly estimates of population size (estimated on the basis of captures during the breeding migration) reflect real changes in abundance or changes in the proportion of the population migrating in any given year.

The long life span and large body size of *A. maculatum* suggests the importance of quality upland habitat to sustaining the population. *A. maculatum* must live and feed in upland habitat for 10 - 11 months of the year, and the resources in the upland must be sufficient to support the adult population. Currently, there are no published studies of the importance of upland habitat quality to the population dynamics of *A. maculatum*. However, in a study of a congener, *A. talpoideum*, clearcutting of the upland habitat effectively reduced the number of migrating adults (Chazal and Niewiarowski 1998). Concomitantly, there was a reduction in the size (indicator of reproductive output) of the migrating adults, an indication of a future decline in population size.

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In this chapter, we present the findings of four years of population monitoring of the Penn State Erie populations of *A. maculatum*. The information from this study describes the current status and yearly fluctuations of the population levels and quality of WC1 and WC2 migrating adults. In addition, we examine the impact of the loss of habitat surrounding WC2 on the abundance and quality of migrating *A. maculatum*.

METHODS

As described in chapter 2, a system of drift fences and pitfall traps was established around both wetland complexes. All pitfalls were checked each morning prior to the beginning of and throughout the entire migration period. For each fence on each day, a minimum of 10 individuals were sexed, weighed to the nearest 0.1 g, measured from snout to vent to the nearest 1 mm, and toe clipped prior to release. All *A. maculatum* were counted, checked for marks, and released on the side of the fence opposite the one at which they were captured. Because toes grow back within a year of clipping, marks from previous years were not assessed. Thus, reliable estimates of individual survivorship from year to year are not available.

The size of the population around each wetland was estimated using the Schnable method (Krebs 1989). This mark-recapture method is used to estimate population size for mobile individuals when there are multiple sampling dates, and it assumes a constant population size during the sampling period. For each wetland on a given day, we pooled the salamanders collected among fences to calculate the population estimate. Drift fences did not completely surround wetlands, and only a fraction of the migrating salamanders were intercepted. To account for the area sampled by the drift fences, which comprised only a fraction of the area of

the wetland system, we multiplied the calculated population estimate by a factor of 10. This is a conservative estimate, as the fences blocked less than 10 percent of the perimeter of the wetlands.

Two types of size data were analyzed: snout vent length (SVL) and wet weight (WWT). SVL was used as an indication of the age of the salamander, while WWT was used as an indication of its reproductive quality. Changes in SVL reflect changes in the age structure of the migrating population, while changes in WWT reflect changes in the reproductive condition of the migrating adults. The data were analyzed using factorial design ANOVA (SAS Institute Cary NC). A total of 1245 salamanders were used for this analysis.

RESULTS

Population Estimates

Estimated population sizes differed significantly between wetland complexes (see Table 4 and Figure 10). Wetland 1 had the largest estimated population size, with a four-year average of 13,665 adults.This estimate was 150 percent of the population estimate for wetlands 4, 6, and 7, combined. Wetland 7 had the smallest estimated population size, with a 4-year average of 387 adults (Figure 11). Of the salamanders captured in WC2, 88 percent were captured near wetland 4, suggesting a much larger population in the upland (mature forest) surrounding wetland 4 than in the upland (abandoned vineyard or athletic field) around wetlands 6 or 7. Population estimates for WC1 showed some variation, but they did not differ significantly among years. However, the estimated population size for WC2 was significantly lower in 1999 than in the three previous years.

TABLE 4. Mark and recapture data. Total number captured (#T), marked (#M), and recaptured (recap) salamanders at each fence for the four years of the fences.

	1996			1997			1998			1999		
	#T	#M	Recap	#T	#M	Recap	#T	#M	Recap	#T	#M	Recap
F1	77	17	0	46	40	2	8	8	4	59	59	2
F2	106	23	1	135	79	17	59	59	4	106	106	4
F3	395	19	2	258	80	9	102	102	8	150	150	3
F4	35	9	2	60	49	4	44	44	4	59	59	6
F5	0	0	0	1	1	0	0	0	0	1	1	0
F6	0	0	0	0	0	0	0	0	0	1	1	0
F7	4	3	1	2	2	0	8	8	1	1	1	1
F8	10	8	1	2	2	3	3	3	1	11	11	2
F9	6	0	0	4	4	0	3	3	1	8	8	3
F10	77	15	2	63	54	3	39	39	1	29	29	0
F11	256	34	3	111	86	14	86	86	13	137	137	7
F12	59	4	1	42	36	6	20	20	0	36	36	6
F13	66	8	0	11	10	0	7	7	2	21	21	3
F14	5	1	0	8	6	1	4	4	0	13	13	0
F15	3	2	0	5	5	1	10	10	1	7	7	0
F16	6	1	0	4	4	0	6	6	1	7	7	0
Sum	1105	144	13	752	458	60	399	399	41	646	646	37

FIGURE 10. The migrating *A. maculatum* population by wetland complex. Bars represent estimated population sizes. Error bars are ± 95% confidence intervals (Krebs, 1989).
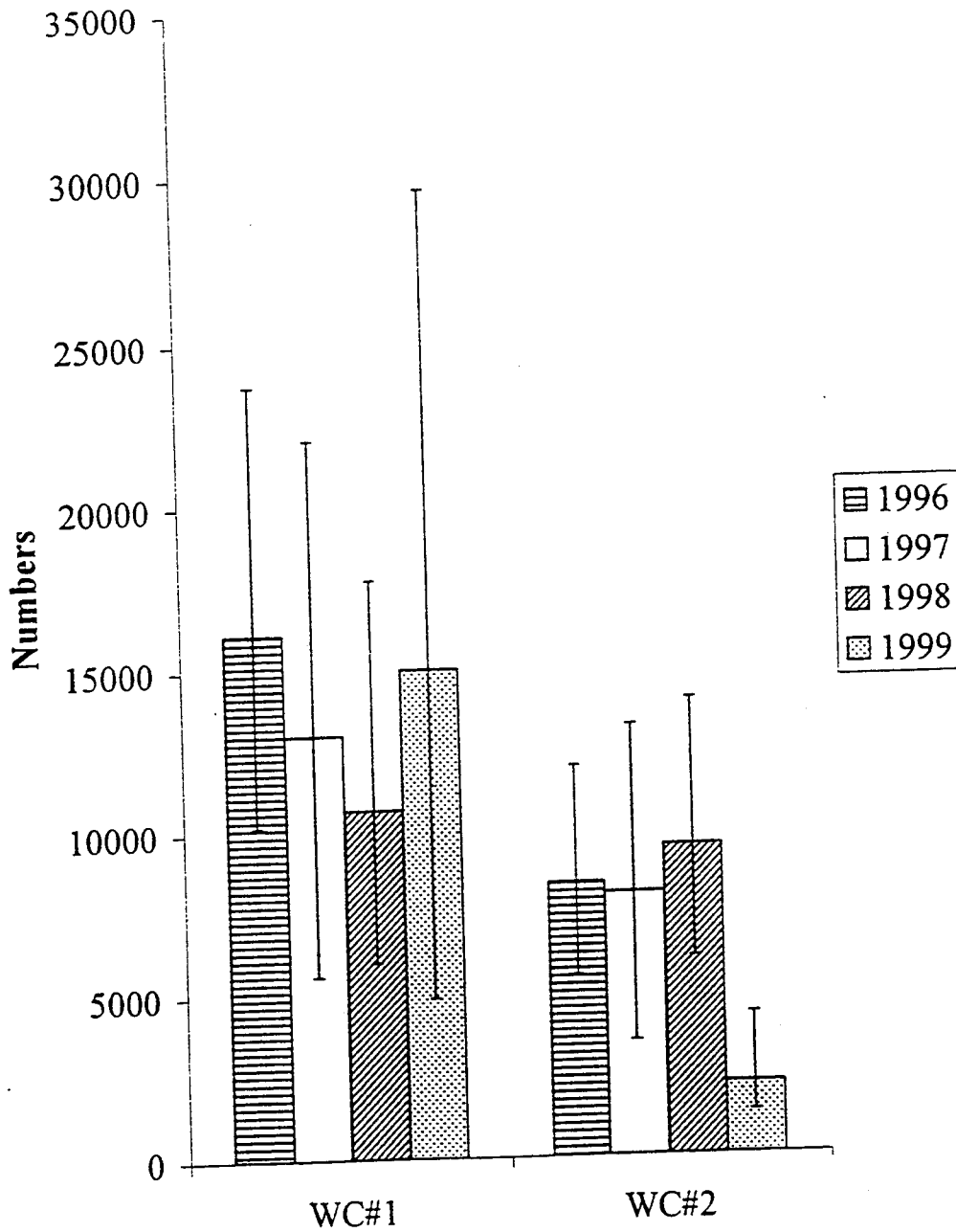
FIGURE 11. **The migrating** *A.* *maculatum* **population by wetland.** Bars represent estimated population sizes. Error bars are ± 95% confidence intervals (Krebs, 1989).
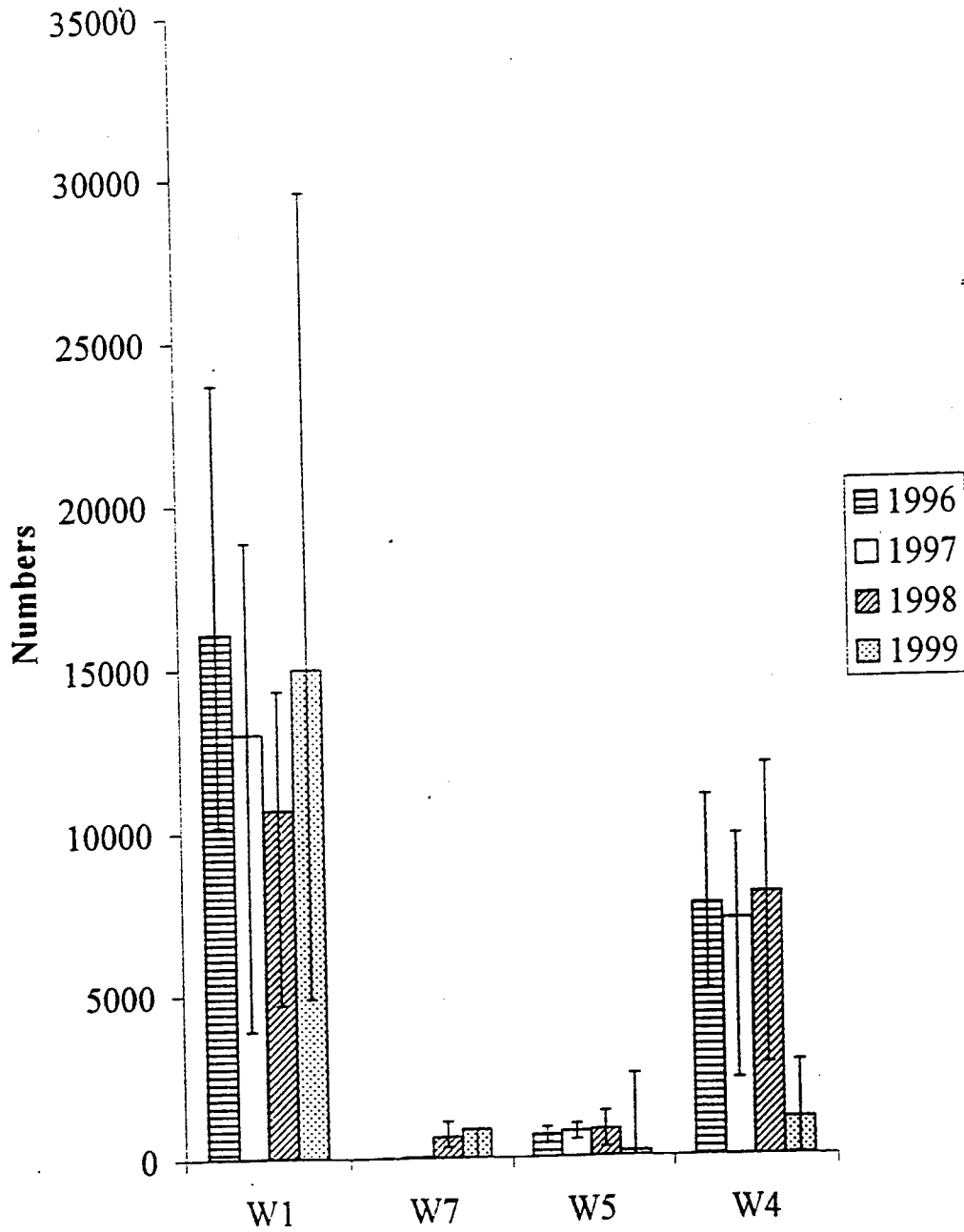
A sharp decline (25 percent) in the estimated number of salamanders living near WC2 occurred one year after completion of the construction of athletic fields and reduction of mature forest surrounding the complex. Much of the reduction is associated with wetland 4, which was originally surrounded by forest. The estimated reduction in the number of adults migrating into wetland 4 is approximately 6500. Wetland 7 also experienced a precipitous decline (80 percent) in migrating adults.

Condition of Migrating Salamanders

The age structure of the populations, as indicated by SVL, did not differ between years or wetland complexes (see Table 5 and Figure 12). Mean wet weights of the migrating adults fluctuated during the study (see Table 6 and Figure 13). In general, the males and females from WC2 weighed the same as, or were slightly larger than the WC1 adults. However, during 1999, WC2 adults were significantly smaller than the WC1 adults. The reduction in wet weight of migrating adults captured in WC2 was first evident in 1998 and continued in 1999. Wet weight of adults is correlated with reproductive output for individuals of the same length. Because reduced wet weight was not associated with reduced SVL length, the change in weight is interpreted to indicate that the migrating adults were of the same age but lower reproductive quality than migrating adults in 1996 or 1997. A similar change did not occur in WC1.

The construction of the Penn State Erie athletic fields was completed in summer 1997. Construction was accompanied by a loss of mature forest, an important source of shelter and food for adult salamanders during the 10 - 11 months of the year when they are not breeding. Loss of high quality upland habitat may have reduced food availability, causing the observed pattern of lower wet weight without accompanying change in SVL.

TABLE 5. Factorial ANOVA of snout vent length.

Source	DF	Type III SS	F	P
Year	3	2578	0.27	0.84
Wetland Complex	1	578	0.18	0.66
Sex	1	3616	0.39	0.76
Year*Wetland Complex	3	1110	0.36	0.55
Year*Sex	3	2942	0.31	0.82
Wetland Complex*Sex	1	578	0.19	0.67
Year*Wetland Complex*Sex	3	3128	0.33	0.80

FIGURE 12. Snout vent length variation by sex, wetland complex, and year. Plots are means ±sem.
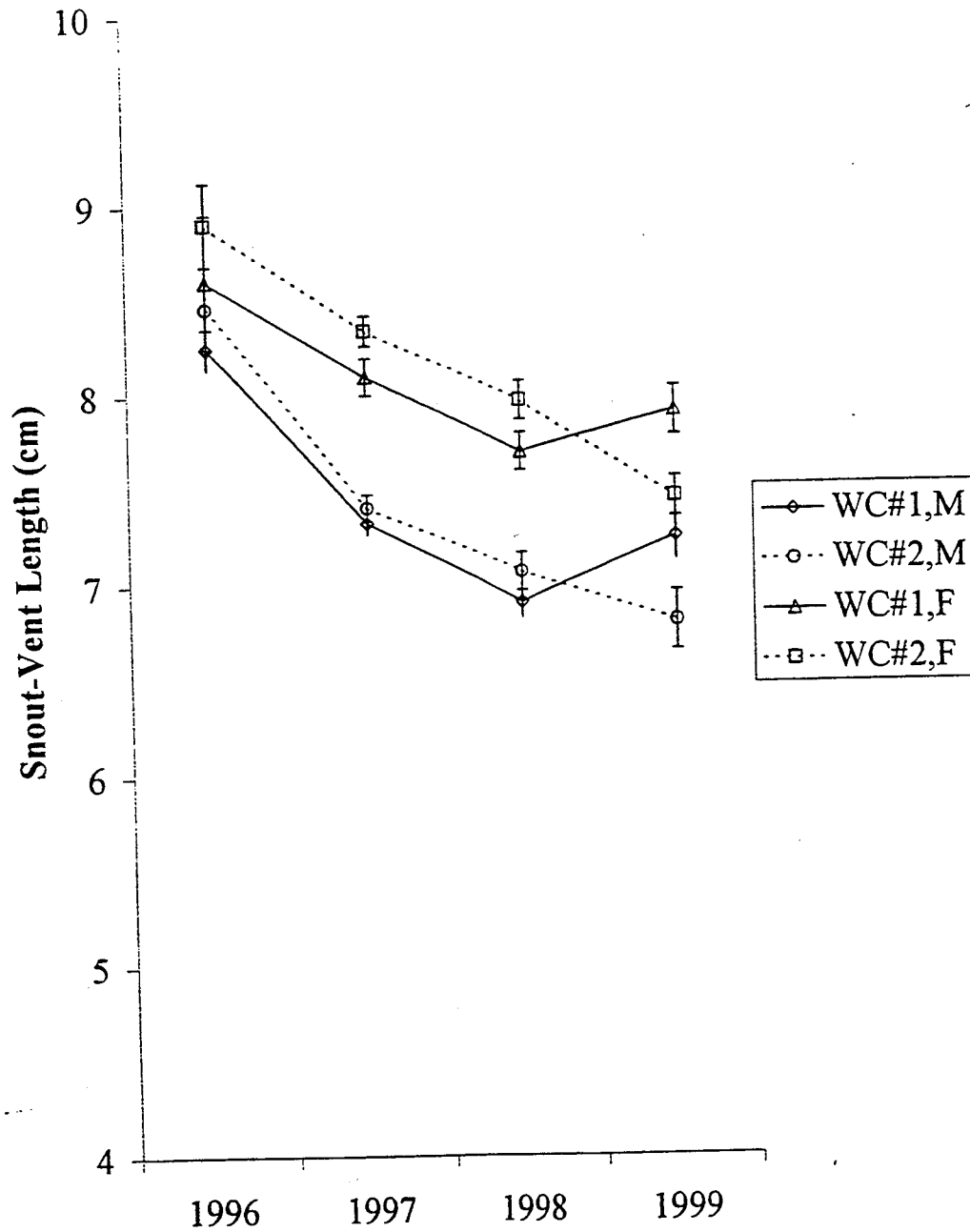
TABLE 6. Factorial ANOVA of wet weight.

Source	DF	Type III SS	F	P
Year	3	7324	68.75	0.0001
Wetland Complex	1	146	4.14	0.0422
Sex	1	6039	170.09	0.0001
Year*Wetland Complex	3	1110	9.71	0.0001
Year*Sex	3	511	4.81	0.0025
Wetland Complex*Sex	1	3	0.09	0.7700
Year*Wetland Complex* Sex	3	29.6	0.28	0.8400

FIGURE 13. Wet weight variation by sex, wetland complex, and year. Plots are means ± sem.
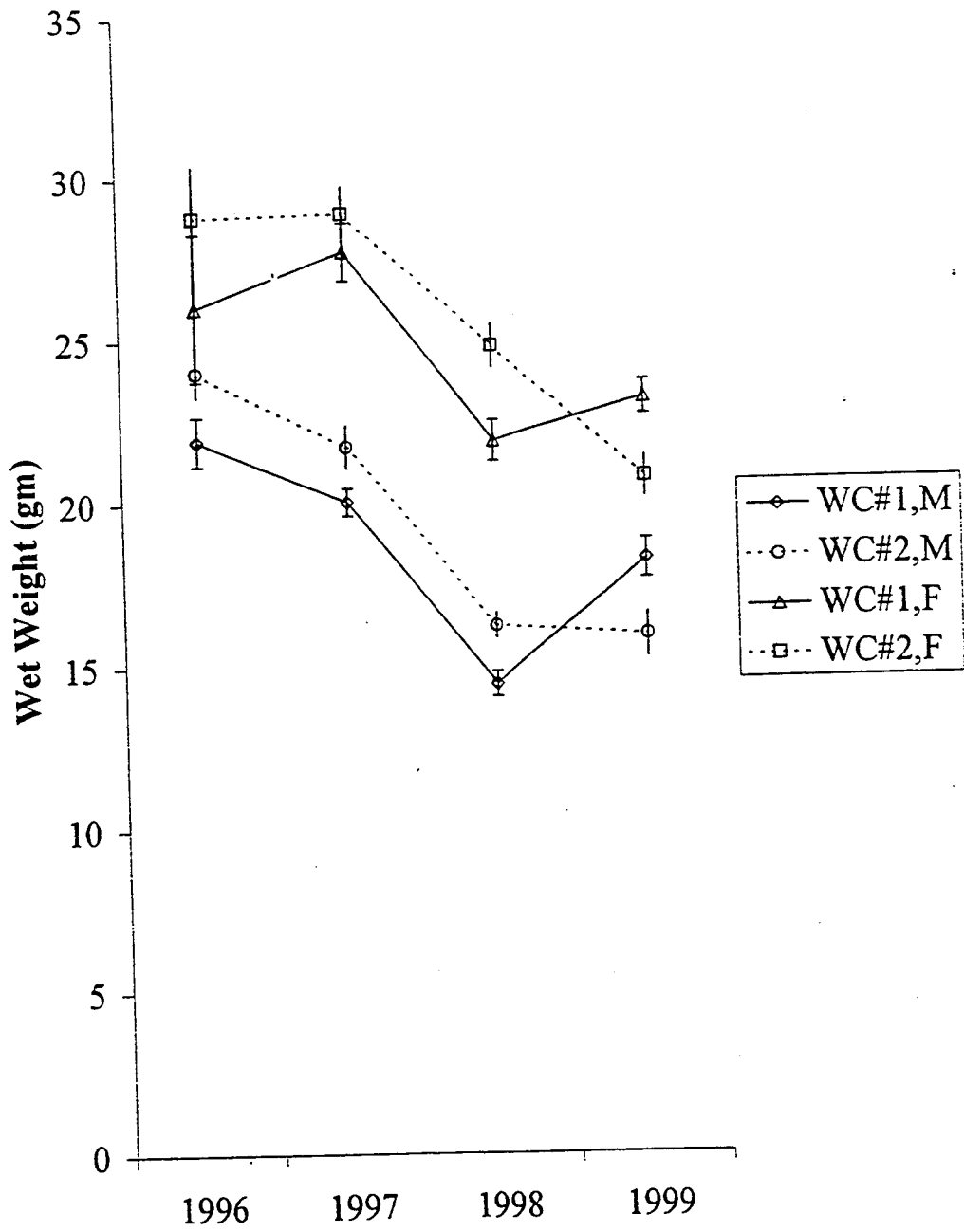
CONCLUSION

The result of the population survey strongly suggests that the area and the quality of the upland habitat affect the number and reproductive quality of salamanders supported by a wetland complex. Even before construction of the athletic fields, WC2 supported fewer salamanders than WC1, in spite of the larger wetland area in WC2. More than 50 percent of the upland habitat around WC2 was abandoned vineyard, and virtually no salamanders were captured at fences adjacent to these areas. On the other hand, WC1 was surrounded by mature forest, and it supported a significantly larger population despite its smaller size. Following construction of the athletic fields around WC2, the area of mature forest was significantly reduced. In the years after the change in upland habitat type, the number of salamanders and the size of the captured individuals decreased significantly. Because a similar deterioration of the population was not observed in WC1, the changes at WC2 cannot be attributed to a "bad year." The conclusion reached on the basis of this survey is that upland habitat quality around WC2 declined and the decline had a strong negative effect on the salamanders.

6. CONCLUSION AND PROSPECTUS

From this study, we can conclude that the wetlands on the Behrend campus support a large, genetically homogenous population of *A. maculatum*. However, sufficient variability is present for us to conclude that some degree of reproductive isolation exists between populations in different wetland complexes. The population is genetically distinct from others in the eastern United States. We also can conclude that the *A. maculatum* populations are dependent upon the area and quality of the upland habitat surrounding the breeding wetlands. The salamanders require continuous tracts of mature upland forest around the wetlands for survival and for reproductive success. Destruction of upland forest or reduction of the area of forest adjacent to the wetlands results in fewer and smaller adults migrating to the wetlands and reduced recruitment of new individuals, ultimately leading to the extinction of populations in isolated small tracts of trees as the existing adults die of old age. Taken together, these results provide important insights into the potential effects of the construction of the East Side Access Highway on the population of salamanders.

7. RECOMMENDATIONS

On the basis of this study, we feel confident making the following recommendations regarding mitigation of the effects of road construction on the salamander population on the Penn State Erie campus.

1. **The mitigation wetlands must be designed in such a way as to ensure that standing water is present from March through July** to accommodate the period of time required for metamorphosis. **The depth of the wetlands should not exceed 1 m and most of the bottom area should be under less than 0.5 m of water.** If possible, woody twigs should be present in the wetland to facilitate egg laying.

2. The analysis of the genetic structure of the salamanders around WC1 and WC2 suggests that all of the salamanders on campus belong to a single large breeding population, rather than to two separate breeding populations. Historically, gene flow has existed between the ponds despite their geographic separation. However, a consistent genetic difference between salamanders around WC1 and WC2 can be identified, and this suggests that some degree of reproductive isolation now exists between the subpopulations associated with each wetland complex. **We recommend that mitigation wetland complexes be established in a way that minimizes barriers to movement of salamanders** from existing breeding populations into the new wetlands. Moreover, to prevent inbreeding depression, **we recommend transplanting eggs from both wetland complexes into mitigation wetlands at the time the wetlands are ready to support breeding salamanders**

3. The results of this study show a clear link between upland destruction and a decline in the number and quality of adults moving into wetlands to breed. Upland destruction has the potential to disrupt salamander breeding in at least two ways. First, destruction of upland habitat reduces living space and decreases food availability for the population during the non-breeding months. In this study, the effect of construction of the athletic fields was to decrease the wet weight of migrating adults, suggesting that there was inadequate foraging for the population in the remaining upland forest. Second, destruction of upland forest is likely to disrupt migration pathways, thereby reducing the number of adults that successfully reach a breeding wetland. In this study, the effect of construction of the athletic fields was to decrease the number of adults entering WC2 to breed. Our observation that some fences consistently were favored, even after construction of the athletic fields, strongly suggests that salamanders try to use old pathways even when they are no longer viable. To ensure that salamanders do become established in the mitigation wetlands, **we recommend transplanting eggs**. Any juveniles emerging from the mitigation wetlands will then view them as natal habitat, and it is to be hoped that the new populations will establish new migration pathways back into the mitigation wetlands. **We also strongly recommend maintaining large intact tracts of upland forest around the new wetlands. Moreover, we recommend ensuring that the tracts are contiguous with older upland habitats** that no longer provide access to the existing wetland complexes in hopes that the recently-isolated adults will find their way into the new wetlands.

4. Habitat loss stemming from development and constructions of new roads is an important factor in the reduction of amphibian abundance and diversity (Blaustein et al. 1994). When roads interrupt migration pathways, they further contribute to the loss of amphibian populations by creating conditions that result in death of large numbers of individuals caused by automobiles (Fahrig et al. 1995). Mitigation efforts should address both replacement and restoration of damaged wetland and upland habitat and provision of safe migration corridors for amphibians moving into or out of breeding habitat. **We recommend placing mitigation wetlands as far as possible from the new road,** and **we recommend consideration of measures to prevent salamanders from crossing the road itself** (fences to funnel salamanders into new habitats or tunnels under the road with fences guiding migrating salamanders into the tunnels). **These efforts should be accompanied by a concerted attempt to identify migration pathways.** However, such a study will require tagging and following cryptic non-breeding adults throughout the year, a labor-intensive and potentially expensive project

5. Evaluation of the effects of habitat alteration, both destructive and mitigative, on populations requires long-term monitoring of populations prior to the habitat alteration, as well as long-term monitoring after the alteration. Many populations experience natural fluctuations stemming from ecological interactions and climate conditions. *A. maculatum* are long-lived and iteroparous, but they do not breed every year. However, virtually all of our inferences must be drawn from data collected during breeding migrations, and it is not clear whether the adults captured while migrating accurately reflect the condition of the entire population. Thus, **it is critical to have many years of continuous study of the**

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population if we are to have confidence in our assessment of the health of the population

and the effects of mitigation efforts.

8. APPENDICES.

APPENDIX A.   List of student researchers who participated in this research project.

<u>Vegetation Survey of the Wetlands on the Campus of Penn State Erie</u>

Terry Shrout

<u>Water Chemistry of the Wetlands on the Campus of Penn State Erie</u>

Marlese Schlott, Christine Huff, and Stacey Casbolm

<u>Genetic Analysis of *Ambystoma maculatum*</u>

Holly Berry, Jennifer Colvin, Carley Gwin, and Jennifer Osmanski

<u>Demography of *Ambystoma maculatum*</u>

Susanne Prescott,* Terry Shrout, Matt Fulton, Heather Lerch, Tammy Marsh, Daniel Thum,

Taher Patwa, Terence Brand, and Margie Goatly*

* Students from Mercyhurst College, Erie, Pennsylvania.

APPENDIX B. Primers used for amplification of mtDNA sequences.

THR	5'-AAA CAT CGA TCT TGT TGT AAG TCG-3'
REV007	5'-CAA GAA TTT TTT TGG CTT GGG GTG C-3'
SALLYSEQ	5'-CCT AAA AGG GCA AGA AAG CC-3'

APPENDIX C. Sequence generated using SALLYSEQ primer for Virgina and PSU Erie populations of *Ambystoma maculatum*. The total length of the sequence is 225 base pairs. A total of 49 individuals were sequenced.

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PSUE572    GCCTATGCCT  CTACAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE576    GTTTATGCCT  CTACAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE7104   GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE158    GCCTATG.CT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE458    GCCTATG.CC  TCTAAAACAG  GCATTGCCAC  TTTTTCCCAA
V791       GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
V796       GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
V792       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
V794       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
V788       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
V787       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
V789       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
V795       GCCTATGCCT  CTAAAACCAG  GCGTTGCCAC  TTTTTCCCAA
PSUE563    GCCTATGCCT  CTAAAA.CAG  GCATTGCC.C  TTTTTCCCAA
PSUE786    GCCTATGCCT  CTAAAA.CAG  GCATTGCC.C  TTTTTCCCAA
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PSUE5110   GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE532    GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE781    GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE719    GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE766    GCCTATGCCC  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
PSUE778    GCCTATGCCT  CTAAAACCAG  GCATTGCCAC  TTTTTCCCAA
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PSUE178    GCCTATG.CT  CTAAAA.CAG  GCATTGCCAC  TTTTTCCCAA
PSUE111    GCCTATG.CT  CTAAAA.CAG  GCATTGCCAC  TTTTTCCCAA
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PSUE137    TCCTATG.CT  CTAAAACCAG  GCATGGACAC  TTTTTCCCAA
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