Seasonal Biomass and Carbohydrate Allocation in a Southern Population of Eurasian Watermilfoil

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ABSTRACT

Seasonal biomass and carbohydrate allocation patterns of Eurasian watermilfoil (*Myriophyllum spicatum* L.) were evaluated to elucidate potential points in the seasonal phenological cycle for improved control. Eurasian watermilfoil was sampled from outdoor 0.3 ha ponds in Lewisville, TX from January 1991 to December 1993 and analyzed for free sugars, starch and total nonstructural carbohydrates (TNC). Root crowns stored up to 30% TNC, followed by autofragments (25%), lower stems(22%), and upper stems and inflorescences (12%). Low levels of stored carbohydrates occurred in July (1991), April (1992), and May (1993). Low levels of stored carbohydrates also were observed in October for all three years. Low points in carbohydrate storage might be exploited to improve management of Eurasian watermilfoil.

Key words: Myriophyllum spicatum, phenology, starch, total nonstructural carbohydrate, autofragment, inflorescence.

INTRODUCTION

Perennial plants of the temperate zone typically store carbohydrates as starch in roots, rhizomes, and specialized structures (e.g., tubers) during late summer and autumn, for winter survival and regrowth in spring (McAllister and Haderlie 1985, Cyr et al. 1990). Without these reserves, the plant may not be able to survive winter or to reestablish growth in the spring. Management of terrestrial weed species has exploited this knowledge, either by repetitive mowing to reduce stored carbohydrates (Klingman et al. 1975) or by timing herbicide applications to coincide with periods of low starch reserves (Schirman and Buchholtz 1966).

Aquatic plants have many structures for storing starch or other nonstructural carbohydrates (TNC) for overwintering or regrowth; including roots (Garza et al. 1994), rhizomes (Gallagher et al. 1984), stems (Best 1977), stembases in rosette species (Tucker and DeBusk 1981), tubers (Hodgson 1966), winter buds (Titus and Adams 1979), and turions (Winston and Gorham 1979). In most aquatic macrophytes, a distinct seasonal cycle is observed in which carbohydrate storage reaches a peak in late summer or fall and is depleted as spring regrowth occurs (Madsen 1991). The typical cycle can be disrupted through disturbance, stress and herbivory.

A knowledge of carbohydrate storage has proven beneficial in previous aquatic plant management applications. For instance, several studies of harvesting effects on Eurasian watermilfoil have examined carbohydrate depletion to predict long-term success of harvest operations (Kimbel and Carpenter 1981, Perkins and Sytsma 1987, Painter and Waltho 1985, Painter 1988). Herbicide treatments were found to be more effective when applied at times of lower carbohydrate storage in alligatorweed (Weldon and Blackburn 1968) and sago pondweed (Hodgson 1966). Feeding of *Neochetina* weevils on waterhyacinth leaves reduced leaf carbohydrate content, even at low densities (Center and Van 1989). Thus, the effectiveness of some biological, chemical and mechanical control techniques on aquatic plants might be interpreted by their effects on whole plant carbohydrate storage.

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a herbaceous perennial submersed aquatic plant that grows in water from 0.3m to 7m, most commonly in the 1 m to 3 m water depth interval (Aiken et al. 1979). A native of Eurasia, it was simultaneously introduced to four states in the 1940's (Couch and Nelson 1985). Eurasian watermilfoil has been documented as occurring in thirty-seven of the United States and three Canadian provinces (Engel 1993). Eurasian watermilfoil is highly adventive and productive, resulting in rapid spread and dense monospecific growths (Grace and Wetzel 1978, Smith and Barko 1990). Vegetative propagation is predominantly via self-formed stem segments (or autofragments) produced through the development of an abscission layer (Madsen et al. 1988). Dense monospecific stands not only create a nuisance to humans for flood control, navigation, and recreation, but also displace native vegetation, causing declines in native species abundance and diversity (Madsen et al. 1991).

A better understanding of the seasonal cycles of plants might produce insight into the timing of biological, herbicidal and mechanical control techniques, to maximize their effectiveness. Specifically, management techniques timed to coincide with reductions in stored carbohydrates may reduce the ability of the target plant to regrow after treatment, or to survive an overwintering period.

The goals of this study were to 1) identify the seasonal dynamics of biomass and carbohydrate allocation patterns of Eurasian watermilfoil, and 2) use those carbohydrate allocation patterns to identify periods of low carbohydrate levels in the annual cycle of the plant at one geographical location.

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MATERIALS AND METHODS

Outdoor experimental ponds with a surface area of 0.3 ha were used at the Lewisville Aquatic Ecosystem Research Facility (LAERF) near Lewisville, TX (latitude 33°04'45"N, longitude 96°57'30"W). Average pond depth was 1.0 m, with a maximum depth of 1.5 m (Smart et al. 1995). Meteorological data was monitored continuously using an Omnidata Easylogger (Omnidata Corp., Logan, UT) and thermistors to record air and pond water temperature every 5 minutes, with hourly averages.

During 1991, twelve biomass samples were collected each month from one pond. During 1992 and 1993, two ponds were utilized. Samples were collected every six weeks from October through March, and every three weeks from April through September. Six samples were collected per pond in 1992, and twelve samples per pond in 1993. Biomass samples were collected in a random pattern, with a 0.1 m^2 quadrat. Samples were consistently collected between 10 am and 2 pm during sample days.

Biomass samples were separated into root crowns, upper and lower shoots, autofragments, and inflorescences. Upper and lower shoots were separated at one-half the height of the total shoot. Root crowns, shoots, autofragments and inflorescences were also counted. Samples were dried at 55 C in a forced air oven for at least 48 hours before weighing. Dried samples were ground in a Cyclone mill to pass a 1 mm mesh filter prior to analysis.

A minimum of at least six replicates per plant part were analyzed for each sample time. Plant samples were analyzed for TNC using a modification by Swank et al. (1982). Extracts of TNC (starch, hydrolyzable sugars, reducing sugars) were incubated for 15 minutes at 55 C with one unit of amyloglucosidase (Sigma A-3042) per milliliter of extractant to completely hydrolyze starch before assaying for reducing sugars (Nelson 1944). Free sugars were determined on extracts not incubated with amyloglucosidase. Starch content was calculated from TNC and sugar content.

RESULTS AND DISCUSSION

Biomass allocation. Total biomass values for 1991 do not show any distinct pattern, but in both 1992 and 1993 peak biomass was observed in May (Figure 1B), which is substantially earlier than observed for northern populations such as Lake Wingra, WI (Adams and McCracken 1974) or Lake Washington, WA (Perkins and Sytsma 1987). Peak biomass was followed by senescence, with the annual biomass minima observed in August of each year; northern populations such as Lake Washington, WA (Perkins and Sytsma 1987) or Buckhorn Lake, ONT (Painter 1988) typically are at or near their biomass peak during late summer. A midsummer biomass minimum may be due to the combination of normal postflowering senescence and high water temperatures typical of shallow ponds in Texas (Figure 1A). Eurasian watermilfoil has a relatively high temperature optima (25-30 C) for temperate aquatic plants (Grace and Wetzel 1978), but does not appear to tolerate daily maximum water temperatures that may reach 35 C, as has been observed for these ponds (Smart et al. 1995). Significant leaf senescence was also observed in midsummer, which may be a symptom of temperature stress.
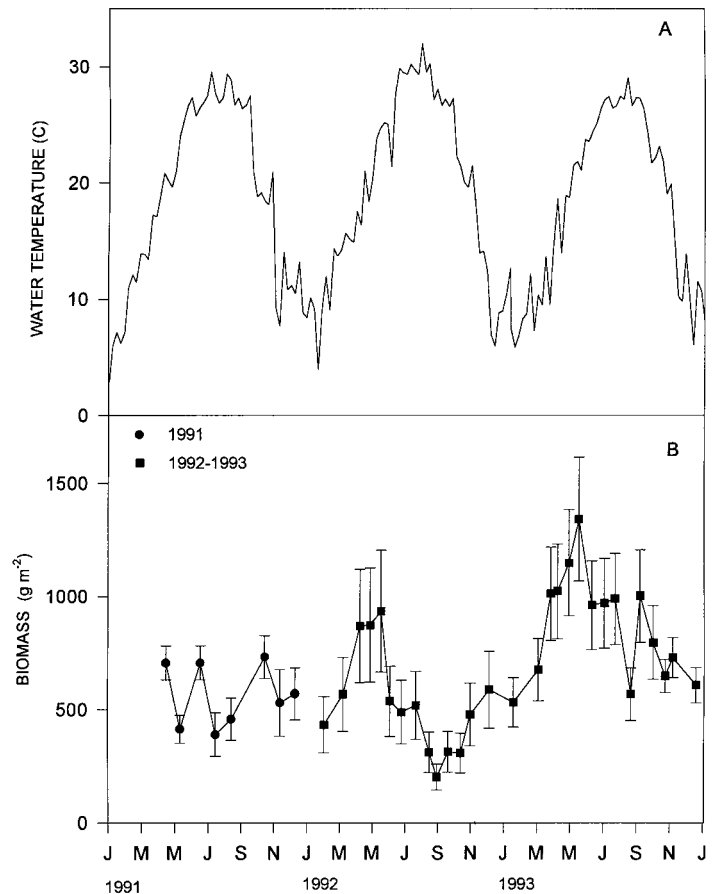

Figure 1. A) Average water temperature (C) in experimental ponds in Lewisville, TX from 1991 to 1993; and B) total Eurasian watermilfoil biomass (g m^{-2}) in experimental ponds in Lewisville, TX from 1991 to 1993. Bars indicated ± 1 standard error of the mean.

Regrowth initiates in September, slows or even decreases slightly during midwinter (December to February), and then increases again in March.

Biomass allocation to various plant components also followed distinct seasonal patterns (Figure 2). Although a small amount of flowering occurred throughout spring and early summer, the most pronounced peaks appeared in June and October of each year (Figure 2A). This peak preceded the period of greatest senescence each year. Coinciding with, or slightly later than, the flowering peak each year was a peak in autofragment production (Figure 2B).

Upper stem, or canopy, biomass increases each spring to a peak in May, then decreased as autofragmentation occurred and senescence began (Figure 2C). Autofragments are formed entirely in the upper canopy, their production significantly reduced upper stem biomass. The lower stems are largely unaffected by autofragment formation, so the percent allocation of biomass to lower stems and root crown appears to increase. Lower stem biomass reached an earlier peak, in February or March, but also reached a minimum in August (Figure 2D). This earlier peak may be related to reallocation of carbohydrates during spring regrowth, and production of new branches and buds. Root crown biomass exhibited peak biomass in May and December for both 1992
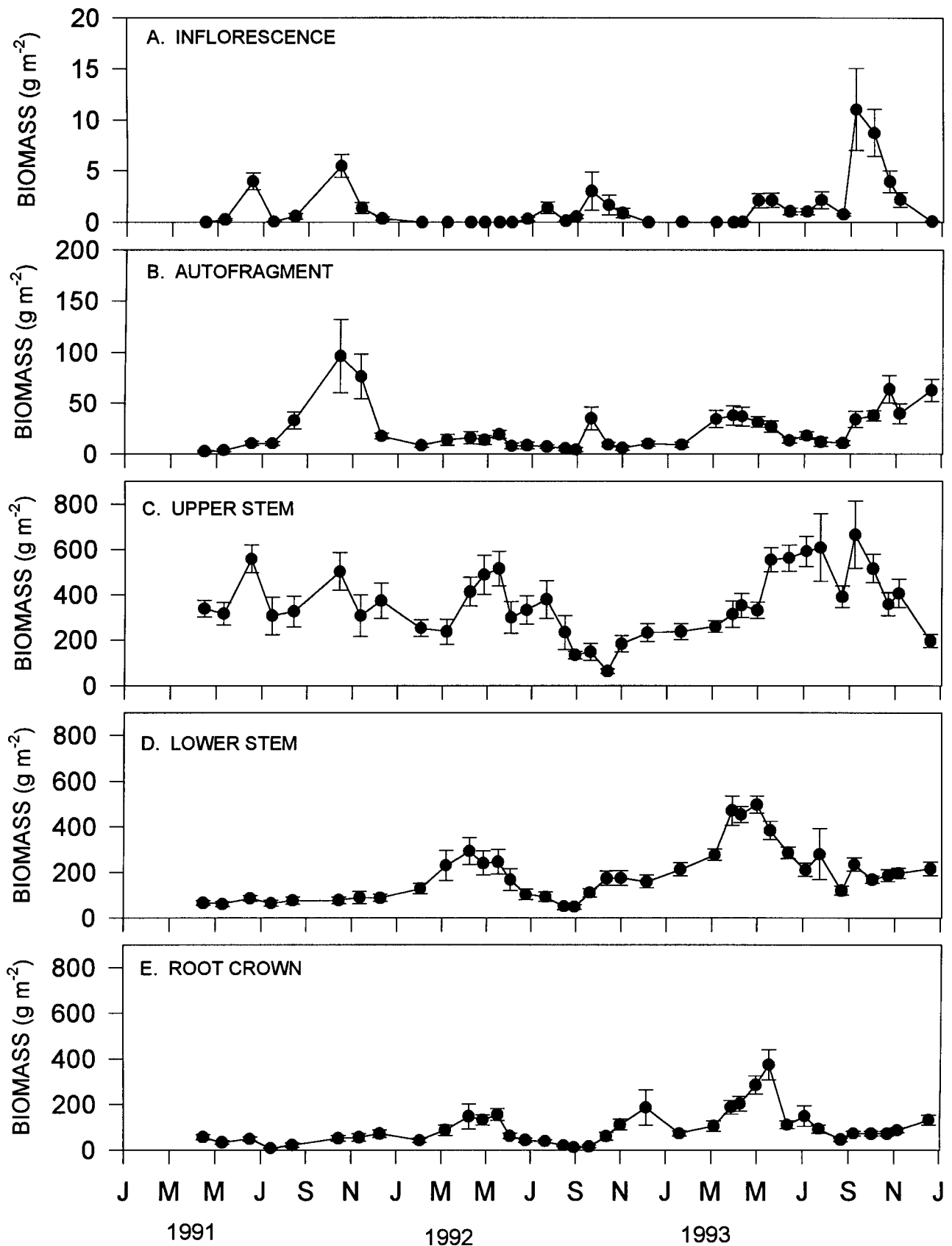
Figure 2. Eurasian watermilfoil plant component allocation, biomass (g m²), in experimental ponds at Lewisville, TX from 1991 to 1993. A) Inflorescences, B) Autofragments, C) Upper stem, D) Lower stem, E) Root crown. Bars indicated ± 1 standard error of the mean.

and 1993. This may be related to high TNC levels and the production of new root buds and spread of the plant vegetatively during the winter and early spring in this locality (Madsen, unpubl. data).

Biomass allocation can also be expressed as a percentage of total biomass. Eurasian watermilfoil, like many perennial submersed aquatic plants, allocates very little to sexual propagation (Madsen 1991). In the Texas populations, maximum allocation to sexual propagation was less than 1%. In contrast, allocation to autofragments, which are the primary vegetative form of propagation, was as high as 11%. Overwintering was predominantly as root crowns (10 to 22%), and lower shoots (15 to 45%). Upper shoots ranged from 20% (winter) to 75% (summer) of total biomass.

Carbohydrate Allocation. Plant components varied greatly in proportions and total amounts of carbohydrates, and in seasonal allocation patterns (Figure 3). Inflorescences (Figure 3A) had high amounts of free sugars (up to 5%), and usually an equivalent amount of starch. Autofragments also had high concentrations of sugars (5% or more), but also large stored concentrations of starch (up to 15%), for TNC concentrations of up to 25% (Figure 3B). The storage of large quantities of starch in stem autofragments indicated these are stems adapted for propagation, not simply accidental breakage of the stem. Autofragments detach from the stem following formation of an abscission zone, frequently forming adventitious roots, and are specifically produced for vegetative propagation of this species (Patten 1956, Aiken et al. 1979). Autofragments, which typically have more than twice the concentration of TNC than allofragments (formed through breakage due to an external force), are significantly more likely to overwinter successfully and are less likely to die during the growing season than are allofragments (Kimbel 1982).

Upper stem tissues typically had less than 5% reducing sugars and a comparable amount of starch for TNC levels of up to 10% (Figure 3C). Maximum levels of TNC in upper and lower stems appeared to occur in midsummer, at the same approximate times as root crown minima of TNC.

Lower stem carbohydrate concentrations varied widely over the year (Figure 3D). Lower stems not only stored large amounts of starch (up to 15%), but the highest levels of reducing sugars for all plant components appeared in these tissues during the time carbohydrates were being reallocated from the root crown to upper stems during rapid growth in the spring and early summer.

Root crown tissues are the main storage area for carbohydrates in overwintering Eurasian watermilfoil. Starch concentrations can reach 20%, with TNC concentrations of up to 30% (Figure 3E). Total nonstructural carbohydrate concentrations consistently reached a peak in January or February. Springtime minima varied from April through July of the three study years. Although root crowns are not specialized for storage, they can hold a significant reserve of carbohydrates for regrowth. In studying sympatric populations of water celery (*Vallisneria americana* L.) and Eurasian watermilfoil in Lake Wingra, Titus and Adams (1979) found that water celery winter bud TNC levels could reach 46%, as compared to Eurasian watermilfoil root crown TNC concentrations of 25%. However, water celery winter bud biomass was

only 18 g m^{-2}, as opposed to Eurasian watermilfoil root crown of 39 g m^{-2}. Therefore, total carbohydrate storage is 8.3 g m^{-2} for water celery, and 9.8 g m^{-2} for Eurasian watermilfoil root crowns. If the evergreen shoots which also store carbohydrates are included, total Eurasian watermilfoil overwintering biomass is 54 g m^{-2} and TNC concentrations are 20%, for a total carbohydrate storage of 10.8 g m^{-2} in Lake Wingra. Eurasian watermilfoil is not at a disadvantage in competing with this tuber-forming species by not having specialized winter buds or tubers, based solely on total mass of carbohydrates stored. However, specialized propagules may confer other advantages, such as greater resistance to environmental fluctuations, decreased maintenance respiration during overwintering, and interannual dormancy of the propagule (Madsen 1991).

Carbohydrate Low Points. A carbohydrate low point is defined as a seasonal minimum in stored carbohydrates. Carbohydrate low points occur when carbohydrate usage from storage equals carbohydrate production. The primary low point is that which occurs during spring regrowth, which is common for all temperate zone perennial plant populations. A secondary low point may occur before reallocation to storage components in the fall; not all populations will exhibit this. The secondary low point is not necessarily another minima, but often appears as the end of a low plateau in carbohydrate levels, before carbohydrates are reallocated to storage. Since root crowns are the primary overwintering storage site in Eurasian watermilfoil, minima in TNC levels in this tissue will indicate minima in whole-plant carbohydrate storage. For the Eurasian watermilfoil populations in the LAERF ponds, early-season minima occurred at different times each year (Figure 4), ranging from April (1992) to May (1993) and July (1991). In contrast, the secondary low point was consistently found in October of each year. Resumption of Eurasian watermilfoil growth in the fall may also be temperature-related. Autumnal weather patterns were more uniform between years than winter weather patterns, which may account for the similarities in secondary carbohydrate low points in the three years.

The occurrence of these seasonal low levels of carbohydrates for northern Eurasian watermilfoil populations can be compared to those of the Texas population using other published studies (Table 1), with populations having significantly different low points among years. In fact, different locations within a given lake can have significantly different timing of carbohydrate minima within a year, as noted in data for Lake Wingra (Titus and Adams 1979, Kimbel and Carpenter 1981).

None of the northern populations had secondary low points, including those shallow lakes that exhibited bimodal biomass growth curves (e.g., Lake Wingra; Table 1). The secondary low points characteristic of southern Eurasian watermilfoil populations may be related to higher water temperatures, which may be causing a mid-summer reduction in growth due to temperature stress and high dark respiration rates.

Information on carbohydrate storage may be useful in planning or evaluating the timing of control technique applications, and evaluating the results of these applications. Consideration of the timing of carbohydrate low points
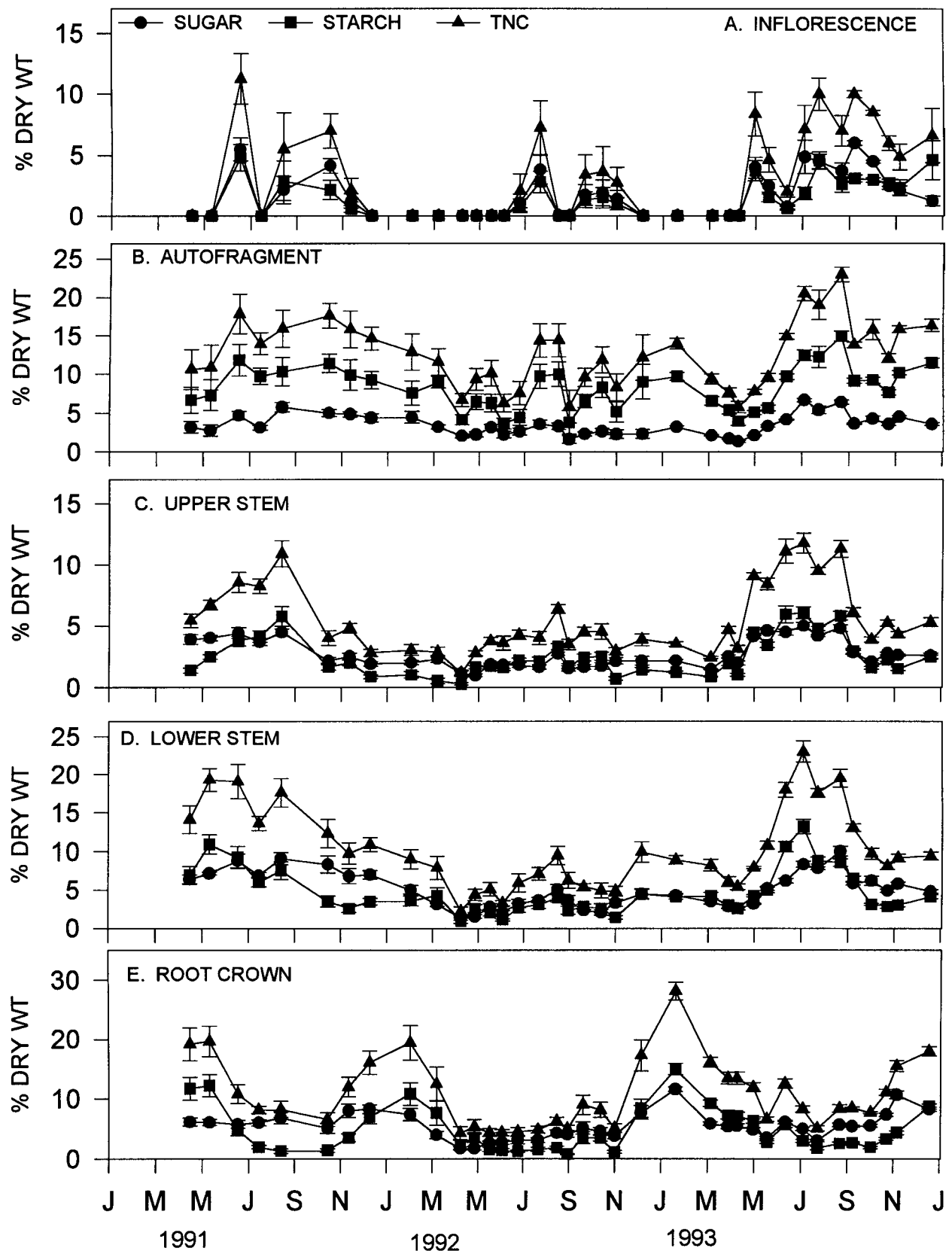
Figure 3. Eurasian watermilfoil plant component concentrations of free sugars (SUGAR), starch (STARCH), and total nonstructural carbohydrates (TNC) for experimental ponds in Lewisville, TX from 1991 to 1993; in percent of dry weight. A) Inflorescences, B) Autofragments, C) Upper stem, D) Lower stem, E) Root crown. Bars indicated ± 1 standard error of the mean.

TABLE 1. CARBOHYDRATE LOW POINTS IN THE SEASONAL GROWTH CYCLE OF EURASIAN WATERMILFOIL POPULATIONS FOR NORTHERN AND SOUTHERN POPULATIONS.

| Lake	State or Prov.	North or South	Date of Low Point	Reference
Lewisville Pd	TX	S	04-15-92	This Study
Lewisville Pd	TX	S	05-21-93	This Study
Lk Wingra (A&B)	WI	N	05-30-75	Titus & Adams 1979
Lk Wingra (C&D)	WI	N	05-30-76	Kimbel & Carpenter 1981
Lk Washington	WA	N	05-30-81	Perkins & Sytsma 1987
Buckhorn Lk	ONT	N	05-30-82	Painter 1988
Lk Wingra (C&D)	WI	N	06-15-75	Kimbel & Carpenter 1981
Buckhorn Lk	ONT	N	06-15-79	Painter 1988
Buckhorn Lk	ONT	N	06-15-80	Painter 1988
Buckhorn Lk	ONT	N	06-15-81	Painter 1988
Buckhorn Lk	ONT	N	07-15-83	Painter 1988
Buckhorn Lk	ONT	N	07-15-84	Painter 1988
Lk Wingra (A&B)	WI	N	07-15-74	Titus & Adams 1979
Lewisville Pd	TX	S	07-15-91	This Study
Lk Washington	WA	N	07-30-80	Perkins & Sytsma 1987
Lewisville Pd	TX	S	10-04-93	This Study
Lewisville Pd	TX	S	10-15-91	This Study
Lewisville Pd	TX	S	10-30-92	This Study
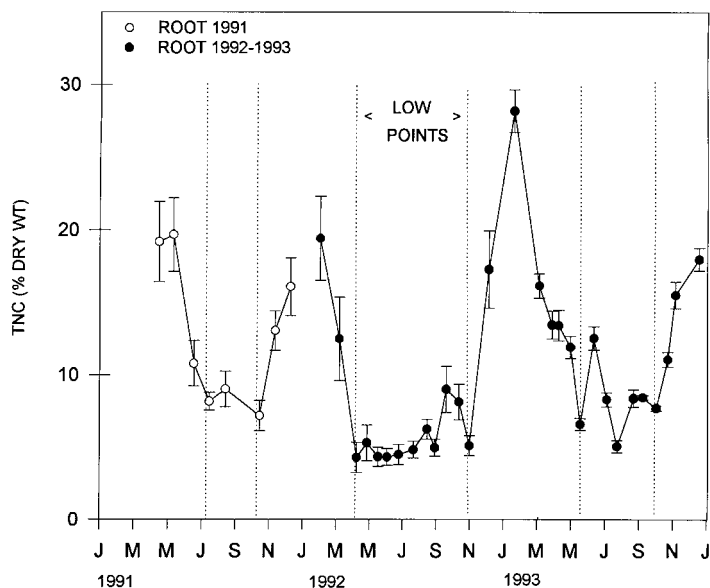
Figure 4. Eurasian watermilfoil root crown concentrations of total nonstructural carbohydrates (TNC) for experimental ponds in Lewisville, TX from 1991 to 1993; in percent of dry weight. Bars indicated ± 1 standard error of the mean. Vertical dotted lines indicate the timing of primary and secondary low points of carbohydrate levels for each year.

might be particularly useful for management with harvesting and contact herbicides, where regrowth is a significant concern. However, carbohydrate storage is also important for understanding the success, or failure, of other management techniques.

ACKNOWLEDGMENTS

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