# Life history aspects of the mayfly *Siphlonurus typicus* (Ephemeroptera: Siphlonuridae) with a new application for measuring nymphal development and growth

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## Abstract

Little is known about the life history of mayflies that inhabit temporary ponds. The purpose of this study was to observe and quantify life histories attributes of *Siphlonurus typicus*, which is known to reside in temporary ponds. Weekly samples of nymphs were taken from two temporary ponds in eastern Connecticut from March to June, 1999. Individuals were graded into 11 maturity classes, based on developmental characteristics, as a means of predicting population maturity rates. Head capsule widths (HCWs) were used to determine if growth occurred between maturity classes. Degree days were found to be an excellent predictor for population maturity ($r^2 = 0.963$, $p < 0.0001$). Nymphs that hatched earlier in the year had larger HCWs than nymphs that hatched later in the year, possibly because of different temperature regimes. The University of Connecticut Forest pond (UCF) population matured faster than the Merrow Meadows pond (MER) population, but both sexes of the UCF population tended to be smaller than their counter parts from the MER population. Sex ratios were female bias for the UCF population (1.35:1), but were not different for the MER population. Adult emergence and mating occurred from 27 May to 21 June. There was a positive relationship between number of eggs per female and female body length. Oviposition at both sites occurred directly on the ponds, pond inlets, and outlets, indicating that pond populations were the result of local migrants as well as permanent residents. Temperature was a determining factor of maturity and fecundity for *Siphlonurus typicus*.

## Introduction

Little is known about the life histories of Siphlonuridae. Current studies include basic biology and ecology of *Siphlonurus typicus* (Eaton) *typicus* (Harper & Harper, 1982), *S. mirus* (Eaton) (Voshell, 1982), *S. aestivalis* (Eaton) (Bretschko, 1985, 1990), *S. occidentals* (Eaton) (Eriksen & Moeur, 1990), *Siphlonisca aerodromia* (Needham) (Gibbs and Mingo 1986, Gibbs and Siebenmann 1996), and *Siphloplecton basale* (Walker) (Clifford, 1976). Little work of past studies was directed at species or populations inhabiting temporary habitats.

North American species in the genus *Siphlonurus* are probably univoltine (Edmunds et al., 1976). Nymphs feed on detritus, including invertebrate carcasses, and even their own exuvia (personal observation from lab rearing). Nymphs typically inhabit calm, shallow waters of ponds fed by springs or intermittent streams. Quiet pools and calm areas at the edges of higher order streams also provide suitable habitat (Burks, 1953). Reports on the habitat from which nymphs hatch has been variable. The genus

*Siphlonurus* has been listed as a permanent resident of temporary ponds, aestivating the dry period in the egg phase (Wiggins et al., 1980; Ward, 1992). Bretschko (1990) agreed with this strategy in particular for *S. aestivalis*. Harper & Harper (1982) called *S. typicus* a distinct headwater species. Voshell (1982) observed early instar *S. mirus* in an intermittent pond during January in southwestern Virginia. Although females were never observed ovipositing, it was hypothesized that the eggs were deposited upstream in an inlet of the pond, and that nymphs drifted downstream into the pond after hatching. In a closely related genus, Gibbs & Mingo (1986) reported that *Siphlonisca aerodromia* made lateral migrations from the Tomah Stream to inundated floodplains in Maine. Little has been reported about the adult stage of *Siphlonurus spp.* regarding oviposition or mating behavior.

Sex ratios have been reported as female bias for *Siphlonurus mirus* (Voshell, 1982), *Siphlonisca aerodromia* (Gibbs & Mingo, 1986), and for *Baetis vagans* (Corkum & Pointing, 1979). These studies recognized that sex cannot be determined for early instar nymphs, but the decision of whether or not a nymph was developed enough to be sexed was qualitatively determined by the size of the individual. Because of the variability in the size of same age individuals, early instar nymphs may have been used for calculating these ratios, thus there is a chance that males may have been counted as females if their genitalia were not yet developed.

Any life history study is concerned with the rate of growth, however, choosing how to measure mayfly growth is challenging. Mayflies molt many times during the nymphal stage (Burks, 1953), depending on temperature and food quality (Brittain 1982). Nymphs reared under controlled laboratory conditions mature faster at higher temperatures regimes (Sweeney, 1978; McCafferty & Pereira, 1984). These factors have an effect on the number of instars an individual has (Brittain, 1982) and any particular instar will not always correspond to an individual's stage of morphological development (Clifford, 1970). In many temperate mayfly species, nymphs that hatch earlier in the season are exposed to low temperatures and grow slower than nymphs that hatch later in the season at higher temperatures, resulting in a varied number of instars between individuals. Therefore, measuring population growth by counting the number of instars per individual is inappropriate.

An alternative to using instars is to use size classes. A set of size intervals based on linear measurements of the head, wing pads, body length or a ratio of two of these features has been used successfully in previous studies (Perry et al., 1985; Pritchard & Zloty, 1994; Scrimgeour & Culp, 1994; Huryn, 1996). Growth rate could then be determined by the distribution of one of these features over time. The problems that underlie this method confound the results and interpretation of the population's growth rate; different temperature regimes not only affect the number of larval instars, but also the final size that an individual attains. Nymphs that begin to grow at higher temperatures tend to be smaller adults compared to individuals that begin growing at lower temperatures (Corkum, 1978; Vannote & Sweeney, 1980). Furthermore, males are smaller than females on average and should be separated before analysis.

At this point a distinction will be made between age classes and maturity classes. Age classification relates to how old an individual is, but not necessarily how developed it is. Maturity classification describes how developed an individual is in terms of becoming an adult. Thus, depending environmental factors, an individual may emerge at the same time as another half its age. Therefore, if the time of synchronized emergence is of interest, as it is in mayfly life histories, then maturation should be of more interest than actual age.

Since Pleskot (1962) recognized size differences in nymphs as a result of growing under varied temperatures and altitudes, distinct developmental stages for characterizing growth, usually based on wing pad maturation, have been identified (Clifford, 1969; McCafferty & Huff, 1978; Humpesch, 1979; McCafferty & Pereira, 1984; Bretschko, 1985). Clifford (1969) classified four stages of development for Ephemeroptera in general. Stage I corresponded to nymphs with no wing pads and stage IV included only nymphs with darkened wing pads. This classification suits the general purpose of surveying many species, but does not adequately categorize the different stages of development for a particular species. A maturity classification developed for a single species is the best option for measuring development.

The effects of water temperature on growth and maturity of mayfly nymphs are somewhat complex. Research has shown that mayfly growth is positively influenced by temperature (Humpesch, 1979; Brittain, 1982; Hawkins, 1986; Benton, 1989; Huryn, 1996). Attainable adult size is directly related to the thermal regime to which the nymph stage was exposed (Coleman & Hynes, 1970; Corkum, 1978; Sweeney, 1978;

Vannote & Sweeney, 1980). Second, body size interferes with the effect temperature has on growth in that the growth of larger nymphs is less positively influenced by temperature than that of smaller nymphs (Hawkins, 1986; Benton, 1989). Lastly, there is a temperature threshold that initiates metamorphosis to the adult stages (Vannote & Sweeney, 1980). The combination of these effects creates a complex set of interactions which is challenging to quantify as a single population growth rate.

The purpose of this study was to observe and quantify aspects on the life cycle of *S. typicus*. Two separate populations were examined for similarities and differences in order to characterize general behavior and adaptive response to temporary habitats. The specific objectives of this investigation were to:

(1) develop a set of maturity classes for measuring growth;
(2) determine if temperature affects population growth;
(3) compare the growth rates and size differences of two populations;
(4) determine if sex ratios were uneven;
(5) qualitatively document the swarming and mating behaviors of adults and quantify fecundity; and
(6) determine if nymphs are migrants or indigenous residents of temporary ponds.

## Methods

### Site descriptions

The Merrow Meadows Pond (MER) is approximately 105 m above sea level, located at 41° 49′ 25″ N by 72° 21′ 02″ W in Merrow, Connecticut, and is part of the Willimantic River watershed. The University of Connecticut Forest Pond (UCF) is approximately 100 m above sea level, located at 41° 49′ 04″ N by 72° 14′16″ W in Storrs, Connecticut, and is part of the Fenton River watershed, near the university pump station. Both ponds are fed by ephemeral streams during snow melt and intervals of precipitation, and have outlets that ultimately lead to respective rivers.

The MER site closely resembles a temporary autumnal pond (Wiggins et al., 1980; Ward, 1992), having a dry period that lasts from August to November. This site is bordered on the east side by a steep slope covered by oaks and maples. Immediately to the west is a grassy field, allowing direct exposure of sunlight to the pond.

Water levels in UCF tend to be more sporadic than MER; during a drought, the pond may dry. This site typically remains wet from January to June. Thus it is categorized between a temporary vernal pond and a temporary autumnal pond (Wiggins et al., 1980; Ward, 1992), drying in midsummer, and filling in winter. The UCF site occurs within a mixed hardwood forest and is shaded by canopy after spring leaf growth.

A dry period occurred at UCF in early spring, 1999. On 10 April, no standing water was observed. On 27 April, four separate regions of its outlet were chosen as sample sites for the remainder of the season. These areas were close to the original pond, and were considered to have representative members of the same breeding population.

### Sampling

A simple grab sampling method was employed, consisting of the use of a 20 × 27.5 × 6 cm tray that was filled with water by immersion into the pond. Once a tray-full was obtained, water was carefully poured through a tea strainer to ensure that nymphs were not lost. Individuals were then picked from the tray with soft forceps and placed into a vial of 80% ethanol. Samples were only considered quantitative in a sense to account for emergence by determining catch per unit of effort (CPUE; number of individuals per grab), otherwise, samples were used to assess the maturity of the populations, not overall abundance.

Weekly samples were taken from each site from 9 March to 22 June, 1999. The mean CPUE was recorded for each site from 7 April to 22 June, 1999. After each grab, nymphs were individually removed from the tray, tallied, and placed into a vial containing 80% ethanol. Grabs were made over an interval of 1.5 h. Specimens were brought to the lab and separated by maturity class (see measuring growth, below) and sex. Head capsule width (HCW) was measured for every specimen to the nearest 0.05 mm with an ocular micrometer.

### Measuring Growth

Maturity classes were delineated as a means of measuring growth, using gill and wing pad development to describe maturity. Early instar nymphs are presumably small enough to respire cutaneously, but must develop gills for more efficient respiration as they become larger in later developmental stages. Gill development was thus the main focus for immature stages. Mature

stages were delineated by wing pad size in relation to other features of the body.

Over 1000 specimens from a previous study of the same ponds (Jokinen, unpublished) were examined for morphological differences in order to develop a maturity class criteria. All individuals with darkened wing pads were considered to be in the last maturity class, because this is an advanced stage of development with emergence soon to follow. Maturity classes were then successively determined backwards by categorizing individuals with morphological features that were less developed.

### Influence of temperature on growth

Larval development was negligible at 4 °C (unpublished data), thus degree days were determined in mean daily temperatures >4 °C, starting on 9 March, 1999. Water temperature was recorded for the MER pond site with an underwater data logger that recorded temperature at hourly intervals from 11 March to 30 June, 1999. Data were retrieved with the Boxcar-Pro software (Onset Computer Corporation) and degree days were calculated from mean daily temperature. Nymphal maturity was viewed from the standpoint that emergence is representative of one hundred percent maturity. Thus, population maturity was determined for each date sampled by finding the weighted mean of individuals in all maturity classes:

$$X_j = E_j + \left[ \Sigma \frac{(n_{ij})(w_i)}{N_j} \right] (100 - E_j), \quad (1)$$

where $X$ is the population maturity of the total sample of $N$ individuals at time $j$, with the $n$ number of individuals in maturity class $i$ adjusted by the weighting factor $w = i/11$. The percentage of the population that had already emerged ($E$) is equal to 100 times the quantity of the averaged mean CPUE of all samples before emergence (amCPUEbe) minus the CPUE at time $j$, divided by the amCPUEbe. Thus, time $j$ where $E$ reaches 100 represents the point in time that the last individual in the population has emerged. $E$ was set to zero for dates before emergence was detected. Linear regression was used to find the relationship between population maturity and accumulated degree days.

### Swarming and reproduction

The vicinity of each pond was scouted from 21 to 26 May at various times after nymphs in stages of metamorphosis were detected. This was done to determine when swarming occurred, if there were any obvious swarm markers, and to record swarm behavior. **Swarms** were monitored from 27 May to 21 June, alternating each night between the two population localities. On each visit, swarm starting and ending times were recorded. Eggs were collected by allowing recently mated females to oviposit egg bundles into a jar containing water. Remaining eggs were manually removed from the uterus postmortem. Female subimagos and nymphs with darkened wing pads were collected and dissected for eggs. Fecundity was estimated from the number of eggs per adult female and was compared with reports of other species of *Siphlonurus*.

### Pond residents or migrants?

Qualitative monitoring of the ponds and their inlets and outlets for hatching nymphs was conducted bimonthly during mid-December, 1998 and early January, 1999 and weekly from mid-January to late February, 1999. Four check points were established along inlets and two were established along outlets. Checkpoints were chosen on the basis of accessibility. Substrate at each checkpoint was disturbed while a plankton tow net (150 $\mu$m mesh) was used as a kick net to catch nymphs downstream. The presence of nymphs in early developmental stages was considered to represent new hatches associated with the area sampled. Furthermore, swarming was monitored from 27 May to 21 June, 1999. Females were followed, when possible, immediately after mating to observe oviposition sites.

### Statistical analysis

The Kruskal–Wallis test of rank-transformed mean HCWs was performed to determine if differences in mean HCWs existed between maturity classes. Tukey's HSD test with Kramer's adjustment was used to identify differences among maturity classes. Significant differences in mean HCWs between two consecutive classes signified detectable growth. These procedures were carried out for each sex in more mature stages (classes that could confidently identify sex), and for individuals within immature stages (classes that could not confidently distinguish sex) for each population. Depending on normality, *t*-test or Wilcoxon Rank Sum test was used to test for differences in HCWs between populations for the same class and sex.

Delineated maturity classes were used to separate underdeveloped nymphs that could not be sexually distinguished. Chi-square analysis was used to test for a

deviation from a 1:1 ratio between the sexes for each pond population, separately, over the entire sample period. Linear regression was used to evaluate the relationship between adult female size and the mean number of eggs per female.

Degree days were square root transformed and linear regression was used to determine the relationship between degree days and population maturity for the MER population. Assuming that early hatchlings mature and emerge before later hatchlings, the effect of accelerated growth on late hatching nymphs due to temperature increases was analyzed for each population. Depending on results of normality, 1-way ANOVA of HCWs or the Kruskal–Wallis test of rank transformed HCWs was used to test the null hypothesis that mean HCWs of nymphs in mature stages are the same across all sampling dates for each sex by population. Tukey's HSD test with Kramer's adjustment was used to show differences of mean HCWs between dates. Maturity rates between populations were analyzed with the test for homogeneity of regression coefficients by the General Linear Model using least square means to examine differences between slopes. Statistical procedures were performed with SAS version 8 (SAS Institute Inc., Cary, North Carolina).

## Results

### Measuring growth

Maturity Class Criteria (Appendix) were created for 11 discernible stages. Classes I–V described immature stages of development where gill structure was emphasized. Classes VI–XI were stages with fully developed gills, using wing pad development as the main character.

Distributions of maturity classes for both populations over time is summarized in Fig. 1. Successive significant differences were detected in mean HCWs between all immature classes from MER ($F = 1,231.83$, $p < 0.0001$) and UCF ($F = 599.94$, $p < 0.0001$), females in Classes VI–X from MER ($F = 187.54$, $p < 0.0001$) and UCF ($F = 157.02$, $p < 0.0001$) and for males in Classes VI–X from MER ($F = 230.82$, $p < 0.0001$) and UCF ($F = 152.25$, $p < 0.0001$) (Table 1). No significant differences in mean HCWs were detected between Classes X and XI for both sexes within each population.

### Influence of temperature on growth

Square root transformed degree days predicted population maturity at MER ($r^2 = 0.963$, $p < 0.0001$, $y = 0.0389\sqrt{x} + 0.0299$). Calculations of ANOVA and the Kruskal–Wallis test of mean HCWs of individuals in metamorphosis from 11 May to 22 June showed different results between pond populations. Both sexes of the MER population showed a significant trend of decreasing HCWs over the emergence period ($F = 6.84$, $p < 0.0001$, for females; $F = 6.77$, $p < 0.0001$, for males) (Fig. 2). No significant differences of mean HCWs were detected between dates for both sexes at UCF ($F = 1.18$, $p = 0.3260$, for females; $F = 1.48$, $p = 0.2047$, for males) (Fig. 3).

### Population comparisons

Test for homogeneity of the regression coefficients revealed that the slopes of both population growth rates were not significantly different ($F = 0.85$, $p = 0.3686$) between populations. Comparison of the slopes using time (days) as a covariate showed that the UCF population (*least square mean* $= 54.09$) was significantly more mature than the MER population (*least square mean* $= 48.03$) at any given day ($p = 0.0229$) (Fig. 4).

Wilcoxon Rank Sum test showed that there were no between population differences for mean HCWs of individuals in immature stages of development (Table 1). The *t*-test and Wilcoxon Rank Sum test showed that females and males from MER had significantly larger mean HCWs than individuals from UCF, respective to class and sex (Table 1).

### Sex ratios

Class I–V were designated as underdeveloped nymphs (Appendix). Maturity class V showed near full development of the gills and the initial development of the front wing pads. Male genitalia could be detected in some individuals, but only with careful scrutiny. There was no confidence that all males could be identified in this maturity class, therefore only nymphs of Class VI–XI were sexed to determine the ratio of males to females.

All males and females from MER occurring in Classes VI–XI totaled 256 and 230, respectively. This proportion was not found to deviate from a 1:1 ratio ($X^2 = 1.39$, $p = 0.2382$). Males and females from UCF occurring in the same classes totaled 202 and
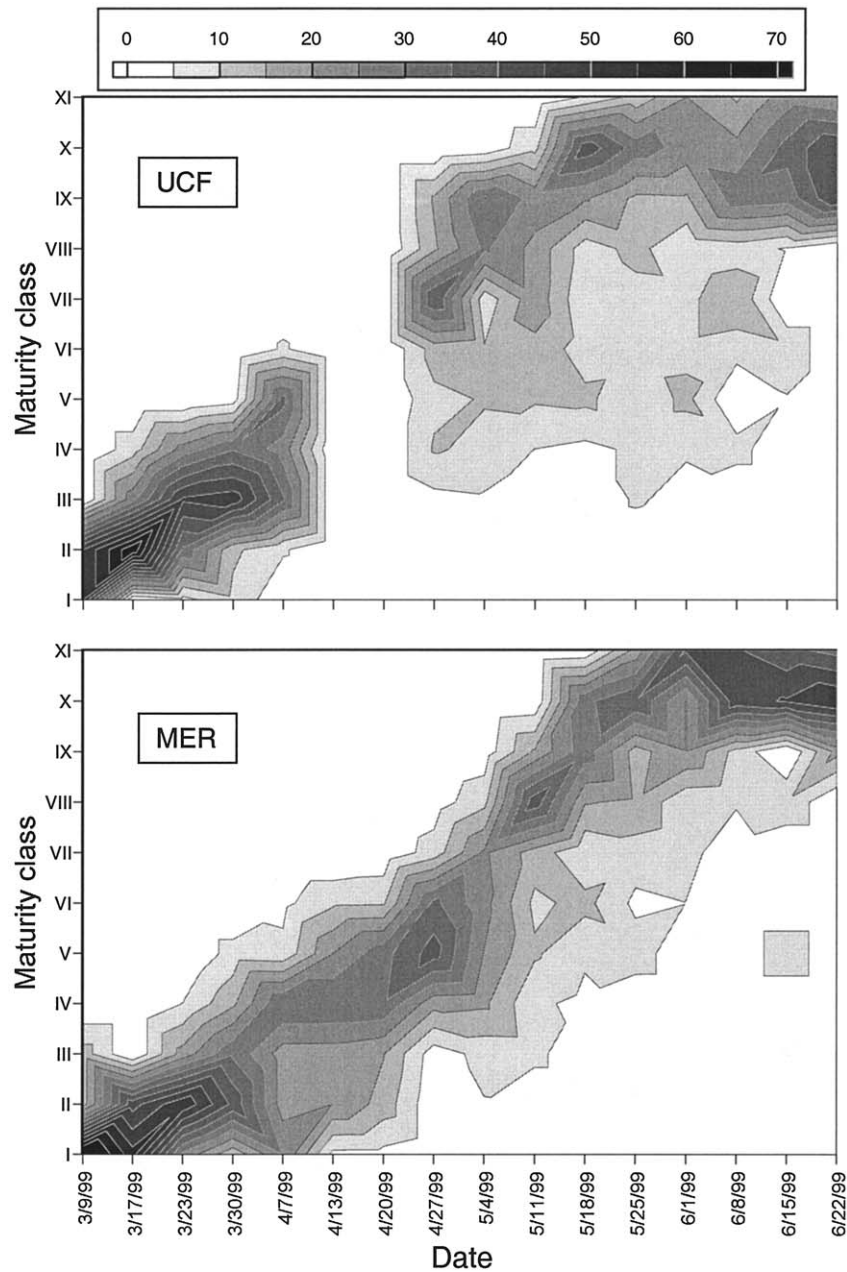
*Figure 1.* The distribution of maturity classes over time for the University of Connecticut Forest Pond Area (UCF) and the Merrow Meadows Pond (MER). Shade intensity depicts percent of individuals. The empty space at UCF indicates a period of no sampling. Prior sampling was in the UCF Pond, thereafter, sampling was conducted in the UCF outlet.

273. This was shown to be a significant female bias of 1.35:1 ($X^2 = 10.61$, $p = 0.0011$).

*Swarming and reproduction*

Nymphs in Class XI were first sampled at both sites on 18 May, 1999. However, a Class X individual was first sampled one week earlier at MER and two individuals of that class were sampled two weeks earlier at UCF. Emergence was presumed to start the week following detection of Class X individuals. Subimagos were not carefully observed in the field, however, lab reared individuals took 24 to 78 hours to transform to imagos. Very few nymphs occurred at both sites on 22
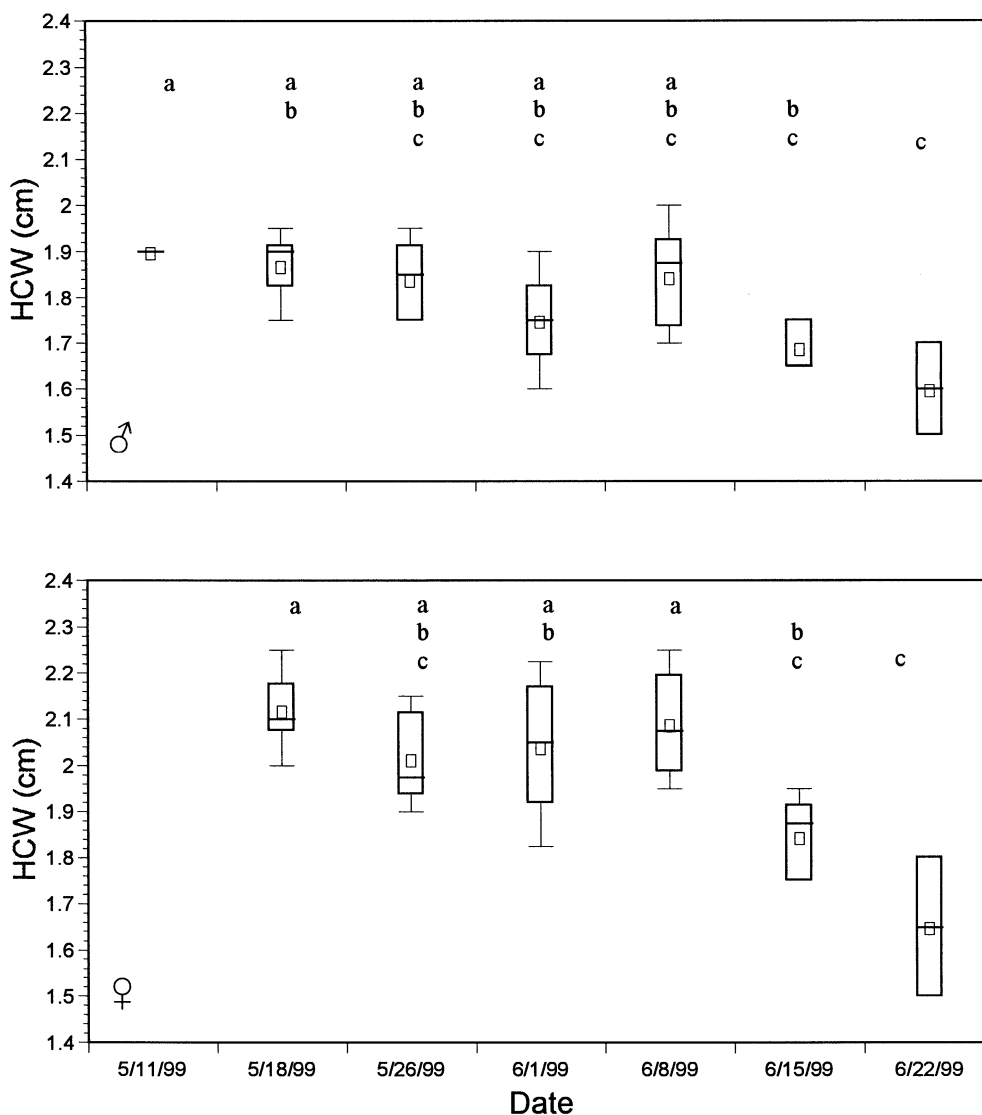


*Figure 2.* Mean head capsule widths (HCW) of males (top) and females (bottom) in stages of metamorphosis from the Merrow Meadows Pond. Whiskers represent 10th and 90th percentiles, the ends of the rectangles represent the 25th and 75th percentiles, the lines in the middle of the rectangles represent the median, and the boxes within the rectangles represent the mean. Letters show significantly different mean HCWs between dates.

June (Fig. 5). Only one of the sampling locations at UCF had standing water at this time and few imagos appeared during the swarming hours at both sites.

Swarming began between 6 and 7 pm and ended between 7:30 and 8:30 pm, at both sites. The shortest and longest swarming durations were recorded at MER, lasting for 25 and 140 min, respectively. The mean swarming intervals for UCF and MER were $91 \pm 10$ and $78 \pm 33$ min ($n \pm SD$). On two occasions, 9 June and 17 June, swarming was not observed at either site. Cool air temperature and overcast conditions may

have inhibited swarming or caused it to occur at an earlier time.

On each night that swarming was observed, commencement of swarming occurred when a few males at a time congregated and began hovering. The swarm grew to hundreds of males within 10 to 20 min. Peak emergence occurred between 30 May and 6 June.

Swarms occurred at specific locations every evening. Most swarms took place over a small, slow moving stream of water; usually an inlet or outlet associated with the pond. Some swarms were high, 10 m or
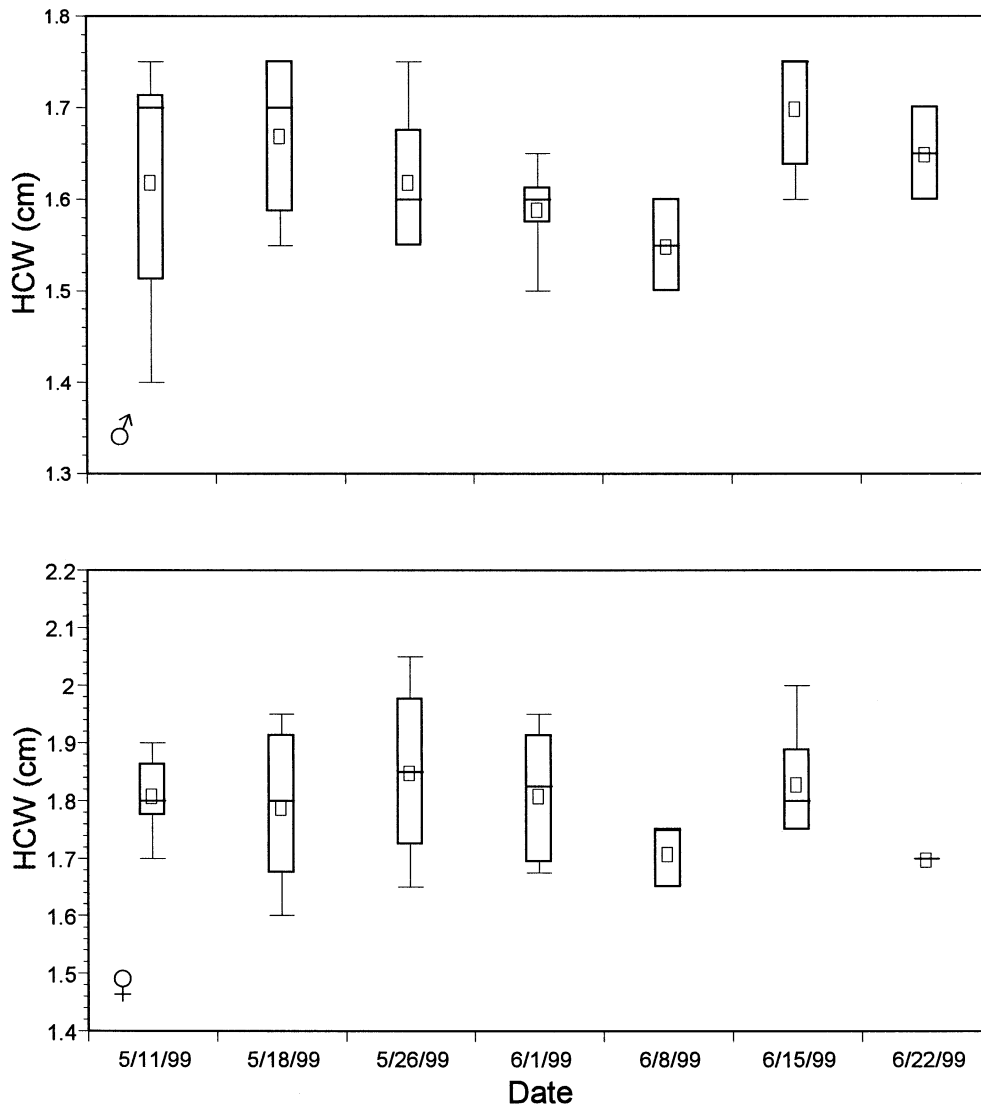
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*Figure 3.* Mean head capsule widths (HCW) of males (top) and females (bottom) in stages of metamorphosis from the University of Connecticut Forest Pond Area. Whiskers represent 10th and 90th percentiles, the ends of the rectangles represent the 25th and 75th percentiles, the lines in the middle of the rectangles represent the median, and the boxes within the rectangles represent the mean. There are no significant differences of mean HCWs between dates.

more above the ground, and others were low, between 0.67 and 2 m above the ground.

In a swarm, males usually did not interact with each other, maintaining at least a small distance between individuals. On occasion, males jostled with each other, but these events were brief and infrequent. Competition between males occurred when a female entered a swarm. Females entered swarms independently of each other with no apparent preference as to where they entered. Upon entering the swarm, several

males immediately tried to seize the female by flying from underneath her.

Immediately after copulation, mated pairs flew to a nearby leaf or branch. The female either landed or grabbed onto the object, supporting the weight of both individuals. Mating lasted from 20 to 300 s ($n = 5$). After separation, the male returned to the swarm, while the female rested in place for about one minute, then flew off to oviposit. Before ovipositing, females landed on the surface of the pond, an object projecting from the pond or an associated inlet or outlet. On
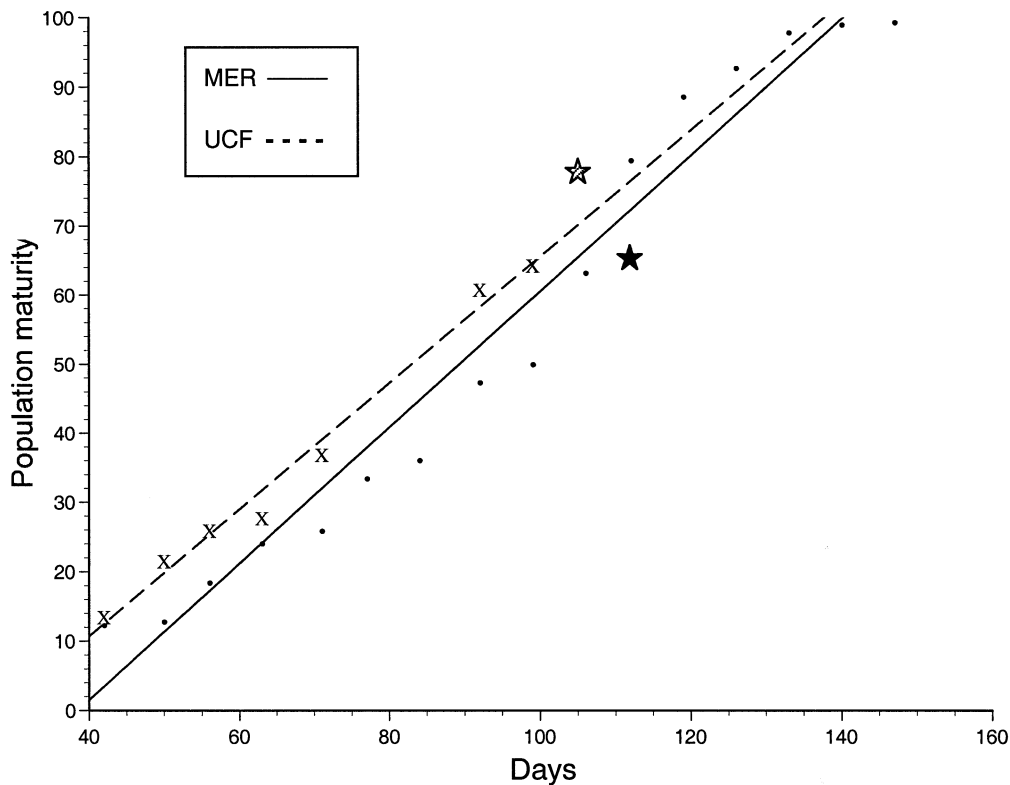
*Figure 4.* Population maturity (from equation 1) for both populations of *Siphlonurus typicus* over days ($\bullet$ = MER, $\times$ = UCF). Slopes were not significantly different ($F = 0.85$, $p = 0.369$) but *y*-intercepts were different ($F = 6.07$, $p = 0.022$). Estimated time of initial emergence is depicted by stars.

one occasion, a female was observed hovering above a small outlet, dipping its abdomen into the running water to release eggs into the current.

Eggs were always oviposited in spherical clumps or 'bundles'. These bundles became sticky upon entering water due to the expansion of the egg casings. The average bundle size was 1.04 mm ($n = 102$, SD = 0.35) having an average of 43.5 ($n = 100$, SD = 18.91) eggs per bundle. Eggs were white to light yellow in color, covered in a filamentous casing. Eggs, including the casing, ranged in size from 0.2 mm to 0.3 mm. Smaller eggs embedded in the uterus were considered to be underdeveloped and therefore were not included in the totals. The total number of eggs per female varied greatly (Table 2) with means of 703 for UCF and 899 for MER (805 together), with standard deviations of 313 and 445, respectively. These results were lower than Clifford and Boerger's (1974) reports for *Siphlonurus alternatus* (range = 884–2023), *S. lacustris* (range = 1712–2499), *S. aestivalis* (range = 933–2678) and for Voshell's (1982) report for *S. mirus* (range = 7706–8256). No significant difference was found in the number of eggs per female between sites using body length as a covariate ($F = 1.71$, $p = 0.2091$). Females of both populations were pooled together for linear regression analysis, yielding a positive relationship between body length and number of eggs per female ($r^2 = 0.53$, $p = 0.0004$) (Figire 6).

Males occasionally attempted to engage with other insects that passed by, or conspecific males that were entering the swarm, but discontinued their pursuit as soon as they recognized that their target was not a female. The reaction to copulate was extremely sensitive to almost anything; males appeared to dart in the direction of birds that flew 2 to 4 m overhead, or even dragonflies that were preying on them. After the active period of mate swarming, the males flew towards the surrounding tree tops. Before, during, and after swarming, some males were noted to dip their abdomens in the water underneath the swarm location.

*Table 1*. Mean head capsule width and standard deviation (SD) for maturity classes of both populations. Letter separations indicate significant differences of classes for each sex within populations. *t*-test was used for normally distributed females and Wilcoxon rank tests were used for non-normally distributed immatures and males to show between site significance at $p < 0.05$. MER = Merrow Meadows Pond. UCF = University of Connecticut Forest Pond Area.

| Class | UCF | | | MER | | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | *n* | Mean | SD | *n* | |
| Immatures | | | | | | | Prob > \|*t*\|[a] |
| I | 0.21 a | 0.04 | 31 | 0.22 a | 0.03 | 59 | 0.6136 |
| II | 0.34 b | 0.06 | 53 | 0.34 b | 0.05 | 119 | 0.8646 |
| III | 0.51 c | 0.08 | 117 | 0.50 c | 0.07 | 95 | 0.3419 |
| IV | 0.66 d | 0.10 | 92 | 0.67 d | 0.09 | 136 | 0.6957 |
| V | 0.90 e | 0.14 | 86 | 0.91 e | 0.12 | 132 | 0.1564 |
| Males | | | | | | | Prob > \|*t*\|[a] |
| VI | 1.04 a | 0.13 | 33 | 1.14 a | 0.10 | 64 | 0.0009 |
| VII | 1.23 b | 0.10 | 22 | 1.35 b | 0.08 | 22 | 0.0008 |
| VIII | 1.36 c | 0.09 | 34 | 1.51 c | 0.13 | 38 | <0.0001 |
| IX | 1.52 d | 0.11 | 54 | 1.73 d | 0.13 | 41 | <0.0001 |
| X | 1.63 e | 0.09 | 44 | 1.84 e | 0.11 | 65 | <0.0001 |
| XI | 1.68 e | 0.06 | 13 | 1.81 e | 0.12 | 30 | 0.0011 |
| Females | | | | | | | Prob > \|*t*\| |
| VI | 1.15 a | 0.11 | 44 | 1.23 a | 0.11 | 33 | 0.0021 |
| VII | 1.34 b | 0.13 | 41 | 1.50 b | 0.12 | 38 | <0.0001 |
| VIII | 1.52 c | 0.15 | 53 | 1.72 c | 0.14 | 47 | <0.0001 |
| IX | 1.67 d | 0.13 | 64 | 1.86 d | 0.13 | 30 | <0.0001 |
| X | 1.79 e | 0.15 | 56 | 2.00 e | 0.15 | 34 | <0.0001 |
| XI | 1.88 e | 0.15 | 20 | 2.04 e | 0.16 | 41 | 0.0003 |

[a]Approximated *t*-values used.

## *Pond residents or migrants?*

Nymphs were not found in the MER inlet during the period of monitoring for hatchlings. The first *S. typicus* nymph was recorded from MER on 26 January and was identified as Class II. A visit to the inlet in early June revealed that there were nymphs present in the inlet. At UCF, nymphs were first found in the pond inlet and outlet on 26 January and were identified as Classes I and II. Standing water at these locations had individuals throughout the sampling season, even though the UCF pond had completely dried up.

It was noted by the presence of Classes I and II representatives that hatching took place all through the season (late January to mid-May). Although the last Class I specimen was sampled on April 27, Class II specimens were sampled well into May (Fig. 1).

## Discussion

### *Measuring growth*

Life history study is concerned with growth rates. This study showed exceptional variability within two populations of *S. typicus* concerning individual size and the time of individual maturation. Actual age of a nymph tends to have little validity when considering development to the reproductive stage. Larval instar is relatively indeterminate of maturity or growth. Size classes can lead to confounding effects resulting from size differences of nymphs exposed to different environmental regimes or simply from genetic variability, thus making it difficult to compare cohorts or populations of different average sizes.

An approach to the problem of quantifying maturation is to identify measurements that can be made
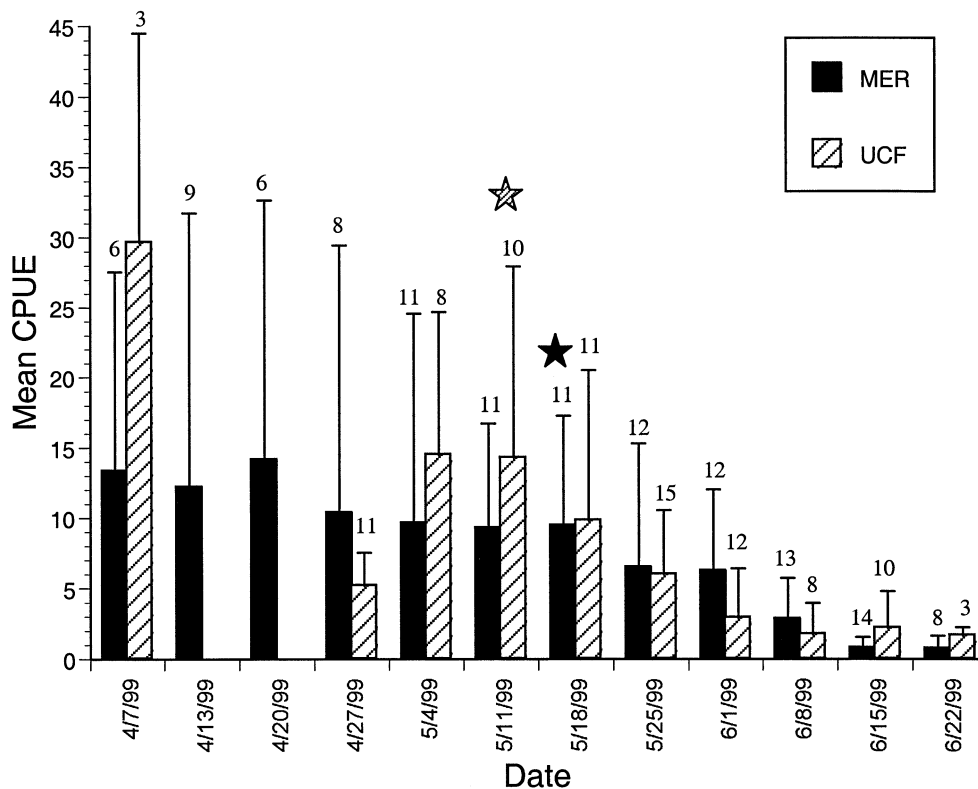
*Figure 5.* Mean CPUE for each sample date at both sites. Stars indicate the estimated date of initial emergence. MER = Merrow Meadows Pond. UCF = University of Connecticut Forest Pond Area. 13–20 April are times during which no sampling occurred at UCF. Error bars show standard deviations. Numbers above error bars indicate the number of grabs taken on that date.

regardless of sex, environmental nurturing, or time of growth. These measurements must be indicative of significant growth regardless of individual sizes. Considering that the overall goal of larval growth is to mature into an adult, maturity classes based on morphological features related to adult characters should be most informative. Not only can comparisons be made among populations or cohorts of different individual sizes, but rate of maturation can be observed with the combination of both sexes.

The pattern of mean separation of HCWs among maturity classes is identical for both populations, regardless of sex (Table 1). Size differences are apparent between males and females and between individuals from separate populations of the same sex and class. However, a distinct succession of growth was observed between classes. Because growth occurs between classes, the classes themselves can be used as a measurement of individual growth or maturity, independent of size, sex or ecological conditions (temperature, nutrition, etc.).

The end result of nymphal development is emergence, soon followed by reproduction, thus development can be expressed in terms of wing pad size with the premise that a certain wing pad length in proportion to the body length is needed before emergence can take place. Clifford (1970) suggested that once nymphs reach a 'mature' stage, the growth of various body parts lessen compared to overall body length. The sexes of both populations did not show any significant differences between Class X and XI, indicating that growth ceased between these two stages of development. Although these classes cannot be separated for purposes of quantifying growth, they are still valid for comparisons of maturity, representing early (Class X) and late (Class XI) metamorphosis.

Examination of *t*-test and Wilcoxon Rank Sum test results showed that the HCWs of both sexes at UCF were significantly smaller than their counterparts at MER for all mature maturity classes (Table 1). Thus, a comparison of these populations using linear measurements as a means of determining growth indicates that the MER population is more mature than the UCF
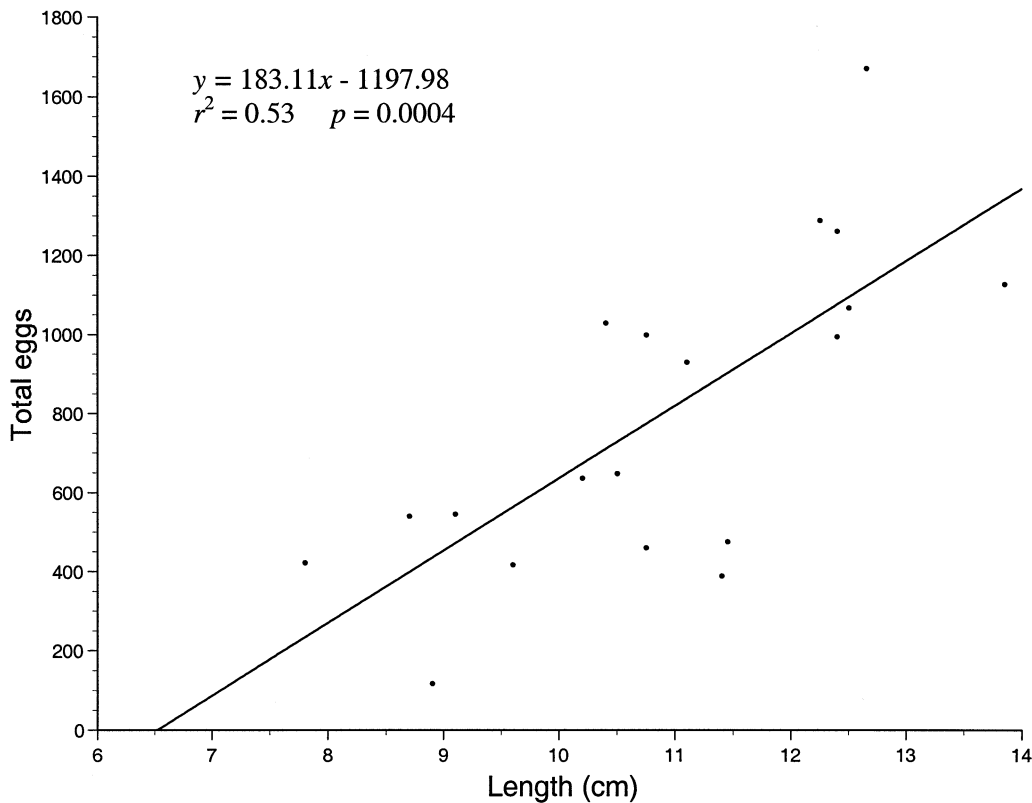
142



$$y = 183.11x - 1197.98$$
$$r^2 = 0.53 \quad p = 0.0004$$

*Figure 6.* Relationship between total number of eggs and total length of adult female *Siphlonurus typicus* from Merrow Meadows Pond and University of Connecticut Forest Pond Area. Females from both populations were pooled together for the analysis.

population, when the opposite was actually the case for the majority of the season (Fig. 4).

Based on the results of this study, the developed maturity classes are shown to be a suitable unit of measurement. Maturity classes eliminate the confounding effects related to individual size differences, and they illustrate a succession of growth which is quantifiable over time.

*Influence of temperature on growth*

Square root accumulated degree days at the MER site were found to be an excellent predictor of the population maturity. Rearrangement of the regression equation predicts that 100% of the population had emerged between 14 and 15 June, 1999. In reality, individuals were present on 22 June and probably persisted into July. At the time of predicted 100% emergence, approximately 90% of the population had actually emerged. The prediction accounts for the majority of the population responding as a function of temperature. Factors such as heterogeneity (individuals are likely to have slightly different temperature

thresholds), predation, and competition are probably some unaccounted predictors. Regardless, temperature is clearly one of the most influential factors affecting maturation.

The MER population showed significant differences in mean HCWs of individuals in stages of metamorphosis over time for both sexes, and comparison of mean values showed a trend of decreasing HCW size over time (Fig. 2). These results are in agreement with other studies (Sweeney, 1978; Vannote & Sweeney, 1980; de Moor, 1982); that winter hatchlings grow and mature to a larger size before spring hatchlings arrive. During the spring, the warmer temperatures increase growth rates and because the winter recruits are more mature at this time, they are the first to emerge. The spring recruits pass through early stages of development more quickly than the winter hatchlings, and thus attain a smaller overall size. Because winter recruits actually lived longer than spring recruits, they had more opportunity to develop at the optimal temperature regime as described by Vannote & Sweeney (1980) and result as larger more fecund adults.

| Individual | Site | Maturity | HCW | Total Length | Eggs |
|---|---|---|---|---|---|
| 1 | MER | Imago | 2.10 | 12.65 | 1674 |
| 2 | MER | Imago | 2.00 | 12.25 | 1292 |
| 3 | MER | Class XI | 2.25 | – | 1209 |
| 4 | MER | Imago | 2.15 | 12.50 | 1071 |
| 5 | MER | Imago | 1.95 | 10.40 | 1033 |
| 6 | MER | Imago | 1.90 | 10.75 | 1002 |
| 7 | MER | Imago | 1.95 | 11.10 | 933 |
| 8 | MER | Class XI | 1.80 | – | 616 |
| 9 | MER | Subimago | 1.70 | 8.70 | 544 |
| 10 | MER | Imago | 2.10 | 11.40 | 392 |
| 11 | MER | Imago | 1.65 | 8.90 | 121 |
| 12 | UCF | Subimago | 2.20 | 12.40 | 1265 |
| 13 | UCF | Subimago | 2.05 | 13.85 | 1131 |
| 14 | UCF | Imago | 1.95 | 12.40 | 998 |
| 15 | UCF | Subimago | 1.70 | 10.50 | 652 |
| 16 | UCF | Subimago | 1.75 | 10.20 | 640 |
| 17 | UCF | Subimago | 1.75 | 9.10 | 549 |
| 18 | UCF | Subimago | 1.90 | 11.45 | 479 |
| 19 | UCF | Subimago | 1.95 | 10.75 | 464 |
| 20 | UCF | Imago | 1.65 | 7.80 | 426 |
| 21 | UCF | Subimago | 1.70 | 9.60 | 421 |

The lack of significant differences in mean HCWs of individuals in stages of metamorphosis from UCF at different time intervals (Fig. 3) does not necessarily contradict the notion of smaller adult size as a consequence to growing at higher temperature regimes. The average HCW of individuals in mature stages (Class VI–XI) were consistently larger in the MER population compared to the UCF population (Table 1), indicating that the UCF population may have undergone stress. There was a drought that exposed many nymphs to conditions of dehydration during a time in the season when the temperatures were approaching the optimal thermal regime. The entire pond population was eradicated and individuals inhabiting the outlet were probably limited by a lowered carrying capacity. If temperature initiates adult tissue development (Vannote & Sweeney, 1980), then adult maturation may have hastened due to reduced water levels that led to elevated temperatures. The fact that the UCF population began to emerge earlier than the MER population strengthens this argument. It is

suggested that the temperature threshold for the promotion of adult tissue maturation was reached in UCF about a week earlier than at MER, thus giving way to relatively uniform small sized mature nymphs at UCF.

Another general factor relating to smaller sizes of spring recruits in comparison to winter recruits is the influence of predation at an early stage of development. Laboratory experiments have shown that the presence of predators can have a detrimental affect on growth and fecundity (Scrimgeour & Culp, 1994; Peckarsky, 1996). With this in mind, winter hatchlings may have an advantage over spring hatchlings in that there are fewer predators to avoid during the colder, earlier months. Thus, spring recruits may be smaller as a result of expending energy towards avoiding predators during early stages of their life cycle.

### Population comparisons

The regression slopes of maturity over time are direct representations of maturation rates. Comparison of the slopes over time (days) between the MER and UCF populations indicated that the UCF population was consistently more mature than the MER population (Fig. 4). The difference is believed to be because of elevated temperatures at the UCF site (see above comments). The comparison of HCWs for each maturity class gives an indication of which population was exposed to more stress. If one population has consistently smaller HCWs than another population for all classes, it may be due to stressful environmental factors that the population experienced, or simply from phenotypic variation. Table 1 shows that there are no differences in mean HCW sizes between individuals in the same class of UCF and MER for Classes I–V. However, individuals in all mature classes were significantly larger in MER than UCF. The drought created less available space for the UCF community, increasing competition and reducing retreats to hide from predators. Thus, it is believed that these conditions were contributing factors resulting in the size differences between MER and UCF.

### Sex ratios

Use of maturity classes made it easy to distinguish females with high level of confidence from underdeveloped unidentifiable males, reducing chance of error due to misrepresentation. Conclusions could not be made from the Chi Square analysis. Female bias has been observed in related species (Corkum & Pointing, 1979; Voshell, 1982; Gibbs & Mingo, 1986), however

this study found female bias only with the UCF population. Under more extreme living conditions females may have a better chance of survival, and perhaps stressful conditions induce parthenogenesis.

*Swarming and reproduction*

Swarm locations are typically associated with specific terrain markers such as trees, clearings or water bodies (Needham et al., 1935; Brittain, 1982). This study observed *S. typicus* swarming over slow running water associated with an inlet or outlet of a temporary pond. Additionally, swarm locations appeared to be related to tree markers or clearings. High swarms seemed to be vertically associated with trees or with clearings as have been observed in other species (Flecker et al., 1988; Harker, 1992; Savolainen et al., 1993). Fewer swarms were observed close to the ground and appeared to be associated with the slow moving water and a small tree, or in one instance, a break in the overgrowth that exposed a small area of an outlet. The difference in heights of separate swarms is possibly an adaptation of two mating strategies. Low flying swarms are less likely to be encountered by avian predators, but are also less likely to be encountered by females. High swarms are easier for females to find, but are more exposed to predation. If females are cued to leks via terrestrial markers, as are males, then the latter strategy would be more of a risk with no greater return. Therefore, low swarming may be a more recent adaptation (Savolainen et al., 1993).

The extent of the emergence period reflects a succession of egg hatching events throughout the season. Regardless, swarm sizes were at their largest for about a week. The locations of the swarms were the same each night. Swarming occurred in the evening around sunset. The actual time swarming began and ended was never the same, nor did it appear that there was a pattern during the three weeks of observation. Sometimes there was a difference of several hours on successive nights. Even though light intensity was not recorded, cloud cover did not appear to be a factor. Savolainen et al. (1993) suggested that temperature may influence the time that swarming takes place. Although air temperature was not recorded, it is possible that the time swarming began was related to both sunset and a change in temperature. On two cool nights no swarming took place, suggesting that temperature may play a role as a limiting factor.

Because males responded to any winged insect entering the vicinity of the swarm by approaching them in the copulatory position, it is apparent that *S. typicus* males cannot detect conspecific females by vision alone. It is possible that males ignore each other within the swarm because of similar flight patterns. This would explain why males newly entering the swarm are first approached by other males in the copulatory fashion. This initial interaction may be for the purpose of sex recognition, or it could be an act of aggressive competition for choice positioning within the swarm (Harker, 1992).

It is suggested here that male dipping of the abdomen into water during and after swarming might be an adaptation for surviving to successive nights. Although it was never confirmed where on the male's abdomen contact with water was made, it is possible that this action ensures that the penes specifically do not dry, or perhaps it is for the purpose of maintaining body moisture.

Total egg counts per female have been used to determine fecundity in mayflies and have been positively correlated with female size (Clifford, 1970; Clifford & Boerger, 1974; Benton, 1989; Scrimgeour & Culp, 1994; Sweeney et al., 1995). Regression analysis from this study showed that there was a positive linear relationship between the total female body length and number of eggs (Fig. 6). Although individuals from UCF did not show a significant decrease in HCW over time (Fig. 3), harsh conditions of their environment might have led to detrimental conditions for growth (see Population comparisons). If given a more favorable environment, it is expected that size differences would exist as found with the MER population. The mean number of eggs per female in this study was lower than that reported for other species of *Siphlonurus* (Clifford & Boerger, 1974; Voshell, 1982). This observation could be due to differences in climatic regimes and habitat conditions. Even within this study, though, a difference of about 200 eggs was detected between populations.

*Pond residents or migrants?*

It is believed that the pond populations partially consisted of migrants from the inlets. Although some migration via drift was sure to occur, each population was more likely to have originated from direct oviposition. Eggs were directly deposited within inlets, outlets, and ponds. Eggs are sure to aestivate until winter since all of these habitats were dry by late summer. Many other locations within the general area were probably suitable for oviposition, but it is not

known why some females oviposited at the location where they mated while others flew to different ones.

## Acknowledgements

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# Appendix. Maturity class criteria for nymphs of *Siphlonurus typicus*

To use the maturity class criteria some familiarity of the organism and the criteria for each maturity class is needed. On initial inspection of a specimen, follow the proceeding steps:

1. Guess which odd maturity class the specimen might belong (class I, III, V, VII, IX, or XI). Go to step 2.
2. Accept that maturity class if all criteria for that class are met. If not then go to step 3.
3. Follow the guidelines given towards class allocation to an even maturity class above or below the original guess. Go to step 4.
4. Accept the maturity class if the characterizations describe the specimen well. If not start back at step 1.

*Immature stages*

**Class I** (hatchlings):
Criteria 1. No gills appear or gills just start to appear as little hooks that do not extend to half of the preceding tergite. If gills appear half the length of the preceding tergite or longer, then the nymph is in Class II.

**Class II** is characterized as having gills with primary lamellae, only.

**Class III**:
Criteria 1. Secondary lamellae of gills I and II do not reach to the end of the preceding tergite. If secondary lamellae reach the end of the preceding tergite, then the nymph is in Class IV. If the secondary lamellae do not exist, then the nymph is in Class II.
Criteria 2. Primary lamellae do not reach one and a half tergites, but do at least cover tergite I. If primary lamellae reach or pass one and a half tergites, then the nymph is in Class IV. If the primary lamellae do not cover tergite I, then the nymph is in Class II.

**Class IV** is characterized by front wing pads appearing as small buds of the posterior lateral of the mesonotum. Primary lamellae of gills I and II extend to or pass one and a half tergites. The secondary lamellae of gills I and II extend to about the end or slightly past the preceding tergite.

**Class V:**
Criteria 1. Front wing pads pass the mesonotal plate, but do not reach the end of the metanotal plate. If the front wing pads reach the end of the metanotal plate, then the nymph is in Class VI. If the front wing pads do not extend beyond the mesonotal plate, then the nymph is in Class IV.
Criteria 2. Hind wing pads do not extend beyond the metanotum. If the hind wing pads extend beyond the metanotum, then the nymph is in Class VI.
Criteria 3. Secondary lamellae of gill I reach past abdominal tergite II and often extend halfway or more through tergite III. If secondary lamellae of gill I reach to the end of tergite II but do not extend much beyond it, then the nymph is in Class IV.

*Mature stages*

**Class VI** is characterized by front wing pads that reach to the base of the hind wing pads or reach to the end of the metanotal plate. Hind wing pads may reach past the metanotal plate, but do not usually extend to half of tergite I. The male sex can be detected.

**Class VII:**
Criteria 1. Front wing pads pass the metanotal plate. If the front wing pads do not pass the metanotal plate, then the nymph is in Class VI.
Criteria 2. Front wing pads do not reach to the end of tergite I. If the front wing pads reach to the end of tergite I then the nymph is in Class VIII.
Criteria 3. Hind wing pads pass the metanotal plate, but do not reach to the end of tergite I. If the hind wing pads reach to the end of tergite I, then the nymph is in Class VIII.
Criteria 4. The length of the interior margin of the front wing pads (starting from where the wing pads extend beyond the mesonotum and ending at the wing tip) is about half of the width between them (the space of mesonotum between the front wing pads). If the length of the front wing pads is less than half of the width between them, then the nymph is in Class VI. If the length of the wing pads is greater than half of the width between them, then the nymph is in Class VIII.

**Class VIII** is characterized with front and hind wing pads that touch or just pass tergite I. The length of the front wing pads is usually less than the width between them, but greater than half of the width between them.

**Class IX:**
Criteria 1. Front and hind wing pads distinctly pass tergite I. If the front or hind wing pads do not pass tergite I, then the nymph is in Class VIII.
Criteria 2. The prosternum and metasternum are never sclerotized. If the prosternum and metasternum are sclerotized, then the nymph is in Class X.
Criteria 3. The length of the front wing pads is about equal to the width between them. If the length of the front wing pads is less than the width between them, then the nymph is in Class VIII. If the length of the front wing pads is greater than the width between them, then the nymph is in Class X.

**Class X** (early metamorphosis) is characterized by the sclerotization of the prosternum and metasternum. The hind and front wing pads usually pass tergite II. The length of the front wing pads is greater than the width between them. The wing pads may start to show darkening folds, but are still mostly light.

**Class XI** (late metamorphosis):
Criteria 1. The wing pads are dark.