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Colonization and Persistence of the Freshwater Amphipod, Crangonyx pseudogracilis, in Temporary Ponds: Aspects of its Ecology, Resistance to Desiccation, and Dispersal Abilities

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Colonization and Persistence of the Freshwater Amphipod,

Crangonyx pseudogracilis, in Temporary Ponds:

Aspects of its Ecology, Resistance to Desiccation, and Dispersal Abilities

A Thesis

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THESIS DEFENSE

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ABSTRACT

Crangonyctid amphipods occupy temporary habitats throughout northeastern North America but they are mostly known as permanent water species. *Crangonyx pseudogracilis* is found at high densities in temporary ponds in western New York but the means by which it colonizes and persists in temporary ponds were not well understood before my study. My objectives were to 1) learn more about and quantify the colonization abilities of *C. pseudogracilis* by performing experiments where holes were dug around temporary ponds; 2) explore the ability of the amphipods and other invertebrates to descend through inundated porous substrates in the laboratory; 3) compare the lifecycles of permanent and temporary populations and how the timing of mating and releasing of broods may be related to survival through the dry season; and 4) understand how and where the amphipods find refuge when a pond dries. During periods of inundation, *C. pseudogracilis* was found in the top 15 cm of soil below and at the edges of the pond basin. After the pond basin became dry, they probably descended in the soil to depths greater than 45 cm. *C. pseudogracilis* and planarian flatworms readily colonized holes dug on the perimeter of the pond. In the lab, amphipods, flatworms, and ostracods readily descended through porous substrates. *C. pseudogracilis* has an annual lifecycle; the previous year's generation began dying in May and was gone by the end June. Ovigerous females were found from 23 March until 28 May. In the laboratory, amphipods survived in soil with an average moisture content of 51% for 15 weeks. My results suggest further studies. 1) Populations of *C. pseudogracilis* in permanent waters migrate to deep water during the same time of year as the temporary ponds I studied dried up. Whether amphipods in permanent waters burrow into bottom sediments during the dry season should be studied. 2) Determine how deep amphipods descend into pond sediments of temporary waters during the dry season. 3) Examine in detail the importance of the soil/water ecotone for organisms living in temporary waters.

Biographical Sketch

I was born in Rochester, New York on   . I earned a B.S. at the State University of New York College at Brockport in 2001 with a major in Biological Sciences, a minor in chemistry, and a concentration in Aquatic Ecology. I went on to pursue a M.S. degree in Biological Sciences at SUNY Brockport. As a Teaching Assistant, I taught laboratory sections of General Biology and Anatomy & Physiology for majors and non-majors. I also served as a field crew leader for grant-funded fisheries work. Currently, I work as a Chemist for an environmental laboratory in Phoenix, Arizona. In the future, I plan to instruct in higher education and pursue a doctorate in the field of Biological Sciences.

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Chapter 1: Background Information

This thesis reports on an experimental and observational investigation of the ecology of the freshwater amphipod *Crangonyx pseudogracilis* in regard to its occurrence in temporary ponds and its ability to persist in them without a known anti-desiccation, resistant stage in its life cycle. Many invertebrates that evolved primarily in lakes, rivers or streams or in upland habitats will opportunistically and successfully exploit wetland environments. In some cases, amphipod and isopod crustaceans, without any apparent adaptation for resisting or avoiding drought, can flourish in temporary water wetlands (Sharitz and Batzer 1999). Such occurrences by *C. pseudogracilis* and other crangonyctids in vernal and autumnal pools have been observed, but their means have remained an enigma (Higgins and Merritt 1999). In this section, I review the biology of Amphipoda (Crustacea: Malacostraca), *C. pseudogracilis* in particular, and conclude with an outline of my research.

AN INTRODUCTION TO THE AMPHIPODA

Amphipoda are chiefly marine; only about 150 of the described American species live in fresh waters (Bousfield 1973, Pennak 1989). Worldwide about 900 freshwater species occur in unpolluted lakes, streams, brooks, springs and subterranean waters. They are thigmotactic (confined to the substrate), except for one species that may swim as a plankter in lakes, and cold stenotherms. Many species are restricted to seeps, springs and subterranean waters in one or a few states, or to a single cave system (Pennak 1989).

The order Amphipoda is divided into three, sometimes four, suborders. These are the Caprellidea, Hyperiidea, Gammaridea, and the Ingolfiellidea, the latter of which some

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authors choose to include with the Gammaridea. Some consider the ingolfiellids to be highly specialized gammarids that do not merit recognition as a separate suborder (Bousfield 1973, Holsinger 2003). The Gammaroidea are the only suborder in American fresh waters. Gammarids are mostly bottom dwellers, and most can swim, even if infrequently (Barnes 1987).

With respect to probable origin, the North American freshwater amphipod fauna are classified in two groups: (1) those of ancient freshwater lineage, having no morphologically close marine relatives, and (2) those of relatively recent marine origin. The first (and largest) group contains the Crangonyctid section of family Gammaridae, represented in epigean waters by the primarily U.S.-endemic genera *Crangonyx* and *Synurella*, and in subterranean waters by an even larger number of U.S.-endemic genera and species. This group occurs mainly in the east-central United States (Bousfield 1958).

The presence of sternal gills, the lengthening of peraeopod 4, and partial reduction of the pleopods in crangonyctids are expected in animals that have long frequented oxygen-deficient subterranean waters, which crawl about upright on or in the substratum, and are feeble swimmers (Bousfield 1958). A true burrowing habit has evolved in some marine, but not in freshwater, species (Pennak 1989). This is an important point for this thesis because *C. pseudogracilis* demonstrates an ability to descend through porous substrates and interstitial environments without moving material. Pennak (1989) reports that a few species of amphipods burrow in the substrate during times of drought and high temperatures; with the onset of normal conditions they resume activities in the water. Holsinger and Dickson (1977) reported this behavior for *Crangonyx antennatus* living in

caves. Another characteristic that distinguishes *C. pseudogracilis* from other species of amphipods is its upright walking gait (MacNeil *et al*. 2000).

Respiration takes place partly through the body surface but mainly through the thin wall of the coxal gills of amphipods (Bousfield 1973). Unlike most amphipods, crangonyctids tolerate low levels of dissolved oxygen. In field transplantation experiments *C. pseudogracilis* tolerated low oxygen content in which other freshwater amphipods such as *Gammarus pulex* and *G. duebeni* cannot survive (Dick 1999, MacNeil *et al*. 2000).

C. pseudogracilis (Figure 1, picture) is found throughout eastern North America in rivers, lakes, sloughs, quarry ponds, reservoirs, and other fresh waters that tend to be turbid and warm in the summer (Bousfield 1958). It has a one-year life cycle with breeding from November to May. Adults begin die off in May and are gone by June. This species is sexually dimorphic with females averaging almost 3 mm more in length than males (Henry and Tarter 1997). Females produce several broods per year (Bousfield 1958 and 1973, Henry and Tarter 1997), and ovigerous females occur from November until May (Henry and Tarter 1997).

CRANGONYX TEMPORARY POND ECOLOGY

Many aquatic invertebrates inhabiting temporary ponds employ specialized strategies for survival and reproduction, such as resting eggs or a dormant juvenile stage (Kenk 1949, Wiggins *et al*. 1980, Williams 1987, Dietz 2001), but amphipods may not possess these characteristics (Kenk 1949, Wiggins *et al*. 1980, Batzer and Sion 1999, Magee *et al*. 1999, Taylor *et al*. 1999). Therefore, amphipods may not be particularly

well adapted to the dry and frozen conditions that characterize vernal ponds during the autumn and winter (Wiggins *et al.* 1980, Batzer and Sion 1999). Species living in temporary water bodies which lack desiccation resistant stages must move to permanent water before the dry season and re-colonize the following spring; they are characterized by excellent colonizing abilities and rapid larval development (Wiggins *et al.* 1980).

Research involving amphipods in temporary water bodies is sparse. *Crangonyx* spp. have been reported in temporary environments by Creaser (1931, cited by Kenk 1949 and Wiggins *et al.* 1980), Hubricht and Mackin (1940), Kenk (1949), Holsinger and Dickson 1977, Wiggins *et al.* 1980, and Jass and Klausmeier (2003). Creaser reported that a *Crangonyx* sp. survived by persisting in underground refugia (crayfish burrows) during the dry season. Wiggins *et al.* (1980) stated that Kenk's amphipods (*C. gracilis*) were most likely *C. pseudogracilis*. With this in mind, Kenk reported that specimens of *C. gracilis* were abundant in both of the ponds he studied, first appearing in early winter when both ponds were frozen. Their numbers gradually increased and peaked in April. During May and June, the numbers of mature animals decreased rapidly until, shortly before the ponds dried, only small specimens of the new broods were seen. Kenk stated, "These young animals apparently dig into the ground when the pond dries out." He also recovered two small specimens from a bottom soil sample long after the pond had dried. Based on these findings, Wiggins *et al.* (1980) said it is not surprising that *C. pseudogracilis* is found in temporary ponds, although their own data are equivocal on this point. The ecology of invertebrates in an intermittent stream was studied by Clifford (1966). His account of the amphipod *C. forbesi* is very similar to the life cycle and ecology reported for amphipods living in temporary ponds. Clifford found *C. forbesi* in

interstitial pore spaces during the dry season, something temporary pond ecologists have attempted to do with little success.

THE FOCUS OF MY THESIS

Crangonyx spp. are commonly found in large, permanent water bodies (Bousfield 1958, Holsinger 1972, Batzer and Sion 1999) rather than small, temporary pools or ephemeral wetlands, due to having relatively few adaptations for drought resistance (Magee *et al.* 1999). Therefore, their presence in high densities in temporary ponds with no connection to permanent water sources is unusual (Batzer and Sion 1999). Although finding these animals in temporary water bodies, as well as persisting from year to year seems unlikely, there are many places in North America, and more specifically in western New York, where this occurs (Batzer and Sion 1999, Harris *et al.* 2002, personal observations by the author). My thesis focuses on how *C. pseudogracilis* is able to colonize and persist in temporary water bodies where they survive for up to six months each year with no surface water, no free water to depths of up to 4.6 m (pers. comm., J. Zollweg, SUNY Brockport), and no known adaptations for such an existence.

Harris *et al.* (2002) showed that *C. pseudogracilis* has the ability to disperse underground through saturated interstitial habitats to isolated depressional areas or dug holes; this result supported their hypothesis that the ability was a means for colonizing temporary water bodies. They did not address whether the underground movements were a result of passive mechanisms, possibly aided by flow of ground water, or whether the amphipods actively moved through interstitial pore spaces and, if so, how. I felt this was something that needed to be explored further.

Other invertebrates (ostracods, planarians and copepods) also appeared in dug holes and isolated depressional areas with the amphipods. Do these invertebrates also disperse through the interstices of the soil? Answering these questions was problematic in that observing these animals in their natural environment and designing experiments which mimicked natural habitats in the lab are difficult.

FIELD STUDY SITES

Brockport, NY is located 16 km south of Lake Ontario on the Erie-Ontario Plain, a subzone of the Great Lakes Plain (Roosa 2002 and references therein). Areas of poorly draining soils, a high water table, and the low relief of the Brockport region contribute to the abundance of temporary ponds (Figure 2).

Two areas with temporary ponds were chosen for field studies. The SUNY Brockport campus pond (Fig. 3) was the primary site because of its convenient location and the physical structure of the pond was familiar from previous research (Harris *et al.* 2002). Early observations were made at this site in the winter of 2001-2002. A second site located in Northampton Park, Monroe County, New York was added in the spring of 2003 to conduct additional field tests. This site was approximately 8 km southeast of the campus pond (Fig. 2, 4).

The campus pond (Fig. 3) is not quite typical of woodland temporary ponds in the Brockport area. It may be best described as a series of pools with depths from about 1 m to 15 cm connected by wide, shallower regions. This gives it the appearance of being patchy, with numerous small, dry areas distributed throughout (Roosa 2002), especially during the latter stages of the pond cycle when the water table is relatively low. The

pond can reach to depths of 111 cm in the late winter and early spring after snow melts; a single body of water exists during these times. When pond recession occurs, isolated pools form due to the topography of the basin. This can happen in less than a week.

The southern portion of the pond is shallow and receives more shade in spring than the northern portion due to vegetation on the southern slope dominated by white ash (*Fraxinus americana*), bitternut hickory (*Carya cordiformis*), basswood (*Tilia americana*), and willow (*Salix* sp.). The shallower regions and dry areas in the northern portion of the campus pond are thick with downed trees and understory brush dominated by red osier dogwood (*Cornus stolonifera*), honeysuckle (*Lonicera* sp.), and flowering dogwood (*Cornus florida*) (Roosa 2002).

The Northampton Park site is located at the bottom of approximately a 30% slope off the south side of State Rt. 31 (Fig. 2, 4), the base of which is approximately 50 m from the road. Adjacent to Salmon Creek are vernal pools covering an area of approximately 15.2 m^2. In contrast to the Brockport site, this area is more typical of woodland temporary ponds. Pools were not more than 30 cm deep and were about 1.5 m in diameter. The environment around the pools was not nearly as dense with trees and understory vegetation as the Brockport site. The trees on the hill slope (consisting of willow, white oak (*Quercus alba*) and basswood) are distributed more uniformly than at the campus pond site. The understory is less dense than at the campus site and consists of saplings, honeysuckle, and red osier dogwood.

There was considerable variation in weather at the study sites from 2002 to 2004. In spring 2002, the ice on the ponds melted in early March, and ponds dried earlier than in previous years due to a lack of spring rains and unseasonably high temperatures. In

2003, thick pond ice did not melt until the last week of March due to an extremely cold winter. It took three weeks for the edges of the pond to thaw after many days at about 10°C in early March. Intermittent light rain aided in gradual pond recession; ponds were dry by 15 June 2003.

Conditions in the winter of 2003-2004 were less harsh than in the previous winter. Snowfall was greater but ponds thawed earlier due to warmer temperatures and copious, steady rains in the spring and early summer. As a result, pond recession was not gradual as it was the previous year. The pond remained at maximum fill until 11 May 2004 and receded slowly for a week, but after 18 May 2004 precipitation increased. Due to constant recharge from rain through 27 June, the pond remained filled. After 27 June, the pond receded through 5 July, on which day the only remnants of the pond were muddy patches. On 25 July 2004, rain filled the pond to its previous level. Little recession had occurred by the end of July, and the pond remained pocketed and patchy until early fall. Remnants of tropical storms and hurricanes from the Atlantic Ocean caused frequent and heavy rains in the month of August. The amount of rainfall led to a state of emergency in the Village of Brockport, and to the pond being full once again. Small pockets of water remained until 3 October 2004.

SOILS AT THE FIELD SITES

The current soil survey for Monroe County, NY and the Brockport area was published in 1973 (USDA Soil Conservation Service 1973), and Cazenovia-series soils surround the general pond location. I found by digging a soil profile that the soils under the pond were not those of the published soil survey. The surface soil was a loam, much

darker in color than the soil underlying it. The surface of the pond basin to a depth of approximately 30 cm is a dark (higher organic material content) loam, but it is variable throughout the pond due to the topography of the basin. This upper layer is as thin as 10 cm in some sections. Soils of the pond and the surrounding area have been disturbed in the past (Whitney Autin, SUNY Brockport, pers, comm.), and are laden with large rock fragments about 30-50 cm below the surface (personal observation). Therefore, the soil which comprises the pond basin is classified as 'Rockland' (USDA Soil Conservation Service 1973), which is not a soil series. It is an area containing frequent rock outcrops and shallow soils. Rock outcrops usually occupy from 25 to 90% of the area (Soil Survey Staff, Natural Resources Conservation Service 2006).

The soils underlying the pond have characteristics similar to the Ovid series, and Ovid soils are found in close proximity to the pond. The Ovid series is known to be somewhat poorly drained, and permeability decreases with depth (Soil Survey Staff, Natural Resources Conservation Service 2006), which helps with water retention. My observations confirmed that the soils formed from reddish till. Clay content in the soil ranged from 28-35%, not enough clay to create the "hardpan" layer that would exist under a permanent pond, yet enough to allow water to be held for a significant period of time with minimal recharge.

The soils along the shore of Salmon Creek, the second study site, are classified as a silt loam in the Wayland series (Soil Survey Staff, Natural Resources Conservation Service 2006), a poorly drained soil always found on level terrain adjacent to streams or in old oxbows. Permeability is moderately slow to moderate in the A and underlying

horizons. The water table is found more than 15 cm below the surface. Occasional ponding occurs in some pedons (soil types).

The combination of the depressional topography and the characteristics of the underlying soils give rise to the temporary ponds at the respective sites. Both sites have temporary pools for virtually the same amount of time each year despite adjacent Salmon Creek at the Northampton Park site.

OBJECTIVES OF MY THESIS

Considering that there are few accounts of *C. pseudogracilis* persisting in temporary ponds, especially those without crayfish burrows (Creaser 1931, as cited by Wiggins *et al.* 1980 and Harris *et al.* 2002) or other underground refugia, the main question I addressed was how amphipods colonize temporary ponds and are able to persist in these habitats without free water for periods of four to six months.

According to Wiggins *et al.* (1980) amphipods are poorly adapted to disperse into newly created habitat yet Harris *et al.* (2002) found evidence to the contrary. My first objective was to delve further into the dispersal and ascending abilities of these animals to shed light on how the amphipods arrive in the temporary pond environment to begin with and to supplement the work presented by Harris *et al.* (2002) dealing with underground dispersal of *C. pseudogracilis*. I looked at the frequency at which amphipods and flatworms migrated to dug holes on the eastern and northern sides of the campus pond (Harris *et al.*'s experiments only tested the northern side) and at a separate site (Northampton Park) to test whether this was a unique phenomenon at the campus site. This work is presented in Chapter 2.

According to Kenk (1949), Clifford (1966), Kaestner (1970), Holsinger and Dickson (1977), Pennak (1989), Sion and Batzer (1999) and Jass and Klausmeier (2003), species such as *Crangonyx gracilis*, *C. pseudogracilis*, *C. shoemakeri*, *C. forbesi*, and *Synurella bifurca*, "burrow" into the substrate as a means of surviving unfavorable conditions. To demonstrate the ability of the amphipods and other invertebrates to burrow or descend through inundated porous substrates in the lab was my second objective. Laboratory experiments manipulating substrate type (gravel size/pore size) were designed to test overall responses and to see if there were differences in the abilities of amphipods of different sizes and among major taxa of invertebrates (Ostracoda, Planaridae, Amphipoda). This work is presented in chapter 3.

Disagreement exists between Bousfield (1973) and Henry and Tarter (1997) on when mating occurs and when ovigerous females are found in permanent water populations. Kenk (1949) reports the smallest *C. gracilis* compose the vast majority of the population just before summer and subsequently burrow into the substrate. Clifford (1966) had similar observations for *C. forbesi* in an ephemeral stream in Indiana. Bousfield (1973) and Henry and Tarter (1997) reported *C. pseudogracilis* having an annual life cycle with adults beginning to die off in May and being completely gone in June. For these reasons, I looked at whether there are differences in the lifecycle of permanent and temporary populations and how the timing of mating and releasing of broods may be related to survival through the dry season. Furthermore, preliminary data from 2002 suggested that average amphipod size changed over the course of the season and a more complete record of samples would help in the understanding of population life history. This work is presented in Chapter 4.

Understanding where the amphipods find refuge when the pond dries was the most fundamental and puzzling question when research began. Batzer and Sion (1999) concluded that amphipods must stay in close proximity to the pond basin due to their rapid appearance after filling of temporary pools. Due to the appearance of amphipods soon after rains, Harris *et al.* (2002) speculated that amphipods are more closely associated with the water table than with the overlying soil. To test this hypothesis, a soil sampling regime which followed a receding pond was designed to "follow" the amphipods as the pond decreased in size. In addition, I tested how long amphipods could survive without free water in the lab because the pond is dry for four to six months in most years. This work is presented in chapter 5.

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Figure 1. Classification of *Crangonx pseudogracilis*

Phylum
Arthropoda

Class
Malacostraca

Subclass
Eumalacostraca

Superorder
Peracardia

Order
Amphipoda

www.vildaphoto.net

Suborder
Gammaridea

Family
Crangonyctidae
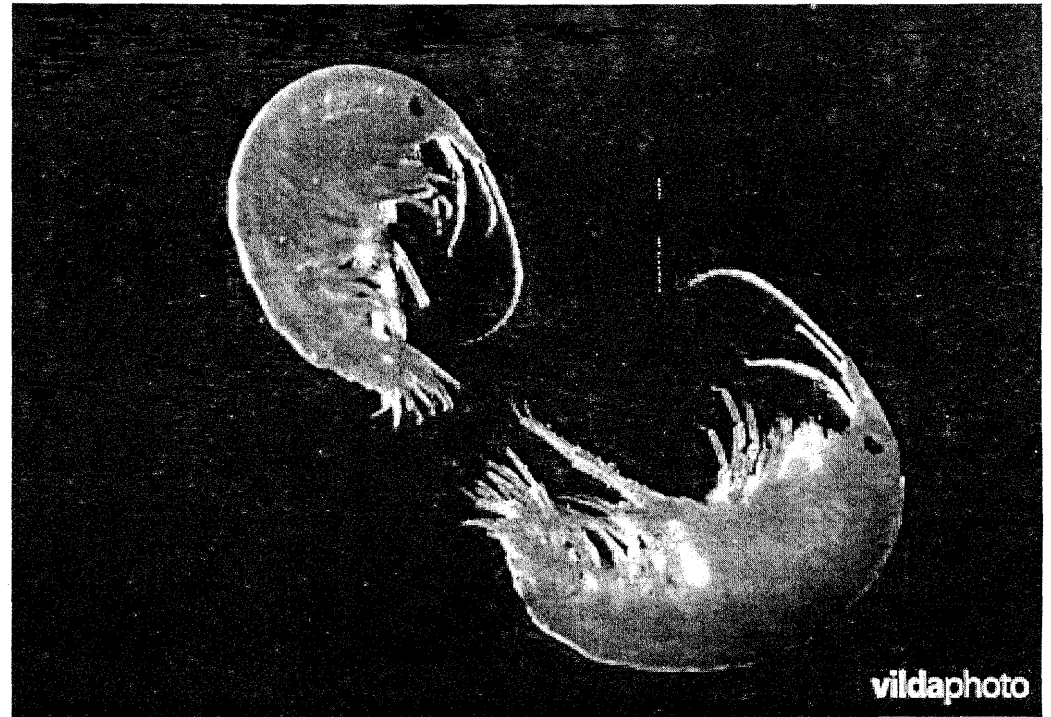
Figure 2. Map (The National Survey 1994) showing study sites (arrows) in Monroe County New York.
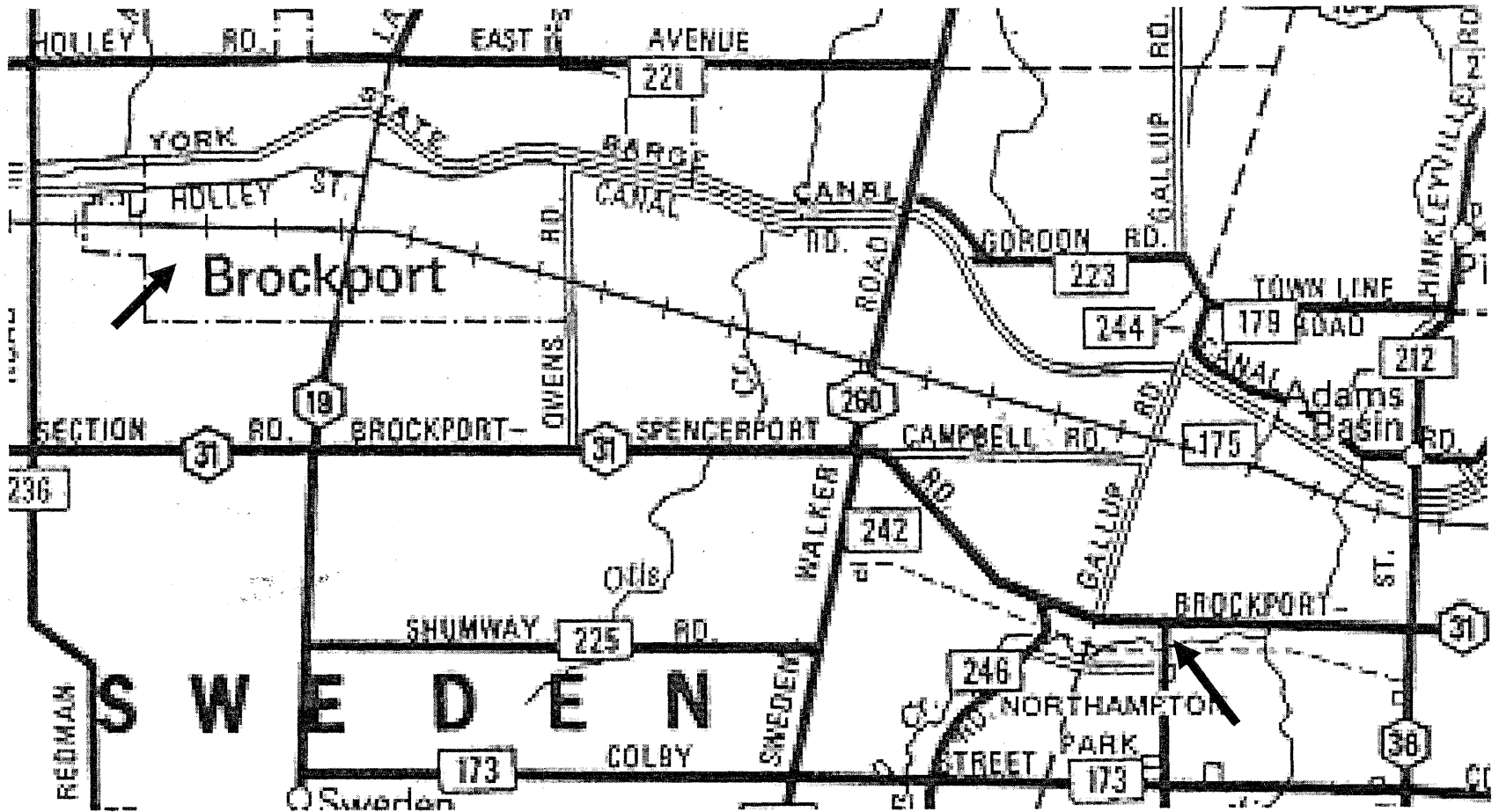
Figure 3.   Detail of the SUNY Brockport sampling site. The hatched area in the center of the diagram with an arrow is the pond area on a topographic map (L. Robert Kimball and Associates 1961).
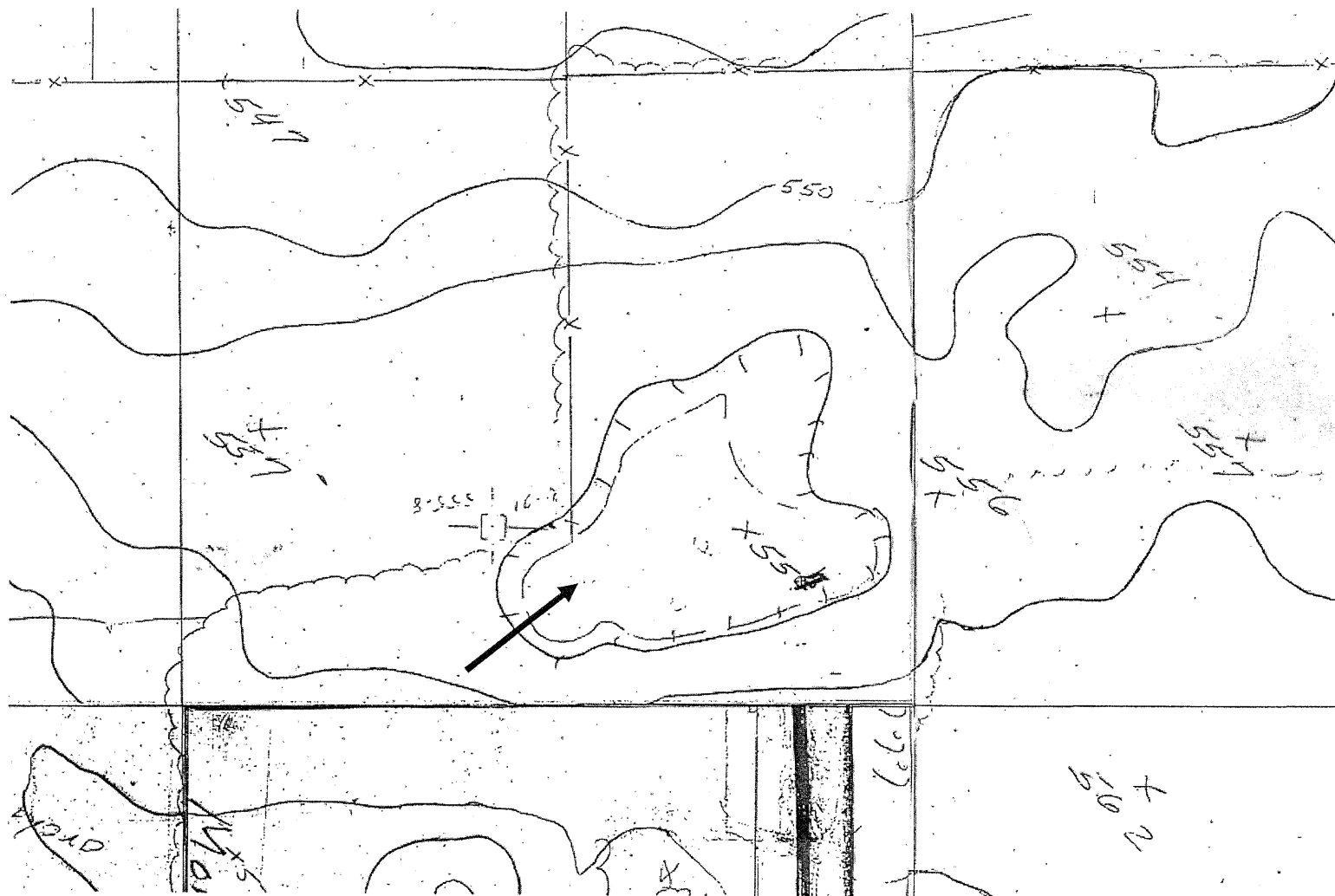
Figure 4.   Detail of the Northampton Park sampling site (see arrow). The low-lying area with an elevation of 548 m is the sampling site located on a topographic map (L. Robert Kimball and Associates 1961).
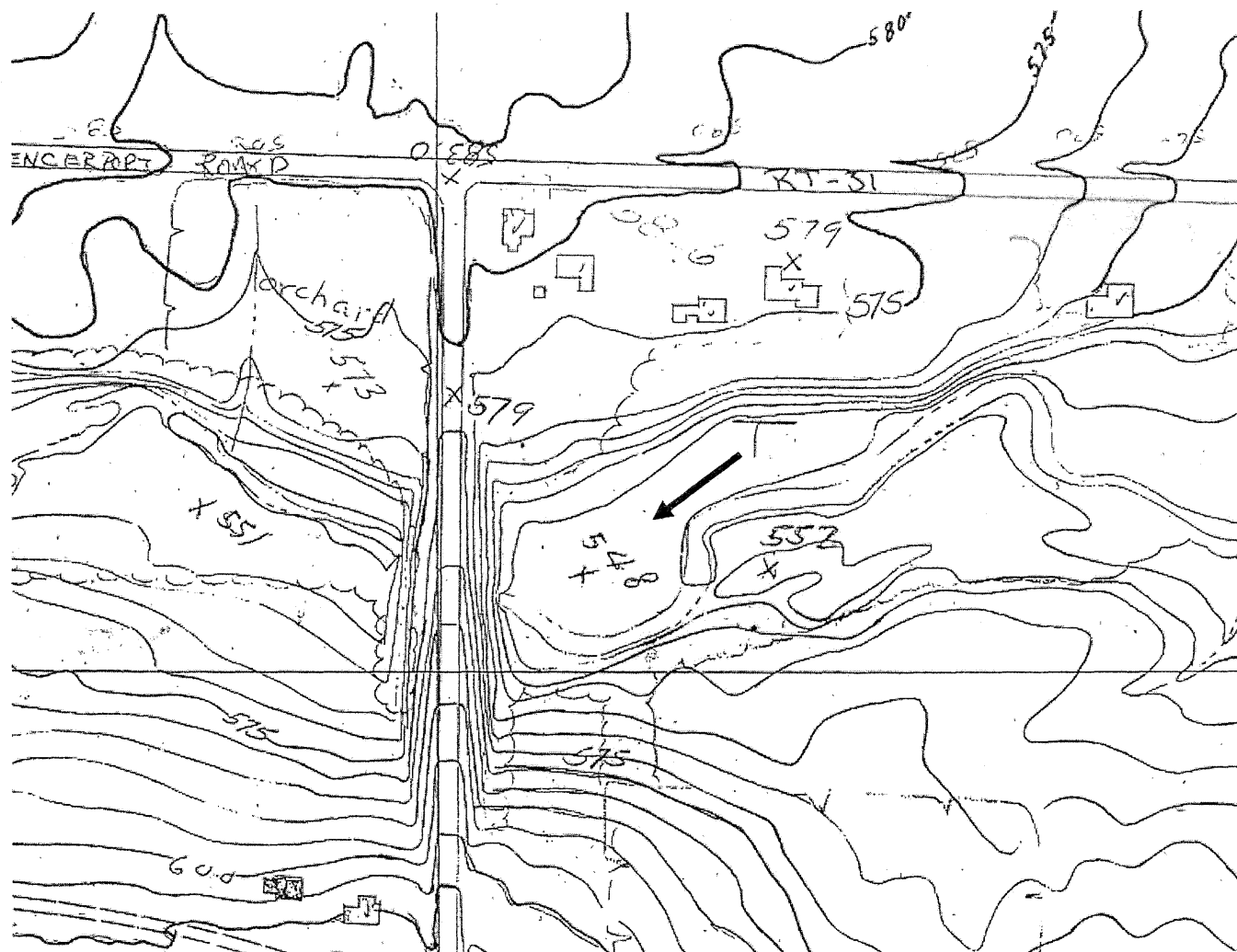
Chapter 2: Underground Dispersal of *C. pseudogracilis*
Through Interstitial Habitat

INTRODUCTION

How amphipods colonize temporary ponds is poorly understood (Harris *et al.* 2002). Amphipods have been collected from the feet and feathers of flying ducks and from the fur of a Labrador retriever (Wiggins *et al.* 1980, Swanson 1984 and references therein). However, waterfowl and semi-aquatic mammals are rare or absent in wooded temporary ponds where crangonyctid amphipods are common and no crangonyctid amphipods have been reported from ducks or mammals. Consequently, transport by vertebrates is an unlikely means of dispersal by crangonyctids.

Harris *et al.* (2002) found amphipods in the SUNY Brockport campus woods (Figs. 1, 2) in small bodies of water, isolated holes (e.g., left by uprooted trees) and depressions that had no above ground connection with the main pond in the area. When they dug holes near the north edge of the pond, amphipods and a species of triclad flatworm occupied the holes within 1-2 days. Based on this information, I tested the hypothesis that *C. pseudogracilis* moving to isolated holes through interstitial habitats was not a site-specific occurrence.

METHODS

At varying distances from the north and east edges of the temporary pond at the SUNY Brockport campus site I dug several holes (45 cm wide by 45 cm deep) (Figs. 2, 3). Because steep banks would have placed holes at higher elevations than the pond, no holes were dug on the west and south edges. I covered holes with plastic container lids

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(two holes at the Brockport site were left without lids, Fig. 3), and glued a fine mesh screen over a hole cut in the center of each lid that would not allow the smallest 1st instar individuals to pass through. This allowed light to reach the holes and for gas exchange, while keeping amphipods out from above. Lids were positioned flush with the soil surface and held in place with stones. In Northampton Park (Fig. 4), I dug holes in the same fashion, but they were fewer in number due to the smaller area containing temporary pools (Fig. 5).

I monitored holes bi-weekly and after precipitation events from 23 December 2002 to 12 June 2003. I inspected the holes by removing the lid and lying on the ground. To reveal invertebrates, I used a stick to move obstructions such as leaves and other debris and a flashlight as needed. This type of inspection eliminated the need for nets or other sampling gear that might have inadvertently acted as dispersal agents to other holes. Due to turbidity, holes could not be inspected for amphipods immediately after digging.

RESULTS

At both field sites (Figs. 1, 3, 5), 14 of 16 dug holes were colonized by amphipods at some point during the field season; eight of 14 holes (2 holes were dry) were still occupied when ponds dried up (Tables 1, 2).

At the SUNY Brockport pond (Figs. 2, 3), I dug five holes on 19 December 2002. They contained water through the end of March but no invertebrates. Temperatures decreased and holes froze at the beginning of March 2003 through 8 April 2003. On 13 April 2003, visibility in holes was poor and observations could be made in only one of the five holes (Table 1). Flatworms appeared in some holes by 20 April 2003, and were

present in all holes after 20 April 2003 to 12 June 2003. I dug two more holes on 20 April 2003 (Table 1). Amphipods were present in one of the seven holes three days after the first flatworms were found. The hole with amphipods subsequently dried up and they did not return to it until 16 May 2003. Amphipods appeared in one of the new holes on 1 May 2003 (Table 1) and remained there until 12 June 2003. The presence of amphipods increased after 20 May 2003. Amphipods appeared in all holes for at least 5 days except for # 2 (Table 1). After constant amphipod presence (Table 1), amphipods disappeared from three holes just before the pond and holes dried up.

At the Northampton Park site (Figs. 4, 5) I dug three holes on 6 April 2003. Within 11 days, all contained flatworms. On 27 April, I dug a fourth hole and amphipods colonized it within 4 d. By the end of the sampling period, six holes were dug at this site. Four of the six were colonized by amphipods (all of which contained amphipods upon drying up) and all were colonized by flatworms (Table 2).

Of the 13 holes at the two study sites, only two never had amphipods (Table 3). Also, amphipod movements in and out of holes were not random (two tailed runs up and down test, Zar 1999) in 10 of 11 holes with 10 or more observations ($p < 0.05$).

DISCUSSION

The hypothesis that amphipods moving to dug holes is not site-specific was supported. Amphipods and flatworms moved in and out of dug holes at both study locations, similar to the findings of Harris *et al.* (2002) but in contrast to those of Wiggins *et al.* (1980) who stated that amphipods in temporary pools exhibit passive dispersal only. Holes in my study were covered, ruling out above-ground sources of colonization.

Passive transfer by hydrostatic pressure gradients is not supported because in some holes amphipods were present one day, gone the next, and returned another day. Sometimes amphipods were found in a hole with 2-3 cm of water one day and the next day the same hole was dry with no amphipods, showing their ability to move into soils as water levels dropped. As part of soil sampling (described in detail in Chapter 5), soil from holes where amphipods were absent but active the day before was sampled. Amphipods were not found in the top 45 cm of underlying soil, suggesting horizontal movement or moving deeper in less than a day. In 2003 and 2004, soil sampling took place in May and early June during the time of year when ponds begin to dry out and amphipods are nearing the time when they descend to the underlying interstitial environment (Kenk 1949, Batzer and Sion 1999). Therefore, I cannot say if amphipods would have remained in sediments below dried holes if I had sampled earlier in the season. If amphipods are able to colonize a hole only to leave and return, it would lend support to the hypothesis that they engage in active dispersal.

This portion of my research showed that amphipods and flatworms colonize temporary waters by moving through soil interstices, but the ultimate reasons as to why they move from one body of water to the next are unknown. Exploratory behavior would be advantageous for an animal living in a highly variable environment. What induces them to penetrate deeper into the sediment remains to be answered (Henry and Danielopol 1999). Knowing that colonization of holes was not random, it is important to realize that amphipods likely are not just using the interstices for migratory purposes, but for everyday activities such as feeding, escaping predators, etc. MacNeil *et al.* (1999) proposed another possibility involving micro-distributions of *Gammarus* and *Crangonyx*

spp.; when the two co-exist, *Gammarus* will prey upon *Crangonyx*. *Crangonyx* could use the abilities presented here and in Chapter 3 (rapid movements through soils) as an escape mechanism from predators.

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Table 1. Colonization of dug holes at the SUNY Brockport site by amphipods and flatworms. Boxes containing the word, "dug", denote the day the hole was created. "A" and "F" denote the PRESENCE of amphipods or flatworms, respectively. The symbol, "---", denotes the absence of both amphipods and flatworms. Specific reasons are given for holes that could not be inspected visually due to unfavorable conditions. "Flooded" denotes an area where the main body of the pond made a connection to the dug hole, making the source of invertebrates inconclusive. Sampling began on 19 December 2002 and concluded on 12 June 2003. All sample holes were dry by 15 June 2003.

Hole	19-Dec	23-Dec	31-Dec	23-Mar	1-Apr	17-Apr	20-Apr	23-Apr	27-Apr	1-May	2-May
1	Dug	turbid	---	---	Frozen	Turbid	turbid	A F	dry	F	F
2	Dug	turbid	---	---	Frozen	Turbid	turbid	F	dry	F	F
3	Dug	turbid	---	---	Frozen	---	F	F	F	F	F
4	Dug	turbid	---	---	Frozen	Turbid	F	F	dry	F	F
5	Dug	turbid	---	---	Frozen	Turbid	F	F	F	F	F
6							dug	F	F	F	F
7							dug	F	F	F	F
8									dug	A F	A F
9									dug	---	F

Hole	6-May	9-May	14-May	16-May	20-May	23-May	28-May	2-Jun	5-Jun	12-Jun
1	F	dry	F	A F	A F	dry	dry	---	dry	dry
2	F	dry	F	F	F	---	dry	---	---	dry
3	A F	A F	F	A F	A F	---	F	A F	A F	F
4	F	F	F	F	F	---	A F	A F	F	F
5	F	A F	F	A F	A F	A F	A F	A F	A F	A F
6	F	F	F	F	F	A F	A F	A F	F	F
7	F	A F	F	F	F	A F	A F	A F	F	F
8	A F	A F	flooded	A F	F	A F	A F	A F	A F	A F
9	F	F	flooded	F	F	turbid	A F	A F	A F	A F
10						dug	A F	A F	A F	A F

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Table 2.   Colonization of dug holes at the Northampton Park site by amphipods and flatworms. Boxes containing the word, "dug", denote the day the hole was created. "A" and "F" denote the PRESENCE of amphipods or flatworms, respectively. The symbol, "---", denotes the absence of both amphipods and flatworms. Specific reasons are given for holes that could not be inspected visually due to unfavorable conditions. "Flooded" denotes an area where the main body of the pond made a connection to the dug hole, making the source of invertebrates inconclusive. All samples are from 2003. All sample holes were dry by 15 June 2003.

Hole	6-Apr	14-Apr	17-Apr	20-Apr	23-Apr	27-Apr	1-May	2-May	6-May	9-May	14-May
1	Dug	F	F	F	F	F	F	F	F	F	F
2	Dug	---	F	turbid	F	F	F	F	A F	A F	F
3	Dug	F	F	F	F	F	F	F	F	F	F
4						dug	A F	A F	A F	A F	F

Hole	16-May	20-May	23-May	28-May	2-Jun	5-Jun	12-Jun
1	Flooded	F	F	F	A F	A F	A F
2	Turbid	A F	A F	A F	A F	A F	A F
3	F	F	F	F	F	F	F
4	F	F	F	A F	A F	A F	A F
5	Dug	A F	F	F	F	F	F
6					Dug	A F	A F

Table 3. Colonization of dug holes analyzed for randomness. This table contains data for holes from Tables 1 and 2 that had 10 or more observations. The column titled "# of changes" lists how many times the observation changed from present to absent or vice versa for that hole. In the significance column, N/A refers to a hole that had a single run of absence for the duration of the experiment.

Hole	n	# of changes	Amphipods present	Significant (p<0.05)
Brockport 1	10	4	Yes	No
Brockport 2	12	0	No	N/A
Brockport 3	16	6	Yes	Yes
Brockport 4	14	2	Yes	Yes
Brockport 5	17	3	Yes	Yes
Brockport 6	14	2	Yes	Yes
Brockport 7	14	4	Yes	Yes
Brockport 8	11	2	Yes	Yes
Brockport 9	10	1	Yes	Yes
Northampton 1	16	1	Yes	Yes
Northampton 2	15	3	Yes	Yes
Northampton 3	17	0	No	N/A
Northampton 4	12	2	Yes	Yes

Figure 1.   Map (The National Survey 1994) showing study sites located within Monroe County New York. Arrows indicate sampling locations.
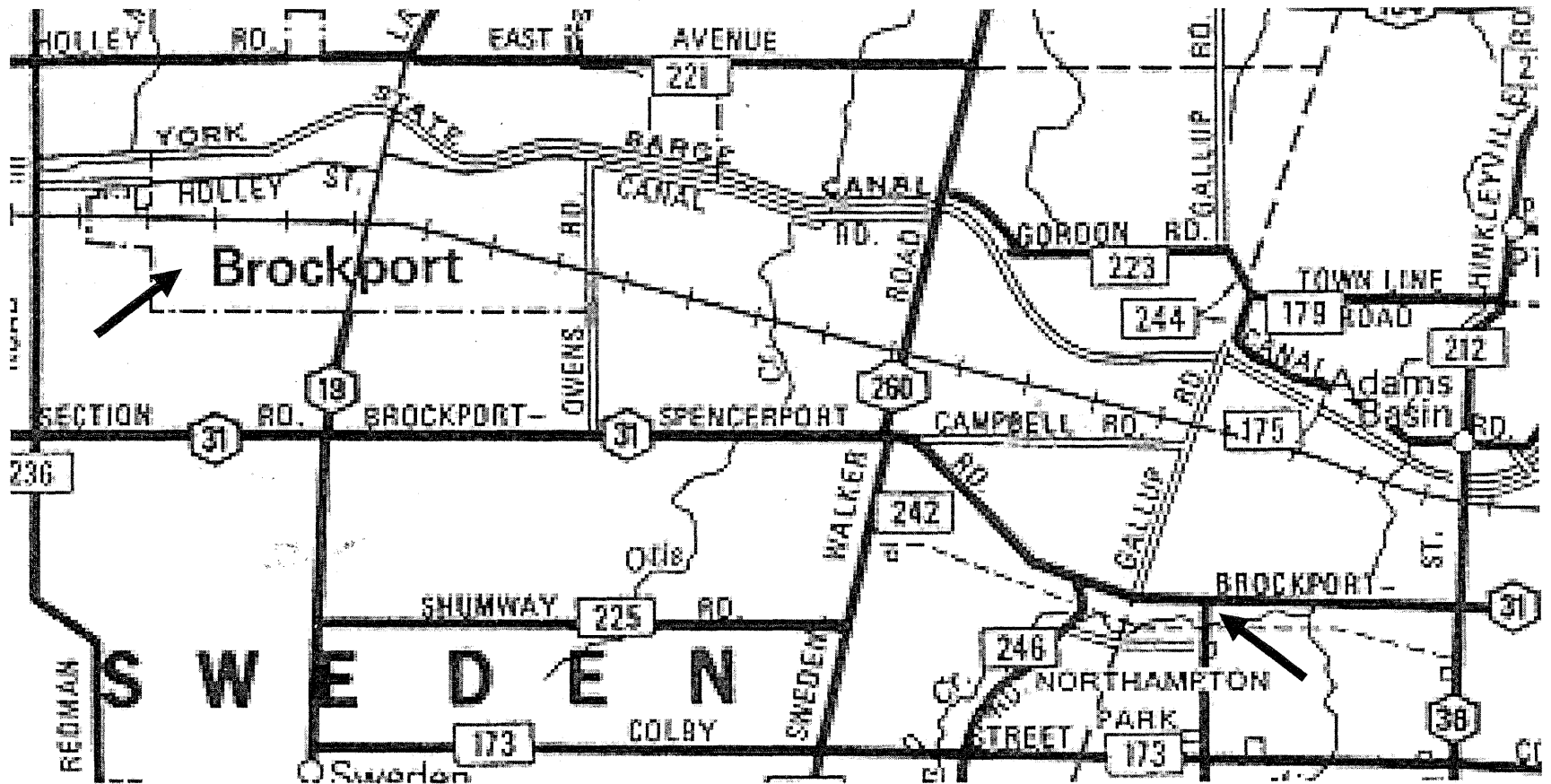
Figure 2.   SUNY Brockport sampling site for dug holes. The hatched area in the center of the diagram is the pond area on a topographic map by L. Robert Kimball and Associates (1961).

North ↓

10 m

*Tree not drawn to scale

1   2   3

6

4

7

10

5

9

8

Tree fall pit

☐ = Lidded Hole

○ = Open Hole
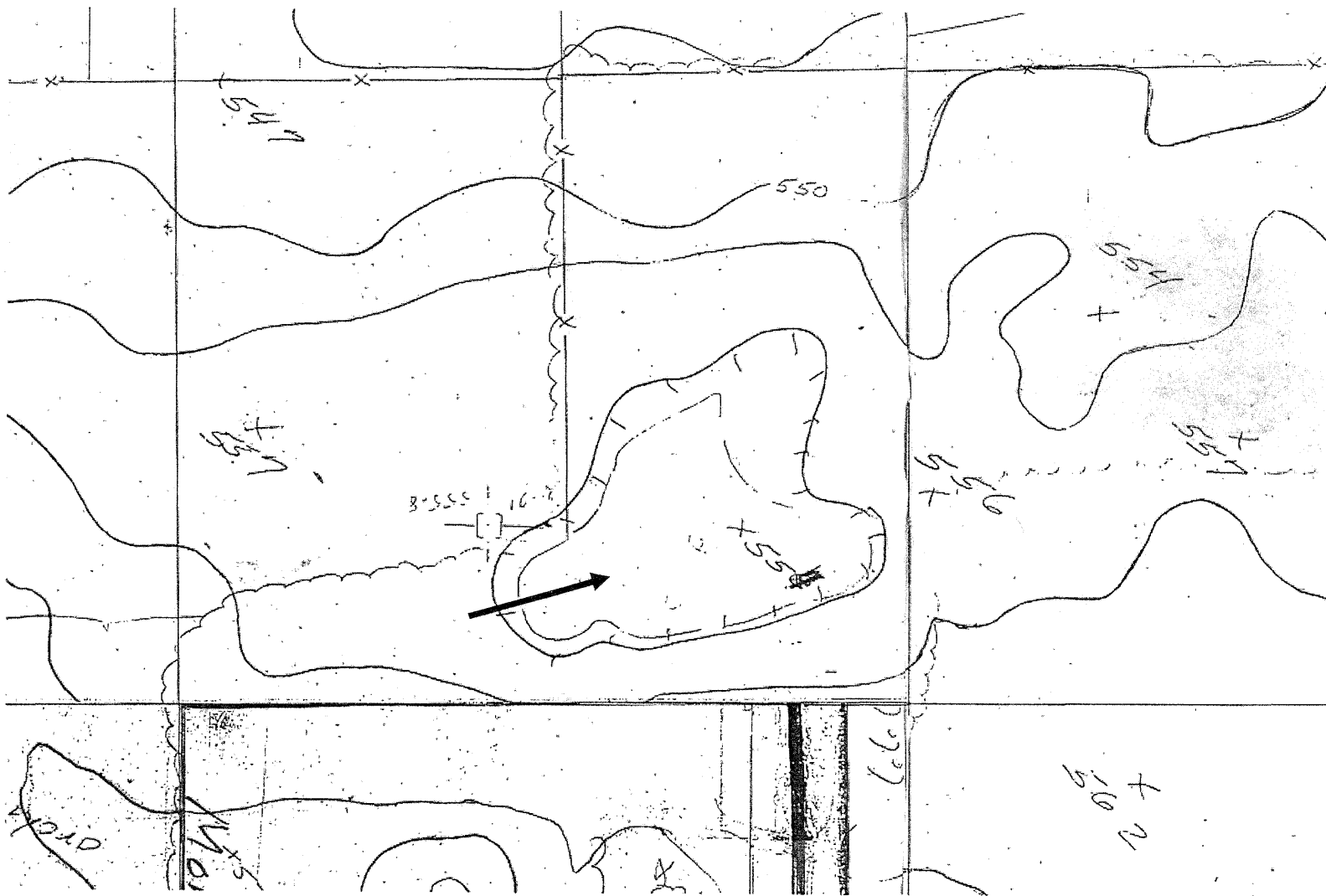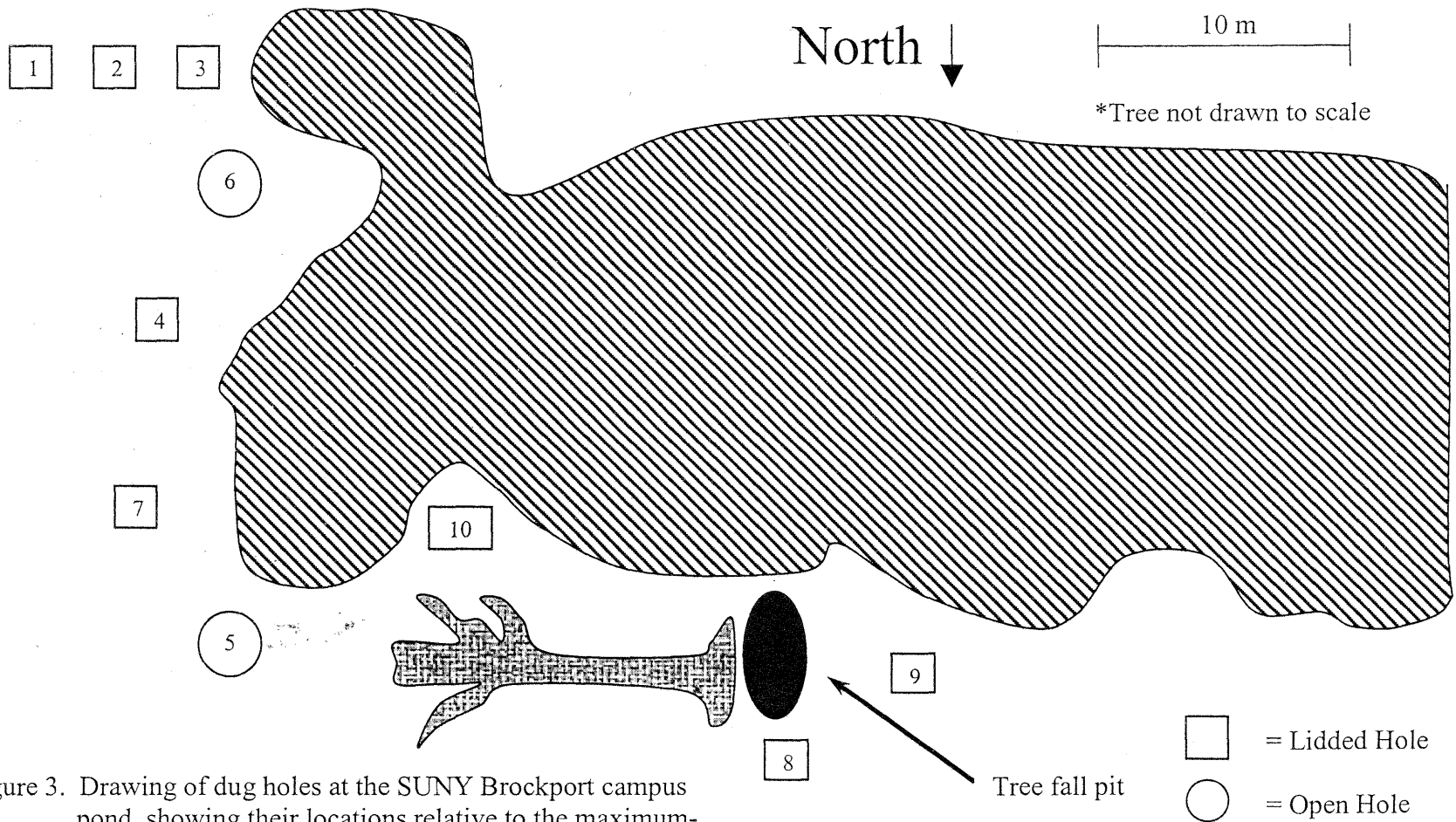
Figure 3. Drawing of dug holes at the SUNY Brockport campus pond, showing their locations relative to the maximum-fill shoreline. The striped area represents the pond and the surrounding area is forested. The pond continues to the west but is not shown in its entirety.

Figure 4.   Northampton Park sampling site for dug holes. The low lying area with an elevation of 548 ft (arrow) north of Salmon Creek was the sampling site (L. Robert Kimball and Associates 1961).
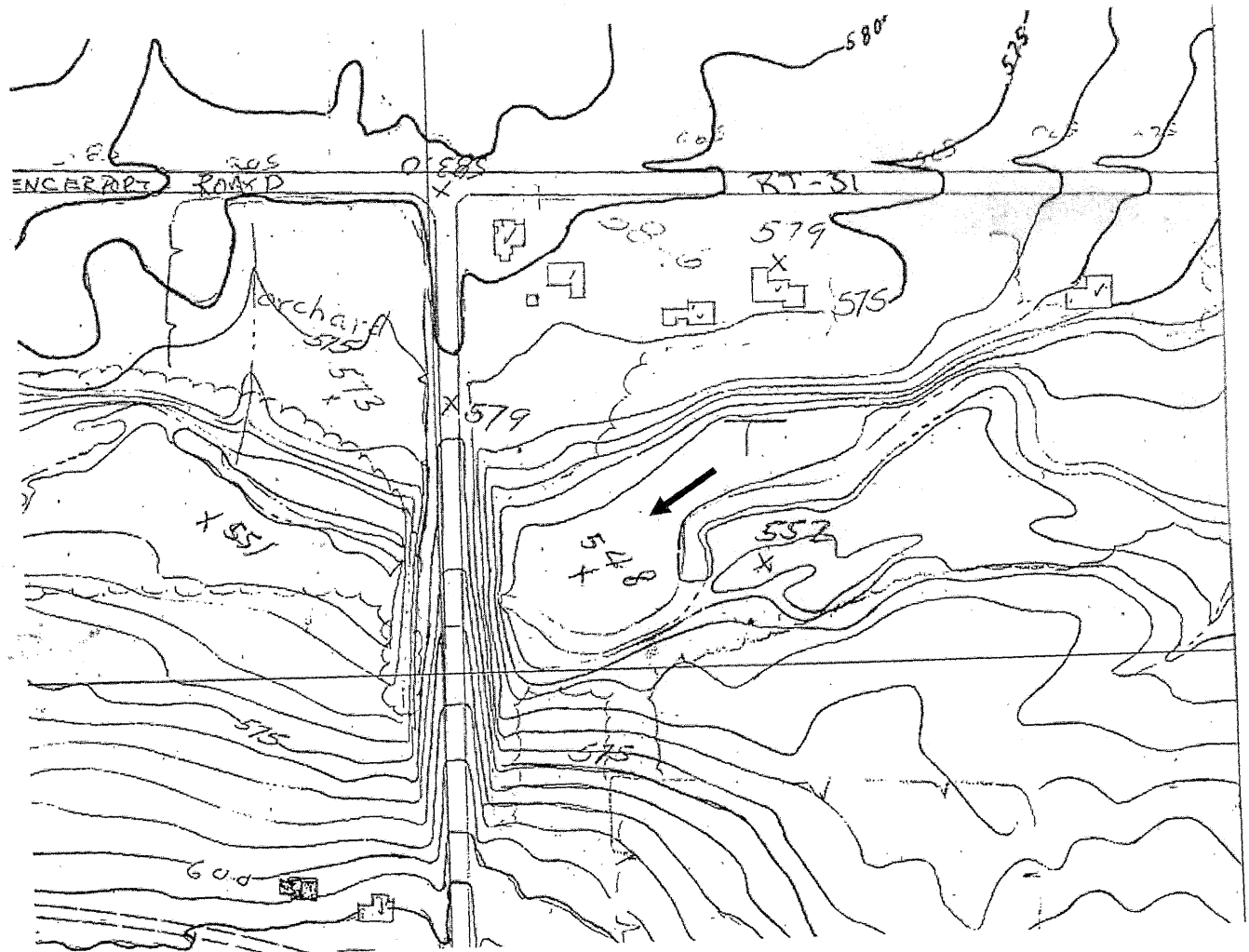
Figure 5. Drawing of dug holes at Northampton Park, Monroe County, NY, showing their locations relative to the maximum-fill shoreline. All holes were lidded. Unshaded area is forested ground.

Chapter 3: Vertical Movement through Substrate

by *C. pseudogracilis*

INTRODUCTION

After showing in Chapter 2 that *C. pseudogracilis* can move underground from depression to depression, this chapter explores its ability to move through porous, inundated substrates in the laboratory. Wiggins *et a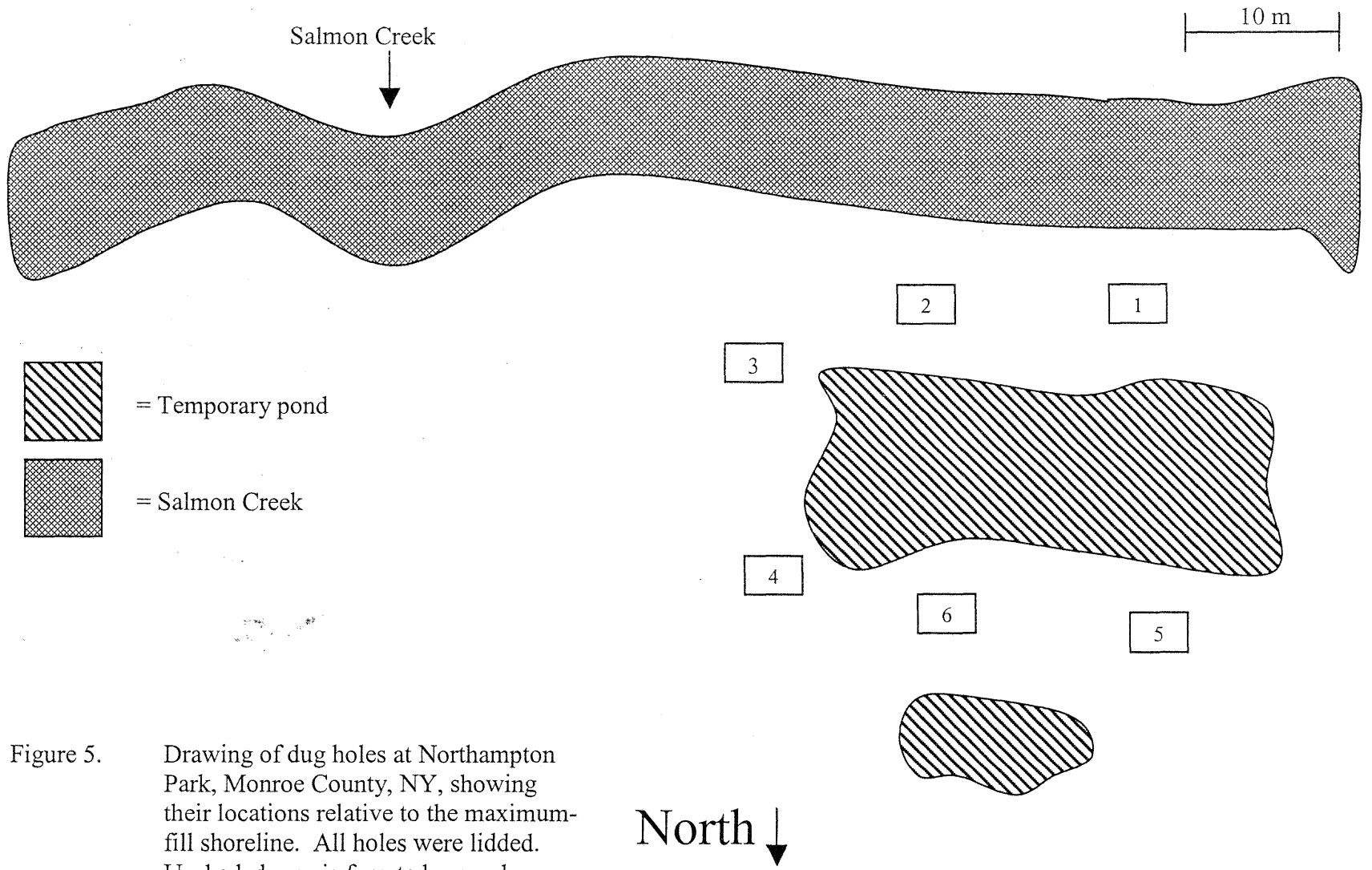l.* (1980) reported that species of *Hyalella* and *Crangonyx* burrow into bottom materials and the interstices of vegetation when surface water is absent for short periods during summer and autumn. In addition, Sniffen (1981) found young individuals of *C. gracilis*, *C. obliquus richmondensis*, *C. serratus*, and *Stygobromus* sp. (undescribed) that survived the dry season by migrating vertically and remaining in moist or wet subterranean habitats in a flood plain in North Carolina. Only a few papers dealing with ecology of amphipods in temporary ponds explored their abilities to burrow or, more properly, descend into soils. Because *C. pseudogracilis* does not possess appendages like those of a crayfish (Pennak 1989) it cannot move substrate materials; therefore, they technically cannot burrow. However, my preliminary observations suggested that the beating action of the appendages of *C. pseudogracilis* is sufficient to allow passage through silt-sized material with a sufficient amount of water.

Kenk (1949) found small specimens of *C. gracilis* in soil samples of a temporary pond, and reported that "these animals apparently dig into the ground when the pond dries out." Holsinger and Dickson (1977) observed a cave-dwelling amphipod (*C. antennatus*) that burrows into mud substrates when water levels are reduced or absent.

Clifford (1966) sampled and observed *C. forbesi* in subsurface seepage and water-saturated air spaces of soil below an intermittent stream. Jass and Klausmeier (2003) reported on a population of *C. gracilis* surviving in temporary ponds in southeastern Wisconsin but performed no further tests. Pennak (1989) stated that a few species of amphipods burrow (i.e., actively move sediment) into the substrate during times of drought or high temperatures; with the onset of normal conditions they resume activities in the water. Henry and Danielopol (1999) suggested that motile animals such as *Gammarus* spp. move actively through interstitial spaces to colonize deeper habitats as flood events drive oxygenated water further into the subsurface. They also noted that *Gammarus roseli,* highly thigmotactic like *C. pseudogracilis*, tends to migrate below cobble-sized into the interstitial regions of finer sediments during laboratory exposures. In addition, the upright walking gait of *Crangonyx* spp. (MacNeil *et al.* 2000), as opposed to the side scuttling or swimming of other amphipods such as *G. faciatus*, may enable *C. pseudogracilis* to orient so as to explore crevices, back up, or walk in another direction.

With evidence suggesting that *Crangonyx* amphipods can move through interstitial habitat below ground, and preliminary lab experiments showing that *C. pseudogracilis* descends upon being placed on a porous, inundated substrate, I designed experiments to test the ability of *C. pseudogracilis* to descend through inundated interstitial habitat in the lab. I tested hypotheses that smaller amphipods would have greater success at descending through porous substrate than larger amphipods and that amphipods would be more successful than ostracods and flatworms (also found in dug holes, Chapter 2) due to their contrasting styles of locomotion.

METHODS

Approximately 95 L of unsorted gravel of various sizes was acquired from a local quarry (Moscow Road Pit, Monroe County, Hamlin, NY) and sorted into four sizes (0.3, 0.5, 0.7 and 1.3 mm) using brass sieves. I rinsed the gravel several times to rid it of dust and the smallest particles. To distinguish between gravel sizes, average pore radii, which are approximately 1/5 of grain diameter (Fetter 1994), were calculated (Table 1). Four gravel/grain sizes were tested with invertebrates.

I used Rubbermaid® wastebaskets (19.8-L capacity) to hold gravels to a depth of 35.6 cm. A solid piece of plastic (35.6 cm x 27.9 cm) was fit into the container and fastened in place using GE® Clear silicone II sealant), which resulted in a container divided into two sections on the long axis of the rectangular opening (Fig. 1). About one-third of the bucket was on one side and the remaining two-thirds were on the other. The small section was filled with gravel and water filled the large section. A 1-cm space was left along the bottom of the divider to provide a place for amphipods to cross from the gravel to the water side.

Containers were filled with deionized water to the height of the substrate. For amphipod-only tests, ten amphipods of each size class (small, $\leq$ 3 mm; medium, 3-7 mm; large, $\geq$ 7 mm; 440 total over 44 replicates per gravel size) were placed into the setup on the gravel surface. For trials using multiple invertebrate species, 10 amphipods (4 small, 4 medium, 2 large), 10 flatworms and 10 ostracods were placed in each bucket (all invertebrates were collected from the Brockport campus pond). Buckets were monitored for 2 h, and animals were counted as they 'crossed' under the divider into the water-only side of the apparatus. After a replicate was completed, gravel was rinsed with extremely

hot water to kill invertebrates not accounted for. Data were analyzed using Sigma Stat®
and Minitab® using Kruskal-Wallis one-way ANOVA, Nemenyi's and Dunn's tests of
independence, and Mann-Whitney tests.

RESULTS

The two smallest pore sizes (0.3 and 0.5 mm) were replicated six times each for
amphipod-only and multiple invertebrate tests. No invertebrates passed through these
gravel sizes, so no further tests were conducted.

Amphipod tests

Non-parametric ANOVAs of ranked data showed significant differences among
the abilities of large, medium, and small amphipods to descend through 1.3 mm and 0.7
mm pore radius gravels (df = 2, H = 89.862, p < 0.001; df = 2, H = 91.872, p < 0.001,
respectively). Nemenyi's tests for multiple comparisons showed no difference between
the abilities of small and medium amphipods to pass through the gravels, but large
amphipods were significantly less able to descend (p <0.001) (Table 2).

Mann-Whitney tests revealed no significant difference in the ability of small
amphipods to descend in the 1.3 mm pore size vs. the 0.7 mm pore size (df = 1, W =
2005.5, p = 0.682; Table 3). Significant differences were detected for the medium (df =
1, W = 2204.0, p = 0.034) and large amphipods (df =1, W = 2821.0, p < 0.001), with both
having more success with the 1.3 mm pore size (Table 3).

Multiple species tests

There were significant differences in the frequency at which amphipods, flatworms and ostracods descended through 1.3 mm pore gravel (df =2, H= 38.25, p < 0.001; Table 4). Flatworms had the greatest success followed by ostracods and amphipods; all were significantly different from each other (Nemenyi's test). The same was true for the 0.7 mm pore gravel (df = 2, H= 51.73, p < 0.001; Table 4), except that there was no significant difference in the ability of flatworms (df = 1, W = 1914.5, p = 0.708) or ostracods (df = 1, W = 1981.0, p = 0.848) to descend through the 0.7 substrate (Table 4).

DISCUSSION

All of the invertebrates tested were adept at descending through the two largest porous, inundated substrates. For amphipods, in particular, the smallest individuals had the greatest success, especially in sediment with the smallest pore size (0.7 mm) that allowed passage. Pore size limited the body sizes that passed through the substrate, a finding supported by Clifford (1966) and Sniffen (1981). Sniffen reported that vertical migration is highly dependent on porosity of soil or sediments. Large amphipods had the greatest difficulty; often remaining at the surface for the duration of a test. This finding is supported by Clifford (1966) who reported that moist leaf litter served as the final temporary 'oasis' for the larger individuals (*C. forbesi*) in dried up pools (this habitat was not available to invertebrates in my experiments). The animals Clifford (1966) found were chiefly large adults; many were ovigerous and too large to follow a subsiding water table. Clearly, amphipods have the ability to move through pore spaces in the laboratory (this chapter) and in nature (Chapter 2). Because pore spaces in the field were not

measured, it was not possible to determine if the particle sizes and associated pore spaces in the laboratory reasonably represented field conditions.

In the multiple species tests, flatworms were the most successful at moving though porous media, and each invertebrate taxon was significantly different from the other. Therefore, my hypothesis that amphipods would be the most successful at descending through porous substrates was rejected.

Based on the life history of amphipods (Henry and Tarter 1997, personal observations), only individuals of the newest generation are able, by virtue of size, to make a full descent into sediments to survive the dry season (Kenk 1949, Clifford 1966); the oldest, largest individuals from the previous year/generation perish with the onset of the dry season. Therefore, my finding that the smallest individuals are able to descend makes sense. In an annual species, large individuals will not live into the next season; therefore, their descending abilities are irrelevant. The only time they may have to descend into pore spaces would be if there is a season with low precipitation, and then damp leaf litter (Clifford 1966, Batzer and Sion 1999) should suffice for short-term survival when free water is not available.

The fact that flatworms and ostracods are able to make the same movements as the amphipods shows that in a temporary pond environment it is important to be able to descend through interstitial spaces during periods of drought. Clifford (1966) observed a community of invertebrates in an intermittent stream, including insects and isopods, that also showed abilities to get into subsurface habitats during dry periods, even if it was only a few centimeters for a few days. Similarly, Holsinger and Dickson (1977) found that cave-dwelling amphipods (*C. antennatus*) burrowed into the mud substrate of cave

pools during dry periods. Descending behavior occurs in many invertebrates (Clifford 1966, Wiggins *et. al* 1980, Wissinger 1999); water always seeks the lowest point and these animals must follow it.

Whether or not the information presented here is transferable to field conditions is debatable, because pore spaces were not measured in the field. *C. pseudogracilis* and other invertebrates descended actively through pore spaces in a setup that had no flowing water. Amphipods actively moving through gravel fits with the results of Chapter 2 where they were shown to move through interstitial habitats (soil pore spaces) to isolated holes and depressions and with the walking behavior reported by (MacNeil *et al*. 2000).

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Table 1. Relationship between grain diameter and pore size. Pore size is approximately 1/5 of grain diameter (Fetter 1994).

Grain diameter	Average Pore Size	Rock Fragment Class
6.0	1.3	Pebble
3.3	0.7	Granule
2.0	0.4	Course sand
0.5	0.1	Medium sand

Table 2. Percent passage rates of large (> 7 mm), medium (3-7 mm) and small (< 3 mm) amphipods through two gravel pore sizes. Data from 44 replicates of each gravel size (10 of each amphipod size class per replicate) were analyzed with Kruskal-Wallis one-way ANOVA and Nemenyi tests (***, $P < 0.001$).

Gravel pore size	Amphipod size			Result
	Large	Medium	Small	
1.3 mm	29	87	88	S = M > L***
0.7 mm	6	82	87	S = M > L***

Table 3. Comparative descending abilities among invertebrate taxa and amphipod sizes in relation to gravel sizes (0.7 and 1.3 mm). (***, Mann-Whitney W-test < 0.001).

Mann-Whitney Test	Significance	P-value	Median 0.7mm	Median 1.3mm
Small Amphipods	NS	0.682	9	9
Medium Amphipods	***	0.034	8	9
Large Amphipods	***	0.000	0	3
Ostracods	NS	0.848	9	9
Flatworms	NS	0.708	8	8

Table 4. Percent passage rates of mixed species through two gravel pore sizes. Data from 44 replicates of each gravel size (10 individuals from each taxon per replicate) analyzed by Kruskal-Wallis and Nemenyi tests (***, $P < 0.001$).

Size	Invertebrate species			Result
	Ostracod	Flatworm	Amphipod	
1.3 mm	74	88	66	A<O<F***
0.7 mm	75	88	63	A<O<F***

Figure .1   Experimental apparatus for invertebrate vertical movement experiments. Cross sectional view (left); top-view (right).
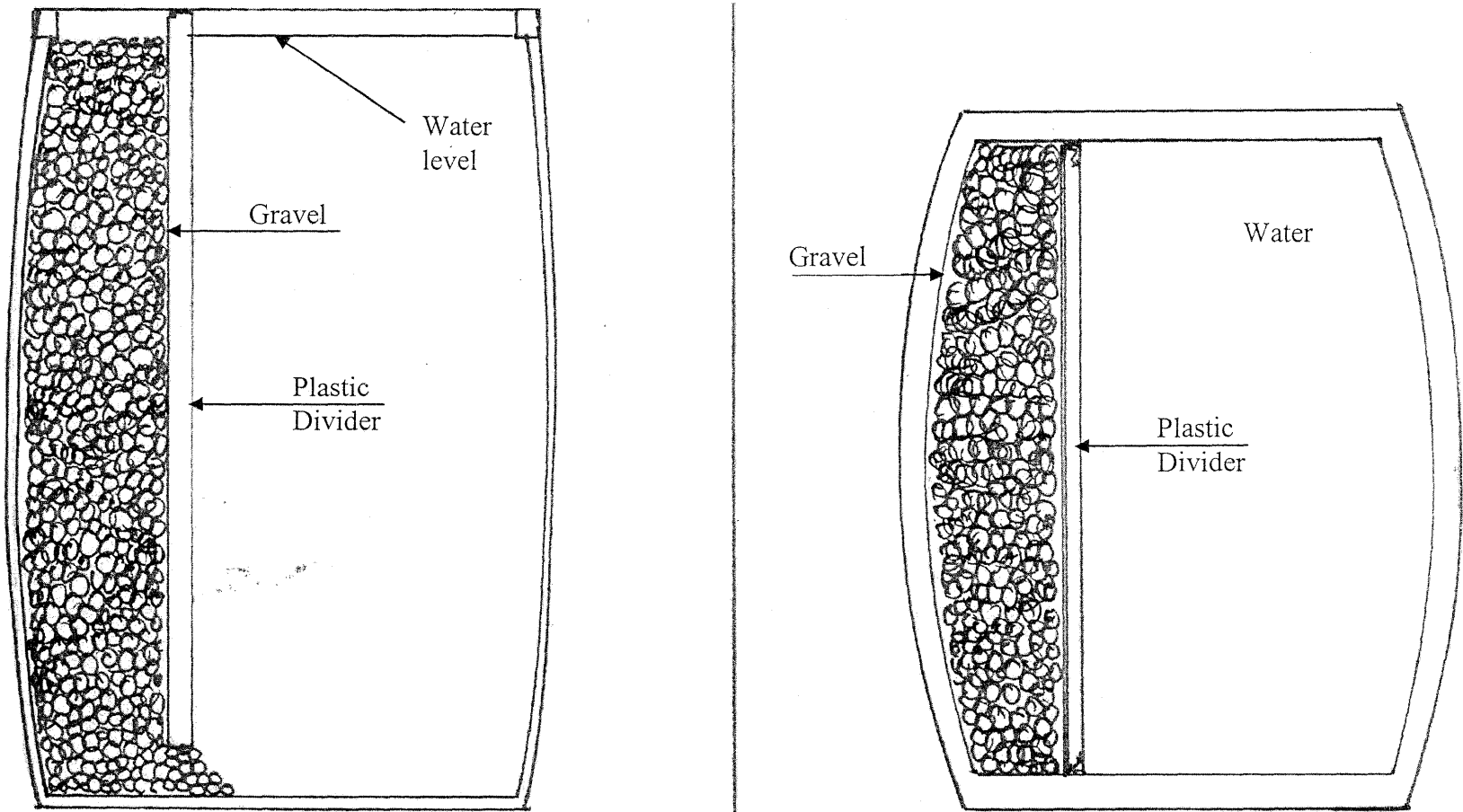
Chapter 4: Growth and Reproduction of *C. pseudogracilis*

INTRODUCTION

The life history of *C. pseudogracilis* is not well studied (Holsinger 1972). Bousfield (1973) sampled brood-bearing females from September through April and reported on its annual life cycle, but did not say when mating and die-off occur. Henry and Tarter (1997) also reported an annual life cycle for *C. pseudogracilis*, finding brood bearing females from November to May in a permanent pond in West Virginia; adults began to decline in May and were gone in June. Both studies reported females producing several broods per year. Other crangonyctids (*C. richmondensis, C. gracilis, C. forbesi*) are reported to have a similar life cycle (Kenk 1949, Clifford 1966, Bousfield 1973).

The purpose of this chapter is to report on mating and growth of *C. pseudogracilis* populations in temporary waters and compare those findings to published information pertaining to populations in permanent waters. Since there is a limited period for reproduction in a temporary pond environment, I also tested the hypothesis that the occurrence of brood bearing females is seasonal in temporary waters.

METHODS

Five 1-m^2 quadrats, arranged diagonally from northwest to southeast, were placed in the temporary pond at SUNY Brockport (Fig. 1). Each quadrat was sampled weekly using a plastic wastebasket with the bottom removed, and a small dip net. The bucket was placed firmly on the substrate and the net was scooped into the sediment ten times. Samples were placed in plastic containers and live amphipods were removed in the lab. The total number of amphipods and females bearing broods were counted before

preservation in 95% ethanol. At the end of the field season amphipods were measured using an ocular micrometer on a compound microscope (40X). Due to their tendency to curl upon preservation, total amphipod lengths were not measured; they were measured from the base of antenna one to the posterior edge of the second pereonite (body segment). Data were analyzed with a one-way, unstacked ANOVA (Minitab®) (amphipod size) and a G-test of independence (frequency of brood bearing females). Figures were constructed using Microsoft Excel® and Sigma Plot®.

RESULTS

Size

The average measured amphipod was 1.4 mm upon thawing of the pond (23 March 2003) and 0.8 mm on the final sampling date (12 June). Average length was greatest on 20 April 2003, after which length decreased markedly (Fig. 2). Amphipods measured from samples on 9 May and before (7 sample dates, n = 437) were significantly larger than those taken on 16 May and after (5 sample dates, n = 467) (df = 11, F = 111.54, p < 0.001). As average size decreased, the number of brood-bearing females also decreased (Fig. 3). The number of amphipods in larger size classes decreased as the field season progressed; by late spring samples contained few to no late instar animals (Fig. 4).

Reproduction

The presence of brood-bearing females was related to time of season (Table 1; G-test of independence, df = 15, G/q = 94.95, p < 0.001). Brood-bearing females were

collected from 23 March until 28 May, with the greatest numbers sampled in mid to late April (Fig. 5, Table 1).

DISCUSSION

C. pseudogracilis has an annual life cycle in the temporary pond at SUNY Brockport, a result consistent with findings in permanent waters by Bousfield (1973) and Henry and Tarter (1997). Averages and frequencies of size over time (Figs. 2, 4) showed the previous year's generation dying and a new generation entering the population. The incubation period for *C. pseudogracilis* is unreported, but for amphipods in general the time ranges from one to three weeks and is largely dependent on variations in molting time due to temperature and age (Bousfield 1973, Pennak 1989).

The hypothesis that finding brood bearing females is seasonal was supported. The span of time (late March to late May) for brood-bearing females in the campus pond was shorter than reported by Henry and Tarter (1997; early November to early May). My samples were collected immediately after ice-out, which suggests that breeding probably occurred under the ice during the winter months.

Amphipods are not microhabitat specialists; they exhibit phenotypic plasticity in growth and survival in relation to environmental parameters (Savage 1996). Successful inhabitants of a temporary pool must synchronize their life histories with the annual cycle of the pool (Wiggins *et al.* 1980). Life history adaptations for surviving the dry phase of the pool may involve desiccation-resistant stages, including eggs, immature stages or adults; timing of oviposition (in the case of amphipods, timing of mating and brood release); or adaptations for rapid growth and development (Wiggins *et al.* 1980).

Because of their obligate univoltine (annual) life cycle, all *C. pseudogracilis* entering the dry season were very small; therefore, they can inhabit small interstitial spaces available at this critical time in their life cycle (Clifford 1966). It appears that the life cycle is synchronized with the onset of the dry season. Henry and Tarter (1997) reported that *C. psuedogracilis* in permanent waters descend to deep water in June and do not return to shallow water until November. This is the same period during which the only habitat where amphipods living in temporary pools can survive is damp sediment. Despite very different habitats, it appears that the life cycle of *C. pseudogracilis* includes a migratory phase in permanent and temporary waters.

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Table 1.   Frequency of brood-bearing *C. pseudogracilis*, 2002-2003.

Sampling dates	Brood-bearing female *C. pseudogracilis*
13-Dec-02	0
19-Dec-02	0
2-Mar-03	0
23-Mar-03	2
1-Apr-03	9
11-Apr-03	6
20-Apr-03	9
27-Apr-03	21
5-May-03	1
9-May-03	3
16-May-03	1
23-May-03	1
28-May-03	1
5-Jun-03	0
12-Jun-03	0
20-Jun-03	0

Figure 1.   Placement of 1-meter sampling quadrats at the SUNY Brockport pond. Drawing is not to scale.

1-meter quadrats

Approximate maximum fill line

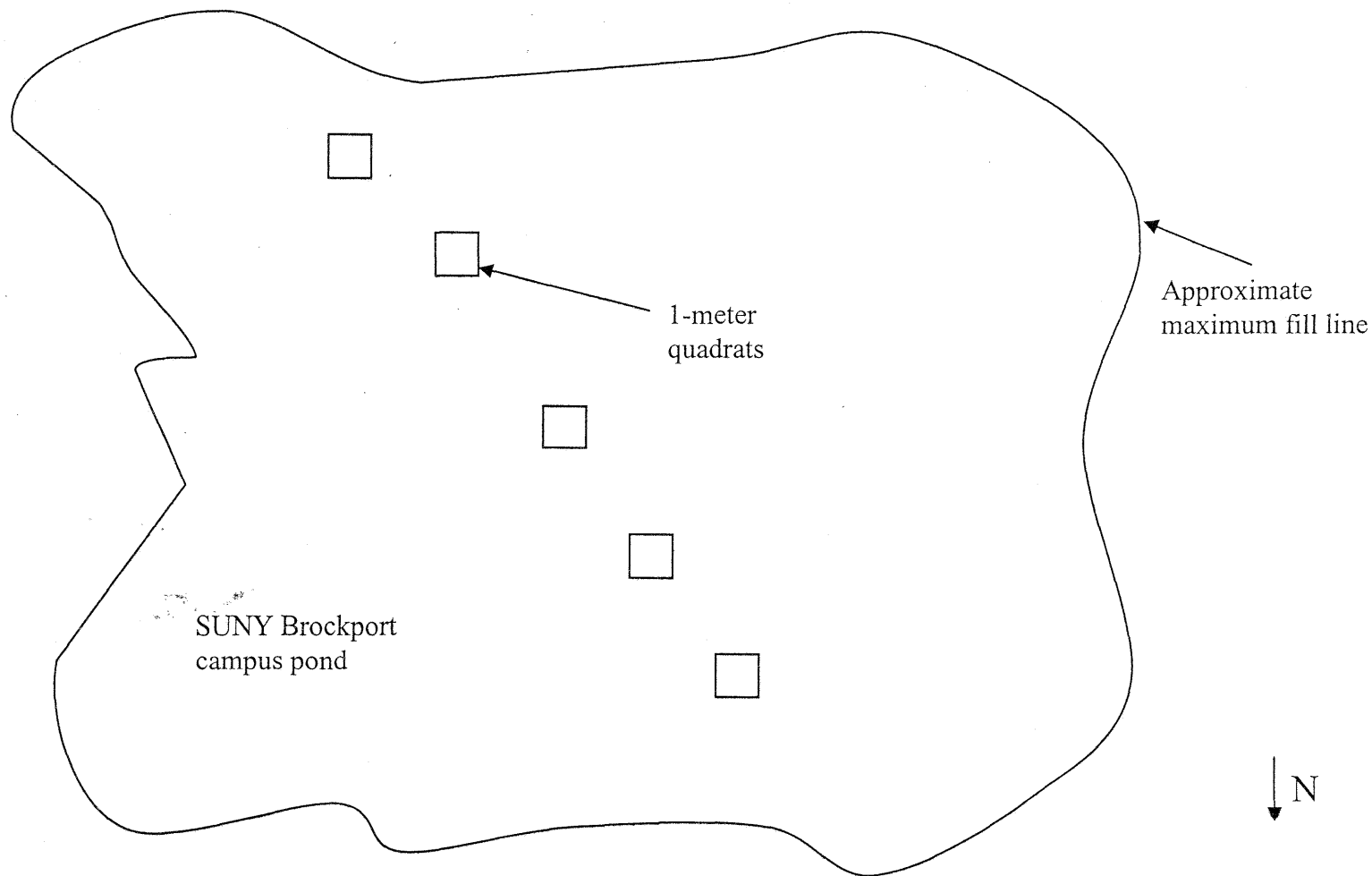SUNY Brockport campus pond

N

Figure 2.	Changes in average amphipod size for *C. pseudogracilis*, 2003. Sizes were measured from the base of antenna 2 to the posterior of segment 2. Error bars represent ±1 SD. Data collection began upon the thawing of the SUNY Brockport campus pond and ceased when no water or amphipods were observed in the pond basin.

Temporal Distribution of Average Amphipod Size: 2003
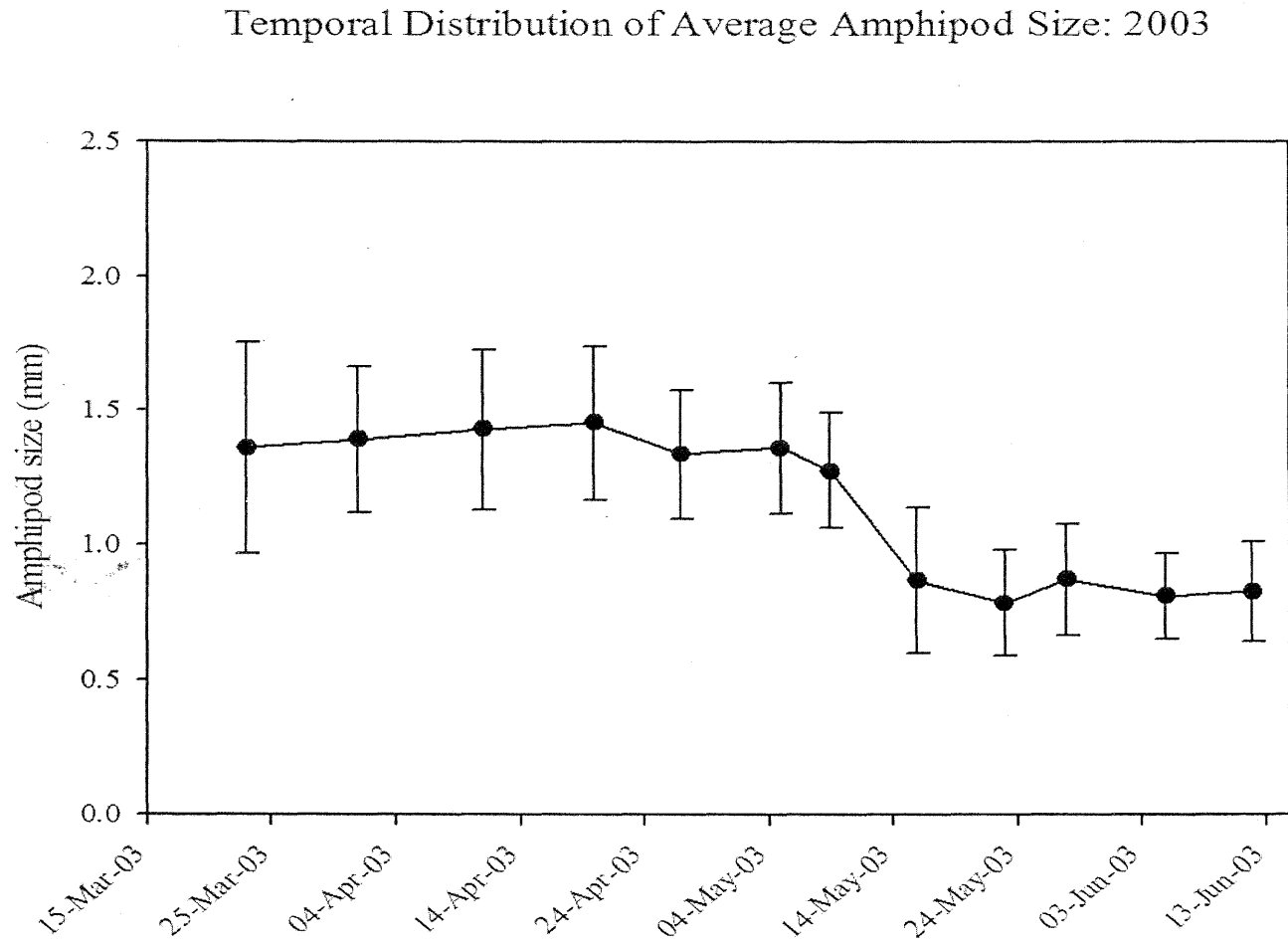
Figure 3.   Average length (base of antenna one to the posterior edge of the second body segment) and the percentage of brood-bearing female *C. pseudogracilis* in the SUNY Brockport campus pond with respect to date in 2003. Error bars represent ±1 SD. The peak of brood-bearing females in late April led to an influx of first-instar individuals and a reduction in the average size of amphipods after mid-May.

Proportion of brood-bearing *C. pseudogracilis* and average measured size of all individuals sampled: 2003
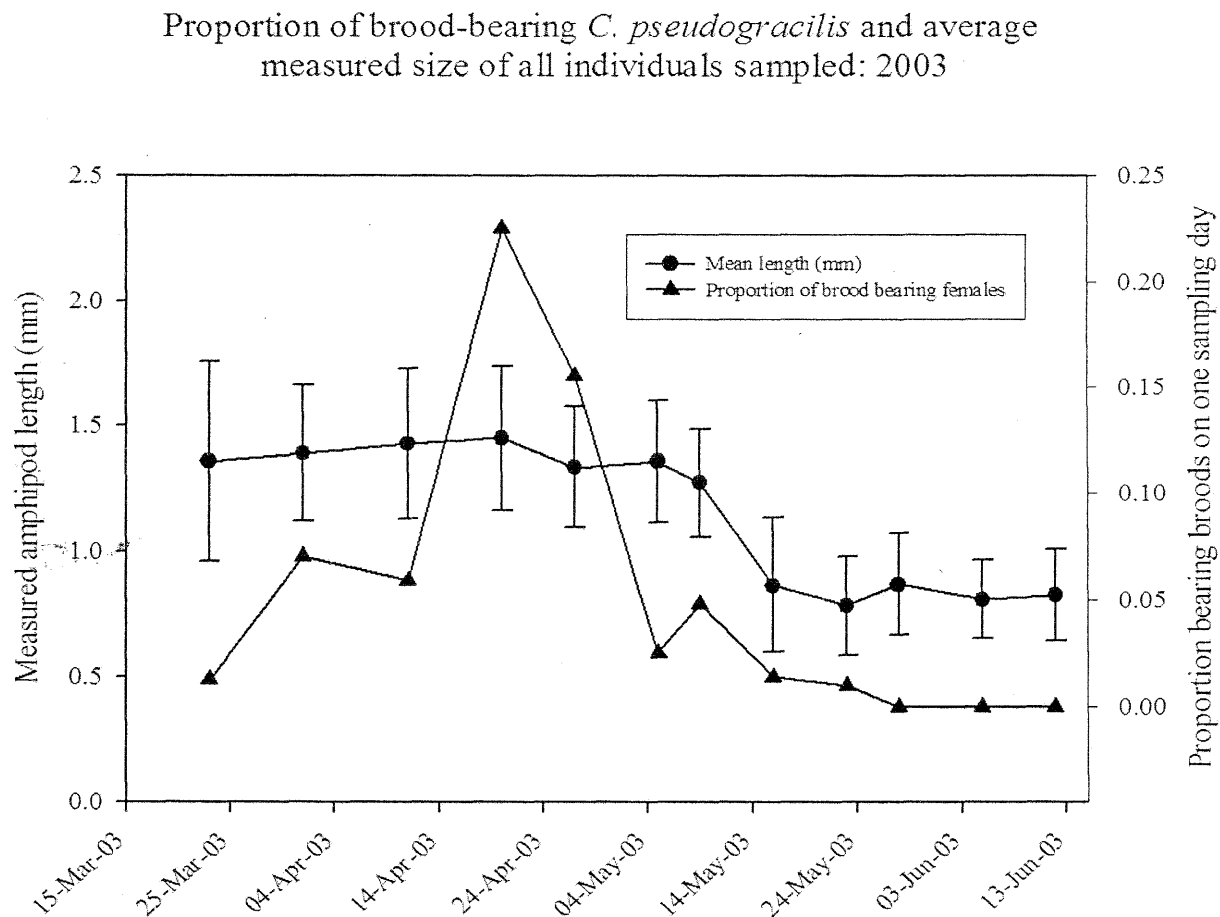
Figure 4   Length frequency distributions of *C. pseudogracilis* in the SUNY Brockport campus pond: 2004. The x-axis is measured size groupings in mm. Note the reduction of the larger size groups and the increase of the smallest size groups as the season progressed.

Figure 4. Continued.

9 May 2003

16 May 2003

23 May 2003

28 May 2003

5 June 2005

12 June 2003

Figure 5.   Changes in the percentage of brood-bearing *C. pseudogracilis* found in box samples taken at the SUNY
Brockport campus pond: 2002-2003.

Temporal Distribution of Amphipods bearing broods: 2002-2003
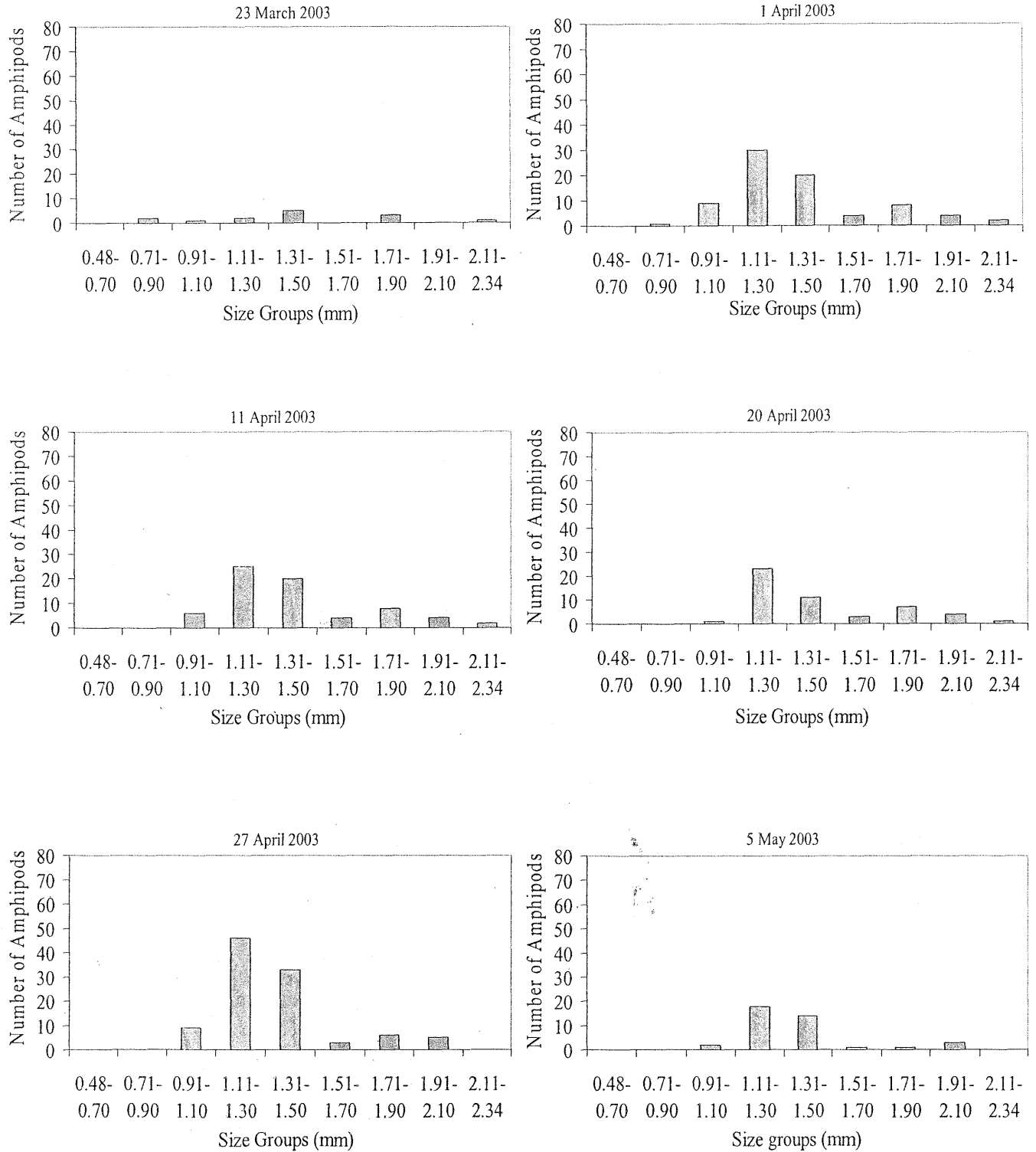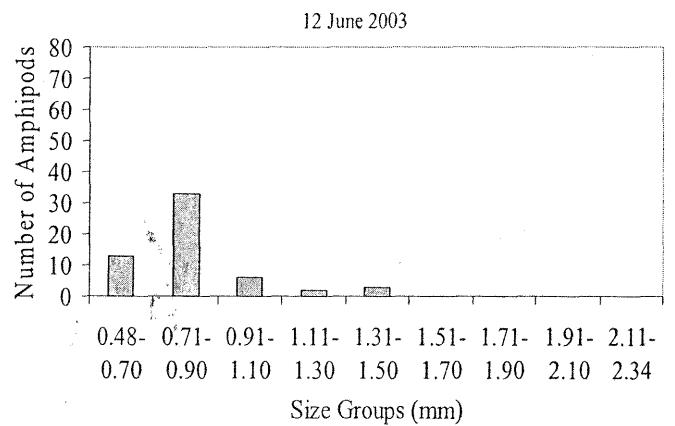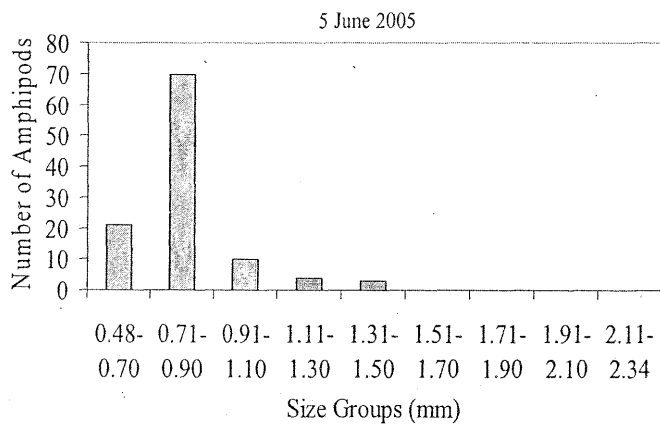
Chapter 5: Investigations into the Dry Season Ecology of

C. pseudogracilis in Temporary Ponds
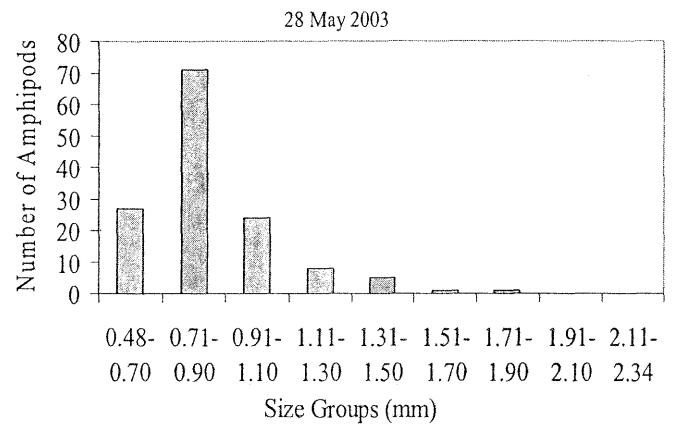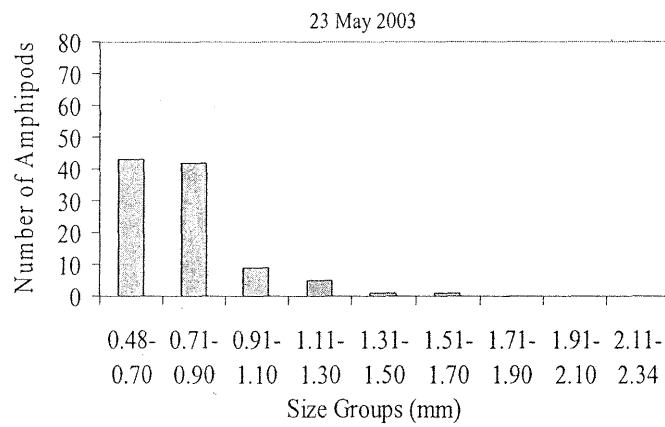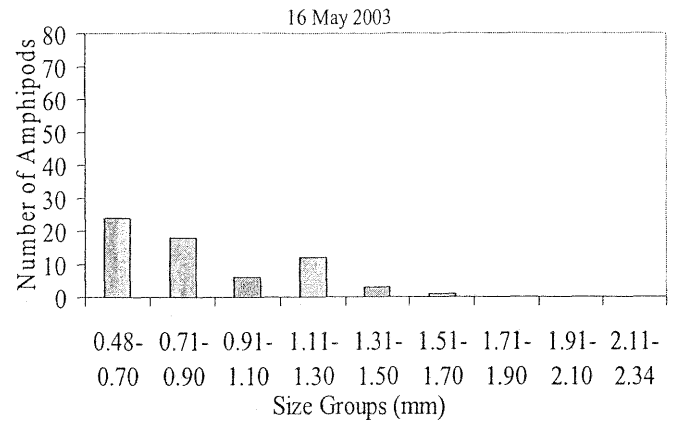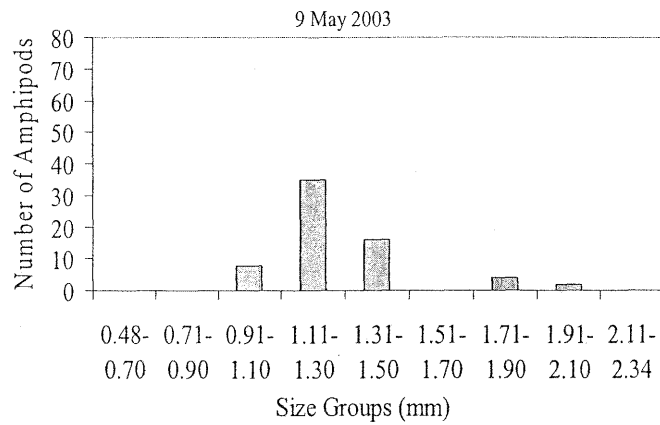
INTRODUCTION

Some invertebrates are behaviorally adapted to survive the dry season by burying in mud, crawling under rocks or logs, or living in crayfish burrows (Holsinger and Dickson 1977, Wiggins *et al.* 1980, Batzer and Wissinger 1996). According to Taylor *et al.* (1999), amphipods generally lack special adaptations for surviving desiccation, such as resting eggs, but have some capacities to persist in moist substrates. Sniffen (1981) reported that the young of three *Crangonyx* spp. survived the dry season by moving to moist or wet subterranean habitats in a flood plain in North Carolina. Similarly, Clifford (1966) found *C. forbesi* residing in damp soil and water-saturated interstitial spaces under a dry, ephemeral stream. Animals living in moist soil or subsurface seepage must either be small enough to inhabit interstitial spaces or capable of burrowing (Clifford 1966); *C. pseudogracilis* probably does the former.

Laboratory experiments have shown that crangonyctids can survive up to 21 days with minimal moisture (Clifford 1966, Batzer and Sion 1999). Furthermore, Batzer and Sion (1999) suggested that *C. pseudogracilis* lie dormant in damp soil in pond basins during the dry season. Based on these findings, and hoping to shed light on how these animals can persist without free water for months, I designed an experiment to test the hypothesis that *C. pseudogracilis* can survive in moist soil without free water for longer than 21 days. In addition, Sion and Batzer (1999) stated that amphipods *must* reside in

soil just beneath the pond basin during dry periods. I tested the hypothesis that upon drying of the pond, amphipods take refuge within 45 cm beneath the bottom of the pond.

METHODS

Soil Sampling

I collected soil samples (approximately 45 x 45 x 45 cm) with an 18 x 30-cm spade from July 2002 through July 2004. I took dry-period samples randomly across the pond basin in 2002 and at random locations within 15 cm of the pond's edge during the period of inundation in the spring of 2003. In 2004, I collected samples along a transect as the pond receded to test if amphipods were following the water table horizontally or staying in damp soil at their previous locations. In 2004, I took the first soil sample within 15 cm of the pond's northwest edge. On the second and subsequent sampling days, I dug samples along the same line within 15 cm of the 'new' pond edge and at the previous pond edge until the pond dried.

I divided each 45-cm sample vertically into three, 15-cm layers, and each layer was placed in a plastic bag for transport to the lab. In the lab, I spread each layer on a tray to a depth of about 1 cm and flooded it with deionized water. Samples stood at room temperature while soil particles settled (usually within 48-72 h). I counted invertebrates visually, sometimes with the aid of a flashlight.

Soil Moisture Experiment

I filled thirty plastic containers (Gladware®, 710 mL, no lids) with soil collected near the temporary pond in the SUNY Brockport woods. I cut a 1-cm^2 hole in each

container for a 'wick' (40 x 8-cm strip of nylon stocking) to protrude through. An upside-down wire rack for drying glassware (46 x 91.5 x 10 cm high) was the platform for the containers. Placing containers on top of the inverted rack allowed their wicks to hang below into beakers of deionized water. To act as a control for mortality that might have occurred from leaving amphipods at room temperature, I placed one container with soil from the SUNY campus woods covering the bottom, and amphipods of varying sizes in an overlying layer of deionized water, next to the experimental setup.

I filled the containers partially with soil and monitored them for one week to ensure the wicks were distributing water to the soil; I checked this by touching the soil. With a plastic pipette, I then added amphipods (5 small—first instar, 5 medium, 4 large) to each of 18 containers partially filled with soil, and more soil was gently placed over the amphipods. Twelve containers had soil only to assess soil moisture content without disturbing amphipods. I refilled the trays with deionized water beneath the containers periodically. At 2, 4, 6, 8, 11 and 15 weeks, three containers with amphipods and two with soil only were sampled. I placed soil with amphipods in a flat pan, flooded the pan with deionized water, and counted amphipods after about 24 h. The containers with soil only were weighed, dried in a hood (by evaporation, not oven-dried), and weighed again to estimate moisture content.

Survival rates of the soil moisture experiment were evaluated with a chi-square goodness of fit test in Minitab® and figures were constructed in Microsoft Excel® and Sigma Plot®.

RESULTS

Soil Sampling: 2002-2003

I found invertebrates only in the upper 15-cm layer of soil. The first soil sample from the dry pond basin, taken on 17 July 2002, contained live seed shrimp (Ostracoda), snails (Pelecypoda: Sphaeriidae), and water mites (Hydracarina), but only one of the other six samples collected during the dry season in 2002 contained invertebrates (Table 1). Three of eight soil samples (18 and 30 May and 11 June 2003), taken during the period of inundation, contained early instar *C. pseudogracilis*. I found no amphipods in samples collected on 23 May and 30 June 2003, the latter was the day after surface water disappeared from the pond (Table 1).

Soil Sampling: 2004

First instar amphipods were collected only in the upper 15-cm at the pond's edge on each sampling date, beginning May 11, until no surface water remained on 4 July (11, 67, 10 and 3 amphipods on May 11, 18, June 13 and 29, respectively). No amphipods were found in samples where a previous edge sample was taken (n = 7). After heavy rains, the pond filled again on 6 July 2004, dried, and filled again 27 July, and new transects were started near the original one. In total, 27 more 45-cm deep samples were collected at the pond's edges (but not at immediately preceding edges) in the summer of 2004; none contained amphipods.

Soil Moisture Experiment

Average soil moisture was 51%, with a range of 42-58%, during the 15-week experiment (Fig.1). After 11 weeks, 52.3% (34 of 65) of the small, 33.9% (22 of 65) of the medium-sized, and none of the large amphipods survived (chi-square = 25.99, df = 2, p < 0.001) (Table 2, Fig. 2). Only one first instar *C. psuedogracilis* survived the entire 15-week experiment (Table 2, Fig. 2).

DISCUSSION

Soil Sampling

The number and kinds of organisms in soil will depend on the time of year they are collected in relation to their life cycles (Clifford 1966). In April, for example, when the Brockport campus pond was inundated, amphipods were abundant in the pond; as the pond retreated they were abundant in the top 15 cm of soil at its edge. During periods of inundation, before the end of June, *C. pseudogracilis* appears to live at the ecotone between the water and soil. The colonization of dug holes reported in Chapter 2 and by Harris *et al.* (2002) illustrates their presence in the ecotone at the pond's edge.

I consistently found *C. pseudogracilis* in the upper 15 cm of saturated soil at the pond's edge until it dried in late June or early July, after which I did not find them to a depth of 45 cm. In addition, *C. pseudogracilis* did not reappear in the top 45 cm of moist soil in the pond after it refilled twice in July 2004, a finding consistent with that of Harris *et al.* (2001). Kenk (1949), Clifford (1966) and Batzer and Sion (1999) also reported not finding amphipods in significant numbers after the end of June.

I found amphipods only in soil samples taken before the end of June, when they were still active in the ecotone of soil and water. By the end of June, amphipods apparently descended to soil depths greater than 45 cm, a finding similar to the migratory behavior of a permanent water population studied by Henry and Tarter (1997). In their study, amphipods left shallow water sampling sites for deeper water in July, and did not return to the edges of the pond until November. Their findings were consistent with arrival of amphipods in the SUNY Brockport campus pond in 2004, a very wet year in which the pond was nearly always filled with water. The temporary water population descends into soils, seemingly out of necessity. The permanent water population also descends (to deeper water), and in both cases this occurs sometime in June. Both populations return to their original locations (shallow water for the permanent population and pond/saturated soil ecotone for the temporary population) sometime in November.

It is unknown how hydro-periodicity influences invertebrates that use desiccation resistance (Dietz 2001), but it seems that the presence of water is not enough to cause amphipods to remain within 45 cm of the soil surface after June. During the period of inundation in the spring (through June), amphipods live in the ecotone of soil and water, an observation that led others (cf. Batzer and Sion 1999, Harris *et al*. 2002) to conclude that they also reside there during the dry season. The summer and fall of 2004 was revealing in that the Brockport pond held a significant amount of water for the vast majority of the summer yet amphipods were not found to a depth of 45 cm until November. Thus, hydro-period does not appear to dictate the migration of these animals.

Where does *C. pseudogracilis* go during the dry season? Harris *et al*. (2001) proposed that they are more closely associated with the water table than with overlying

soil. This seems to be true before the end of June. Given the demonstrated ability of *C. psuedogracilis* to use interstitial habitats in the field (this chapter) and laboratory (Chapter 3), the only place they can be during the dry season must be dry basin soil deeper than 45 cm—the only habitat not sampled at the Brockport campus pond site. In hindsight, I should have sampled this habitat.

Soil Moisture Experiment

Many small and medium-sized but no large *C. pseudogracilis* survived 11 weeks; this result was not surprising. As shown in Chapter 4 and by Henry and Tarter (1997), adult amphipods begin to disappear in May and are gone in June after completing their annual life cycle. Clifford (1966) reported that organisms restricted to surviving in moist soil must be of small size during the dry season due to the physical nature of soil and pore spaces. Also, first instar amphipods are poorly sclerotized (softer and thinner exoskeletons, Pennak 1989), enabling easier gas exchange. In addition, Sniffen (1981) reported that immature crangonyctids of four species were better at surviving drought conditions than mature individuals.

Sniffen (1981) measured soil moisture in the field while sampling invertebrates in a floodplain. After no inundation for more than a month, dry season moisture content ranged from 28-39%, whereas inundated moisture content ranged from 76-83%, which Sniffen termed "complete saturation" Soil moisture during my 15-week experiment ranged from 42-58%, values between Sniffen's (1981) dry and wet conditions.

Clifford (1966) reported survival of *C. forbesi* during the dry season in water-saturated air spaces of a streambed. He subsequently experimented with putting

amphipods in a humidor-type apparatus in the lab and tested the survival of two crangonyctid species in water-saturated air; 50% survived for 7 d, while maximum survival was 11 d. His experiments eliminated free-water and soil as important factors for amphipod survival during the dry season and, in combination with results from my study, establish the importance of the moisture content of the air spaces in the interstitial environment for survival in soil during the dry season.

C. pseudogracilis can endure low oxygen levels (MacNeil *et al.* 2000); field transplantation experiments showed they were able to thrive in areas of low oxygen and low water quality where other gammarids did not. Others have shown that gammarids can lower their metabolic rate during unfavorable conditions (Clifford 1966, Hervant *et al.* 1996). Clifford (1966) also reported that the lowered metabolic rate of *C. forbesi* led to a period of arrested growth when they were residing under the streambed. He hypothesized that this is necessary because if amphipods kept growing they would not be able to re-emerge from interstitial pore spaces.

My moisture experiment, along with the research of Clifford (1966), Sniffen (1981) and Batzer and Sion (1999), shows that amphipods can live without free water. This is important for survival during the dry season and in years of low pond recharge when amphipods must still be active in order to complete their life cycle. These results may explain how amphipods are able to colonize and survive in temporary ponds.

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Table 1.   Soil samples from the SUNY Brockport campus pond: 2002-2003. X indicates presence and --- indicates absence. Numbers of *C. pseudogracilis* are given in parentheses. A soil moisture description (SMD) is given for each sampling date and can be found at the bottom of its respective column. Note: samples from 11 April 2003 through 30 June 2003 were taken more than 30 cm from the pond's edge; earlier samples were taken from a dry pond basin.

Taxon	17-Jul-02	1-Aug-02	29-Aug-02	16-Sep-02	30-Sep-02	7-Nov-02	26-Nov-02	11-Apr-03
C. pseudogracilis	---	---	---	---	---	---	---	---
Planariidae	---	---	---	---	---	---	X	X
Copopoda	---	---	---	---	---	---	X	X
Ostracoda	X	---	---	---	---	---	---	X
Gastropoda	---	---	---	dead	---	---	---	---
Pelecypoda	X	---	---	dead	---	---	---	---
Hydracarina	X	---	---	---	---	---	---	---
SMD	2	2	3	2	3	3	3	3

Taxon	20-Apr-03	27-Apr-03	18-May-03	23-May-03	31-May-03	11-Jun-03	30-Jun-03
C. pseudogracilis	---	---	X(15)	---	X(7)	X(4)	---
Planariidae	X	X	X	---	---	X	---
Copopoda	X	X	X	---	X	X	---
Ostracoda	X	X	X	---	X	X	---
Gastropoda	---	---	---	---	---	---	---
Pelecypoda	X	---	X	---	---	---	---
Hydracarina	X	---	---	---	---	---	---
SMC	1	1	1	1	1	1	2

Soil Moisture Description	
1	complete saturation, soil within the water table, interstitial environment
2	damp soil, dark color from moisture, but not inundated with water, recently inundated
3	dark color from moisture but not recently inundated
4	dry, low moisture content

Table 2.   Amphipod survival (raw counts) in the soil moisture experiment. Amphipods were placed into the setup on 6 June 2004 and destructive sampling dates are shown at right. * denotes containers that had wick problems, resulting in very dry soil. Note: totals at the bottom of the table do not include the last three containers sampled at 15 weeks.

Container	Amphipod Size			Sample Date	Time spent in situ
	S	M	L		
1	2	2	0	20-Jun	2 weeks
2	3	1	0		
3	3	2	0		
4*	0	0	0	4-Jul	4 weeks
5	2	2	0		
6	3	0	0		
7	4	4	0	18-Jul	6 weeks
8	1	2	0		
9	2	1	0		
10	3	2	0	1-Aug	8 weeks
11	2	1	0		
12	4	3	0		
13	2	2	0	22-Aug	11 weeks
14*	0	0	0		
15	3	0	0		
16*	0	0	0	21-Oct	15 weeks
17*	0	0	0		
18*	1	0	0		

Totals

	S	M	L
# Surviving	34	22	0
# Used	75	75	60
% Survival	45	29	0

Figure 1.   Changes in soil moisture content during the 15 week moisture experiment. Each point represents one sampling day where soil moisture content was an average of three containers of soil. Error bars represent ±1 SD.

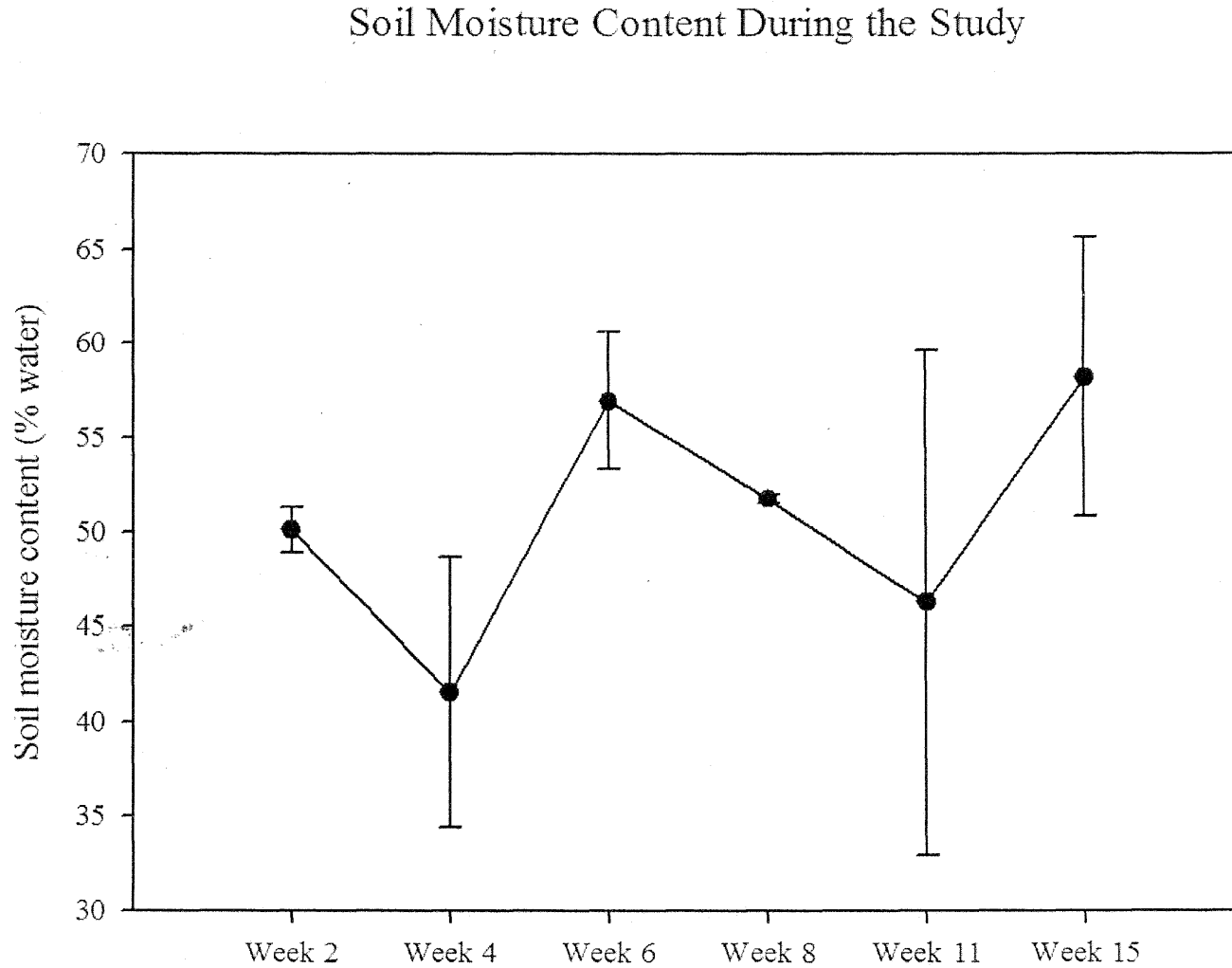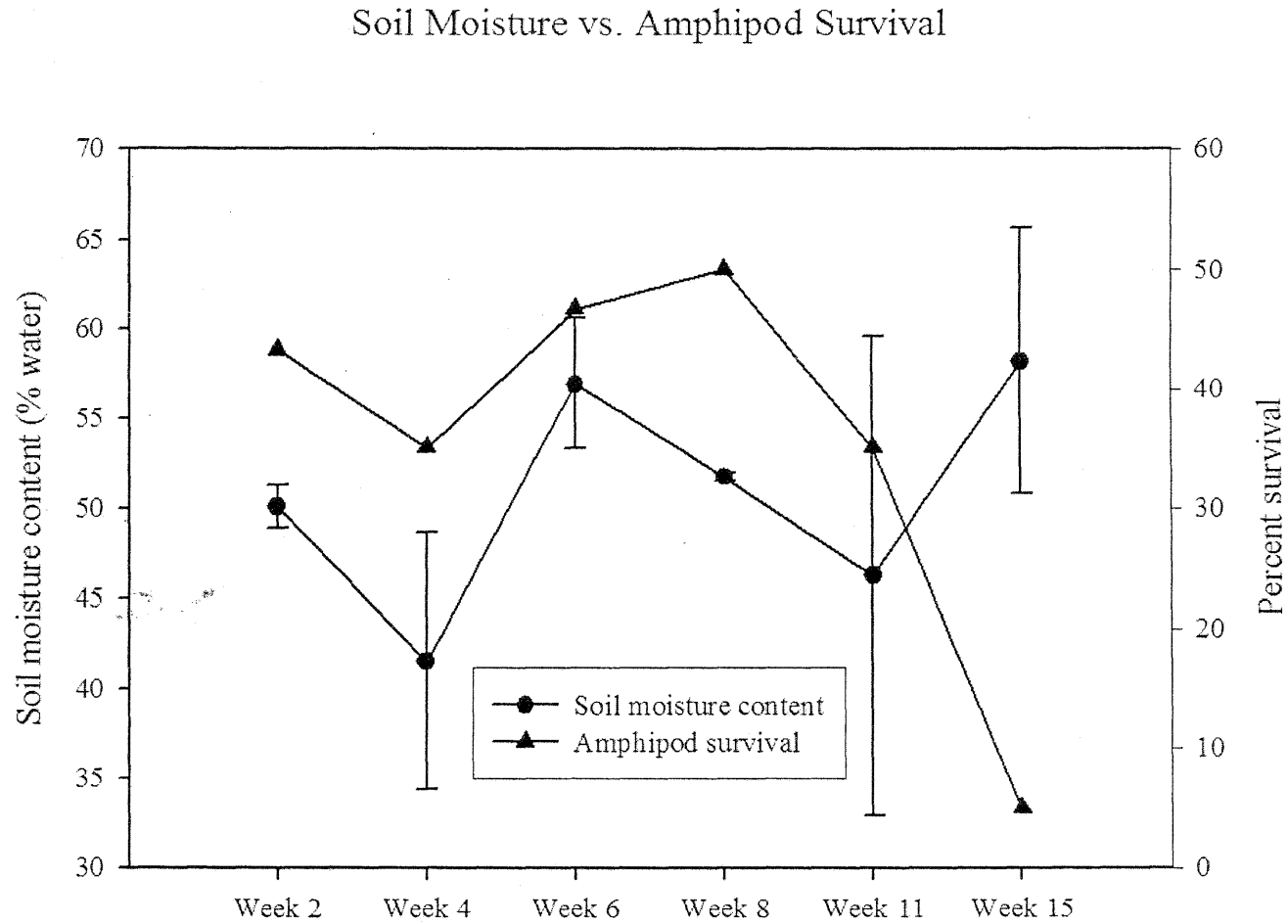Soil Moisture Content During the Study

Figure 2.   Comparison between changes in soil moisture content and survival of amphipods. Each point represents one sampling day where at most 20 amphipods could have survived and soil moisture content was an average of three containers of soil. Error bars represent ±1 SD.

Soil Moisture vs. Amphipod Survival

Chapter 6: Wrapping Up

SUMMARY OF FINDINGS

1)   *C. pseudogracilis* and flatworms moved to dug holes and depressions through interstitial habitats. The underground activity by *C. pseudogracilis* suggests they inhabit interstices of pond sediment at the ecotone of open water and sediments in pond basins.

2)   Amphipods, ostracods, and flatworms are adept at descending through porous inundated substrates to a depth of 35.6 cm. Small amphipods were more successful than large amphipods, and among the three taxa, flatworms were the most successful.

3)   The number and proportion of amphipods in larger size classes decreased after late April; by late spring samples contained few to no late instar or ovigerous individuals. Older instars died as a result of their annual life cycle, leaving only first instars in the population entering the dry season. Presence of brood-bearing females also was related to time of season.

4)   *C. pseudogracilis* survives well in soil with an average soil moisture content of 51% for up to 11 weeks in the lab (maximum survival 15 weeks). During the dry season, amphipods presumably reside in soil at depths greater than 45 cm, although this was not shown empirically. Although the ponds were nearly permanent during the summer and fall of 2004, amphipods did not return to soil less than 45 cm deep until November.

CONCLUSIONS

My research dispels the notion that *C. pseudogracilis* has few adaptations for drought resistance. Thus, it should no longer be a surprise to find these animals in high densities in temporary habitats (Batzer and Sion 1999). Underground colonization abilities, annual life cycle, tolerance for low amounts of oxygen (Dick 1999, MacNeil *et al.* 2000), and ability to survive for up to 5 months (in the field; 15 weeks in the lab) without free water allows *C. pseudogracilis* to thrive in temporary habitats.

C. pseudogracilis readily colonizes dug holes and depressions during the winter and spring (Harris *et al.* 2002; this study). Colonization of dug holes by amphipods and descending behavior through porous substrates provides a possible mechanism as to how amphipods may have arrived in temporary environments.

Although *C. pseudogracilis* readily move through inundated pore spaces through-out its life cycle, the most important time for descending underground is when first instar individuals comprise the population with the dry season approaching. Amphipods likely descend to depths greater than 45 cm sometime in late June and remain there until November, even if water remains in the temporary pools through this time.

C. pseudogracils can survive up to 15 weeks with minimal moisture (51% soil moisture content) in the lab. This is done through tolerance of low levels of dissolved oxygen and the ability to obtain oxygen through both their exoskeleton (Pennak 1989) or gills from moist air in pore spaces (Clifford 1966).

DIRECTIONS FOR FUTURE RESEARCH

My research shows that amphipods and flatworms colonize temporary waters by moving through soil interstices, but the ultimate reasons as to why they actively move from one body of water to the next, and the locomotory mechanism by which they do it, are unknown. Exploratory behavior by *C. pseudogracilis* would be advantageous for an animal living in a constantly changing environment. What proximate cues induce them to penetrate deeper into the sediment remains to be answered (Henry and Danielopol 1999). After the end of June, *C. pseudogracilis* could not be found in inundated soil samples < 45 cm deep. This suggests that amphipods reside at depths greater than this during the dry season. Sampling at these depths with a shovel is difficult, not to mention the amount of soil to transport and analyze. Deeper bucket experiments could supplement a deeper soil sampling strategy. If there was a way to view amphipods descending in porous substrates, it could help guide the sampler as to where or how deep to sample in the field during the dry season.

Similarities between the life cycle of permanent water and temporary water populations raise an interesting question. The temporary population descended into soil in June, seemingly out of necessity to escape dry surface conditions, and returned to the surface in November. Henry and Tarter (1997) observed *C. pseudogracilis* migrating to deep water in a permanent pond at the same time the temporary population descends underground. These amphipods returned to shallow water in November, the same time the temporary population ascended to ponds in SUNY Brockport in 2004. It would be interesting to know if amphipods in permanent water descend to interstitial habitats as well. This behavior raises the possibility that *C. pseudogracils* was originally a

temporary water species that has adapted to living in permanent water rather than vice versa. This hypothesis is consistent with speculation by Batzer and Sion (1999) who found *C. pseudogracilis* only in temporary pools near permanent waters in the same woodland. They speculated that temporary water may be more conducive to survival.

Further research regarding the ecotone that comprises pond benthos and the underlying interstitial habitat is required for a more comprehensive understanding of temporary pond ecology. The experiments presented in this thesis show that amphipods and other invertebrates frequent interstitial habitats and epigean (above-ground) habitats simultaneously and it is unknown how much time is spent in these underground habitats and whether their activities underground differ from those above ground.

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