Detritus Processing of Four Species of Leaves in Three North-Central PA Streams

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Abstract

Leaf processing of four riparian plant species [sugar maple (*Acer saccharum*), blue beech (*Carpinus caroliniana*), red oak (*Quercus rubra*), and sycamore (*Platanus occidentalis*)] was studied in three North Central PA streams. Processing rates (k), percent organic content, and macroinvertebrate colonization were measured after 2, 7, 14, and 55 days. The effects of stream order, time, and levels of acidity were assessed. In general, the acidic first order stream revealed an overall significantly slower decomposition rate (k= 0.0034 days^{-1}). *Carpinus caroliniana* and *Acer saccharum* revealed faster average decomposition rates for all streams, with k-values of 0.0204 (days $^{-1}$) for both. Mayflies (Ephemeroptera: Heptageniidae) dominated in abundance in the third order stream. Stoneflies (Plecoptera: Taeniopterygidae) and net-spinning caddisflies (Trichoptera: Hydropsychidae) dominated in abundance in the second order stream. Only four invertebrates (Trichoptera: Hydropsychidae) were found in the first order acidic site. Percentage abundance of shredders was greatest in the October sample in the second order stream. A second study completed in March and April, 1998 assessed fungal growth via a chemical index measuring ergosterol content on *Acer saccharum* and *Platanus occidentalis* after 2, 7, 14, and 28 days of incubation in two streams of differing levels of pH. *Platanus occidentalis* exhibited the highest concentration of ergosterol after 28 days of incubation with values of 0.28 μg ergosterol/mg detritus for Mill Creek (pH = 6.69) and 0.14 μg ergosterol/mg detritus for the Morris study site (pH = 3.04). In conclusion, this study revealed significantly slower decomposition rates due to increasing acidity. Differences in stream order and time between September and October did not reveal any significance. A general trend of increased fungal biomass with increased time of incubation was found, although no definitive conclusions could be made due to small sample size.

Introduction

Leaf decomposition can be defined as the process by which organic matter is catabolized into its constitutive inorganic forms or incorporated in living biomass, such as during assimilation by bacteria or invertebrates (Boulton and Boon 1991). Allochthonous (terrestrial input of detritus; i.e. leaf material) resources entering a stream are rapidly colonized within the first few days by fungi, bacteria, and macroinvertebrates, mainly by the group collectively known as shredders. The microbial conditioning that occurs, especially by a group of fungi commonly referred to as aquatic hyphomycetes, is important because they initially structurally soften the leaf to allow more active feeding by invertebrates. Figure 1 shows a conceptual model of stream structure and function.

To understand more fully the actions of fungi in the decomposition process, recent research has developed a method to quantify fungal biomass via a chemical index. The detection of a fungal membrane sterol, ergosterol, is currently the best-known technique. Ergosterol is not a vascular plant sterol, so its detection in leaf litter indicates fungal colonization (Newell 1992).

The importance of leaf litter decomposition as a major supplier of imported energies into most stream ecosystems throughout the annual cycle is well documented (Cummins 1974; Maloney and Lamberti 1995; Wallace et al. 1997). Estimates of processing efficiencies, typically measured based on the leaf mass loss over time, have exhibited variability not only between species (leaf processing continuum) but also within a single species due to differences in stream order, time, and water chemistry.

Differences in the rates at which leaves decompose are due in part to the initial physical and chemical components of the leaves (Webster and Benfield 1986). Petersen and Cummins (1974) categorized decomposition rates as being fast when the k-value is greater than 0.01 $days^{-1}$. Leaves that are considered fast decomposers generally have higher nitrogen

content, higher total nonstructural carbohydrates, lower fiber, tannin, and lignin content (Godshalk and Wetzel 1978). It is also suggested that the interactions between phenolics, lignin, and nitrogen containing compounds form resistant complexes and may limit the availability of nitrogen to microbes, thereby slowing the decomposition rate (Suberkropp et al. 1976).

Previous studies (Horton and Brown 1991) have shown faster processing values with increased stream order. However, Chauvet et al. (1993) found that a seventh order lowland river exhibited slower processing rates than did a third order mountain stream. The slower rates found in the river was attributed to differences in composition, abundance, and activity of the microbial communities, as well as less fragmentation, which normally occurs due to abrasion against rough streambeds.

Differences in decay rates have also been exhibited between spring, autumn, and summer studies. Gardner and Davies (1988) found autumn rates to be higher than those in the spring, mainly due to decreases in microbial and macroinvertebrate feeding activity in the spring months. A similar study by Maloney and Lamberti (1995) found higher decay rates in the summer months. This discovery was mainly attributed to higher temperatures found in stream waters leading to increase amounts of microbial activity. This study also concluded that physical and microbial degradation was more important in the decomposition process than macroinvertebrate shredding.

Variable processing rates also exist due to differences in water chemistry, specifically differing levels of pH. Increased acidity has shown to reduce decomposition rates (Frigberg et al. 1982; Burton et al. 1985). This finding is connected to decreases in microbial and invertebrate activity and biomass. Lowered decomposition rates could reduce nutrient and energy supplies, leading to lowered productivity in headwater streams.

The objectives of this study were to characterize any differences in processing rates, percent organic loss, and macroinvertebrate and fungal colonization due to variance in stream order, levels of pH, and time for four species of leaves.

Materials and Methods

Site Description

The study was conducted in three North Central PA streams of varying stream order. As defined by Cummins (1974), stream order varies from 1-12 with headwater streams given a value of 1 (first order). Two first order streams combine to make a second order stream, and two second order streams combine to make a third order stream and so forth. The Larry's Creek study site is a third order stream located in Salladasburg, PA. The study site at Mill Creek is a second order stream located below the town of Warrensville, PA. The Morris study site, located near Morris, PA in Tioga County, is a first order, acidic stream due to coal mine drainage.

Physical and Chemical Water Measures

Stream water samples were analyzed in the lab for pH, alkalinity, nitrates and phosphorus content. Alkalinity (by titration) and pH were analyzed using a Corning 440 pH meter. Nitrates and phosphorus were analyzed following Standard Method procedures (American Public Health Association 1992) using a DR 3000 spectrophotometer. Temperature (°C) and dissolved oxygen (D.O.) in ppm were measure using a hand held YSI model 55 D.O. meter. Velocity (m/sec) was measured using a Swoffer model 210 meter and conductivity (mS) was measured with a Hanna, HI 8633 meter. Depth (cm) and width (m) were measured with a meter stick and tape.

Leaf Litter Decomposition

Leaf litter decomposition was measured using four species of leaves. *Acer saccharum* (fast decomposer), *Carpinus caroliniana* (fast decomposer), *Quercus rubra* (slow

decomposer), and *Platanus occidentalis* (slow decomposer) were utilized for this study (Petersen and Cummins 1974). Leaves were handpicked from trees before abscission in early September, air-dried flat (> 48hr), and initial surface areas were taken using a LI-COR model LI-3000A portable area meter. Packs of five leaves were strapped onto bricks with rubber bands and placed in the riffle areas facing upstream for incubation periods of 2, 7, 14, 55 days. Forty-eight packs were placed in each stream (4 incubation periods x 4 species of leaves x 3 replicates). Packs were placed in Larry's and Mill Creek on September 19, 1997 and a second set were put in Morris and Mill Creeks on October 2, 1997. Three packs were collected after each incubation period, transported in Ziploc bags to the lab, and leaves were then rinsed with tap water over a mesh screen to remove inorganic debris and invertebrates. Invertebrates were preserved in 70% ethanol. The remaining leaf biomass was again air-dried flat (> 48 hrs), and final surface areas were taken.

After taking leaf area, five leaves for each sample were ground and dried at 70°C (>24 hrs) and weighed to a constant weight (DM). Organic matter content was determined as weight loss on ignition (1 hr @ 550°C). Controls for organic content consisted of ten leaves collected before abscission, air dried flat (>48hrs), and organic content was then determined using the before mentioned process. Processing rates (k) for each species were determined by the slope of the linear regression following the form of the equation $\ln (W_d/W_o)/d$: where W_d is the final surface area of the leaf, W_o is the initial surface area, and d is the time in days (Petersen and Cummins 1974).

Invertebrate Analysis

The macroinvertebrates retained by a US #30 mesh screen were sorted and identified to the lowest possible taxon according to Merrit and Cummins (1984), and assigned to a functional feeding group according to specifications set by PA's Department of Environmental Protection.

Determination of Fungal Biomass

A separate study was conducted in early March, 1998 to locate and utilize a satisfactory technique for quantifying fungal biomass. *Acer saccharum* and *Planatus occidentalis* species were used. Leaves were collected post-abscission in late October and dried flat. Packs consisted of two leaves banded to bricks and placed in the riffle areas of Morris and Mill Creeks for periods of 2, 7, 14, 28 days. Sixteen packs were placed in each stream on March 12, 1998 (4 incubation periods x 2 species x 2 replicates). Two replicates were collected after each incubation period. Collected leaves were placed in Ziploc bags and transported to the lab on ice. Leaves were rinsed with tap water to remove inorganic debris and invertebrates. For each sample, ten 13mm discs and one replicate of five 13mm discs were cut, placed in methanol, and stored in the dark at 4°C. For each sample date, two parallel replicates (ten and five discs) were used for determination of mean dry weight per replicate by drying (> 24 hr) at 70°C. Controls consisted of leaves collected post abscission in late October, air-dried flat (>48hrs), and replicates of ten and five 13mm discs were cut and placed in methanol and stored at 4°C. Ergosterol extraction was completed following the procedures of Newell et al. (1988), using the reflux method (see flow diagram, Appendix 1). Redissolved samples and standards were filtered through a 0.45 um nylon membranes (Acrodisc; Gelman Sciences, Inc.) before injection into the following HPLC system: Waters 510 pump; 100ul sample loop; Waters 991 photodiode array detector; Whatman Partisil 5 ODS-3, 25cm column and a Waters μBondapack C18 precolumn. At a flow rate of HPLC-grade methanol eluant (Fisher Scientific) of 1.5-ml · min^{-1} and a detection wavelength of 282nm, erogsterol eluted between 5.2 and 5.6 min. Due to low concentrations of ergosterol, several samples were spiked with the lowest standard to ensure correct peak identification. Ergosterol (Sigma-Aldrich) was dissolved in HPLC-grade methanol and serial diluted to

form standard concentrations. Concentrations of unknowns were calculated by comparing the lowest standard's concentration (20 μg ergosterol/ml methanol) and peak area with the unknown's peak area. Pentane was purchased from Fisher Scientific Co.

Statistical Analysis

Analysis of organic data followed simple rank sum testing to determine significance from the control. Processing rates (k) between streams for each species were compared using two sample hypothesis testing using an α-level = 0.05 (Sprechini 1992).

Results

Physical and Chemical

The results of all stream physical and chemical analysis are shown in Tables 1 and 2. The Morris study site had the lowest pH of 2.58 for the initial study and 3.04 for the March, 1998 fungal study. Conductivity readings at the Morris site were higher than the other two streams at 1.32mS. Nitrate and phosphorous contents were also higher at the Morris site.

Leaf Processing Rates

Processing rates (k), shown in Table 3, exhibited some significant differences between streams. The Morris site had the lowest average k-value for all four species (0.0034). Significant differences were found between the Morris and Mill streams for *Acer saccharum* (0.0048, 0.336) and *Platanus occidentalis* (0.0019, 0.0087). Significant differences were also found between Larry's and Morris streams for *Quercus rubra* (0.010, 0.0026) and *Platanus occidentalis* (.0071, 0.0019) and between Morris and Mill (Oct.) for *Carpinus caroliniana* (0.0043, 0.0106). For all streams, *Acer saccharum* and *Carpinus caroliniana* processed at faster rates (0.0204, 0.0204) than did *Quercus rubra* and *Platanus occidentalis* (0.0074, 0.0062). *Acer saccharum* and *Carpinus caroliniana* are considered

"fast" decomposers ($k > 0.01$) according to processing categories developed by Petersen and Cummins (1974). No significant k-values were found for differences in time between September and October as well as second and third stream orders.

The percent organic content is depicted in Figures 3a-d. Significant differences from the controls were found for all incubation periods for all species in each stream except in the Morris site. Significance within the Morris site was found for the 2-day sample only for *Carpinus caroliniana* and *Platanus occidentalis*, and the 2, 14, and 55-day samples for *Acer saccharum,* and 14 and 55-day samples for *Quercus rubra*. For all species incubated in the Morris site, the 7-day sample showed an increase in the percent organics, as shown in Figure 4a. This trend also occurred between the 14 and 55-day sample for *Platanus occidentalis* within the Morris site. *Carpinus caroliniana* revealed lower percent organics for the longer incubation period for all streams except the Morris site. Figures 4b-d show that *Carpinus caroliniana* and *Acer saccharum* had larger percent organic content loss than *Platanus occidentalis* and *Quercus rubra*. The greatest percent reduction in percent organics was found in Mill Creek (October sample) for *Carpinus caroliniana* with an initial value of 95.1% and a 14-day value of 70.5%. The Morris site had higher percent organic values for all species and streams for each incubation period.

Macroinvertebrate Colonization of Leaf Packs

Figure 5 shows the mean number of invertebrates per pack for Larry's and Mill Creeks. Larry's Creek numbers revealed a possible trend with the greatest average of inverts found on the 7-day samples. Mill and Larry's Creeks revealed similar average numbers for all four species. The October sample for Mill Creek showed an increase for all species in number of inverts per pack compared to the September samples.

Figure 6 represents percent of total inverts for each species by stream categorized into their functional feeding groups. The greatest percent of shredders was found in the October

sample in Mill Creek. Leaf packs in Larry's Creek were dominated by the Family Heptageniidae (Order Ephemeroptera), hence portraying the higher percent of scrapers shown in Figure 6. Mill Creek was dominated by net-spinning caddisflies (Trichoptera: Hydropsychidae). The October sample for Mill Creek revealed higher numbers of Hydropsychidae and stoneflies (Plecoptera: Taeniopterygidae). The Morris site only produced 4 total invertebrates for the entire study (Trichoptera: Hydropsychidae). Identification of all invertebrates collected and total numbers are shown in Table 4.

Fungal Characteristics

Figure 7 shows ergosterol content of leaf detritus for *Acer saccharum* and *Platanus occidentalis*. The Morris site showed increases in ergosterol concentration with time for *Platanus occidentalis*, the highest value at 28 days was 0.14 μg ergosterol/ mg detritus (n=1). With the exception of the 2-day sample, *Platanus occidentalis* revealed higher concentrations of ergosterol than *Acer saccharum* for the Morris site. The greatest amount of ergosterol found in Mill Creek was the 28-day sample of *Platanus occidentalis* (0.28μg/mg detritus).

Discussion

The results revealing significantly slower decomposition rates under acidified conditions within the Morris site, shown in Table 3, are consistent with several other reported studies (Leivestad et al. 1976; Burton et al. 1985). The lowered k-values could be due to several factors. First, physically the Morris site exhibited colder water temperatures, which could possible effect microbial activity (Maloney and Lamberti 1995; McArthur and Barnes 1988). Second, the absence of a macroinvertebrate shredding community could possibly reduce processing rates. And third, Palumbo et al. (1987) found that under acidified conditions, bacterial activity was significantly reduced, resulting in lowered decomposition rates.

The decomposition rate results for Larry's and Mill Creeks were inconsistent with a study done by Horton and Brown (1991) who found that decomposition rates increased as stream order increased. Mill Creek, a second order stream, revealed faster processing rates than did Larry's Creek, the third order site. However, the Morris (first order) site did reveal lowered rates, but this could be attributed more to the acidified conditions.

Low percentages of shredders, exhibited in Figure 6, is consistent with several studies (Maloney and Lamberti 1995; Chauvet et al. 1993). The October study in Mill Creek revealed higher densities of shredders, but overall the decomposition rates were lower. This could be due to the colder temperatures in October. This is also consistent with the study by Chauvet et al. (1993), in which it was found that a stream with lower numbers of shredders actually had higher decomposition rates. Also, a study by Maloney and Lamberti (1995) found that the relatively low abundance of shredder taxa did not reduce decomposition rates. This tempts me to assume that differences in composition and activity of the microbial community and physical factors dominate in importance over shredder abundance.

The rise in the percent organic content for the 7-day sample for all species and between the 14 and 55-day sample for *Platanus occidentalis* within the Morris site (Figure 4a) could be due to microbial colonization and/or mineral deposition. This trend was not found for any other stream.

The relatively low amounts of ergosterol concentration (Figure 7) found on *Acer saccharum* and *Platanus occidentalis,* as compared to other studies (Gessner and Chauvet 1997), is consistent with the study done by Suberkropp (1997). This study found that in the spring and summer months, fungal biomass and activity revealed lower amounts than in the autumn months.

In conclusion, this study revealed significant lowering effects on leaf decomposition due to increasing acidity. Stream order and time between September and October did not

reveal any significant differences. I feel that a study solely focused on the microbial aspect of leaf decomposition in an acidic stream compared to a control site needs to be done. It was noted in a study completed by Palumbo et al. (1987) that the importance of fungi in the decomposition process could possibly increase in acidic conditions. The sample size used in this study was too small to make a definitive conclusion but does demonstrate a general trend of increased fungal biomass with increasing time of incubation.

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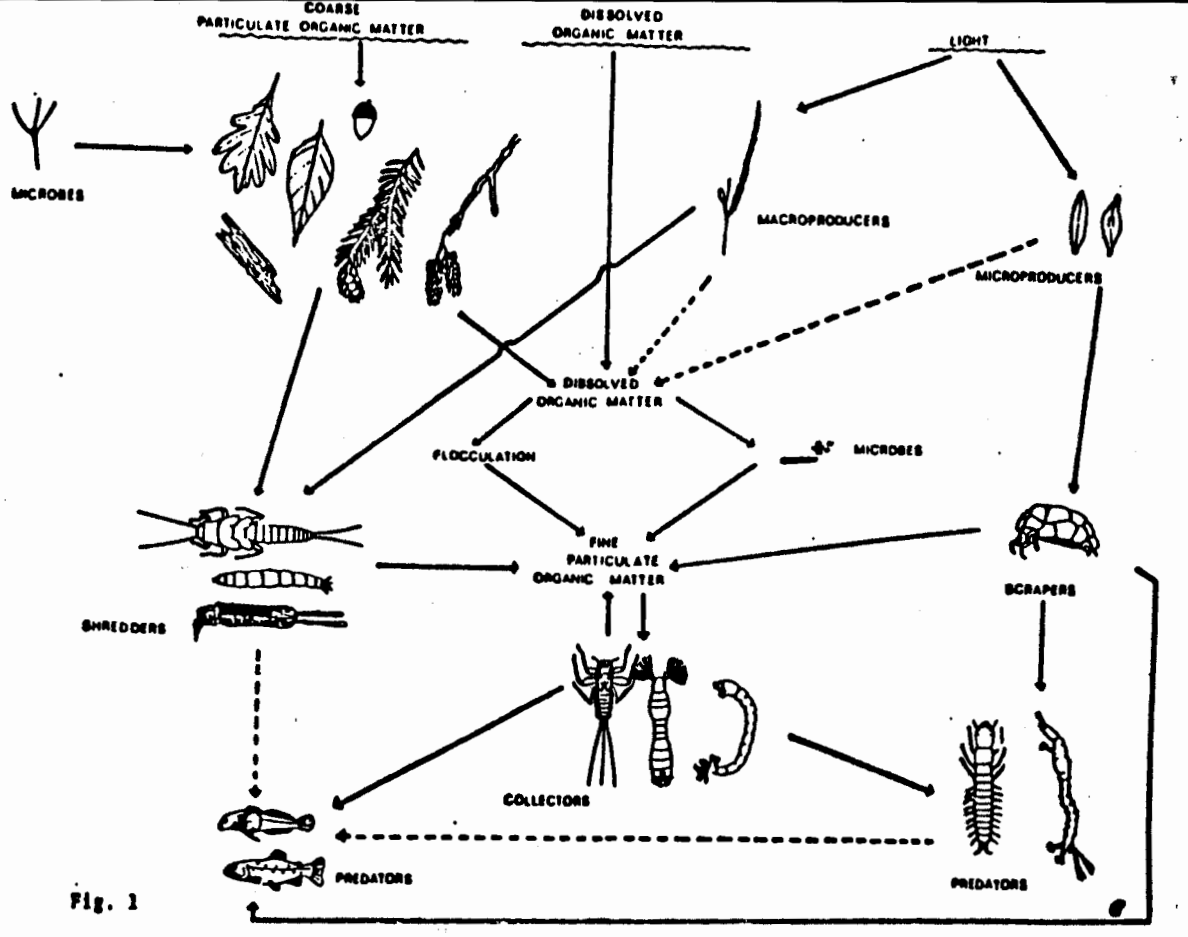


Figure 1: A model of stream structure and function (modified from Cummins 1973).

Stream	Temp. (°C)	D.O. (ppm)	Depth (cm)	Width (m)	Velocity (m/s)	Conductivity
Larry's	16.2	9.79	18	17.1	0.35	0.07
Morris	8.4	11.14	4.2	0.88	0.10	1.32
Mill	17.1	10.15	13.6	7.1	0.37	0.09
Morris (3/12/98)	1.1	14.49	18.3	1.03	0.56	N/A
Mill (3/12/98)	1.2	9.74	35.3	8.7	0.70	N/A

Table 1: Physical readings for all three streams.

Stream	pH	Alk (ppm $CaCO_3$)	NO_3 (ppm)	NO_2 (ppm)	P_{tot} (ppm)	P_{ortho} (ppm)
Larry's	6.72	27	1.3	3	0.10	0.22
Morris	2.58	----	4.8	12	0.65	0.34
Mill	6.35	17	1.2	2	0.11	0.18
Morris (3/12/98)	3.04	----	3.3	2	0.24	0.18
Mill (3/12/98)	6.69	3	1.2	2	0.09	0.17

Table 2: Chemical analysis for all three streams.

Stream Site	*Acer saccharum*	*Carpinus caroliniana*	*Quercus rubra*	*Platanus occidentalis*
Morris	0.0048 [a] (n=3)	0.0043 [b] (n=3)	0.0026 [c] (n=3)	0.0019 [d,e,f] (n=3)
Larry's	0.0173 (n=2)	0.0315 (n=2)	0.0100 [c] (n=3)	0.0071 [e] (n=3)
Mill	0.0336 [a] (n=3)	0.0353 (n=2)	0.009 (n=2)	0.0087 [d] (n=3)
Mill (Oct.)	0.026 (n=2)	0.0106 [b] (n=3)	0.0079 (n=3)	0.0070 [f] (n=3)

Table 3: Comparison of processing rate coefficients (k) for four species located in three streams. Matching letters denote significant differences between the study sites within a single species. Sample sizes are displayed (n) due to their importance in understanding significant differences.

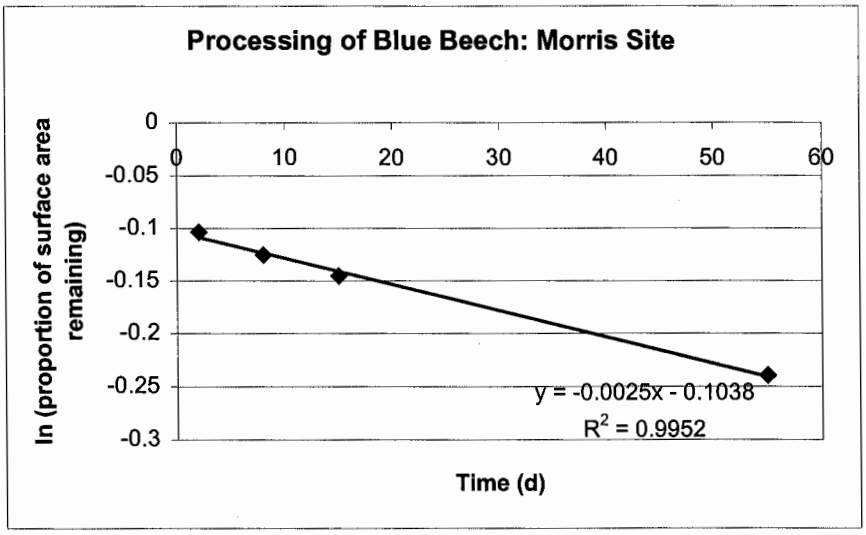


Figure 2: An example of processing (surface area loss) of one replicate of Carpinus caroliniana vs. time. The slope of the linear regression is taken to be the processing rate (units/d).

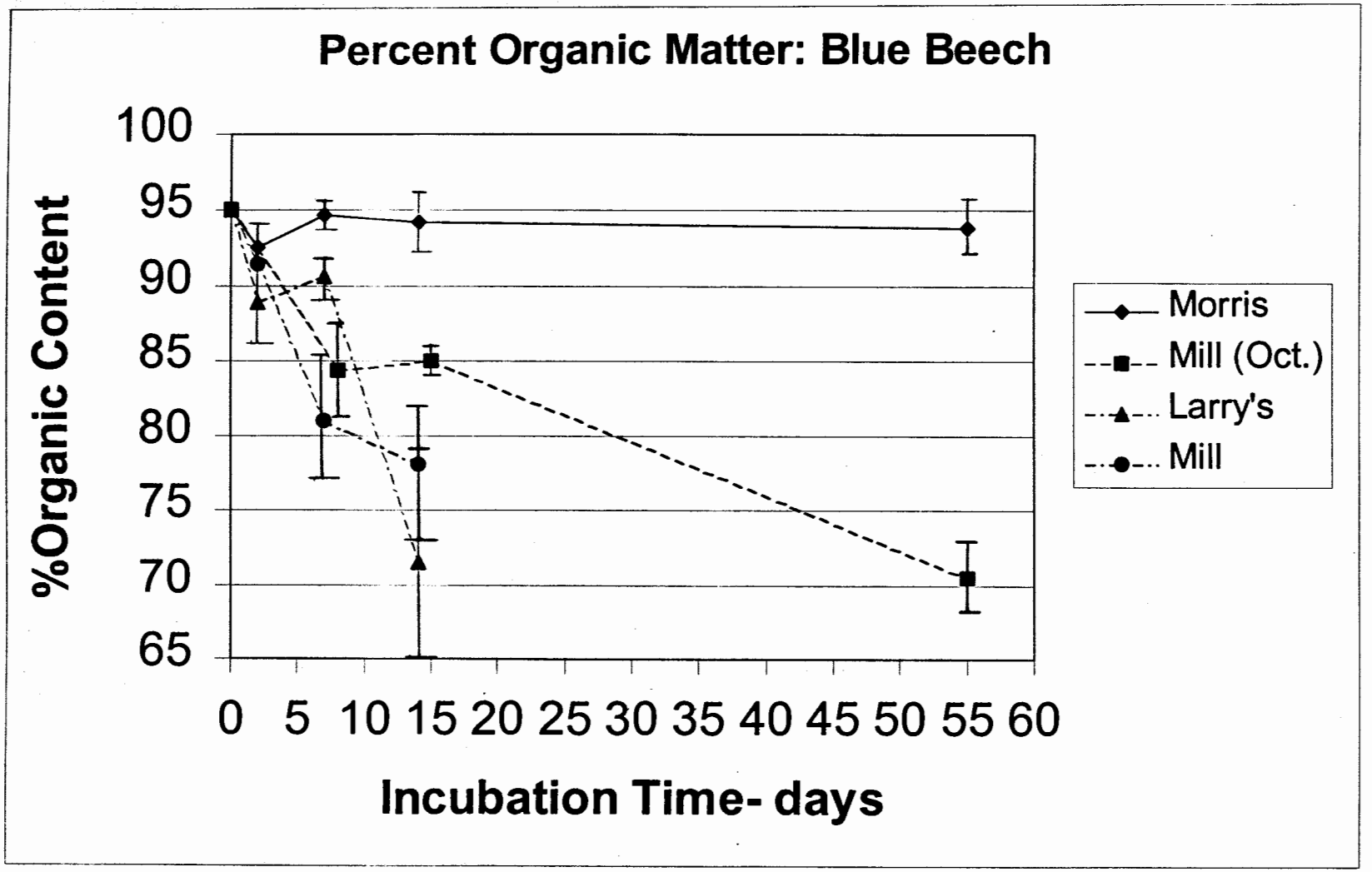


Figure 3a: Percent organic content for *Carpinus caroliniana* (Blue Beech). Significant differences from the control were found for all samples except for the 7, 14, and 55 days for the Morris site. Larry and Mill Creek's 55-day samples were lost. Error bars denote standard deviations.

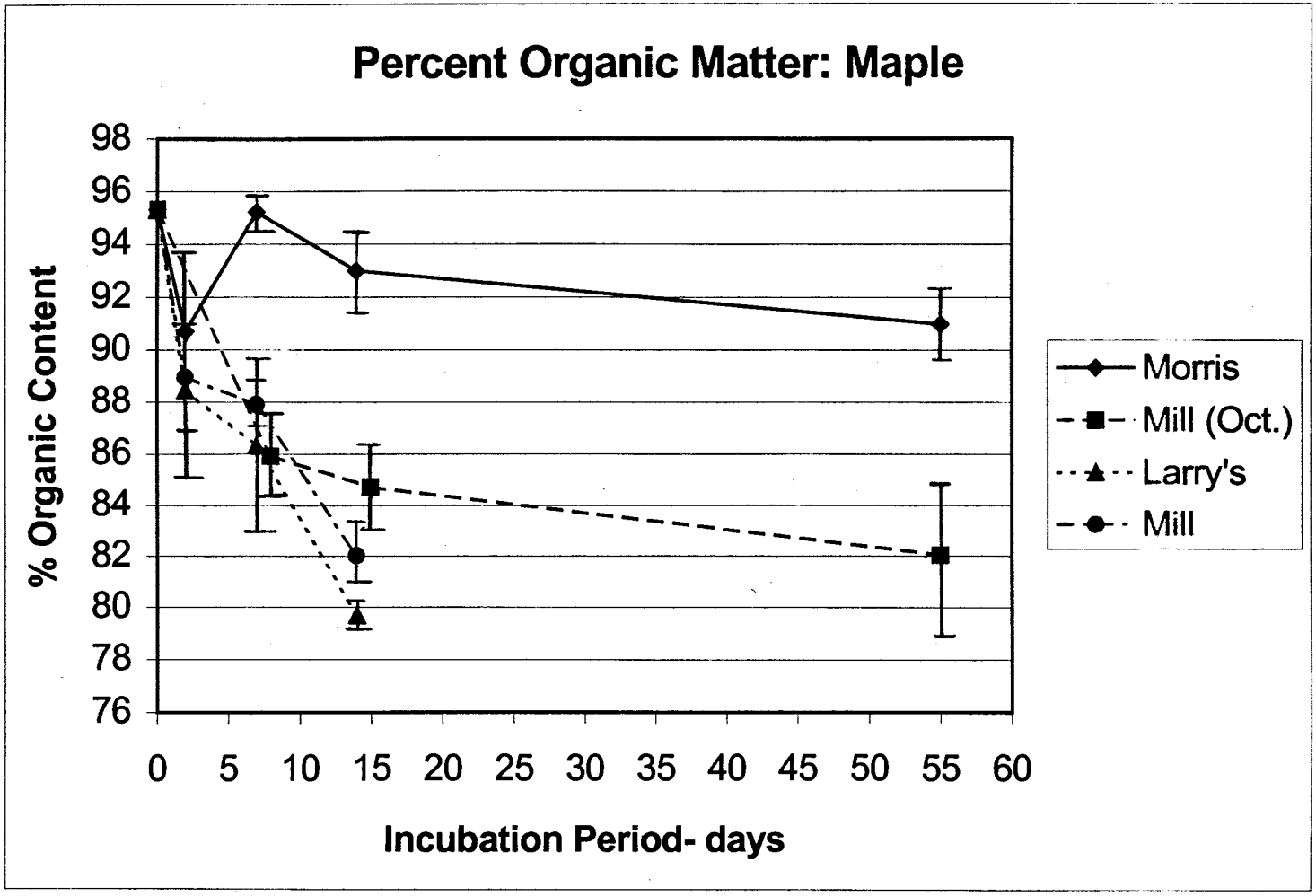


Figure 3b: Percent organic content for *Acer saccharum* (Maple). Significant differences from the control were found for all samples except for the 7-day sample at the Morris site. Larry and Mill Creek's 55-day samples were lost. Error bars denote standard deviations.

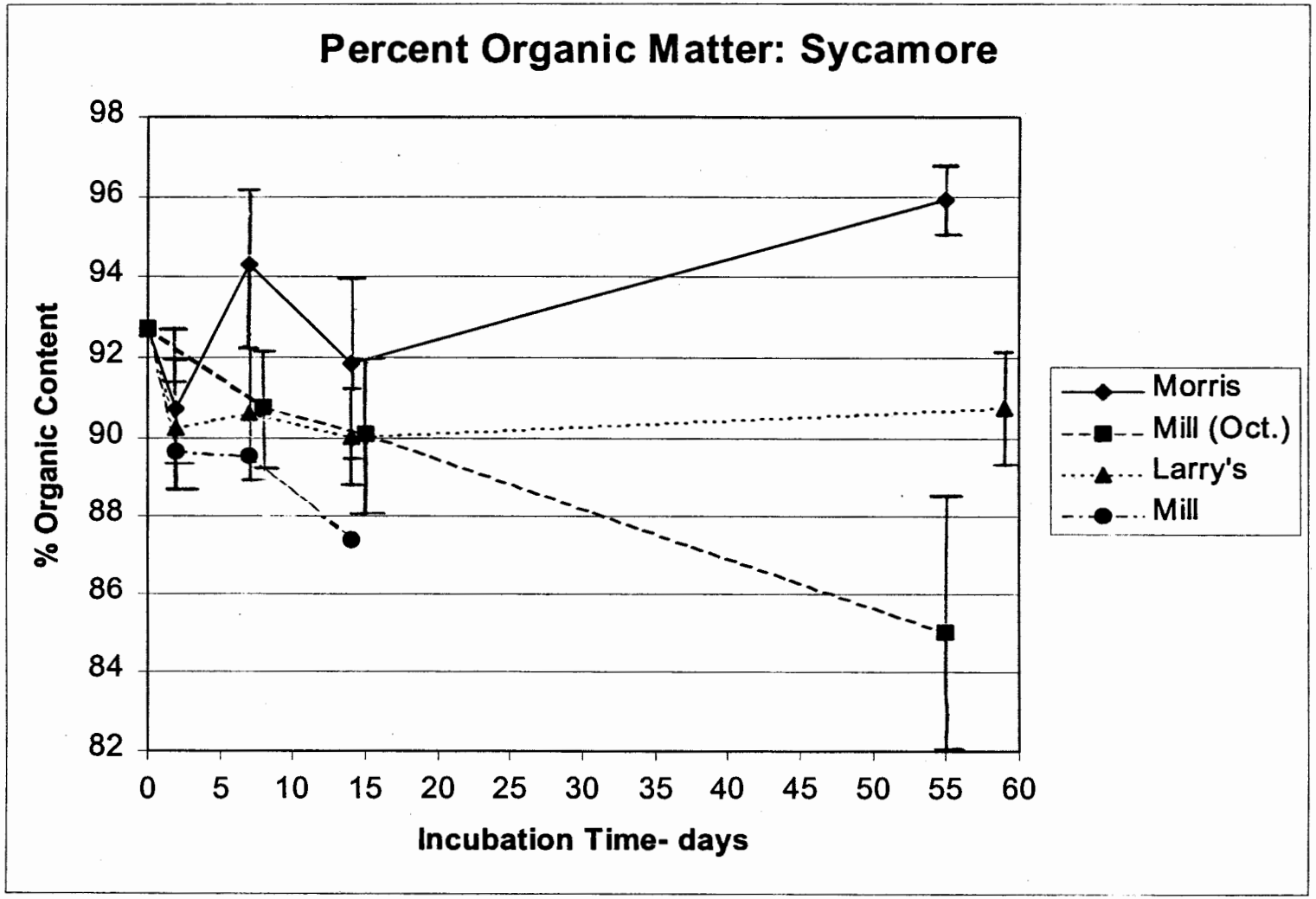


Figure 3c: Percent organic content for *Platanus occidentalis* (Sycamore). Significant differences from the control were found for all samples except for the 7, 14, and 55 days for the Morris site. Mill Creek's 55-day sample was lost. Error bars denote standard deviations.

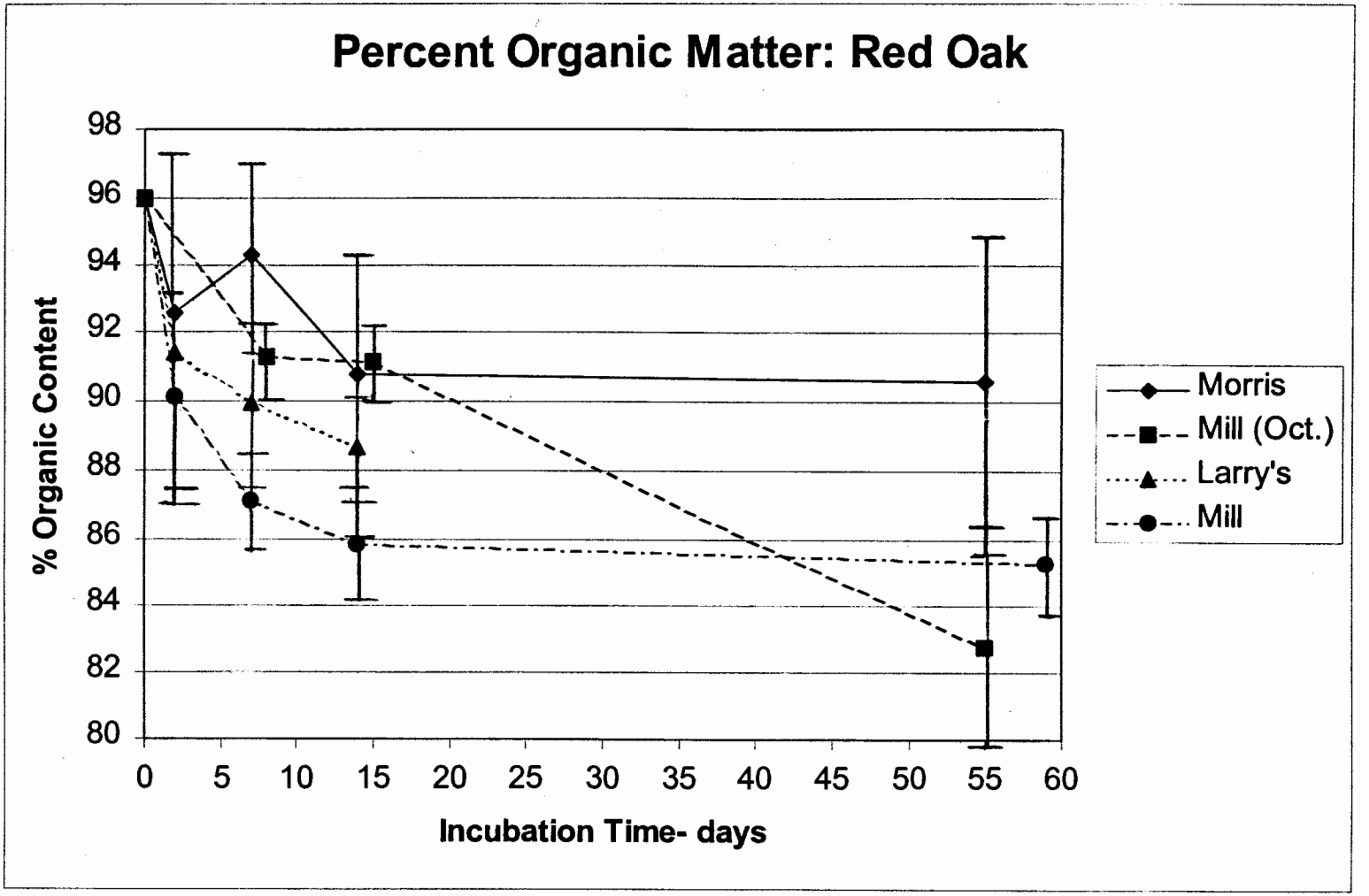


Figure 3d: Percent organic content for *Quercus rubra* (Red Oak). Significant differences from the control were found for all samples except for 2 and 7-days for the Morris site. Larry Creek's 55-day sample was lost. Error bars denote standard deviations.

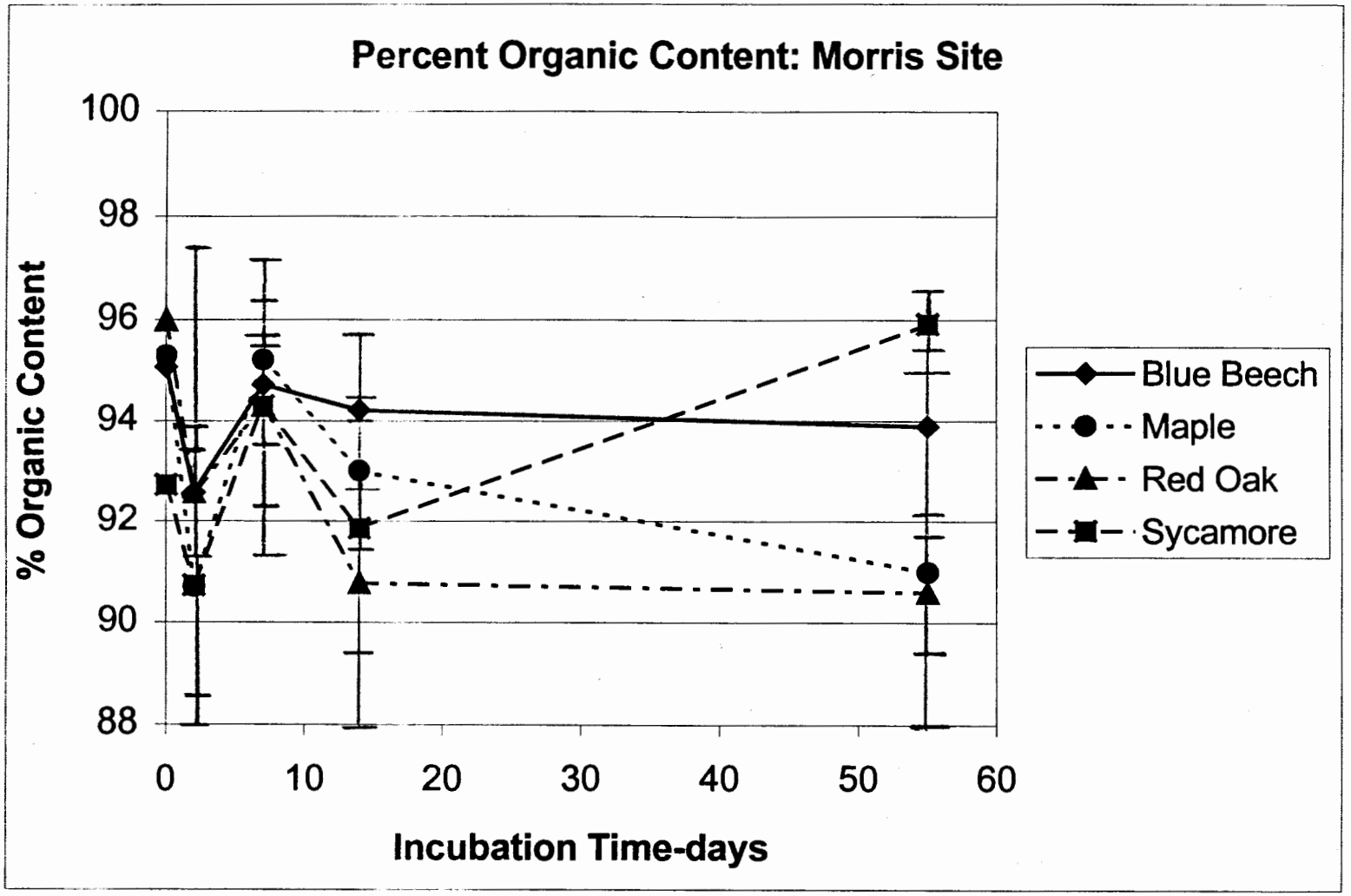


Figure 4a: Percent organic content for the Morris site for all four species. Significant differences from the control were found for the 2-day sample for Blue Beech, Maple, and Sycamore, the 14-day sample for Maple and Red Oak, and the 55-day sample for Maple and Red Oak. The 7-day sample showed an increase in percent organics for all four species. This increase is probably due to microbial colonization or possibly from mineral deposition. Error bars denote standard deviations.

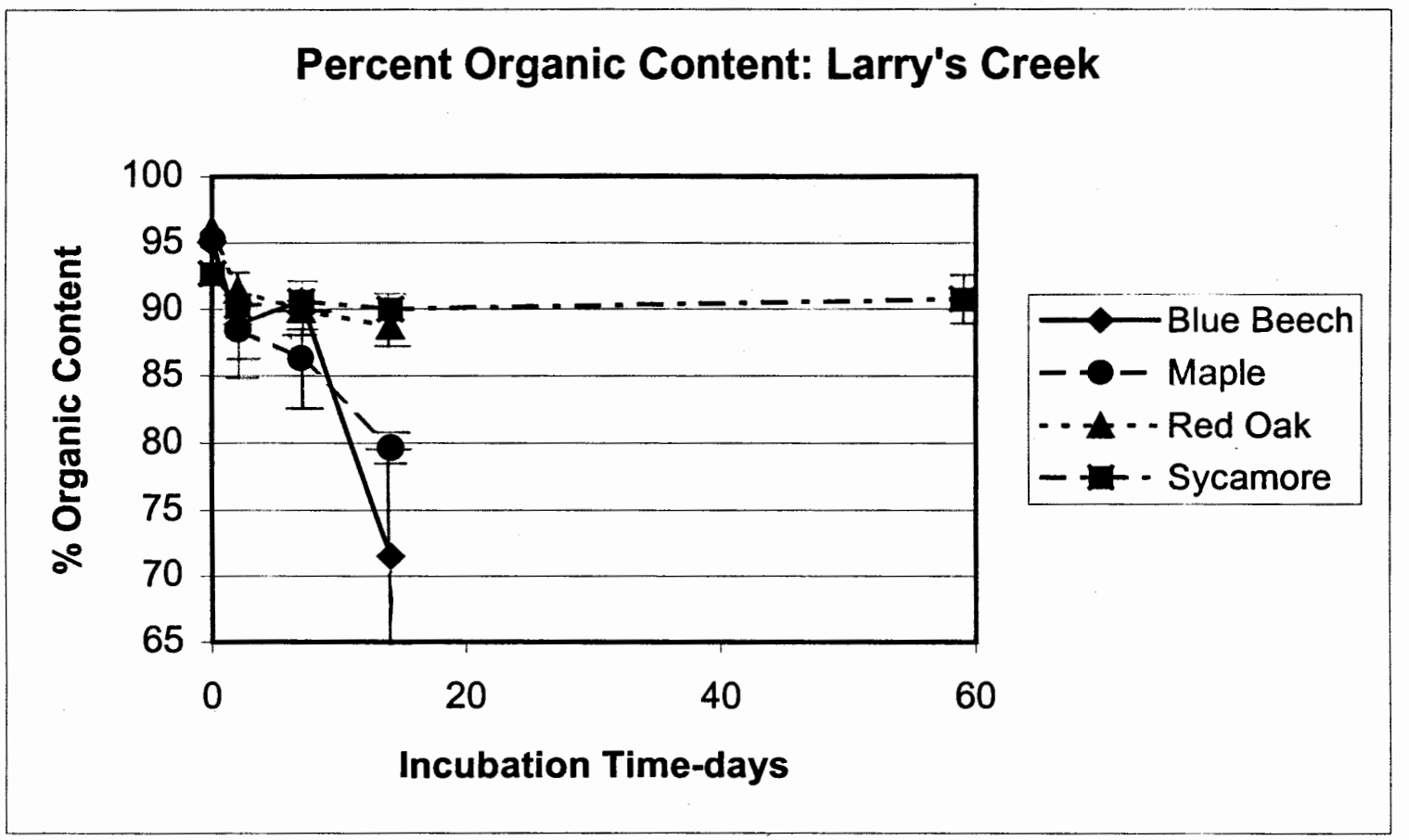


Figure 4b: Percent organic content for Larry's Creek for all four species. Significant differences from the control were found for all samples. Error bars denote standard deviations. The 55-day samples for Blue Beech, Maple, and Red Oak were lost.

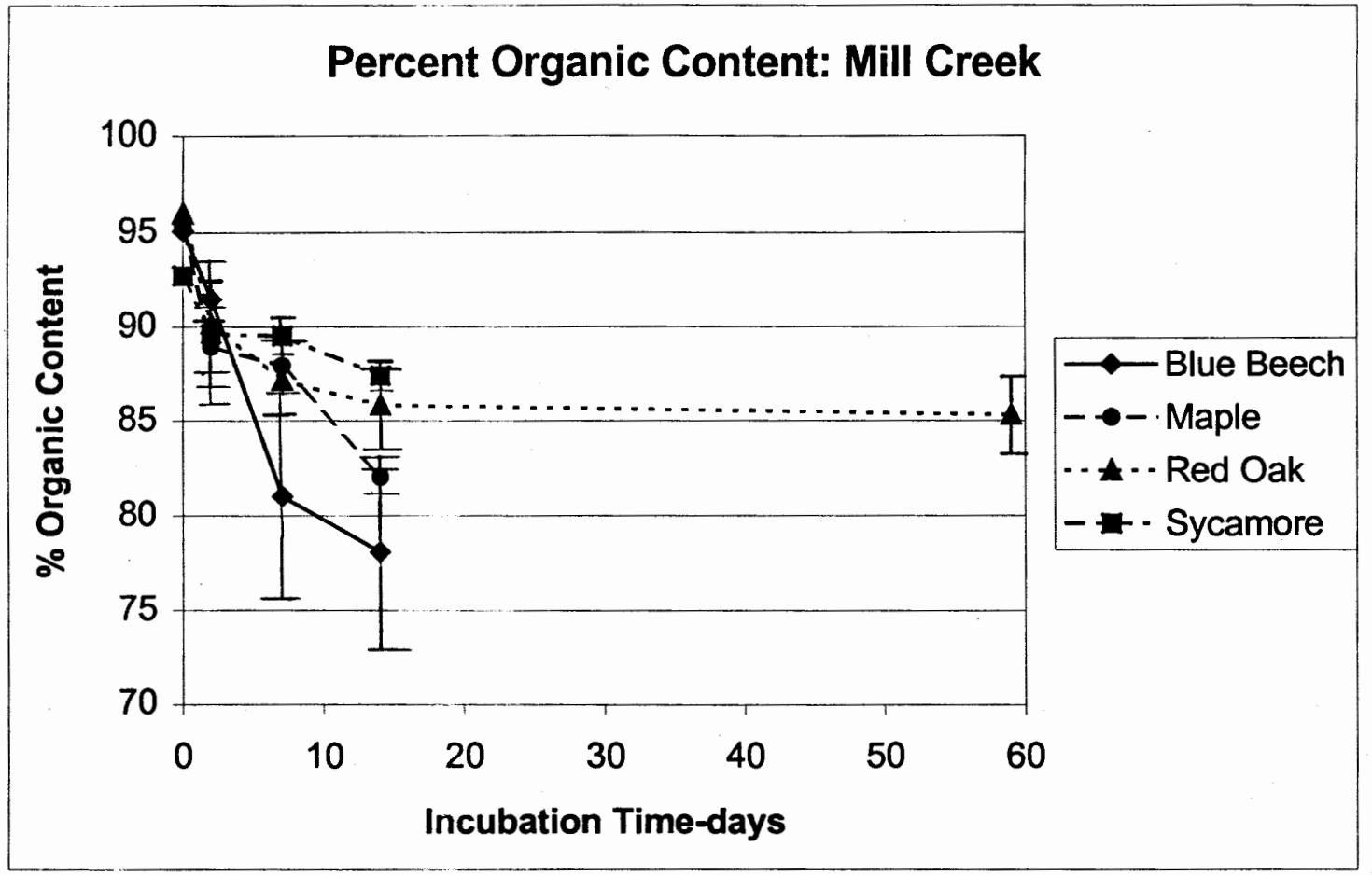


Figure 4c: Percent organic content for Mill Creek for all four species. Significant differences from the control were found for all samples. Error bars denote standard deviations. The 55-day samples for Blue Beech, Maple, and Sycamore were lost.

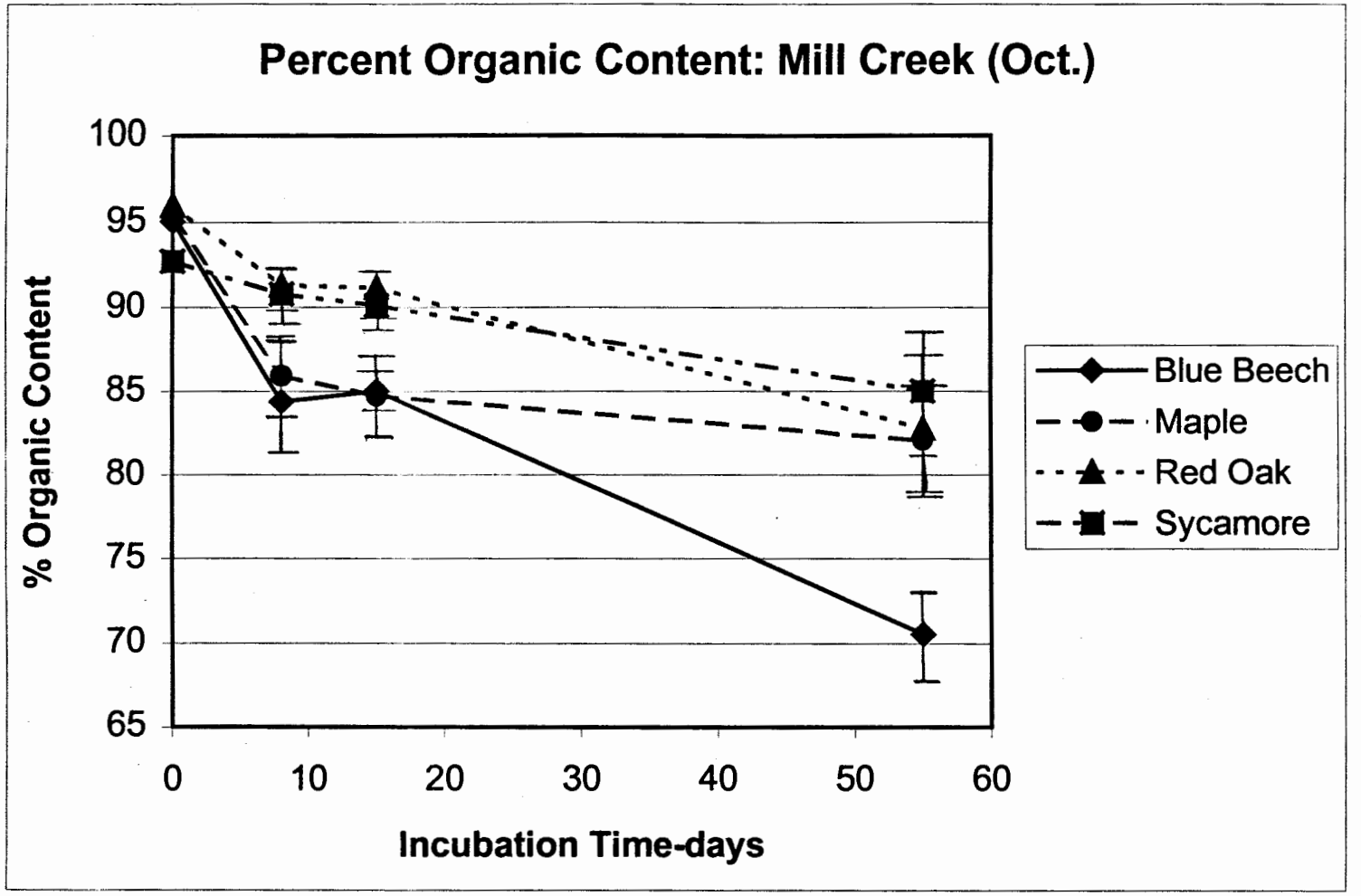
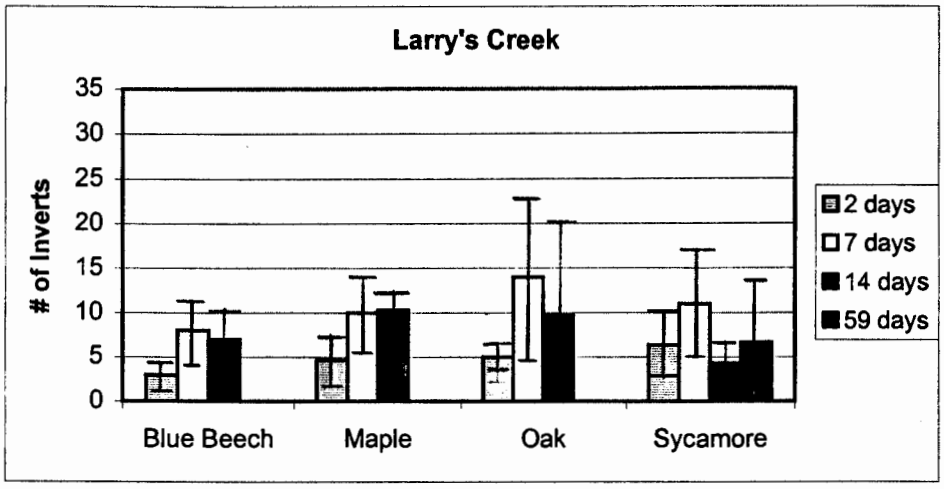
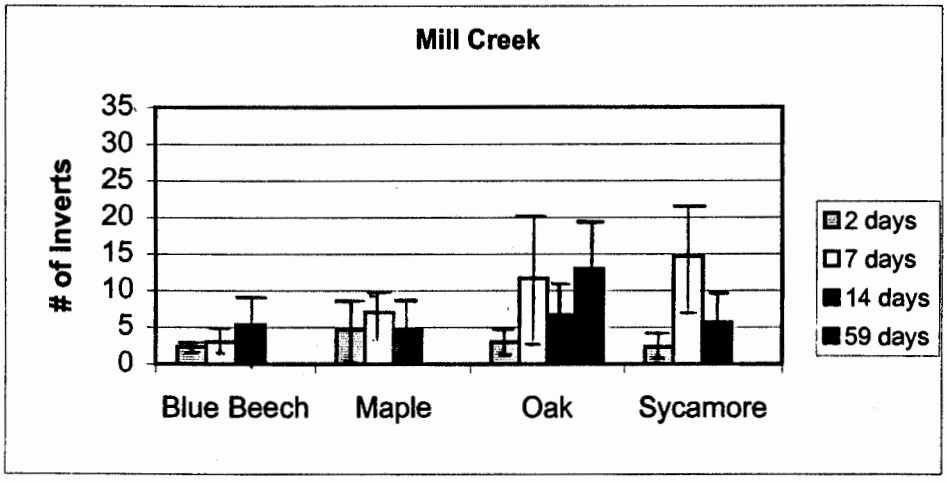


Figure 4d: Percent organic content for Mill Creek (Oct.) for all four species. Significant differences from the control were found for all samples. Error bars denote standard deviations.





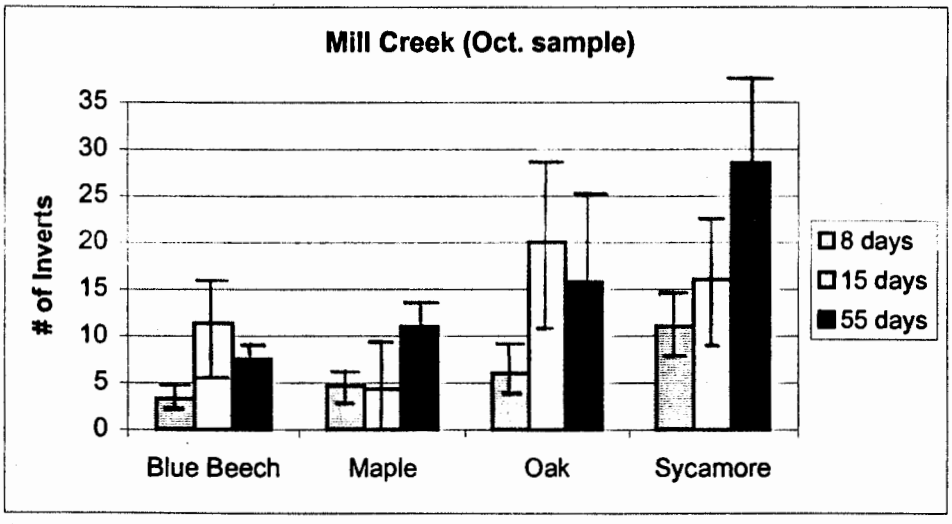
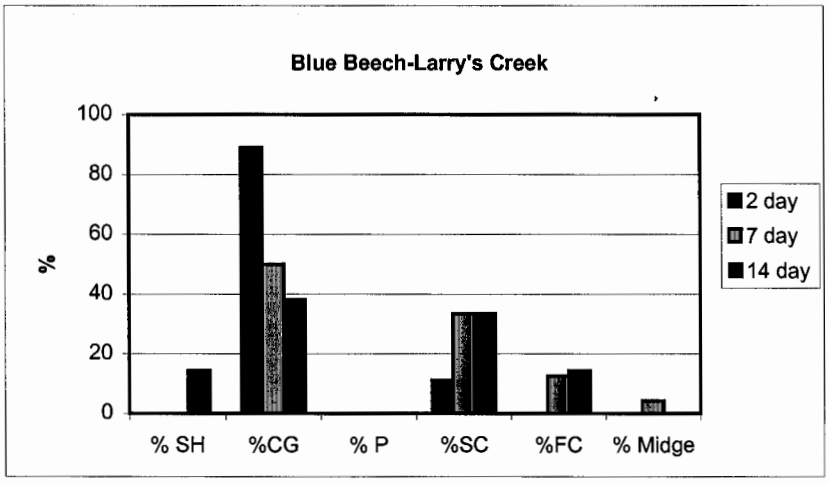
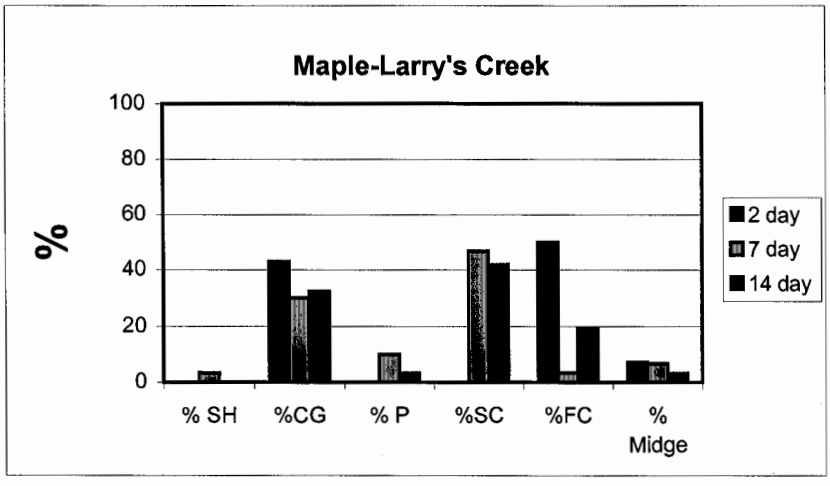


Figure 5: Mean number of invertebrates per pack by stream for all four species. The Morris site was not depicted because only four total inverts were found over all incubation periods. Error bars denote standard deviations.





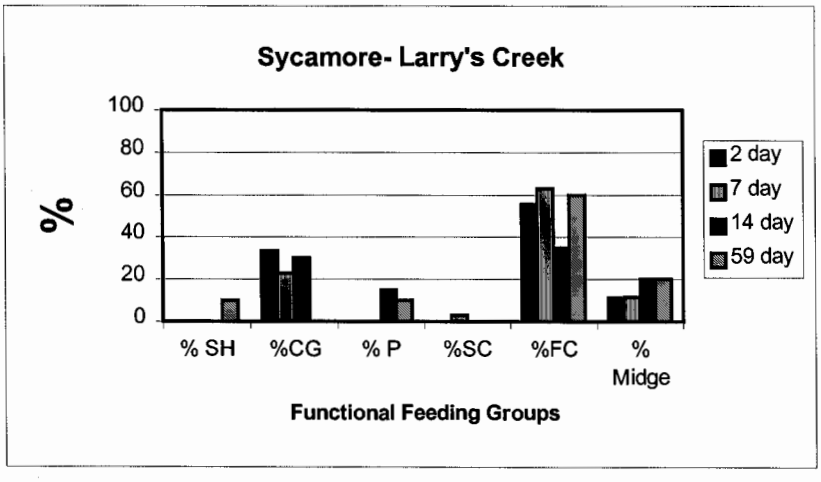
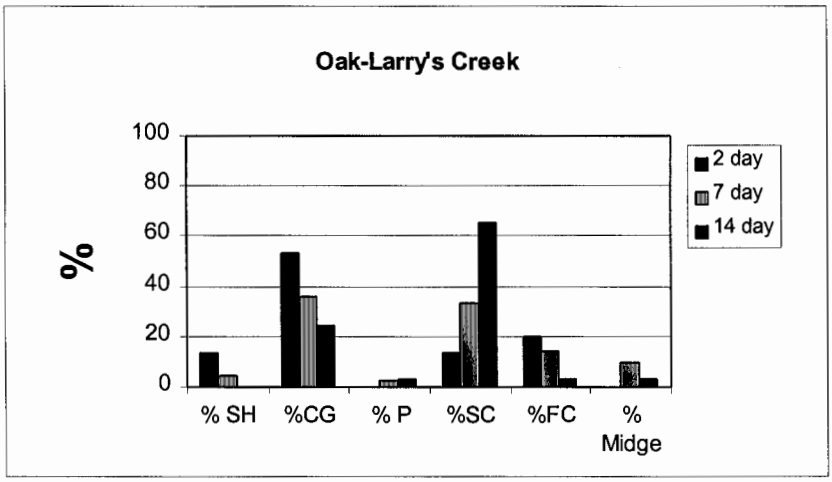
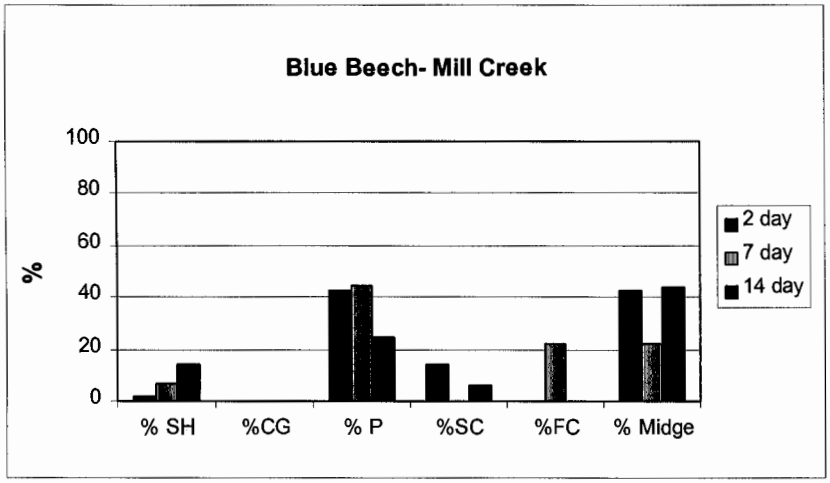


Figure 6: Percent of total number of invertebrates categorized into their functional groups. [SH-shredder; CG-collector-gatherer; P-predator; SC-scraper; FC-filtering-collector]





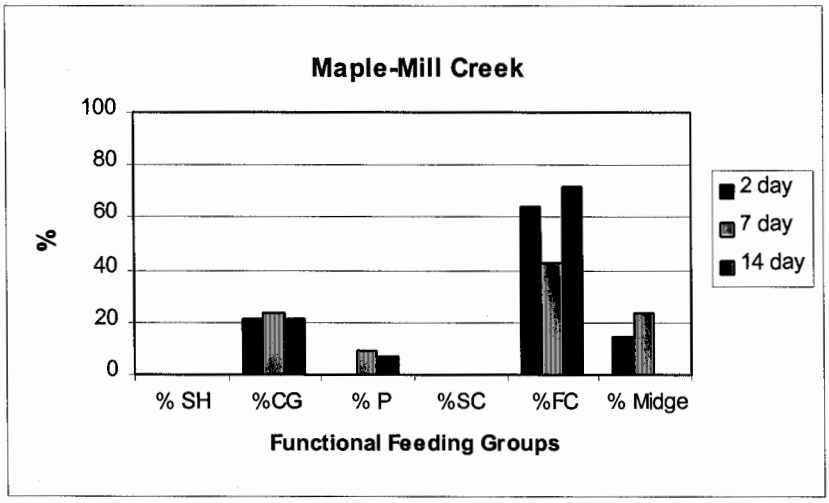
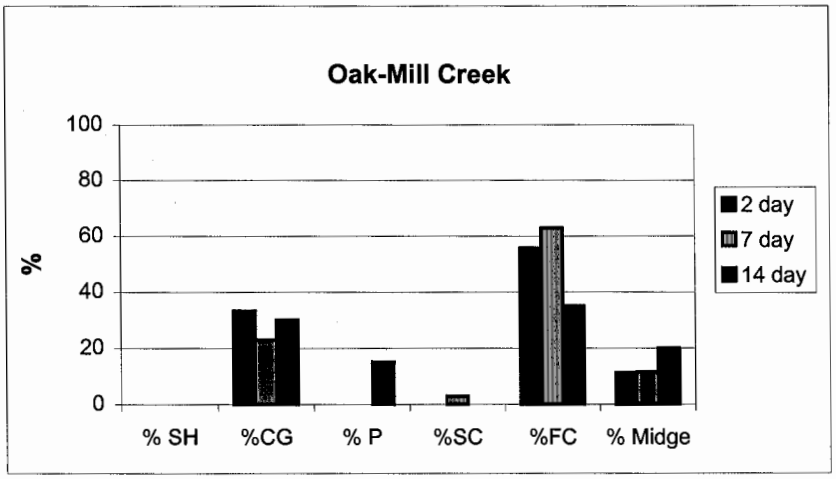
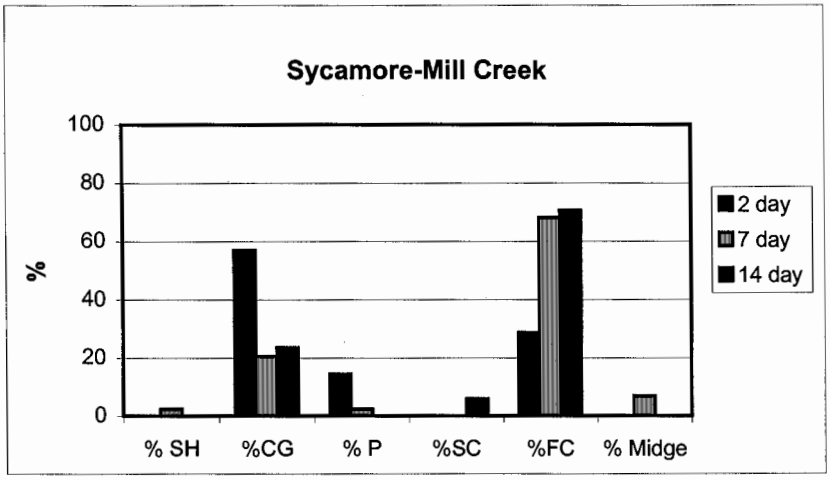


Figure 6: Continued





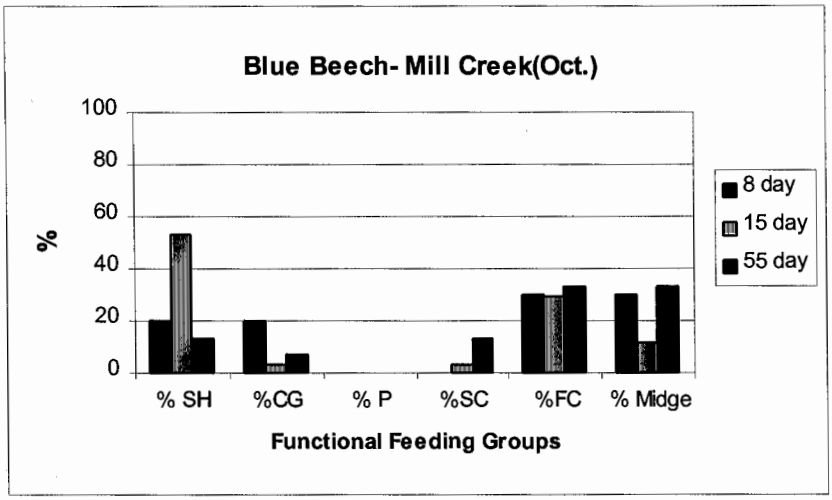
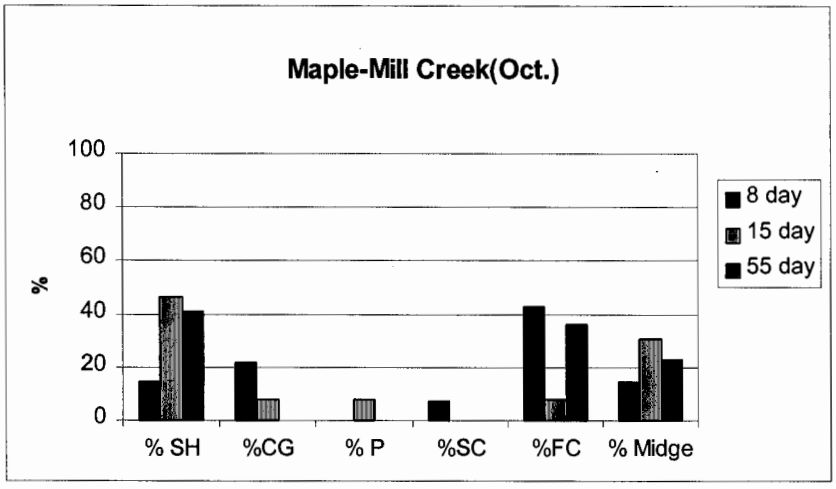
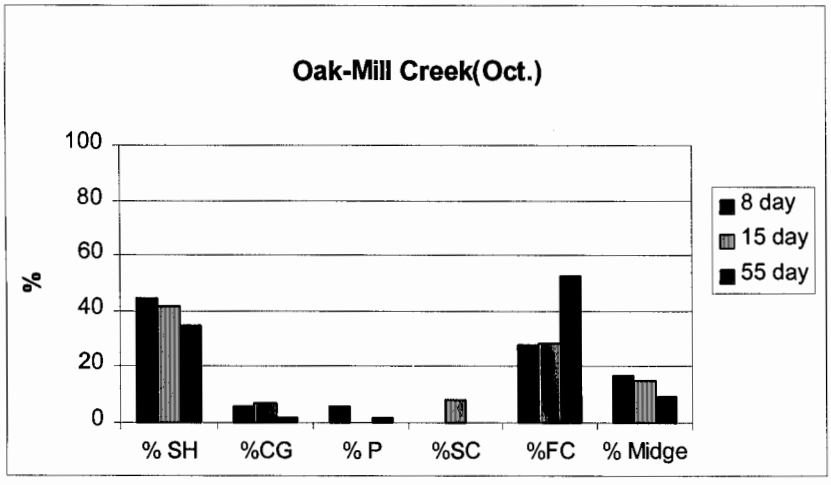


Figure 6: Continued





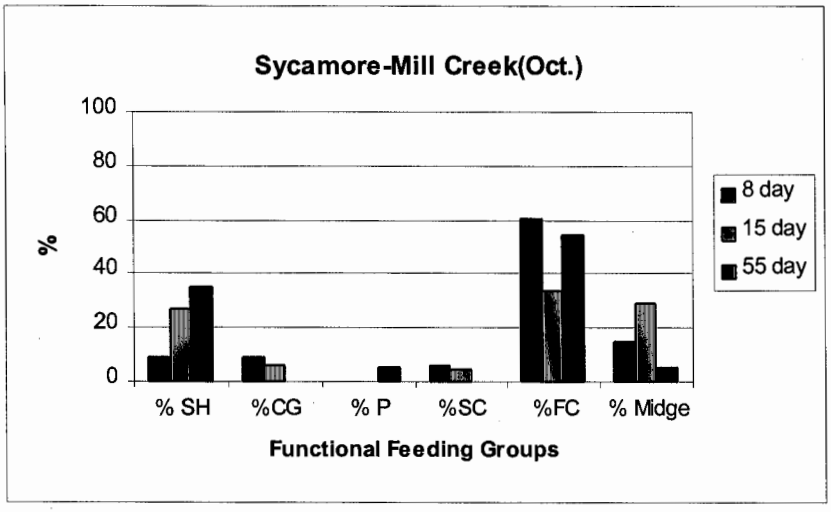
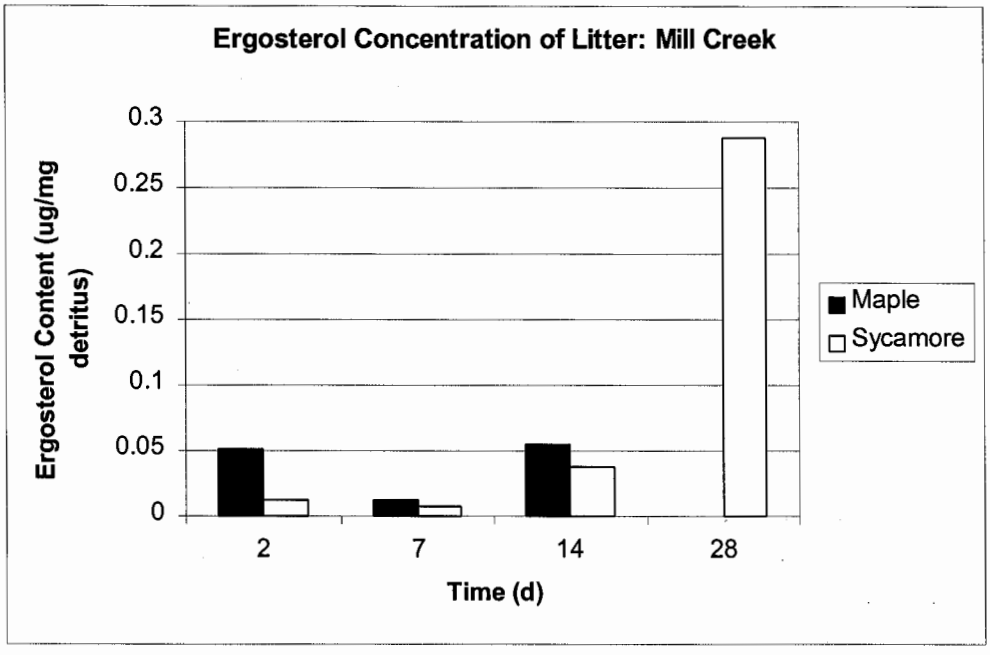


Figure 6: Continued



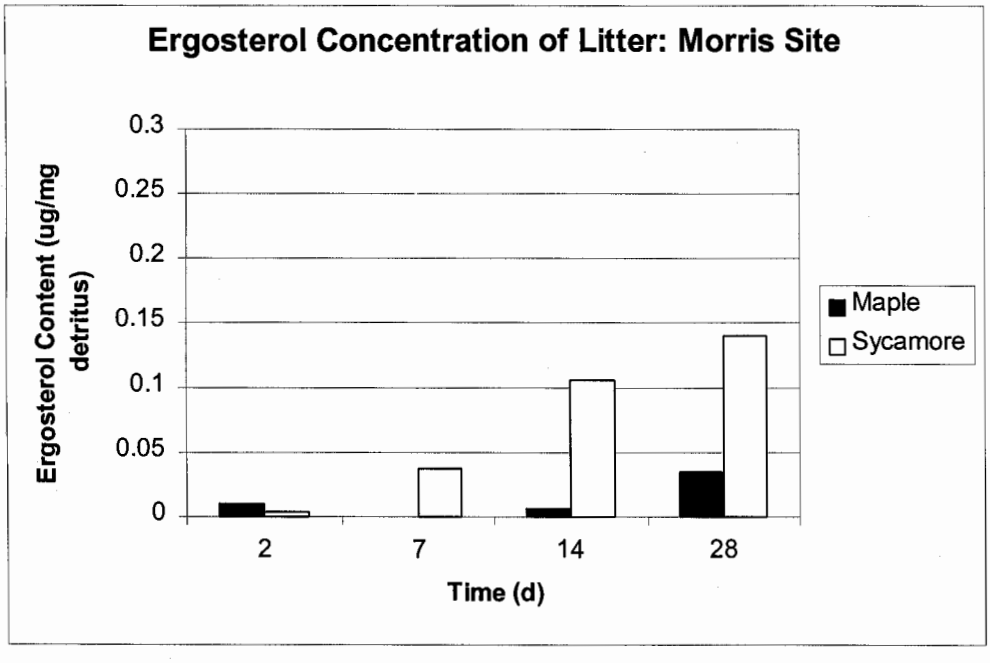


Figure 7: Concentration of ergosterol (μg ergosterol/mg detritus) for *Acer saccharum* (Maple) and *Platanus occidentalis* (Sycamore) in Mill and Morris Creeks. The 28-day sample for *Acer saccharum* for Mill Creek was lost.

Table 4: Number of macroinvertebrates collected per leaf pack on all four species. Numbers for each sample date are totals from all three replicates.

Acer saccharum

2 days- Larry's

Order Ephemeroptera
- Family Baetidae – 4
- Family Leptophlebiidae – 1

Order Trichoptera
- Family Hydropsychidae – 2

Order Diptera
- Family Chironomidae – 1
- Family Simuliidae – 4

7 days- Larry's

Order Plecoptera
- Family Taeniopterygidae – 3

Order Ephemeroptera
- Family Baetidae – 7
- Family Heptageniidae- 14
- Family Leptophlebiidae – 2

Order Trichoptera
- Family Hydropsychidae – 1

Order Diptera
- Family Chironomidae – 2
- Family Tipulidae – 1

14 days- Larry's

Order Plecoptera
- Family Taeniopterygidae – 1

Order Ephemeroptera
- Family Baetidae – 6
- Family Heptageniidae – 13
- Family Leptophlebiidae – 2

Order Trichoptera
- Family Leptoceridae
  - Genus Setodes – 2
- Family Hydropsychidae- 3

Order Diptera
- Family Chironomidae – 1
- Family Simuliidae – 3

Carpinus caroliniana

2 days- Larry's

Order Ephemeroptera
- Family Baetidae- 8
- FamilyHeptageniidae-1

Order Ephemeroptera
- Family Baetidae- 8
- Family Heptageniidae- 8
- Family Leptophlebiidae- 3

Order Trichoptera
- Family Hydropsychidae- 3
- Family Leptoceridae
  - Genus Setodes- 1

Order Plecoptera
- Family Taeniopterygidae- 3

Order Ephemeroptera-
- Family Baetidae- 7
- Family Heptageniidae- 7

Order Trichoptera
- Family Hydropsychidae- 2
- Family Leptoceridae
  - Genus Setodes- 1

Order Diptera
- Family Simuliidae- 1

2 days- Mill

Order Ephemeroptera
- Family Baetidae- 2

Order Trichoptera
- Family Hydropsychidae- 7
- Family Leptoceridae
  - Genus Setodes –1

Order Diptera
- Family Chironomidae –2
- Family Simuliidae- 2

Order Ephemeroptera
- Family Baetidae- 3

Order Trichoptera
- Family Hydropsychidae- 3
- Family Rhyacophilidae
  - Genus Rhyacophila- 1

7 days- Mill

Order Plecoptera
- Family Taeniopterygidae –1

Order Ephemeroptera
- Family Baetidae –4
- Family Siphlonuridae- 1

Order Trichoptera
- Family Hydropsychidae- 9
- Family Rhyacophilidae
  - Genus Rhyacophila –1

Order Diptera
- Family Chironomidae- 4

Order Ephemeroptera
- Family Baetidae- 3
- Family Heptageniidae- 1

Order Trichoptera
- Family Hydropsychidae- 2
- Family Leptoceridae
  - Genus Setodes- 1

Order Diptera
- Family Chironomidae- 1

14 days- Mill

Order Ephemeroptera
- Family Baetidae – 3

Order Trichoptera
- Family Hydropsychidae – 9
- Family Rhyacophilidae
  - Genus Rhyacophila – 1

Order Diptera
- Family Simuliidae –1

Order Ephemeroptera
- Family Baetidae- 2
- Family Leptophlebiidae- 1
- Family Siphlonuridae- 1

Order Trichoptera
- Family Hydropsychidae- 7
- Family Rhyacophilidae
  - Genus Rhyacophila- 1

Order Diptera
- Family Chironomidae- 3
- Family Blephariceridae-1

8 days- Mill (Oct.)

Order Plecoptera
- Family Taeniopterygidae –2

Order Ephemeroptera
- Family Baetidae –2
- Family Heptageniidae –1

Order Trichoptera
- Family Hydropsychidae – 3

Order Diptera
- Family Chironomidae – 1

Order Plecoptera
- Family Taeniopterygidae- 2

Order Ephemeroptera
- Family Baetidae- 2

Order Trichoptera
- Family Hydropsychidae- 3

Order Diptera
- Family Chironomidae- 3

15 days- Mill (Oct.)

Order Plecoptera
- Family Perlidae –1
- Family Taeniopterygidae –6

Order Trichoptera
- Family Hydropsychidae –1
- Family Leptoceridae
  - Genus Setodes –1

Order Diptera
- Family Chironomidae – 4

55 days- Mill (Oct.)

Order Plecoptera
- Family Taeniopterygidae –7

Order Trichoptera
- Family Hydropsychidae – 8

Order Diptera
- Family Chironomidae –5
- Family Tipulidae- 2

8 days- Morris

Order Trichoptera
- Family Hydropsychidae –2

Quercus rubra

2 days- Larry's

Order Ephemeroptera
- Family Baetidae- 4
- Family Heptageniidae- 2

Order Trichoptera
- Family Hydropsychidae- 3
- Family Leptoceridae
  - Genus Setodes- 4

Order Diptera
- Family Tipulidae-1

Order Plecoptera
- Family Taeniopterygidae- 17

Order Ephemeroptera
- Family Heptageniidae- 1

Order Trichoptera
- Family Hydropsychidae- 10
- Family Leptoceridae
  - Genus Setodes- 1

Order Diptera
- Family Chironomidae- 4
- Family Tipulidae- 1

Order Plecoptera
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Siphlonuridae- 1

Order Trichoptera
- Family Helicopsychidae- 2
- Family Hydropsychidae- 5

Order Diptera
- Family Chironomidae-5
- Family Tipulidae-1

Platanus occidentalis

Order Ephemeroptera
- Family Baetidae- 7
- Family Heptageniidae- 1
- Family Leptophlebiidae- 1

Order Trichoptera
- Family Hydropsychidae- 4
- Family Leptoceridae
  - Genus Setodes- 1

Order Diptera
- Family Chironomidae- 2
- Family Simuliidae- 2

7 days- Larry's

Order Plecoptera
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Baetidae- 10
- Family Heptageniidae- 14
- Family Leptophlebiidae- 3

Order Trichoptera
- Family Hydropsychidae- 6
- Family Leptoceridae
  - Genus Setodes- 2

Order Diptera
- Family Chironomidae- 4
- Family Tipulidae- 2

14 days- Larry's

Order Plecoptera
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Baetidae- 5
- Family Heptageniidae- 19
- Family Leptophlebiidae- 2

Order Trichoptera
- Family Hydropsychidae- 1

Order Diptera
- Family Chironomidae- 1

59 days- Larry's

2 days- Mill

Order Ephemeroptera
- Family Baetidae- 3

Order Trichoptera
- Family Hydropsychidae- 3

Order Diptera
- Family Chironomidae- 1
- Family Simuliidae- 2

Order Plecoptera
- Family Perlidae-1
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Baetidae- 8
- Family Heptageniidae- 15
- Family Leptophlebiidae- 4

Order Trichoptera
- Family Hydropsychidae- 4
- Family Leptoceridae
  - Genus Setodes- 2

Order Diptera
- Family Chironomidae- 3

Order Plecoptera
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Baetidae- 5
- Family Heptageniidae- 2

Order Trichoptera
- Family Hydropsychidae- 2

Order Diptera
- Family Simuliidae- 1
- Family Tipulidae- 1

Order Trichoptera
- Family Hydropsychidae- 12
- Family Rhyacophilidae
  - Genus Rhyacophila- 2

Order Diptera
- Family Chironomidae- 4
- Family Tipulidae-2

Order Ephemeroptera
- Family Baetidae- 4

Order Trichoptera
- Family Hydropsychidae- 1
- Family Rhyacophilidae
  - Genus Rhyacophila- 1

Order Diptera
- Family Simuliidae-1

7 days- Mill

Order Ephemeroptera
- Family Baetidae- 5
- Family Heptageniidae- 1
- Family Siphlonuridae- 3

Order Trichoptera
- Family Hydropsychidae- 19

Order Diptera
- Family Chironomidae- 4
- Family Simuliidae- 3

Order Plecoptera
- Family Taenioptergidae-1

Order Ephemeroptera
- Family Baetidae- 4
- Family Leptophlebiidae-2
- Family Polymitarcidae-1
- FamilySiphlonuridae-2

Order Trichoptera
- Family Hydropsychidae- 26
- Family Psychomyiidae-1

Order Diptera
- Family Chironomidae- 3
- Family Simuliidae- 4
- Family Culcidae-1

14 days- Mill

Order Plecoptera
- Family Taeniopterygidae- 1

Order Ephemeroptera
- Family Baetidae- 5
- Family Siphlonuridae- 1

Order Trichoptera
- Family Hydropsychidae- 7
- Family Leptoceridae

Genus Setodes- 2
- Family Rhyacophilidae
  - Genus Rhyacophila - 2

Order Diptera
- Family Chironomidae- 4

Order Ephemeroptera
- Family Baetidae- 4
- Family Heptageniidae- 1

Order Trichoptera
- Family Hydropsychidae- 10

Order Diptera
- Family Simuliidae-2

59 days- Mill

Order Plecoptera
- Family Taeniopterygidae- 11

Order Trichoptera
- Family Glossosomatidae-1
- Family Hydropsychidae- 7
- Family Rhyacophilidae
  - Genus Rhyacophila - 2

Order Coleoptera
- Family Elmidae- 3

Order Diptera
- Family Chironomidae- 11
- Family Tipulidae- 4

8 days- Mill (Oct.)

Order Plecoptera
- Family Perlidae- 1
- Family Taeniopterygidae- 7

Order Ephemeroptera
- Family Siphlonuridae- 1

Order Trichoptera
- Family Hydropsychidae- 5

Order Diptera
- Family Chironomidae-1
- Family Tipulidae-1

Order Plecoptera
- Family Taeniopterygidae- 3

Order Ephemeroptera
- Family Baetidae-3
- Family Heptageniidae- 4

Order Trichoptera
- Family Hydropsychidae- 19

Order Diptera
- Family Chironomidae- 5
- Family Simuliidae- 1

15 days- Mill (Oct.)

Order Plecoptera
- Family Taeniopterygidae- 25

Order Ephemeroptera
- Family Heptageniidae- 4
- Family Siphlonuridae- 4

Order Trichoptera
- Family Hydropsychidae- 19

Order Coleoptera
- Family Elmidae- 1

Order Diptera
- Family Chironomidae- 9

Order Plecoptera
- Family Taeniopterygidae- 13

Order Ephemeroptera
- Family Baetidae-1
- Family Heptageniidae- 2
- Family Leptophlebiidae- 1
- Family Siphlonuridae-1

Order Trichoptera
- Family Hydropsychidae- 16

Order Diptera
- Family Chironomidae- 14

55 days- Mill (Oct.)

Order Plecoptera
- Family Taeniopterygidae- 12

Order Ephemeroptera
- Family Baetidae- 1

Order Trichoptera
- Family Hydropsychidae- 22
- Family Rhyacophilidae
  - Genus Rhyacophila - 1

Order Diptera
- Family Chironomidae-6
- Family Tipulidae- 3

Order Plecoptera
- Family Taeniopterygidae- 22

Order Ephemeroptera
- Family Heptageniidae- 1

Order Trichoptera
- Family Hydropsychidae- 42
- Family Rhyacophilidae
  - Genus Rhyacophila -3

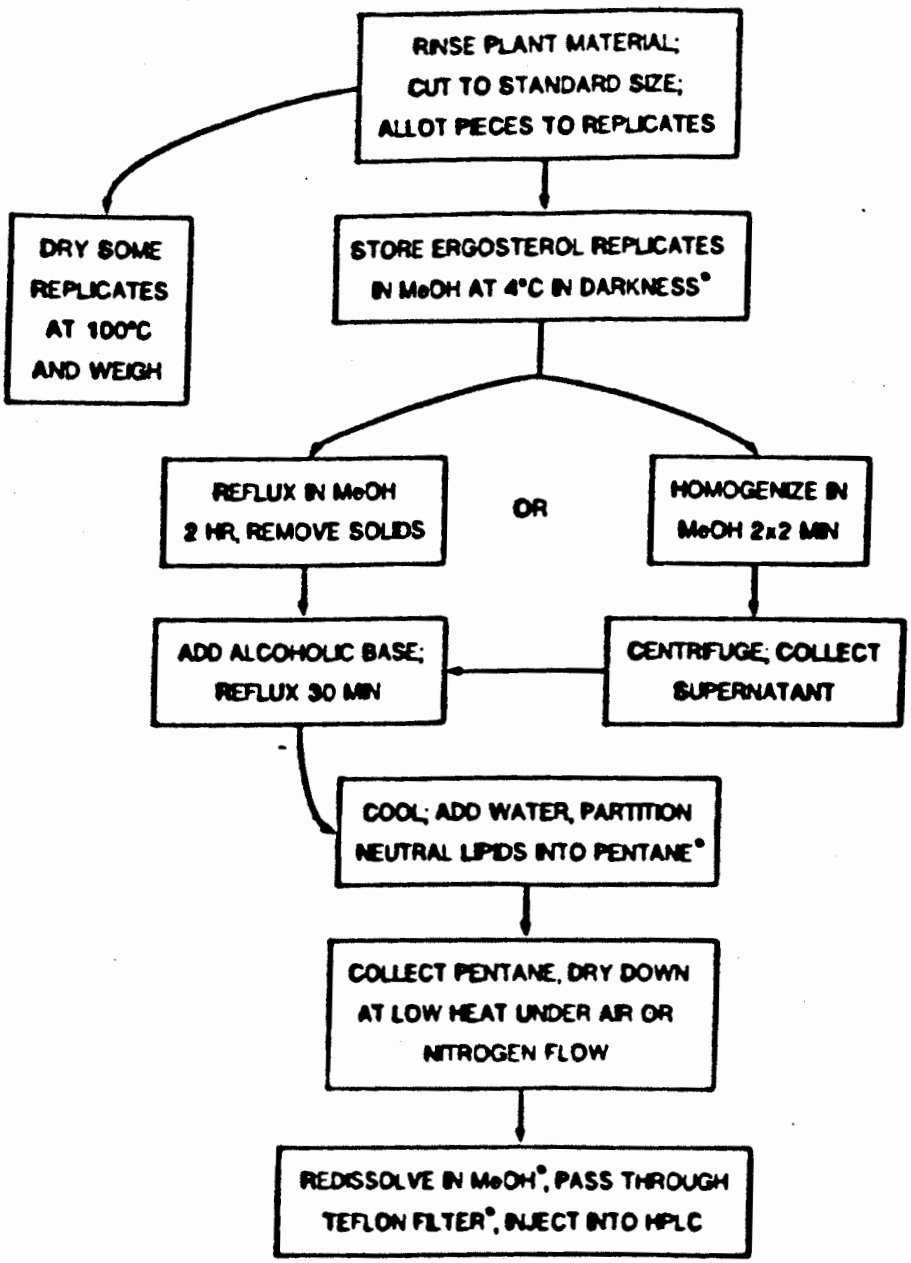
Order Diptera
- Family Chironomidae- 3
- Family Tipulidae- 5

2 days- Morris

Order Trichoptera
- Family Hydropsychidae-1

Order Trichoptera
- Family Hydropsychidae-1

Appendix 1



A flow-chart displaying the ergosterol extraction procedure (Newell et al. 1988).