Community and ecosystem-level impacts of a vascular macrophyte on the Ventura River, California

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This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to Hydrobiologia.

Abstract

In Mediterranean climates, light and nutrient concentrations are high and the lack of summer rain results in long, disturbance-free seasons for plant growth. *Ludwigia hexapetala* (hereafter, *Ludwigia*), an exotic invasive, dominates the Ventura River of southern California downstream of a wastewater treatment plant (WWTP), and its presence has strong impacts on the river's biogeochemistry and community ecology. Green macroalgae biomass is reduced in the presence of *Ludwigia*, whereas that of shade-tolerant diatoms is increased. *Ludwigia*'s growth and nitrogen uptake rates increase with increases in nutrient availability, and it appears to be responsible for considerable decreases in dissolved nutrients downstream of the WWTP. *Ludwigia*'s presence and dominance varies considerably from year to year as the plant is scoured from the river during winters with high rainfall and large flooding events and it may take several consecutive years of low flows for it to re-establish dominance. On shorter time scales (months to years), the presence of the WWTP and *Ludwigia* reduce some of the natural variability inherent in this system by establishing a relatively homogenous monoculture and altering seasonal patterns of algal growth and succession. Over longer time scales (years to decades), however, *Ludwigia* responds to variations in regional rainfall with dramatic differences in the physiognomy of the primary producer community and in patterns of nutrient cycling and retention.

Introduction

(I played with the first paragraph and then decided it was probably unnecessary)

Although the role of aquatic plants in lakes has been well-studied, vascular plants in streams and rivers have received considerably less attention, although they can have significant influences on biogeochemistry (Hamilton et al. 1995, Howard-Williams et al. 1982, Vincent and Downes 1980) and community ecology (Gregg and Rose 1982). Plants have been shown to increase deposition and trapping of sediments and particulate organic matter (Fritz et al. 2004, Horvath 2004, Koetsier and McArthur 2000) and can both increase and decrease flow velocities (Clarke 2002, Cotton et al 2006) and dissolved carbon and oxygen concentrations (Sand-Jensen and Mebus 1996). They often increase stream bed stability, thus decreasing disturbance in response to high storm flows (Fritz and Feminella 2003). Plants also have been shown to decrease periphyton biomass (Fritz and Feminella 2006), and both positively (Toet et al. 2003, Eriksson 2001) and negatively (Schaller et al. 2004) affect bacterial denitrification in high-nutrient streams and wetlands.

The distribution of vascular plants in streams is often constrained by a combination of resource supply and flow regimes (Riis and Biggs, 2001, Westlake, 1973). Because plants grow and recover more slowly after disturbance than algae, streams which are frequently disturbed by storm flows may have few to no vascular plants. Conversely, vascular plants can reach high biomass in streams with high resource availability (primarily light and nutrients) and less frequent storm flows (Riis and Biggs 2003).

Streams in Mediterranean-type climates (i.e., coastal Chile, western Australia, western South Africa, coastal southern California, and the actual Mediterranean region), characterized by long, dry summers, with flood-free growth periods of six months or more, are capable of supporting extensive plant growth. 80-90% of annual precipitation usually falls during brief, moderate to severe winter storm events (deCastri and Mooney 1973). Additionally, resource availability can be high as a result of human activities such as the use of fertilizers and reductions in riparian habitat (Walsh et al. 2005, Carpenter et al. 1998). Increased nutrient loading can have strong effects because, being semi-arid, these systems have low dry season flows, which minimize the dilution of nutrient inputs (Sabater et al. 1998; Gasith and Resh 1999; Sabater et al. 2000).

Human populations in Mediterranean-climate regions are growing rapidly, particularly in southern California (Beighley et al. 2003), where a 37% increase by the year 2025 is predicted (SCAG 2004). The

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resulting increase in resource availability will have predictable effects on primary producer communities such as nuisance blooms of algae (Paul and Meyer 2001) and is likely to stimulate plant populations as well. However, the impacts of the interacting factors of climate and resource availability on vascular plant communities, and through them on the entire system, has rarely been addressed (but see Rejmankova 1992).

Vascular plants are present in many southern California streams and tend to be more densewhere resource availability has been increased by human impacts (Rejmankova 1992). (this might be an appropriate place for a sentence or two on the complications introduced by invasive species) *Ludwigia hexapetala* (Onagraceae; (Hook. & Arn.) Zardini, Gu & Raven) is an emergent aquatic plant native to South America (hereafter, *Ludwigia*). *Ludwigia* is presently rare in southern California (USDA 2006), but there is a dense population growing downstream of a wastewater treatment plant on the Ventura River (Ventura County, CA) which has persisted since the 1960s (Smith 1998). The wastewater treatment plant discharges effluent high in nitrogen into the river. Other populations of this plant species exist in nearby, anthropogenically-influenced streams (e.g., in Mission Creek, Santa Barbara, CA), suggesting that it has the potential to spread to further locations with high light and nutrient availability.

This study was conducted to determine the effects of *Ludwigia hexapetala* on the nutrient chemistry and community ecology of the Ventura River. Specifically, we asked the following questions: 1) What are the realized and potential growth rates of this plant, how does its biomass change over time, and is its growth nutrient-saturated in this location? 2) Does the presence of *Ludwigia* alter the biomass and/or community composition of algae? 3) How much of the longitudinal loss of dissolved inorganic nitrogen from the Ventura River below the wastewater treatment plant can be attributed to uptake and incorporation by *Ludwigia*? 4) Does the *Ludwigia* population have effects on seasonal and diel changes in nutrient levels in the Ventura River? We monitored nutrient concentrations and the growth and biomass of plants and algae over a growing season (April – October 2003), and conducted greenhouse experiments to observe the growth response of *Ludwigia* to the range of nutrient levels present in streams in southern California which could potentially be colonized by *Ludwigia*.

Methods

Site description and study species

The Ventura River, located in southern California (river mouth at 34°15'50 N, 119°18'29 W) approximately 100 km north and west of Los Angeles (Fig. 1) has a catchment area of 487 km², with headwaters in the Santa Ynez Mountains and the main stem of the river draining a coastal plain for about 24 km before emptying into the Pacific Ocean. About 20% of the watershed is forested, and 75% scrub/brushland; half of the catchment lies within the Los Padres National Forest. Land use in the remaining 5% is human dominated, devoted to either urban or agricultural (cattle, orchards, vineyards) uses. The majority of human land use occurs on the coastal plain and foothills in the lower portion of the watershed.

Average annual precipitation is 40 cm and falls almost exclusively between October and April. The interannual variation is high: 12 to 98 cm over the 132-year City of Buenaventura (Ventura) record. During the dry season, much of the main stem has no surface flow as a result of water diversion to Lake Casitas; however, a high water table in the region of Foster Park (nr. site U, Fig.1) usually furnishes surface water for the last 10 km of the river. Approximately 8 km from the ocean a sewage treatment plant, with a maximum capacity of 11,000 m³ day⁻¹, discharges effluent into the river,. Although this discharge represents only a small amount of the total river flow during wet years (discharge measured at USGS gauging station 11118500 at site U averaged 734,300 m³ day⁻¹ during the 2003 dry season) it can be the dominant source of summer flows during drought. For example, average 2002 dry-season discharge at the upstream gauging station was 1,950 m³ day⁻¹, less than 18% of the discharge capacity of the treatment plant.

Ludwigia is an emergent aquatic plant which can be found growing at high densities on the lower main stem of the Ventura River. *Ludwigia*, native to South America, has been introduced to North America and is considered a nuisance aquatic species in the southeastern and northwestern U.S (SEEPPC 1996, State of Washington 2003). *Ludwigia* plants grow clonally according to a form classified by Rejmankova (1992) as "creeping emergent." Clones are usually rooted in sediments at the edge of the river, whereas new shoots grow out from the edge towards the center of flow. Because of extensive air spaces in the stem tissues, even a dense and tall (up to 1m) network of *Ludwigia* can float on the surface of the water, with shoots emerging and growing upright, and roots dangling in the water column. Growth can be rapid, and within one growing season the plants may extend out to cover the entire surface of the river.

To monitor plant and algal biomass and dissolved nutrient chemistry, we established four sites on the main stem of the river. Three sites were located downstream of the wastewater treatment plant outflow,

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where nutrient concentrations are typically high and *Ludwigia* is present (sites D1, D2, and D3; 0.25, 3.2, and 4.4 km upstream of the tidal boundary, respectively). An additional site was located upstream of the plant, where *Ludwigia* was entirely absent (site U, 8.9 km upstream of the tidal boundary). Nutrient concentrations were lower at this upstream location than at sites below the wastewater treatment plant (Table 2), and other aquatic plants common to the area were present, although sparse (e.g., *Rorippa nasturtium-aquaticum, Potamogeton* sp., *Veronica* sp.). Plant and algal cover, algal biomass, nutrient concentrations, discharge and current velocity were measured bi-weekly at these four sites between April 10 and October 21, 2003.

Estimation of plant and algal biomass

A 50 m study reach was defined at each site and, on each sampling date, five randomly selected crossstream transects were monitored within each reach. The percent cover of plants and algae was visually assessed at five evenly-spaced points along each transect using a modification of the EPA Rapid Bioassessment Protocol (Barbour et al. 1999), which entails the use of a viewing bucket with a grid (28 cm x 28 cm) of 50 dots on the clear, bottom surface. The grid was used to measure plant cover above the water's surface, or algal cover below. Plants were identified to species where possible, macroalgae identified to genus, and diatom films were measured and classified by thickness, as thin (0 - 1 mm), medium (1 - 5 mm), or thick (> 5 mm).

At each sampling point, algal biomass was sampled from hard substrata with a 26 mm diameter periphyton sampler, consisting of an open ended syringe cylinder with removable scouring pads on the bottom of the central plunger (described by Davies and Gee 1993). After algae had been scrubbed from the substrata with the scouring pads, the pads were removed and stored at 10°C for subsequent laboratory extraction and analysis. If floating macroalgae were present the outer cylinder of the sampler was used to bore a 2.6-cm diameter hole through the floating mat and these algae also were retained for analysis. All five samples from each transect were combined into a single composite sample.

The standing stock of algae growing on the roots of *Ludwigia* was measured on October 28, 2003. *Ludwigia* plants across a range of heights were selected, and three individual shoots of *Ludwigia* were selected within each 10 cm height range (i.e., three within 0 - 10 cm height, three within 10 - 20 cm, etc.), to represent the entire above-water height range (2 - 70 cm). (I don't fully understand this. How were plants selected? Random? Whatever? And what's the difference between plants and shoots.) All *Ludwigia* and attached diatom biomass was removed from a 20 x 20 cm sample quadrat centered on the selected shoot. These samples were taken back to the lab and all diatoms gently flushed from the surface of the roots with deionized water, captured on filters and frozen until analysis.

In the laboratory, algae samples were rinsed from the scouring pads with deionized water. Floating macroalgae and benthic algae samples were homogenized in 850 ml of deionized water using a commercial blender (Waring model 31BL91). These composite samples were stirred thoroughly, and sub-samples (usually 100 ml) filtered through Whatman GF/C glass fiber filters. Chlorophyll a was extracted from the filters with 90% acetone and measured according to the EPA rapid bioassessment protocol for streams and wadeable rivers (with phaeophytin correction; USEPA 1992) using a Turner 10-AU Field Flourometer.

In addition to the random transects, five fixed cross-stream transects were established across the wetted width of the river at each sampling site in April. The percent cover of plants and algae was measured along these transects in April, July, and October using a point-contact method, where species present under points every 50 cm along the transect were recorded. Where *Ludwigia* was present, plant height above the water's surface was recorded and biomass estimated (using a height-biomass regression equation derived using the *Ludwigia* samples collected on 28 October 2003). All plant samples were separated into shoots, roots, and leaves, and weighed after drying to constant weight at 60° C.

Samples of *Ludwigia* leaf, root, and shoot tissues were taken in April and July, 2003 to measure carbon and nitrogen content. Samples were taken at each site where *Ludwigia* was present, dried at 60° C, and homogenized in a tissue grinder. The resulting powder was assayed for percent weight of carbon and nitrogen with an accuracy of $\pm 0.3\%$ on either a Control Equipment Corporation 440 Elemental Analyzer or a Leeman Labs CE440 Elemental Analyzer. Nitrogen uptake and incorporation by *Ludwigia* could then be estimated by multiplying the amount of *Ludwigia* biomass produced over the growing season by the nitrogen content of that biomass.

Physical and chemical conditions

Conductivity and dissolved oxygen were measured in the field using a YSI Model 85 dissolved oxygen/conductivity meter. At least two, and up to four, water samples were collected at each site on each sampling date to measure nutrient concentrations. All samples were filtered immediately through glass fiber filters (Gelman A/E), stored on ice during transport and then at 4° C until analysis. Samples for total dissolved N (TDN) and total dissolved P (TDP) were oxidized with persulfate (Valderrama

1981), then measured for soluble reactive phosphorus (SRP) and nitrate ($NO_3 + NO_2$). Ammonium (NH_4), NO_3 , SRP, TDN and TDP were measured spectrophotometrically on a Lachat QuikChem 8000 Flow Injection Analyzer using standard methods (SRP, TDP: QuickChem Method 31-115-01-3-A, reaction with ammonium molybdate and ascorbic acid; NO_3 , TDN: QuickChem Method 31-107-04-1-A, reduction of nitrate to nitrite, nitrite was diazotized with sulfanilamide and coupled with ethylenediamine dihydrochloride; NH_4 : QuickChem Method 31-107-06-5-A, phenolate method). (The methods list appears overly long to me. I'd probably suggest "QuickChem methods 31-115-01-3-A, 31-107-04-1-A and 31-107-06-5-A" Shouldn't there also be a reference as to where to find these methods described?)

Flow on each monitoring date was calculated as the product of cross-stream area and average current speed ,(determined by multiplying average surface float velocity across the width of the stream by 0.8 to correct for bed roughness; Gore 1996). Five separate area-velocity measurements were averaged to estimate discharge at each location. Previous measurements comparing this method to current velocities measured using a Marsh-McBirney model 201D magnetic flowmeter had shown the results of the two methods to be statistically indistinguishable (data not shown). Discharge rates multiplied by nutrient concentrations at each site were used to calculate the total mass of nutrients lost between sites D3 and D1.

Diel patterns

To determine the influence of *Ludwigia* on diel patterns of river chemistry, we monitored pH, dissolved oxygen, conductivity, and nutrient concentrations at all sites over a 24-hour period in September 2003. Sites were visited every three hours from 08:00 hs on September 10 to 08:00 hs on September 11. Dissolved oxygen, conductivity (YSI 85) and pH (ThermoOrion 250A+) were measured and nutrient samples collected. Inorganic N and P were analyzed immediately upon return to the laboratory (storage times varied between 3 and 30 hours); unfiltered samples for total N and P were oxidized with persulfate at this time and analyzed within three days.

Greenhouse experiments

Two greenhouse experiments were conducted in September and October of 2003 to compare the growth and nitrogen storage capacity of *Ludwigia* under a range of nutrient conditions. In each experiment, five recirculating flow-through tubs (11.3L Rubbermaid storage boxes) were set up in a fenced outdoor area that received full sunlight. Each tub was connected to a 60-L reservoir with a water pump (Beckett M350AUL16 Fountain Pump; Beckett Corporation, Irving, Texas) which pumped the water up to one end

of the tub via Tygon tubing; water flowed through the tub, out the other end through a bulkhead fitting, and back to the reservoir at a rate of 4.7 liters per minute. Each reservoir was filled with deionized water and each treatment was amended with a different amount of a modified Hoagland's solution, a complete nutrient solution with an N:P ratio of 12:1 (Hoagland and Arnon 1950). Concentrations were chosen to represent the range of N concentrations observed in anthropogenically-influenced streams and rivers along the south coast of California (Table 1); concentrations in the September experiment were roughly an order of magnitude higher than those in the October experiment.

Plant cuttings were taken from the Ventura River and standardized such that each shoot had either 3 (September experiment) or 5 (October experiment) rooting nodes and 12 mature leaves (defined as > 6 cm in length). Length and wet weight of each cutting was recorded at the beginning and end of each experiment, and at the end of the experiment plants were separated into roots, shoots, and leaves, and weighted after drying to constant weight at 60° C. In September, twelve standardized shoot cuttings were placed in each treatment tub. In October, six shoots were placed in each tub. The September experiment ran for 16 days (9 - 25 Sept.), and the October experiment for 17 days (26 Oct. – 12 Nov.). The 171 μ M N, 15 μ M P treatment was lost in the October experiment due to equipment failure. Three leaves that had formed during the course of the experiment were taken from each shoot and analyzed for carbon and nitrogen content as detailed above.

Statistical analysis

Data from both experiments were combined to determine the response of *Ludwigia* across the entire range of nutrient concentrations tested. A Michaelis-Menten curve was fitted to the growth data using the model $V = (V_{max}N)/(k_m + N)$ where V is the growth rate measured as the percent increase in plant length over the course of the experiment, V_{max} is the maximum potential growth rate, N is the concentration of nitrogen, and k_m is the concentration of nitrogen that supports half the maximum growth rate. V_{max} and k_m were calculated using a Lineweaver-Burk transformation (Lineweaver and Burk 1934).

Changes in river chemistry were analyzed using linear regression. Differences in the percent cover of algae in the presence vs. absence of *Ludwigia* were compared with paired t-tests. All data were transformed to meet parametric assumptions, including ln transformations (or ln (n+0.1) where zero values were present) for chlorophyll a and nutrient concentrations and arcsine square root transformations for proportion plant or algal cover. Residuals and skewness of transformed data were examined for

conformance to parametric assumptions (e.g., normality, homogeneity of variances). Statistical analyses were performed using S-Plus (S-Plus Professional Release 3, MathSoft Inc.).

Results

Plant and algal cover

From April to October, *Ludwigia* grew until it covered most of the river surfacet the three lower locations. There was a bloom of filamentous green algae, primarily *Cladophora*, in late spring/early summer; by June 1, *Cladophora* covered between 73% and 97% of the wetted width of the stream at all sites (Figs. 2 and 3). Coverage by *Cladophora* declined in midsummer, and was replaced by diatom films at sites D1 and D3, by a combination of diatom films and crustose green microalgae at D2, and by diatoms and other filamentous green algae (principally *Enteromorpha, Rhizoclonium*, and *Spirogyra* spp.) at site U. Diatom films were generally thicker at sites where nitrate concentrations were higher. A second *Cladophora* bloom occurred in the fall at site U, similar to the spring bloom in percent cover (paired t-test, p = 0.38). *Cladophora* also appeared in the fall at the downstream sites, but was restricted to areas of open water not covered by *Ludwigia*. As a consequence, percent cover of *Cladophora* during the fall bloom was significantly lower than in spring at all sites where *Ludwigia* was present (paired t-tests, p < 0.01 for each site; Fig. 3).

Peak standing stock of *Ludwigia* at the end of the growing season at the three downstream sites decreased with decreasing mean NO₃ and SRP concentrations (Table 2). When normalized to final standing stock per square meter of wetted width, *Ludwigia* biomass was highest at site D3 (1.03 kg m⁻²) and lowest at site D1 (0.75 kg m⁻²). Mean final standing stock of *Ludwigia*. averaged across all measurements at all downstream sites, was $0.98 \pm .03$ kg m⁻² dry weight (± one standard error).

Benthic chlorophyll was highest and most variable at site U (dry season average $239 \pm 66 \text{ mg m}^{-2}$; Fig. 4), and typically decreased downstream, with dry season averages of 216 (± 39), 112 (± 29), and 94 (± 27) mg m⁻² at sites D3, D2, and D1, respectively (Table 2, Fig. 4).

Algal biomass on the roots of *Ludwigia* plants in October was highest near the center of river flow where plants were < 20 cm in height (223 ± 57 mg chla m⁻², mean \pm s.e.), but was still substantial on plants taller than 20 cm nearer the shore (46 ± 8 mg chla m⁻²). Chlorophyll concentrations for algae growing on *Ludwigia* roots per unit bottom area were comparable to benthic chlorophyll concentrations for the same

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time period (mean 76 \pm 27 mg chla m⁻²). (I may be wrong here. I think your original description was confusing since a cross stream variation – edges/tall plants vs. nr. center of flow/short plants – is not the same as a longitudinal variation – leading upstream edge, etc.)

The nitrogen content of *Ludwigia* leaves was higher at sites with higher dissolved N in the water column,, and was higher in spring than in summer (summer: 4.3% at D3 and 3.9% at D1, one-tailed t-test, p = 0.09; spring: 5.5% at D2 and 4.4% at D1, p < 0.01).

Physical and chemical measurements

For most of the dry season, nutrient concentrations were lowest at U and highest at D3 (immediately downstream of the treatment plant outflow), decreasing downstream from D3 (Table 2, Fig. 5). From 1 June (after the influence of a storm which occurred on 3 May, see Fig. 5) until the end of the dry season, TDN decreased at site U and increased at all three downstream sites; the slope of the increase was greatest at site D3 and least at site D1 (Site U: $\ln(TDN) = -0.01(date) + 4.4$, $R^2 = 0.88$, p < 0.01; Site D3: $\ln(\text{TDN}) = 0.0052(\text{date}) + 4.6, \text{ R}^2 = 0.66, \text{ p} < 0.01; \text{ Site D2: } \ln(\text{TDN}) = 0.0050(\text{date}) + 4.3, \text{ R}^2 = 0.59, \text{ p}$ < 0.01; Site D1: ln(TDN) = 0.0025(date) + 4.3, R² = 0.23, p = 0.10). TDP also increased over time at all three downstream sites, but the slope of this increase did not decrease with distance from the WWTP (Site D1: $\ln(TDP) = 0.0226(date) + 0.05$, $R^2 = 0.47$, p < 0.01; Site D2: $\ln(TDP) = 0.0276(date) - 0.24$, $R^2 = 0.0276(date) - 0.0276(date) - 0.024$, $R^2 = 0.0276(date) - 0.024$, $R^2 =$ 0.40, p = 0.02; Site D3: $\ln(TDP) = 0.0251(date) + 0.12$, $R^2 = 0.33$, p = 0.04). There were no consistent patterns in TDP concentrations over time at Site U (ln(TDP) = -0.0026(date) - 0.30, R² = 0.06, p = 0.53). (I'll probably need to come back to this, but my first response is why is the slope important and why do we need all these equations? Why not just say," the increase was greatest at site D3, least at D1. TDP below the plant also increased over time but the pattern was less consistant." I think the fig. makes things clear without all the regression stuff. I went back and looked over the data and the absence of a similar relationship for TDP comes down to a single data point in early Sept. The error bar on that point is appreciable, and my two samples also show widely divergent results. By the way, where do the error bars come from? Are these samples you took and analyzed?)

Total dissolved N and P were closely correlated with dissolved inorganic N and P (r^2 for both TDN vs. DIN and for TDP vs. DIP ca. 0.95). NO₃ consistently comprised 80% of total dissolved N at all sites, whereas NH₄ concentrations were negligible (never greater than 1% of TDN; see Table 2).

From the discharge and flux calculations made at sites D3 and D1 on each sampling date, the average amount of inorganic nitrogen removed from the water between these two sites was estimated to be 5 kg N km⁻¹ day⁻¹ over the course of the growing season. Using biomass estimates and tissue N measurements, the standing stock of N in *Ludwigia* biomass in October, accrued over the entire growing season, was estimated as 322 kg N per kilometer of river, which would require an approximate uptake rate of 1.5 kg N km⁻¹ day⁻¹. Thus up to 30% of the nitrogen removed from the water column during the dry season could be attributed to direct uptake and incorporation by *Ludwigia*.

Diel patterns

Both pH and dissolved oxygen increased during daylight hours and decreased at night. These patterns were more pronounced at the U site than at the downstream, *Ludwigia*-dominated locations (Figs. 6a and b). At sites D3, D2, and D1, dissolved oxygen ranged from a maximum of 9.8 mg L⁻¹ during the day, to 5.6 during the pre-dawn hours, whereas at U it increased to 14.7 mg L⁻¹ during the day but decreased to similar levels as the other sites at night. At the downstream sites pH ranged between 8.4 and 7.9, but decreased to a night-time low of 7.7 at site U. Conductivity remained relatively constant within each site over the 24-hour period (average of 950 ± 4.3 , 1295 ± 3.9 , 1400 ± 3.5 , and $1417 \pm 4.3 \,\mu$ S cm⁻¹ at sites U, D3, D2, and D1, respectively; mean \pm one standard error).

Nitrate concentrations at sites D3 and D2 decreased during daylight hours, reaching lowest levels soon after dark, then increased over the rest of the night. Nitrate concentrations ranged from 102.2 to 129.4 μ M at D3 and 84.0 to 98.8 μ M at D2 (Fig. 7). Sites D1 and U displayed a different pattern where nitrate was slightly higher at night than during the day, and absolute differences between day and night nitrate concentrations were much smaller (ranging from 53.4 to 63.2 μ M at D1 and 8.7 to 14.2 μ M at U).

Greenhouse experiments

Data from both greenhouse experiments were analyzed together to determine the growth response of *Ludwigia* to the full range of nutrient concentrations tested. Change in stem length was used as a proxy for growth rate, as plant biomass was confounded by algal growth among the *Ludwigia* roots; these algae were tightly attached and could not be removed without damaging or removing root tissue. Root length was negligible in comparison to stem length (typical roots were less than 3 cm in length), and was not included in the growth rate calculation. Growth rates ranged from 0.4 cm day⁻¹ in the lowest nutrient treatment to 4.4 cm day⁻¹ in the highest. Percent increase in cutting length as a function of N

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concentration displayed a Michaelis-Menten pattern, with $V_{max} = a 147\%$ increase in length per 16 days, and $k_m = 376 \mu M$ nitrogen (Fig. 8).

Within each experiment, the plants stored greater concentrations of leaf tissue N in high nutrient treatments than in low nutrient treatments. Molar C:N ratios decreased from low to high nutrient treatments, from 16.4 in the low nutrient treatment to 9.3 and 9.6 in the two highest nutrient treatments in the September experiment, and from 13.1 in the lowest nutrient treatment to 11.3 at the highest nutrient level in October. Differences in C:N ratios among treatments were largely due to changes in N rather than C; % of dry weight of carbon remained relatively constant across treatments (mean \pm standard error = 47.1 \pm 0.2). Leaf nitrogen content also increased linearly with increasing concentrations of dissolved N in each greenhouse experiment ($r^2 = 0.90$, p = 0.01 for the September experiment, $r^2 = 0.98$, p = 0.01 for the October experiment). When both experiments were analyzed together, however, increases in leaf tissue nitrogen content were not significantly related to initial dissolved N concentrations.

Discussion

The results of both field monitoring and the outdoor experiments demonstrate that *Ludwigia hexapetala* has the capacity to grow rapidly under high nutrient conditions. By the end of the 2003 growing season, *Ludwigia* covered almost the entire wetted surface of the Ventura River at sites where it had been initially present. In October, the standing stock of *Ludwigia* on the Ventura River was 0.98 ± 0.03 kg m⁻² dry weight.

Ludwigia's erect growth form is likely one of the factors enabling it to reach high biomass levels. The standing stock measured in this study represents almost twice the amount recorded for a congener (*L. peploides*) growing in high-nutrient agricultural drainage ditches in central California (Rejmankova 1992). *L. peploides* is emergent, but follows more of a creeping than an upright mode of growth; when grown with stem supports which enabled it to grow erect, biomass of *L. peploides* increased four-fold (Rejmankova 1992). Other erect emergent stream plants have been found to reach levels of standing stock comparable to those recorded here (Howard-Williams et al. 1982).

Ludwigia's ability to produce high amounts of biomass is comparable to that of highly productive floating mats of vegetation found in tropical rivers and floodplain ponds: variously measured at 0.7 kg m⁻² (Junk 1970), 0.5 - 3 kg m⁻² (Carignan and Neiff 1992) and 0.6 - 2.3 kg m⁻² (Junk and Piedade 1993). However,

the standing stock of emergent plants in tropical ecosystems has sometimes reached levels which dwarf those from temperate regions (e.g., 8.0 kg m^{-2} , Piedade et al. 1991).

The biomass of plants, when present, is generally much higher than the biomass of primary producers in streams without vascular plants, where external inputs of energy (e.g., leaf litter from riparian plants) tend to exceed in-stream photosynthetic production (Mulholland et al. 2001). Stream plants typically reach higher levels of biomass than submerged vegetation in many lakes, where peak biomass can range from $0.063 - 1 \text{ kg m}^{-2}$, but is more typically $0.4 - 0.5 \text{ g m}^{-2}$ (Stevenson 1988), perhaps because plant production in lakes is often limited by carbon availability and/or reduced light levels caused by attenuation through the water column or by epiphytic fouling (Stevenson 1988). *Ludwigia*'s emergent growth form enables it to avoid both light and carbon limitation experienced by submerged lacustrian plants. Additionally, the presence of a current can increase nutrient delivery and, therefore, accumulation of biomass (Conover 1968).

Effects of Ludwigia on algal communities

The presence of *Ludwigia* decreased the magnitude of *Cladophora* blooms at sites where it was present, probably partly through the interception of light. On the Ventura River, *Cladophora* bloomed at all study sites in late May and early June, before *Ludwigia* had grown to cover substantial areas of the open channel (see Fig. 3). A similar bloom of *Cladophora* occurred in the fall at site U, but was reduced at the other three sites where *Ludwigia* was present; at these locations the bloom was restricted to open water areas where *Ludwigia* was absent. Emergent plants have been found to reduce benthic light levels by 50 - 98% (Fritz and Feminella 2006, Wilcock et al. 2002) and to decrease the biomass of primary producers growing under their canopies (Fritz and Feminella 2003, Wilcock et al. 2002). Although light was not measured in this study, *Ludwigia* did form dense canopies when present, which certainly intercepted incident light and reduced light levels at the stream bottom. Green macroalgae, and particularly *Cladophora* spp., are known to depend on high light conditions (Dodds 1991, Dodds and Gudder 1992).

In contrast to its inhibitory effect on green macroalgae, the presence of *Ludwigia* likely increased diatom biomass. Diatoms are generally more shade-tolerant than green algae, can maintain high rates of growth under a canopy (Sundback et al. 1996) and can switch to heterotrophic metabolism when light becomes limiting (Tuchman et al. 2006). Diatoms growing on *Ludwigia* roots increased the standing stock of algal biomass by almost 40% over benthic diatom biomass in areas without *Ludwigia*. Underwater roots of floating aquatic plants have been shown to be important habitat for periphyton in the floating meadows of

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the sediment-rich Amazon (Engle and Melack 1989, 1993). In streams where nutrient concentrations are high and flood disturbance infrequent, algal biomass may reach high enough levels that further growth is limited by the availability of physical substrata for attachment (Busse et al. 2006). Gregg and Rose (1982) found that the presence of vascular plant roots increased the biomass of diatoms when other appropriate growth substrata were limited. During some parts of the year, diatom mats were thick in the Ventura River (greater than 2 cm in length; see Fig. 3), and likely limited by the scarcity of attachment substrata. During these periods, *Ludwigia* roots probably represented valuable space for shade-tolerant diatoms, and promoted higher levels of diatom biomass than if absent.

Physical and chemical conditions

Upstream of the treatment plant, TDN concentrations and discharge decreased over the summer, suggesting that increases in nutrients at the three downstream sites was a result of an increasing proportion of the flow being composed of treatment plant effluent. (To show increased N inputs from the WWTP you would have to show an increase in the flux at D3, not simply an increase in concentration. I'm ashamed to say I never did look at what happened to the flux at any single point – which might have shown either how consistant was treatment plant output, or how good were the flow calculations – but I have now. In 2003 the flux was relatively consistant, except for noticeably higher values from May through the first week in June – presumably from recessional winter flows and the rain storm you mention. If anything, the values decrease as the summer goes on – perhaps due to increased uptake between the outfall and D3. So your observation about increased WWTP inputs is not correct . It's reasonable to assume that plant output is more or less consistant on a monthly, or even daily, basis – but might vary considerably throughout the day.)

A substantial amount of the nitrogen draw down (up to 30%) between sites D3 and D1 could be accounted for by direct uptake and incorporation by *Ludwigia* plants. Howard-Williams et al. (1982) reported similar rates of N uptake in watercress, and calculated that plant uptake actually exceeded dissolved N loss rates from the Whangamata Stream in New Zealand. The Whangamata Stream however is much smaller than the Ventura River (base flow rate of 0.1 m³ s⁻¹ vs. 8.5 m³ s⁻¹ in the Ventura during the summer of 2003), which probably allowed for greater contact between plants and the flowing water, enabling greater rates of biological nitrogen uptake. In drier years (e.g., 2002: average discharge rate of 0.02 m³ s⁻¹), N uptake by *Ludwigia* in the Ventura River may account for a much greater proportion of dissolved N loss.

Ludwigia may also indirectly increase N withdrawal from the water column by enhancing bacterial denitrification. Aquatic vascular plants can enhance denitrification rates by creating areas of low flow and sedimentation around their roots where dissolved oxygen is reduced, and by providing a source of organic carbon (Eriksson 2001, Toet et al. 2003). Preliminary measurements of denitrification potential in sediments taken from the Ventura River suggest high rates of denitrification (unpublished data), and high rates have been observed in similar systems. For instance, Carignan and Neiff (1992) measured denitrification in floating meadows of *Eichhornia crassipes* which are physiognomically comparable to floating *Ludwigia* mats (i.e., emergent plants with roots dangling in the water column) and found that up to 30% of available nitrate was denitrified within 16 hours when nitrate levels were raised to 98 µM, a concentration in the range of N levels measured in this study.

(I assume you are talking about Craig's measurements of denitrification in the above paragraph. How about including some of those measurements here? I don't think Craig will ever publish them or even use 'em in his disertation, and this might be a good opportunity to get 'em in on record. We could simply thank him in the Acknowledgements section. You might ask him if you think it worthwhile.)

Diel patterns

Ludwigia can influence stream chemistry on daily as well as seasonal time scales. Algal photosynthesis and respiration produce predictable patterns of high dissolved oxygen levels during daylight hours and lower levels at night. Diel patterns in pH are also typically observed, where photosynthesis draws down dissolved carbon dioxide concentrations, driving pH up during daytime hours, and nighttime respiration increases dissolved carbon dioxide, driving pH down. These patterns were observed at all sites on the Ventura River. This diel pattern was strongest at site U, where the daytime peak in O_2 and nighttime reduction in pH were greatest, likely owing to the high biomass of filamentous green algae present at site U. There were little to no filamentous green algae present at any of the three downstream sites after the spring *Cladophora* bloom, where most of the stream was covered by *Ludwigia*. *Ludwigia* releases oxygen into the atmosphere, rather than into the stream water, and its roots also likely draw oxygen from the water. It appears that *Ludwigia* can dampen diel fluctuations in pH and dissolved O_2 by restricting the growth of filamentous algae, as has been seen with other floating macrophytes (Wilcock and Nagels 2001). Working in the Pantanal of Brazil, Hamilton et al. (1995) also found minimal daily fluctuations and low overall daytime O_2 concentrations in aquatic habitats with dense cover of macrophytes, and they concluded that even though the plant roots offered substrata for algal growth, shading by the emergent portions of the plants inhibited photosynthesis below the water's surface. The similarity of these results suggests that the influence of dense canopies of emergent vascular plants on metabolic processes in the

water column may be widespread, and highlights the importance of differentiating growth form when assessing the role of vascular plants in aquatic habitats.

Nitrate concentrations decreased during daylight hours, reaching lowest levels after dark at Sites D3 and D2, and increased at night (Fig. 8). The most pronounced diel changes in nitrate occurred at sites D2 and D3 where *Ludwigia* was most abundant, and diel nitrate patterns suggested that *Ludwigia* primarily takes up nitrate during the day. Other aquatic plants have shown similar patterns. For example, Vincent and Downes (1980) found diel changes in nitrate uptake in watercress (*Nasturtium officinale*) growing on the Whangamata stream in New Zealand, with both uptake rates and cellular concentrations of nitrate reductase being higher during mid-day than at night. Diel patterns of nitrate uptake may be widespread in vascular plants, because the activity of nitrate reductase is regulated by light availability, being rapidly deactivated in darkness and reactivated during the day (Kaiser and Huber 1994). Diel changes in nitrate concentrations at site D1 were likely less influenced by *Ludwigia*, because it had a much lower biomass at this site than Sites D2 and D3.

Temperature variations may also have contributed to diel changes in N concentration. Plant, algal, and bacterial metabolism are all temperature-dependent, and plant uptake and bacterial denitrification were likely slowed during the cooler, nighttime hours (~18°C vs. ~24°C during daylight).

(All this begs a question we've talked about earlier – what was the TDN concentration coming from the WWTP and did it remain constant throughout the 24 hours. Another underlying question is whether or not the flow also remained constant, i.e., what happened to the flux and flux uptake as flow passed each location? We don't know. If we assume that concentration – and flow – remained constant, we could draw some conclusions. If not, then the picture is too clouded for any straightforward statement. I've thought of ways around this, not to include in the paper but to insure ourselves that these kinds of conclusions are valid. One way is to look at how concentrations at D2 varied with respect to D3, and at D1 with respect to D2 – this would remove the effect of any fluctuating WWTP TDN output, however, it still assumes that flow remained constant. I've attached a graph showing the results (including how TDN at U varied with respect to the daily average). At D1 concentrations increased during the night – perhaps substantiating your conclusion that uptake occurred mostly during the day. However, concentrations showed a relative decrease at D2 – so did uptake occur during the nighttime there, and if so, how? To repeat, we don't know anything about flow. At U flow varied by about 14 % during the 24 hours – increasing during the night. Since it also had to increase at the D locations – upper river flows were still dominant over WWTP outflow – this would have decreased concentrations if WWTP outflow

concentrations remained constant, i.e., greater dilution, at the D sites. However, WWTP outflow could presumably have decreased – decreased inflow during the night would have led to decreased outflow (incoming sewage pushes out treated effluent at the other end, however, we don't know how the residence time changes). I believe these kinds of variations – in flow and plant output -- are responsible for the lack of an abrupt day-night nutrient transition like the one we saw at U – variations in flow and TDN concentrations would have been minimized at U giving us the cleanest example of an abrupt increase in TDN once it got dark. So given this, and the totally opposite variation we see at D1 vs. D2, I don't think we can draw any conclusions in this paper - or ever. (You also can't say that the most pronounced nitrate changes occurred at D1 and D2 – sure they did in terms of absolute change, but not in % change; the greatest change actually occurred at U.) Actually, D1 looks a lot like U. I could attribute that to the dominance of algae and say that the difference in that response to what happened at D2 and D3 is the Ludwiga effect, but then it would sure look like most Ludwiga uptake occurred at night – a quite different conclusion than what you state. I think we need to throw the whole section out. There are also problems with the DO conclusions also since they don't consider the relative importance of flow – lower flows at U magnify the diel differences – so, althought I hate to suggest it I think we need to remove all mention of the diel work also. I've been thinking about this a lot lately: how can we use the diel DO variation to estimate algal productivity? Obviously, flow plays a big role since as flow increases the same amount of algal productivity will reduce the magnitude of the diel DO cycle. Then there is the question of why wasn't the pH variation at the D sites as nice and clean as that for DO? Eliminating all the diel stuff will give us more leeway with the length of the rest of the paper. Hey, it helped with the dissertation but it's probably not useable anywhere else – next time we'll know how to do it better – measuring stage and getting data on WWTP output.)

Greenhouse experiments

Results from the greenhouse experiment suggest that the growth rate of *Ludwigia* on the Ventura River was not saturated by ambient nutrient concentrations. *Ludwigia*'s growth rate increased linearly with increasing nutrient supply, then became saturated following Michaelis-Menten kinetics (Fig. 9). The calculated half-saturation concentration of 376 μ M nitrogen is considerably higher than nitrogen concentrations measured in the Ventura River in 2003. This suggests that if *Ludwigia* were to become established in streams or rivers in coastal southern California which have higher nutrient concentrations, such as the Santa Clara River in Ventura County (mean NO₃ concentrations over 400 μ M, with peaks >1600 μ M; California Regional Water Quality Control Board, 2003), or San Diego Creek in Orange County (mean dry season NO₃ concentrations of 1500 μ M, with peak concentrations over 4000 μ M (County of Orange, 2003), its growth could be even more rapid, with profound impacts on physical, chemical, and biological conditions in those systems.

Temporal cycles of change

Ludwigia biomass appears to exhibit multi-year cycles which are driven by climatic variables, particularly variability in magnitude of winter flooding (pers. obs.). Annual rainfall amounts in southern California are highly variable (coefficient of variation = 0.45 for the last 132 years; Ventura County Watershed Protection District, Station 66), especially in contrast with regions with more equitable, year-round precipitation. Surveying 78 streams across North America with multi-decade stream gauge records, Poff and Ward (1989) found higher variability in flooding in southern California streams than in streams in more temperate climates. They reported an annual coefficient of variation of discharge in southern California of 0.86, vs. a mean of 0.42 for all streams surveyed. The typical pattern is of several near- or below-average rainfall years, punctuated by one or more years of heavy rainfall where total precipitation exceeds 150% of the mean (13% of the years in the Ventura record exceed this amount) (Fig. 9). Below-average rainfall years often have little or no major flooding, whereas floods in above-average years can cause substantial movement of bedload and scouring of stream channels (Fisher et al. 1998).

Emergent macrophytes increase the stability of stream sediments such that greater scouring forces are required to dislodge benthic substrata (Fritz et al. 2004). In the absence of scouring floods, riparian and in-stream plant communities become more firmly established, requiring increasingly greater floods to remove them and scour stream bottoms (Fisher et al. 1998). This study was conducted in 2003, after five average to below-average rainfall years (including 2002, one of the driest years on record), and *Ludwigia* was the overwhelmingly dominant primary producer at all sites where it was present, covering virtually every meter of riverbank downstream of the wastewater treatment plant. 2005 was a year of high rainfall and flooding, with peak winter storm flows an order of magnitude higher than in 2003 (2005 = 1,161 m³ s⁻¹ vs. 2003 = 144 m³ s⁻¹; USGS gauging station #11118500 at site U), and more than two orders of magnitude higher than peak flows in the winter of 2002 (5.4 m³ s⁻¹). During 2005 almost all overwintering *Ludwigia* plants were scoured from the stream bed and banks, and in the following summer *Ludwigia* populations were reduced to small, scattered patches of 1 m diameter or less (pers. obs.). Observations in early 2006 show that *Ludwigia* is still a fringe member of the primary producer community, occurring only in small, isolated patches (Fig. 10).

Streams and small rivers are known to be highly variable systems (Cooper et al. 1997), and some human activities reduce this variability, such as when a sinuous stream with complex natural bed structure is channelized for flood control purposes. The Ventura River, however, demonstrates that human impacts can also increase variability. On shorter time scales (months to years), the presence of the wastewater treatment plant and the exotic invasive *Ludwigia* reduce variability, with *Ludwigia* growing as a relatively homogenous monoculture and altering patterns of algal growth and succession; while, on longer time scales (years to decades), the presence of *Ludwigia* increases variability as it responds to high interannual variations in rainfall and flooding with changes in the physiognomy of the primary producer community and in patterns of nutrient cycling and retention.

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Table 1. Initial nutrient concentrations for each of five nutrient levels used in two greenhouse experiments examining the growth of *Ludwigia hexapetala* across a gradient of nutrient concentrations.

	September		
	NO ₃	PO_4	
	(µM)	(µM)	
1	9328	789	
2	4500	422	
3	2286	241	
4	1096	132	
5	511	85	
	October		
	October		
	October NO ₃	PO ₄	
	October NO ₃ (µM)	ΡO ₄ (μΜ)	
1	October NO ₃ (µM) 702	PO ₄ (μM) 59	
1 2	October NO ₃ (μM) 702 350	PO ₄ (μM) 59 30	
1 2 3	October NO ₃ (μM) 702 350 171	PO ₄ (μM) 59 30 15	
1 2 3 4	October NO ₃ (μM) 702 350 171 85	PO ₄ (μM) 59 30 15 7	

Table 2. a) Mean values (± 1 s.e. of the mean) for selected physical and chemical parameters, averaged across the 2003 dry season (April – October), for study sites on the Ventura River. b) Mean values (mean of five transects at each site ± 1 s.e. of the mean) for selected physical and biological parameters for study sites on the Ventura River. Chlorophyll a values were averaged across the 2003 dry season (April – October); *Ludwigia* biomass represents standing stock on October 23, 2003.

(a)							Conductivity
		$NH_4 \left(\mu M \right)$	$NO_3 (\mu M)$	$SRP\left(\mu M\right)$	TDN (µM)	$TDP\left(\mu M\right)$	$(\mu S \text{ cm}^{-1})$
	U	0.3 (0.1)	31.5 (3.7)	1.3 (0.0)	39.0 (3.5)	0.9 (0.1)	941.9 (5.9)
	D3	1.2 (0.1)	115.0 (5.2)	10.5 (0.7)	137.1 (6.6)	10.2 (0.7)	1222.0 (48.9)
	D2	0.6 (0.1)	88.1 (4.2)	9.1 (0.7)	108.3 (5.0)	8.7 (0.7)	1304.7 (61.0)
	D1	0.4 (0.1)	61.9 (2.4)	7.2 (0.6)	83.2 (3.3)	7.1 (0.6)	1333.6 (57.1)

(b)

	wetted width April (m)	wetted width Oct. (m)	benthic chl a (mg m ⁻²)	<i>Ludwigia</i> biomass (kg m ⁻¹ river)
U	19.5 (0.7)	15.4 (1.4)	239.1 (66.3)	0
D3	16.7 (1.6)	10.8 (2.4)	216.1 (38.9)	12.6 (3.1)
D2	10.9 (1.4)	8.0 (1.4)	112.3 (28.6)	11.1 (2.3)
D1	6.9 (0.4)	4.0 (0.6)	94.4 (26.9)	3.2 (0.7)

Fig. 1: Map of the Ventura River drainage basin, showing cities (stars), tributaries and main stem, and sampling sites. Hatched oval between sites U and D3 indicates location of wastewater treatment plant. (You might reduce the size of the California portion of the site map, making the whole figure smaller.)

Fig. 2: Percent cover of the Ventura River by plant and algal taxa during the 2003 dry season: (a) Site U;(b) site D3; (c) site D2; (d) site D1. Thick, medium and thin indicate the presence and thickness of diatom films (see text), filament refers to filamentous green algae, and plants refers to vascular plants. % cover values for each sampling date are the mean values across 5 samples for each of 5 transects at each sample site.

Fig. 3: Percent cover by *Cladophora* sp. at each site during the peaks of the spring and fall *Cladophora* blooms. Asterisks indicate significant differences between the magnitude of spring and fall blooms (t-test, p < 0.01). The heights of histograms represent the average of 5 samples taken from 5 transects at each site, error bars represent ± 1 s.e. of the mean.

Fig. 4: Benthic algal chlorophyll a concentrations at each sampling site across the dry season, 2003. Each data point represents the average of five transects taken from each site, with 5 samples taken from each transect, and error bars represent ± 1 s.e. of the mean.

(You might conbine 3 and 4 into a single figure. It changes nothing but may give the appearance of having less figures in the paper.)

Fig 5: Dissolved nutrient concentrations at four sites in the Ventura River, and discharge at site U between April 10 and October 21, 2006. Error bars represent ± 1 s.e. of the mean for at least two samples taken at each site on each date. a) TDN concentration over time; b) TDP concentration over time.

Fig. 6: Diel changes in pH and dissolved oxygen concentration at each site, measured over 24 hours from September 10-11, 2003. Light areas represent day and dark areas night. a) pH. b) dissolved oxygen concentration.

Fig. 7: Diel changes in nitrate concentrations at all sites on September 10-11, 2003. a) site U; b) site D3; c) site D2; c) site D1. Shaded areas represent the nighttime period. Note the different scales of the y-axes.

Fig. 8: Growth rates of *Ludwigia* cuttings in September and October outdoor experiments across a range of starting nitrogen concentrations (Table 1). Line depicts Michaelis-Menten equation with parameters derived by a Lineweaver-Burk transformation: $V_{max} = 147\%$ increase in length per 16 days, and $k_m = 376$ µM nitrogen.

Fig. 9: Yearly deviation from mean annual precipitation in the City of Ventura, 1885 – 2005. Mean annual precipitation = 39.6 cm. Source: Ventura County Watershed Protection District, Station 66. (If worse comes to worse, this figure could be eliminated and the characteristics of the variation simply described in the text.)

Fig. 10. Looking upstream from the center transect at site D2 in the Ventura River. (a) June 2003 (b) June 2006.

(My only comment here is why not put together a more complete series of photos and make them available to reviewers and readers at some web page (perhaps on the LTER Server)? One of my photo essays might suffice. This might help the paper through review. I think a lot of authors are now making supplemental material available through a web site. We could even show the diel results and describe what went wrong and how we might be able to do it right the next time.)







(b) Site D3



17-Apr 17-May 17-Jun 17-Jul 17-Aug 17-Sep 17-Oct





(d) Site D1















(b)







(b) Site D3







(d) Site D1









(b)

