

SEASONAL INFLUENCE OF LIGHT AVAILABILITY ON METABOLISM IN AN
URBAN, SPRING-FED RIVER

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
AB	Aquarena Springs Drive Bridge
HB	East Hopkins Street Bridge
SMR	San Marcos River

ABSTRACT

In aquatic ecosystems, temperature and light are the primary drivers of ecosystem metabolism, which is comprised of gross primary production (GPP) and ecosystem respiration (ER). However, light availability and temperature are generally seasonally synchronous in aquatic systems outside of the tropics, thus it is difficult to assess the independent influence of each parameter on ecosystem metabolism. I examined the effects of seasonal light availability on both GPP and ER, macrophyte community dynamics, and autotrophic metabolism in the San Marcos River, a physicochemically stable spring-fed system with high perennial macrophyte biomass. I estimated GPP, ER, and net ecosystem production (NEP) for spring, summer, and winter seasons in the upper San Marcos River. Using high-resolution drone imagery and in-field measurements, I determined seasonal biovolume (m^3) across four reaches of varying recreational intensity. Finally, I used metabolic chambers to estimate seasonal GPP, respiration (R), and net primary production (NPP) of three macrophyte species and plastic plant analogues of increasing morphological complexity to determine how structural complexity influences metabolism of macrophytes and their epiphyte communities. I found that both GPP and ER in the upper San Marcos River were strongly correlated with seasonal light availability, indicating autotrophic respiration as a substantial portion of ER. I found that reach-scale macrophyte coverage and biovolume are largely controlled by recreational activity and not seasonal light availability. Finally, macrophyte and epiphyte metabolism were different across seasons and morphotypes, with the most structurally complex

macrophytes exhibiting the greatest epiphyte metabolism. Overall, this study indicates that ecosystem metabolism in thermally stable spring-fed rivers is largely driven by light limitation of autotrophic biomass and that human recreational intensity influences ecosystem functioning.

I. SEASONAL INFLUENCE OF LIGHT AVAILABILITY ON METABOLISM IN AN URBAN, SPRING-FED RIVER

Introduction

Aquatic ecosystem processes (e.g. primary and secondary production, ecosystem respiration, and nutrient cycling) are largely regulated by environmental factors including light intensity, photoperiod length, temperature, and resource availability (Young et al. 2008, Tank et al. 2010). In non-tropical systems, where light availability, temperature, and resource flux are temporally synchronous, seasonal changes in these drivers of ecosystem processes result in greater response variability, particularly with latitude. For example, higher latitude streams and rivers typically experience long, dark, sub-freezing winters, whereas the subtropics have relatively mild winters with only a few days below freezing, more uniform photoperiod, and comparatively warmer temperatures (Huryn and Benstead 2019). Seasonal changes in light availability and temperature influence the timing and quantity of pulses of allochthonous terrestrial resource subsidies to aquatic systems, such as leaf litter (Guo et al. 2021). Additionally, macrophyte gross primary production (GPP) and respiration are driven primarily by higher and lower light availability, respectively (Mulholland et al. 2001, Roberts et al. 2007, Bernot et al. 2010, Burrell et al. 2014, Huryn et al. 2014, Hornbach et al. 2015, Huryn and Benstead 2019), while heterotrophic metabolism in aquatic systems is positively regulated by temperature and resource availability (Allen et al. 2005, Yvon-Durocher et al. 2010, Demars et al. 2011, Yvon-Durocher et al. 2012, Beaulieu et al. 2013, Jankowski et al. 2014, Clapcott et al. 2016, Huryn et al. 2014, Huryn and Benstead 2019).

Lotic systems, which are highly dependent on surface water runoff and shallow

groundwater exchange during baseflow conditions, also frequently experience a high degree of seasonal synchronicity in light availability and temperature, making it difficult to parse out the individual influence of each on ecosystem metabolism. However, systems dominated by groundwater discharge, with stable thermal regimes relative to typical lotic systems (Huryn et al. 2014, Willis et al. 2017, Huryn and Benstead 2019, Nichols et al. 2020, Preiner et al. 2020), can serve as model systems in which the influence of temperature and light can be isolated at larger temporal and spatial scales when compared to smaller experimental systems (i.e., stream mesocosms). In spring-fed ecosystems, ectothermic activity may be sustained at a consistent rate throughout the year due to a lack of significant diel and seasonal variability in water temperature. Indeed, ectotherm metabolic activity is especially high in systems dependent upon relatively warm groundwater, such as geothermal and lower latitude springs (Brown et al. 2004, Woodward et al. 2010, Ferreira et al. 2015, Gossiaux et al. 2020). At the ecosystem scale, heterotrophic respiration should remain relatively high year-round within these systems unless seasonally limited by organic carbon (C) availability (Huryn et al. 2014, Huryn and Benstead 2019).

Perennial, spring-fed streams are often physicochemically stable, with low turbidity and consistent discharge, which facilitates abundant macrophyte growth (Sear et al. 1999). Under stable thermal and hydrological conditions, increased macrophyte growth is due to temperature-dependent allometric growth, within which production of reproductive structures and germination occurs within a specific temperature range (Barko and Smart 1981, Flint and Madsen 1995, Tolley-Jordan and Power 2009) and under a given range of velocities (Hutchinson 2019). Macrophytes and their associated

epiphytic biofilms can contribute significantly to gross primary production (GPP) and ecosystem respiration (ER), especially when areal macrophyte coverage and biomass are high in aquatic systems (Bernot et al. 2010, Beaulieu et al. 2013, Burrell et al. 2014, O'Brien et al. 2014, Alnøe et al. 2016, Preiner et al. 2020). Productivity within macrophyte beds is largely dependent on photosynthetically active radiation (PAR) intensity and photoperiod length, which are positively correlated with GPP (Riis et al. 2003, Sand-Jensen et al. 2007, Willis et al. 2017, Nichols et al. 2020, Preiner et al. 2020, Riis et al. 2020). Seasonal macrophyte production and biomass generally increases with mean PAR intensity and photoperiod length increase (Willis et al. 2017, Nichols et al. 2020); however, as macrophyte densities increase, self-shading within the bed can lead to higher rates of autotrophic respiration from both macrophytes and epiphytes (Alnøe et al. 2016, Riis et al. 2020). As mean daily PAR and photoperiod decline throughout the fall, senescence of macrophyte biomass may cause GPP and ER to decline and increase, respectively (Townsend et al. 2011, Riis et al. 2020). Macrophyte beds are relatively highly productive habitats whose structural complexity provides large surface areas for epiphytic biofilms while also reducing flow velocity, creating refugia and depositional zones for sediment and nutrient-rich organic particles (Sand-Jensen 1998, Pinardi et al. 2009, Levi et al. 2015, Alnøe et al. 2016, Willis et al. 2017, Wolters et al. 2019, Nichols et al. 2020). This may be especially true for communities of structurally complex macrophytes in which the ratio of surface area to biomass is high (Warfe and Barmuta 2006, Ferreiro et al. 2013, Wolters et al. 2019). Spring-fed systems often contain high densities of macrophytes, which can contribute significantly to GPP and autotrophic ER (Alnøe et al. 2016, Riis et al. 2020), but productivity is limited by seasonal light

availability, whereas heterotrophic metabolism is more directly regulated by temperature which is relatively constant year-round (Huryn and Benstead 2019, Preiner et al. 2020).

I assessed the effects of variation in seasonal light availability and macrophyte biomass on GPP and ER in a subtropical spring-fed river of consistent physicochemical conditions and discharge (San Marcos River, Texas, USA). I additionally investigated the influence of seasonal light availability and macrophyte morphological complexity on the metabolism of individual macrophyte morphotypes and their associated epiphytic communities. I also compared stream metabolism estimates from the upper San Marcos River to other rivers in the literature in order to place the rates estimated from a high water clarity and macrophyte dominated system in the context of the diversity of metabolism estimates from a diversity of stream ecosystems. I tested four predictions in this thesis. First, I predicted that variability in stream metabolism in the San Marcos River would be positively correlated with seasonal variability in photoperiod and insolation intensity (and largely independent from temperature), with the highest rates of GPP and ER occurring in summer and the lowest in winter. Second, I predicted that macrophyte biomass would be positively correlated with seasonal light availability and would positively influence ecosystem metabolism. Third, I predicted that productivity of macrophytes would vary among morphotypes, with structurally-complex macrophytes having the greatest and simplistic macrophytes having the lowest productivity. However, I also predicted that epiphytic biomass and contribution to overall metabolism would be greater on complex than on simple morphotypes. Lastly, I predicted that ecosystem metabolism estimates for the San Marcos River would be relatively high when compared to published estimates for other systems, due to the high macrophyte biomass and

extremely high water clarity.

Materials and Methods

Field Study

Site Description

All sampling occurred in the upper San Marcos River (SMR) in a ~680-meter segment from the Aquarena Springs Drive bridge (AB) to the East Hopkins Street bridge (HB) in Hays County, Texas, USA (Fig. 1). The San Marcos River is an urban spring-fed river with consistent temperature and chemistry due to its deep groundwater source from the Edwards Aquifer, and emerges from a large complex of springs located in the headwaters (Spring Lake, approximately 500 m from the upstream end of the study segment). Because it is spring-fed, during baseflow conditions turbidity is relatively low, with high water clarity, low light attenuation in the water column, and thus, high macrophyte biomass. The study segment is primarily populated with the endemic Texas wild rice (TWR; *Zizania texana*), but other species are also present to a substantially lesser extent. Due to its consistent conditions and high water clarity, the upper San Marcos River is also subject to intense seasonal human recreational activity, mainly as swimming, kayaking/canoeing, and tubing in spring and summer months (Owens et al. 2001, BIO-WEST 2020). The study segment has multiple recreational access points including Sewell Park, Dog Beach Park, and City Park, with the latter two being on opposite sides of the same segment of the river. Because the SMR is spring-fed and relatively thermally constant, recreation in the river is nearly year-round, though recreation intensity varies with time of day and season, with the highest recreation levels

occurring in summers.

Ecosystem Metabolism Estimation

A Eureka MANTA multiparameter sonde was mounted vertically on a bridge piling in the middle of the river at the HB site in the spring (4/20/2021 – 5/12/2021), summer (7/17/2021 – 8/23/2021), and winter (12/20/2021 – 1/10/2022). Water temperature (°C), dissolved oxygen (DO), and depth (m) were all measured at 15-minute intervals. PAR was measured by a sensor (LI-190R Quantum Sensor, LI-COR, Lincoln, NE, USA) deployed on the roof of the Freeman Aquatic Building at Texas State University (adjacent to the SMR) during the same periods. Discharge data were downloaded from a United States Geological Survey (USGS) gauging station located at the start of the study segment under Aquarena Drive (USGS 2022; Figure 1).

Seasonal ecosystem metabolism was estimated from sonde data with the *streamMetabolizer* package in R (RStudio, version 4.1.2) for GPP, ER, and K_{600} using DO, temperature, PAR, and depth (Appling et al. 2018, Preiner et al. 2020). GPP and ER were converted from $\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ to $\text{g C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ using a photosynthetic quotient of 1.2 and respiration quotient of 0.85, respectively (Bott 2006). To determine basal ER when GPP was zero, ER:GPP were regressed across all sampling dates and for each individual season using the intercept from these regressions (Figure 3). Relationships between segment-wide GPP and ER with light and temperature for each season were estimated using simple linear regressions and Arrhenius plots of the natural log (\ln) of GPP and ER vs. $1/kT$, where k is the Boltzmann constant and T is the temperature (in Kelvin). However, due to limited temperature variability, the temperature dependence of GPP and

ER were modeled using Arrhenius plots with fixed-slope (-0.35 eV for RuBisCO carboxylation for GPP and -0.65 eV for aerobic catabolism for ER [Allen et al. 2005]), variable intercept, least-squares equations. To determine the greatest departure from predicted activation energies, and to isolate the effects of seasonal changes in light and temperature on these metabolic processes, the standardized residual errors from the Arrhenius plots were then regressed against natural log transformed mean daily PAR.

Macrophyte Community Dynamics in the San Marcos River

To determine seasonal macrophyte areal coverage (m^2), a drone (DJI Phantom 4) was flown from AB to HB each season to collect high-resolution. The height of these macrophyte stands were sampled by kayak within a two-week period before or after these flights by measuring the height (m) of haphazardly chosen points in macrophyte beds of predominant species throughout four reaches (i.e., A, B, C, and D) within the study segment (Figure 1). The areal coverage and height of macrophyte beds were then combined to estimate seasonal macrophyte biovolume for each reach.

Areal coverage for each species was measured using ArcPro GIS (3.0.2) and multiplied by the median macrophyte depth to obtain macrophyte biovolume (m^3) of each species in each reach for spring, summer, and winter (Figure 7b, d, f, h). The areal extent of bare substrate was estimated by measuring the total surface area of each reach and subtracting the total vegetated surface area of that reach for each season.

Autotrophic Metabolism and the Importance of Epiphytes

To estimate metabolism of individual macrophyte morphotypes and epiphytic biofilms, three species of macrophytes, three types of artificial plants (analogues) of comparable structural complexity, and cobbles were utilized. The three macrophyte species selected are the dominant species of the upper 7.4 km of the river and were categorized by their structural complexity: *Zizania texana* – simple, *Hygrophila polysperma* – intermediate complexity, and *Hydrilla verticillata* – greatest complexity. Macrophyte community metabolism was estimated by sealing a randomly selected individual macrophyte in an acrylic cylinder within the water column, in a design similar to Alnøe et al. (2016). The cylinder was fitted with a circulating pump and a hose with a clamp that allowed water to be withdrawn and sampled with an O₂ probe (ProSolo ODO; Yellow Springs Instruments, Yellow Springs, Ohio) to measure DO (mg O₂·L⁻¹), O₂ saturation, and temperature (°C), and returned to the chamber. The pump was sealed inside the cylinder that was placed over an acrylic disc with a gasket to prevent water and air exchange. The cylinders were sampled at 10-minute intervals for 2 hours during midday. After the illuminated incubation period, a dark PVC cylinder was placed over the cylinder to measure respiration. After a 1-hour acclimation period, measurements resumed every 10 min for 2 hours. This was done with four replicates of each macrophyte species each season (i.e., spring, summer, winter). Except for *Z. texana*, which was imaged in the field, macrophytes were removed, imaged, weighed, and had their surface areas measured using imaging software (ImageJ.JS; Schneider et al. 2012).

For estimates of epiphyton metabolism, four of each structural analogue were adhered to an acrylic disc anchored to a stone and placed within a clearing in a shallow

stand of TWR in the river to condition for 5-8 weeks. The site was in a relatively open canopy location in which flow velocities were similar to most of the main channel of the river. After conditioning, production and respiration rates were measured using the same methods used for macrophytes. Analogues were then brought to the lab and scrubbed with a clean nylon-bristled brush and deionized water before filtering the collected slurry through GF/F glass-fiber filter. Filters were extracted in acetone, chlorophyll *a* (chl-*a*) quantified, and chl-*a* was scaled to the analogue surface area ($\text{g chl-}a \cdot \text{m}^{-2}$ plant surface). Metabolic and chl-*a* measurements were performed on $n = 4$ replicate cobbles to measure epilithon metabolism and biomass. Surface area of cobble was estimated using the aluminum foil method (Morin 1987). Epiphytic biofilm and individual plant production and respiration ($\text{g O}_2 \cdot \text{m}^{-2}$ plant surface d^{-1}) were estimated by multiplying the increase or decrease in DO ($\text{g O}_2 \cdot \text{L}^{-1}$) under light and dark conditions, respectively, by the volume of water (L) in the submerged cylinder and dividing by the surface area of each analogue or macrophyte (in m^2). Production and respiration data were converted to $\text{g C} \cdot \text{m}^{-2}$ plant surface $\cdot \text{d}^{-1}$.

To determine the effect of season and species/morphotype on production and respiration for both macrophytes/cobble and macrophyte analogues, a two-way analysis of variance (ANOVA) was used with macrophyte type and season as independent factors and GPP, R, or NPP as the response variable. Before analysis, assumptions for normality and homoscedasticity were assessed. To determine significant homogenous subsets between macrophyte species within seasons and within each macrophyte species across seasons, a Tukey's HSD post-mortem analysis was used. A similar configuration of this analysis (two-way ANOVA) was used for comparisons among plant analogues across

seasons.

To determine the effect of season and morphotype on chl-*a* biomass, a two-way ANOVA was used with chl-*a* as the response variable and season x type as factors, where type includes simple, intermediate, and complex analogues and cobble. Tukey's HSD was used to determine significant homogenous subsets between morphotypes within seasons and within each morphotype across seasons.

Comparison of San Marcos Metabolism Estimates to the Literature

To assess San Marcos River metabolic estimates in the context of the literature, I first accessed a data set of 365 North American rivers from Appling et al. (2018), who present daily estimates of GPP and ER among rivers. I randomly pooled 1,050 paired daily estimates of GPP and ER and groomed the data to remove both negative estimates of GPP and positive estimates of ER, resulting in $n = 847$. I converted the data from $\text{g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ to $\text{g C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (Bott 2006) and estimated NEP by adding negative ER to GPP. An ANOVA, with river (i.e., SMR or literature) as the independent variable and either GPP, ER, or NEP as the dependent variable, determined significant differences between metabolic values. Finally, following Hall and Beaulieu (2013), I estimated the autotrophic respiration quotient of the SMR across all seasons and the pooled values from Appling et al. (2018), comparing the SMR to the pooled estimate and the estimates from Hall and Beaulieu (2013) and Bertuzzo et al. (2022).

Results

Ecosystem Metabolism in the San Marcos River

Water temperature was lowest in the winter and highest in the summer (Figure 2a, Table 1), with mean daily water temperature in summer being 22.76 ± 0.02 °C (\pm SE) and winter being 21.46 ± 0.10 °C. Mean daily PAR followed a similar pattern and was lowest in the winter and highest in the summer (Table 1). Mean daily water temperature and PAR were somewhat positively correlated ($R^2 = 0.40$, $p < 0.0001$). Mean daily discharge was lowest in the spring and highest in the winter (Figure 2c, Table 1), ranging from $3,352 \pm 82$ L·s⁻¹ (\pm SE) in the spring to $6,373 \pm 56$ L·s⁻¹ in the winter. Mean daily K_{600} (the gas exchange velocity) was lowest in the winter, highest in the summer, and intermediate in the spring (Figure 2d, Table 1).

Mean daily ecosystem GPP was significantly lower in winter than in spring or summer (Figure 3a, Table 1). GPP was positively correlated with water temperature ($R^2 = 0.41$, $p < 0.0001$; Figure 5a) and PAR ($R^2 = 0.75$, $p < 0.0001$; Figure 5b). Mean daily ER was lower in winter than in spring or summer (Figure 3b, Table 1). ER was positively correlated with water temperature ($R^2 = 0.42$, $p < 0.0001$; Figure 5c) and PAR ($R^2 = 0.62$, $p < 0.0001$; Figure 5d). ER was positively correlated with GPP ($R^2 = 0.82$, $p < 0.0001$) and the average basal rate of ER predicted when GPP was zero was 1.05 ± 0.31 g C·m⁻²·d⁻¹ (y-intercept \pm 95% CI; Figure 4). Season-specific predicted basal ER was 3.30 ± 0.49 , 0.62 ± 0.50 , and 0.58 ± 0.10 g C·m⁻²·d⁻¹ (Figure 4) for spring, summer, and winter, respectively. GPP/ER_{total} was 0.65, while $GPP/ER_{spring} = 0.89$, $GPP/ER_{summer} = 0.73$, and $GPP/ER_{winter} = 0.31$. Mean daily NEP was significantly lower in the summer than in spring or winter ($F_{2,82} = 11.34$, $p < 0.0001$; Figure 3c, Table 1). NEP was not correlated

with water temperature ($R^2 = 0.01$, $p = 0.38$; Figure 5e) or PAR ($R^2 = 0.01$, $p = 0.41$; Figure 5f).

The greatest departure from predicted values of GPP occurred at lower levels of PAR (GPP residual error = $1.078 \times \ln(\text{PAR}) - 6.279$, $R^2 = 0.50$, $p < 0.0001$; Figure 6b) than lower water temperatures (Figure 6a). The greatest departure from predicted values of ER occurred at lower levels of PAR (ER residual error = $1.029 \times \ln(\text{PAR}) - 6.002$, $R^2 = 0.45$, $p < 0.0001$; Figure 6d) than lower water temperatures (Figure 6c).

Macrophyte Community Dynamics

Vegetation composed a substantial proportion of the channel area within each reach each season, ranging from 54.7% in reach C in summer to 84.0% in reach A in spring. The dominant species in each reach was the native endemic TWR, composing >90% of the vegetated area in each season, except for reach D in spring (86.9%; Table 2). TWR areal coverage declined in highly recreated reaches A and C from 2,409.5 to 1,784.7 m² (25.9% decline) and 4,180.5 to 3,203.0 m² (23.4% decline), respectively, between spring and summer (Figure 7a, e). Areal coverage of bare substrate increased from spring to summer in A, C, and D from 467.2 to 1,106.7 m² (136.9% increase), 1,963.3 to 3,041.6 m² (54.9% increase), and 1,706.2 to 1753.9 m² (2.8% increase), respectively (Figure 7a, e, g). Total macrophyte areal coverage across the entire segment for spring, summer, and winter was 14,188, 12,754, and 14,090 m², respectively, while bare substrate composed 6,009, 7,443, and 6,107 m², respectively.

Macrophyte biovolume was dominated by TWR, with >90% of total biovolume for each reach across all seasons. TWR biovolume ranged from 775.9 m³ in A in summer

to 3,521.6 m³ in B in winter (Figure 8b, d). TWR biovolume declined from spring to summer in both A and C from 1,471.4 to 775.9 m³ (47.3% decrease) and 3,076.0 to 2,157.8 m³ (29.8% decrease), respectively (Figure 8b, f). Total TWR biovolume across the entire reach was 7,552.1, 7,828.2, and 10,570.7 m³ for spring, summer, and winter, respectively.

Autotrophic Metabolism

Mean macrophyte surface area was 357.6 ± 183.4 , 291.2 ± 94.2 , and 236.7 ± 49.4 cm² (mean \pm 1 SD) for TWR (simple), *H. polysperma* (intermediate), and *H. verticillata* (complex), respectively. Mean cobble surface area was 156.9 ± 59.2 cm².

Macrophyte/cobble GPP differed across seasons, type, and the interaction of season and type (Table 3). *H. verticillata* GPP was lower in winter than spring (Tukey's HSD, $p < 0.001$), but summer was similar to spring (Tukey's HSD, $p = 0.18$) and winter (Tukey's HSD, $p = 0.66$; Figure 8a, b, c). GPP across species in spring was higher in *H. verticillata* than TWR (Tukey's HSD, $p = 0.0004$), *H. polysperma* (Tukey's HSD, $p = 0.049$), and cobble (Tukey's HSD, $p < 0.001$), but no other species were different (Figure 8a).

Macrophyte/cobble R was not significantly different across seasons or type (Figure 8d, e, f, Table 3). Macrophyte/cobble NPP was different across seasons, types, and the interaction of season and type (Figure 8g, h, i, Table 3). *H. verticillata* NPP was significantly lower in winter than spring (Tukey's HSD, $p < 0.001$), although summer was similar to spring (Tukey's HSD, $p = 0.58$) and winter (Tukey's HSD, $p = 0.16$; Figure 8g, h, i). NPP across species in spring was higher in *H. verticillata* than TWR (Tukey's HSD, $p < 0.0001$), *H. polysperma* (Tukey's HSD, $p < 0.001$), and cobble

(Tukey's HSD, $p < 0.001$), but no other species were different (Figure 8g). NPP across species was higher in summer for *H. verticillata* than TWR (Tukey's HSD, $p = 0.04$) and *H. polysperma* (Tukey's HSD, $p = 0.02$), but cobble was similar to TWR (Tukey's HSD, $p = 0.80$), *H. polysperma* (Tukey's HSD, $p = 0.69$), and *H. verticillata* (Tukey's HSD, $p = 0.81$; Figure 8h).

Analogue surface area was $1,079.9 \pm 0$, $1,537.6 \pm 1,028.8$, and $1,498.6 \pm 795.4$ cm^2 (mean ± 1 SD) for simple, intermediate, and complex, respectively. Analogue GPP was differed across seasons, type, and the interaction of season and type (Table 3). Simple GPP was lower in winter than spring (Tukey's HSD, $p < 0.0001$), but summer was similar to spring (Tukey's HSD, $p = 0.08$) and winter (Tukey's HSD, $p = 0.52$; Figure 8a, b, c). GPP across analogues in spring was lower in intermediate than simple (Tukey's HSD, $p = 0.0006$) and complex (Tukey's HSD, $p = 0.04$; Figure 8a). GPP across analogues in summer was significantly higher for complex than intermediate (Tukey's HSD, $p = 0.02$), but simple was similar to intermediate (Tukey's HSD, $p = 0.99$) and complex (Tukey's HSD, $p = 0.053$; Figure 8b). GPP across analogues in winter was higher in complex than intermediate (Tukey's HSD, $p = 0.04$), but simple was similar to intermediate (Tukey's HSD, $p = 0.98$) and complex (Tukey's HSD, $p = 0.32$; Figure 8c). Analogue R was significantly different across season and type (Table 3), but not the interaction of season and type. Simple R was higher in spring than winter (Tukey's HSD, $p = 0.04$), but summer was similar to spring (Tukey's HSD, $p = 0.40$) and winter (Tukey's HSD, $p = 0.96$; Figure 8d, e, f). R across analogues in spring was lower in intermediate than simple (Tukey's HSD, $p = 0.01$), but complex was similar to simple (Tukey's HSD, $p = 0.70$) and intermediate (Tukey's HSD, $p = 0.41$; Figure 8d). Analogue

NPP was different across seasons, types, and the interaction of season and type (Table 3). Simple NPP was lower in winter than spring (Tukey's HSD, $p = 0.003$), but summer was similar to spring (Tukey's HSD, $p = 0.12$) and winter (Tukey's HSD, $p = 0.81$; Figure 8g, h, i). NPP across analogues in spring was lower in intermediate than simple (Tukey's HSD, $p < 0.01$) and complex (Tukey's HSD, $p = 0.04$; Figure 8g). NPP across analogues in summer was lower in intermediate than simple (Tukey's HSD, $p = 0.03$) or complex (Tukey's HSD, $p = 0.01$; Figure 8h). NPP across analogues in winter was higher for complex than intermediate (Tukey's HSD, $p = 0.03$), but simple was similar to intermediate (Tukey's HSD, $p = 0.10$) and complex (Tukey's HSD, $p = 0.15$; Figure 8i).

Areal Chl-*a* on analogues and cobbles differed across seasons, type, and the season x type interaction (Table 4). Complex chl-*a* was significantly higher in summer than spring (Tukey's HSD, $p = 0.03$), but winter was similar to spring (Tukey's HSD, $p = 0.97$) and summer (Tukey's HSD, $p = 0.42$; Figure 9a-c). Chl-*a* across analogues and cobble in the summer was higher for complex than simple (Tukey's HSD, $p < 0.001$) or intermediate (Tukey's HSD, $p < 0.01$), but cobble was similar to simple (Tukey's HSD, $p = 0.50$), intermediate (Tukey's HSD, $p = 0.97$), and complex (Tukey's HSD, $p = 0.20$; Figure 9b). Chl-*a* across analogues and cobble in the winter was higher for complex and cobble than simple (Tukey's HSD, complex $p = 0.02$, cobble $p < 0.01$) or intermediate (Tukey's HSD, complex $p < 0.001$, cobble $p < 0.001$; Figure 9c).

Comparison of San Marcos Metabolism Estimates to the Literature

Mean daily GPP across all seasons for the SMR was greater than the pooled literature from Appling et al. (2018; Table 5). Mean daily ER across all seasons was

higher for the SMR than the pooled literature from Appling et al. (2018; Table 5). Mean daily NEP across all seasons was not different than the pooled literature from Appling et al. (2018; Table 5). The mean daily ratio of GPP/ER across all seasons for the SMR was not different than the pooled literature from Appling et al. (2018; Table 5). The proportion of GPP as autotrophic respiration was estimated to be 77% (CI = 76 - 79%) and 49% (CI = 38 - 56%) for the SMR and pooled literature (Appling et al. 2018), respectively.

Discussion

Ecosystem Metabolism

Previous studies have found that stream GPP is largely driven by light availability, at multiple temporal (i.e., seasonal insolation, day length, and cloud cover) or habitat (i.e., closed vs. open canopy, turbidity, etc.) scales (Huryn et al. 2014, Hall et al. 2015, Bernhardt et al. 2018, Bernhardt et al. 2022). The study segment of the SMR is predominantly open canopy, with minimal shading from riparian vegetation, and receives a high amount of light typical of semi-arid, sub-tropical regions. I found that GPP was strongly correlated with light ($R^2 = 0.75$) and that the greatest departure from expected values of GPP (least-square regression with slope constrained to -0.3 eV [approximate activation energy of RuBisCO carboxylation (Allen et al. 2005)]) occurred not at the lowest temperatures, but at the lowest levels of light (Figure 6b). Additionally, ER was strongly correlated with mean daily PAR ($R^2 = 0.62$) with a similar departure from expected values of ER (least-square regression with slope constrained to -0.65 eV [approximate activation energy for aerobic catabolism (Allen et al. 2005)]) occurring at

lower light availability rather than lower temperatures (Figure 6d). This is likely due to large perennial macrophyte biomass significantly contributing to overall ER as autotrophic respiration as GPP and ER were strongly correlated ($R^2 = 0.82$). Hall and Beaulieu (2013) estimated the proportion of GPP as respiration from autotrophs (and their closely associated heterotrophs) to be 0.44 ± 0.19 (SD) using 13 streams with long-term metabolic data. Additionally, using pooled data from daily metabolism measurements across 305 streams, Hall and Beaulieu (2013) found autotroph (and associated heterotroph) respiration to constitute 63% (CI = 53-66%) of GPP. Bertuzzo et al. (2022) found that the autotrophic contribution to ER from 5 geographically and climatically distinct rivers fell within the upper range of the 0.44 ± 0.16 (SD) estimate of Hall and Beaulieu (2013). Using the same method as Hall and Beaulieu (2013), I estimated Autotrophic ER across the sampling period for the SMR to be 77% (CI = 76-79%) of GPP. My estimate is well above the upper limit of Hall and Beaulieu's (2013) pooled metabolic estimate, but given the high autotrophic biomass of the SMR, this is unsurprising. Additionally, Alnøe et al. (2016) found that even at low percentages of total surface cover, a disproportionately large proportion of habitat-scale metabolism (GPP, ER, and NEP) is derived from macrophyte habitats. Given that the SMR has high perennial macrophyte biomass which responds positively to light (i.e., increased GPP), and that I found autotrophic respiration to be a large proportion of GPP, the significant differences in metabolism between high light seasons (spring and summer) and winter were expected.

The SMR was net heterotrophic (i.e., negative values for NEP) across all seasons except for 11 non-consecutive days in spring (Figure 3c). Although high macrophyte

biomass in rivers is associated with high estimates of GPP (O'Brien et al. 2014, Alnøe et al. 2016, Preiner et al. 2020), and thus, autotrophic respiration, macrophyte beds are typically productive habitats whose structural complexity provide surfaces for epiphytic biofilms and decrease flow velocity, acting as refugia as well as depositional zones for sediment and nutrient-rich organic particulates (Sand-Jensen 1998, Biggs et al. 2005, Jones et al. 2011, Levi et al. 2015, Alnøe et al. 2016, Willis et al. 2017, Wolters et al. 2019, Nichols et al. 2020). Reduced flow velocity within macrophyte beds allows epiphytic biofilms to have larger boundary layers (Biggs et al. 2005) and the deposition of organic matter rich sediments (Jones et al. 2011), resulting in increased rates of microbial respiration (*see* Autotrophic Metabolism and the Importance of Epiphytes below). Alnøe et al. (2016) found that community respiration (CR) within macrophyte-dominated habitats greatly exceeded other habitat types in their study (i.e., stone, gravel, and sand) and was the only habitat type that was net heterotrophic. Given that percent macrophyte coverage in the SMR ranged from 63.1% in summer to 70.2% in spring, the observed net heterotrophy across all seasons is unsurprising. I found that NEP was significantly more negative in summer than spring or winter, however, the ratio of GPP:ER was much lower in winter (0.31) than in summer (0.73) despite less negative NEP in winter. This could indicate that due to lower mean daily PAR, GPP and autotrophic respiration decreased, but heterotrophic metabolic activity (i.e., respiration) remained at similarly high levels as in other seasons due to consistency of the water temperature.

Comparison of San Marcos Metabolism Estimates to the Literature

The SMR river had relatively high mean GPP and ER across all seasons compared to the pooled mean daily metabolism estimates from 365 North American rivers (Appling et al. 2018). The higher metabolism of the SMR is likely due to high macrophyte biomass and relatively high light availability associated with its latitude. However, while the magnitudes of GPP and ER were higher in the SMR than the pooled literature (Appling et al. 2018), mean NEP was similarly heterotrophic. A significant number of factors influence the trophic status of streams (e.g., light availability, turbidity, nutrient availability, water temperature, flow velocity, etc.), and, in most cases, the combination of these environmental factors results in net heterotrophic streams (Acuña et al. 2007, Roberts et al. 2007, Tank et al. 2010, Hoellein et al. 2013, Huryn et al. 2014, Alnøe et al. 2016, Hall et al. 2016, Priener et al. 2020, Riis et al. 2020). Additionally, GPP/ER was similar for both the SMR and pooled literature (Appling et al. 2018). GPP/ER (or P/R) can be used to estimate if a river is heterotrophic or autotrophic, but the point at which a river shifts from heterotrophy to autotrophy is uncertain as it falls within 0.5 to 1.0, which encompasses both the SMR and the literature. Overall, the SMR has high GPP and ER, even when compared to other spring-fed, macrophyte dense rivers (Huryn et al. 2014, Preiner et al. 2020, Riis et al. 2020).

Macrophyte Community Dynamics

The effects of recreation on submersed macrophytes and metabolism in freshwater ecosystems is understudied. However, research on these same effects in analogous systems, such as marine seagrass and turtlegrass habitats, and adjacent

terrestrial ecosystems, such as shoreline and riparian habitats, is much more developed. In seagrass and turtlegrass communities with prolonged and intense levels of recreation, sites with high levels of recreation were found to have reduced areal coverage of these macrophytes relative to less disturbed sites (Herrera-Silveira et al. 2010, Travaille et al. 2015). Direct contact (e.g., trampling) within these habitats damages or even completely removes macrophytes (Herrera-Silveria et al. 2010, Travaille et al. 2015, Garmendia et al. 2017, Nadiarti et al. 2021), particularly when the substrate is less firm (Eckrich and Holmquist 2000). Breslin (1997) found that recreational activities in the San Marcos River can negatively impact TWR. Although they found that the percentages of total interactions that resulted in damage to TWR were relatively low, at the time of the study TWR's areal coverage was lower than it is today (BIO-WEST 2020, Poole et al. 2022). Routine annual sampling of TWR for the entire river has shown that coverage has increased from $2,000\text{ m}^2$ in 2001 to $\sim 14,750\text{ m}^2$ in 2020 (BIO-WEST 2020). TWR has elongate leaves that, when submersed, are generally oriented perpendicular to the streambed, easily becoming tangled on people swimming and kayak paddles, often getting ripped or torn off completely during these contact events. Even activities with minimal contact, such as fishing, have been found to be destructive as hooks may get tangled in, tear, or completely remove leaves or entire macrophytes (Breslin 1997, O'Toole et al. 2009, Schafft et al. 2021). The greatest amount of fragmentation of macrophytes in the SMR occurs primarily during the afternoon in summer in areas with the most access points (Owens et al. 2001), which coincides with the peaks in seasonal and diel recreational activity (Breslin 1997) as observed in the loss of TWR coverage and biovolume in highly recreated reaches during summer sampling.

While the total TWR population and areal coverage across the SMR has increased over the last 20+ years (Poole et al. 2022), largely due to active management (e.g., planting TWR, removal of non-native macrophytes, displaying protected habitat signage, cordoned off areas), this study found that reach-scale TWR seasonal phenology is largely driven by human recreational disturbance, particularly in reaches with recreational access points. I found that TWR coverage changed between spring and summer by -26% and -23% for Sewell Park and City Park, respectively, with minimal increases of 2% and 10% between summer and winter, respectively, despite less recreational activity. However, between March and mid-September 2020, all public access points to the SMR were closed due to local COVID-19 restrictions, where TWR coverage changed from 2019 by +27% and +30% for Sewell Park and City Park, respectively (BIO-WEST 2020). Given that recreation clearly has negative impacts the reach-scale distribution of this endangered, endemic species, balancing human recreational and economic use with conservation of protected habitat is logistically challenging.

Autotrophic Metabolism and the Importance of Epiphytes

I found that overall GPP was higher for the structurally complex macrophyte (*H. verticillata*) and the simple and complex analogues per unit surface area. This is potentially due to greater chl-*a* biomass on the complex analogue (and potentially *H. verticillata*; M. Stehle, personal observation) as GPP was positively correlated with chl-*a* (set intercept = 0, $R^2 = 0.82$). However, simple analogue chl-*a* biomass was not higher than any other analogue or cobble at any point. Considering the high standard deviation of simple analogue chl-*a*, this could be the result of low sample size ($n_{\text{season}} = 4$). Cobble

also had high areal chl-*a*, but given the disparity between conditioning duration of analogues (5-8 weeks) and the cobble already found in the river, this may be unsurprising.

Respiration rates were similar across macrophytes and cobble across all seasons, but the magnitude of R was significantly lower in intermediate analogues than simple or complex. The reasons for this consistency in macrophytes and cobble but inconsistency across analogue morphotypes remains uncertain. However, NPP was highest in *H. verticillata* and the complex analogue, indicating structural complexity may improve autotrophic productivity, potentially due to mechanisms associated with slowed water velocity. Alnøe et al. (2016) found that epiphytes contributed 28, 20, and 24% to NEP, community R, and GPP of habitat-scale metabolism, despite macrophyte habitats comprising only 14% of the reach. Rates of epiphytic GPP, R, and NPP occasionally overlapped macrophyte metabolism estimates, but varied seasonally (Figure 8a-i). However, respiration most commonly overlapped across seasons, likely due to higher heterotrophic components within the epiphytic biofilms.

Macrophyte habitats, including the epiphytic community, are disproportionately productive relative to non-vegetated habitats (Alnøe et al. 2016). Slowed flow velocities within macrophyte beds, particularly with increasing macrophyte structural complexity, increase nutrient rich sediment deposition and macrophyte uptake rates (Sand-Jenson 1998, Jones et al. 2011, Levi et al. 2015, Preiner et al. 2020, Riis et al. 2020) while additionally increasing epiphyton boundary layers, allowing for greater and extended contact time with soluble bioavailable nutrients (Biggs et al. 2005). Thus, macrophyte beds can significantly influence nutrient cycling as their epiphytic communities actively

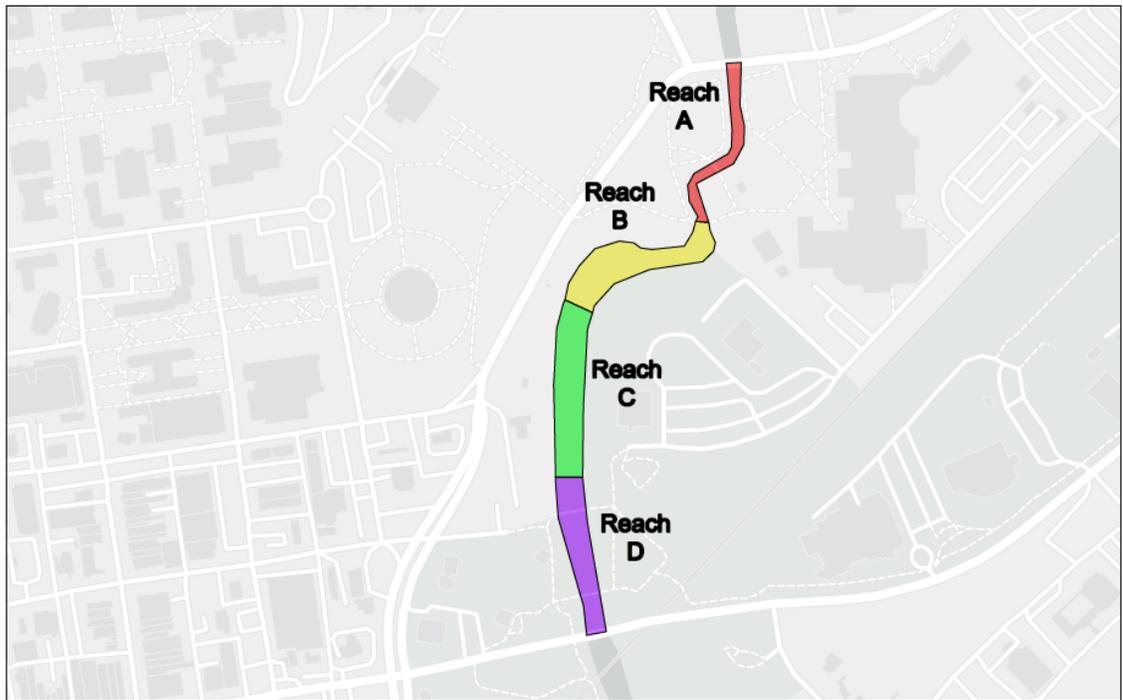
receive nutrients from the water column (Wear et al. 1999, Peterson et al. 2007, Baggett et al. 2010, Wolters et al. 2019). Wolters et al. (2019) found that epiphytic biofilms grown on artificial analogues had higher C:N:P molar ratios than those grown on macrophytes themselves, indicating absorption of nutrient exudates from the plants themselves. Additionally, macrophytes may potentially limit epiphyte colonization by secreting allelopathic metabolites and competition for dissolved inorganic carbon, which is a consideration for future research using artificial substrates conditioned with epiphytic biofilms (Wolters et al. 2019).

Conclusion

The physicochemically stable nature of the SMR allowed me to isolate the effects of seasonal light availability on ecosystem metabolism and individual autotroph metabolism with minimal confounding influence from seasonal difference in water temperature. In summary, I found that light availability was the primary driver of seasonal changes in GPP and ER in the SMR, with the greatest divergence from expected values occurring under low light availability rather than low water temperatures. Additionally, GPP and ER were more strongly correlated with mean daily PAR than water temperature. I found that even with high perennial macrophyte biomass, the SMR was still net heterotrophic across all seasons, indicating the importance of macrophyte habitats and autotrophic respiration on overall ecosystem metabolism. Ecosystem respiration was strongly correlated with GPP, and both were relatively high, falling under the “bright and stable” category of the conceptual model by Bernhardt et al. (2022). However, with seasonal periods of high recreation intensity, macrophyte beds may be

disturbed or entirely removed, and reducing or eliminating critically productive habitat. I found that variation in individual plant metabolism across seasons and species was inconsistent. A similar inconsistent trend was found for epiphyton-colonized artificial plants across seasons and structural complexities. Patterns in metabolism across seasons and structural complexity in macrophytes and artificial analogues could be further elucidated with further research utilizing larger sample sizes. Overall, while spring-fed rivers are uncommon they are useful for investigating the effects of various physicochemical influences on ecosystem dynamics at large experimental scales.

San Marcos River Study Segment



11/14/2022

1:5,011
0 0.04 0.07 0.14 mi
0 0.05 0.1 0.2 km
Esri Community Maps Contributors, City of Austin, City of San Marcos, Texas Parks & Wildlife, © OpenStreetMap, Microsoft, CONANP, Esri, HERE,

Figure 1. Map of study site; the study segment of the San Marcos River, San Marcos, Texas, USA.

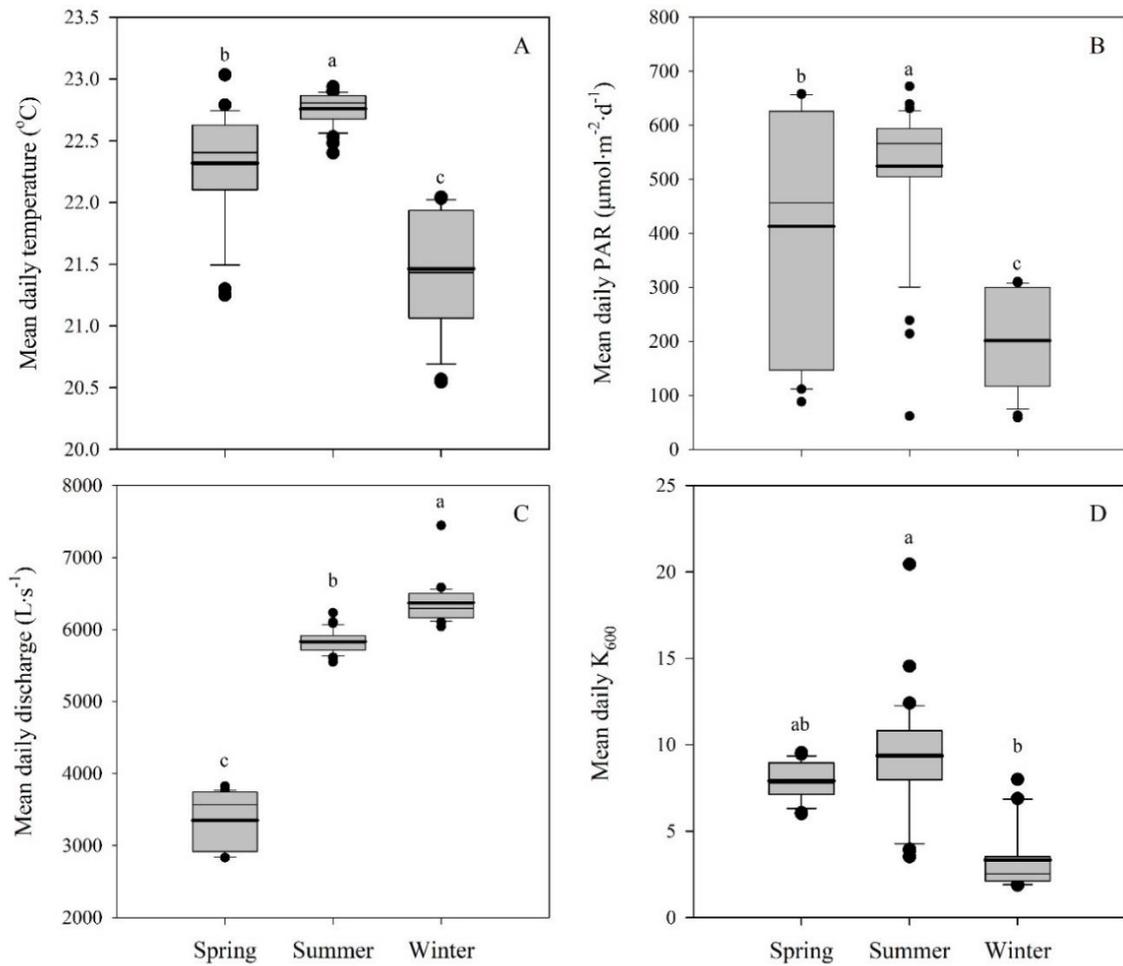


Figure 2. Seasonal environmental variable boxplots; box and whiskers plots of (a) mean daily temperature, (b) mean daily photosynthetically active radiation (PAR), (c) mean daily discharge, and mean daily K_{600} for spring, summer, and winter. Gray boxes represent 25th and 75th percentiles with the thin and thick horizontal lines representing the median and mean, respectively. Whiskers are 1.5x the interquartile range and outliers are represented as outsized points. Letters indicate significant differences ($p < 0.05$) between environmental variables across seasons.

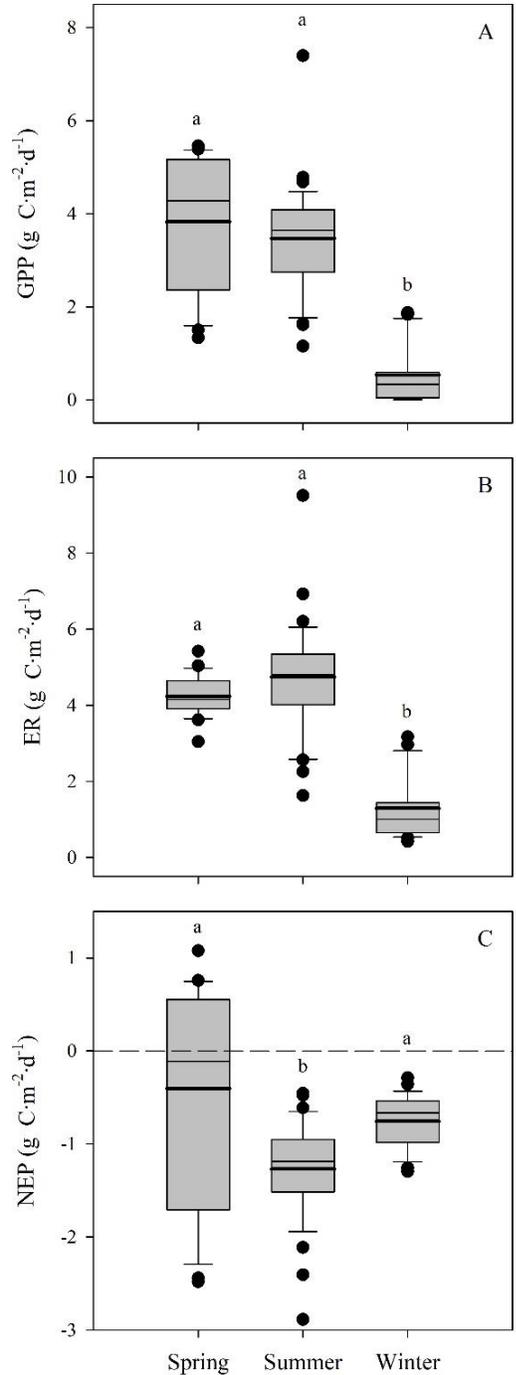


Figure 3. Metabolic output boxplots; box and whiskers plots of (a) gross primary production (GPP), (b) ecosystem respiration (ER), and net ecosystem respiration (NEP) for spring, summer, and winter. Gray boxes represent 25th and 75th percentiles with the thin and thick horizontal lines representing the median and mean, respectively. Whiskers are 1.5x the interquartile range and outliers are represented as outsized points. Letters indicate significant differences ($p < 0.05$) between metabolic variables across seasons.

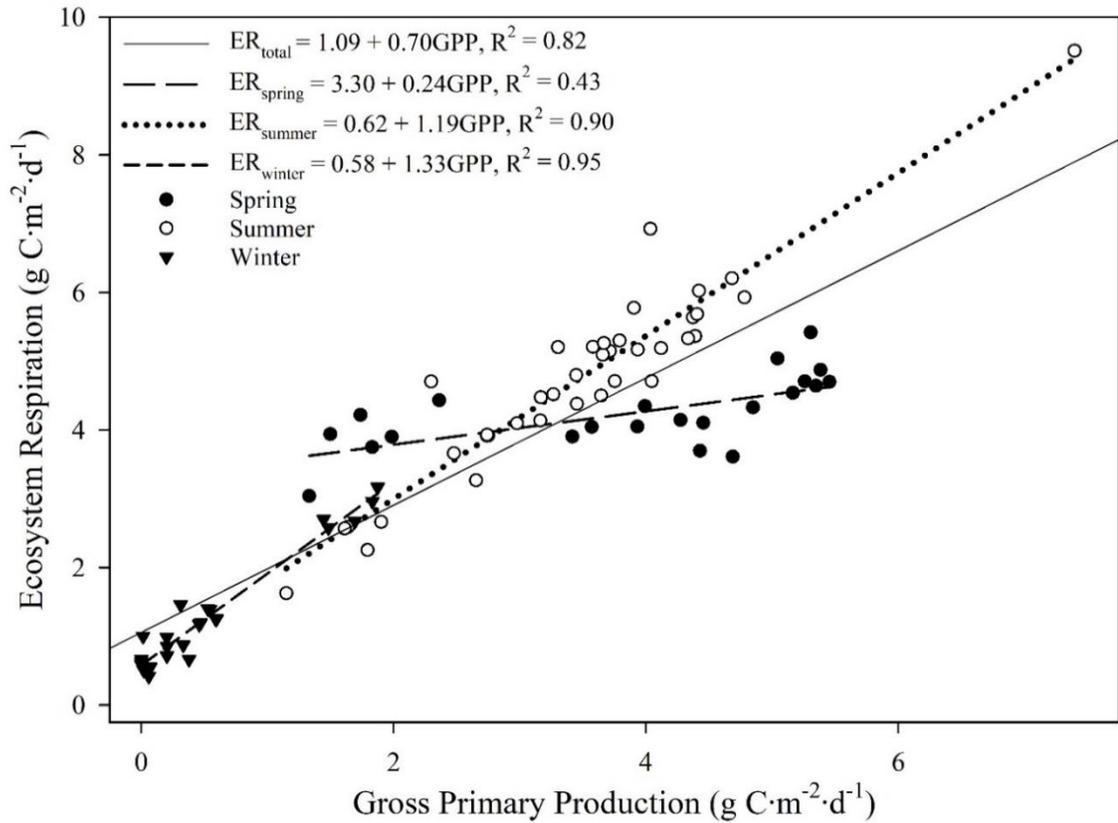


Figure 4. Ecosystem respiration/gross primary production correlation; the relationship between ecosystem respiration and gross primary production with linear regressions for the entire study period and each season (spring, summer, and winter).

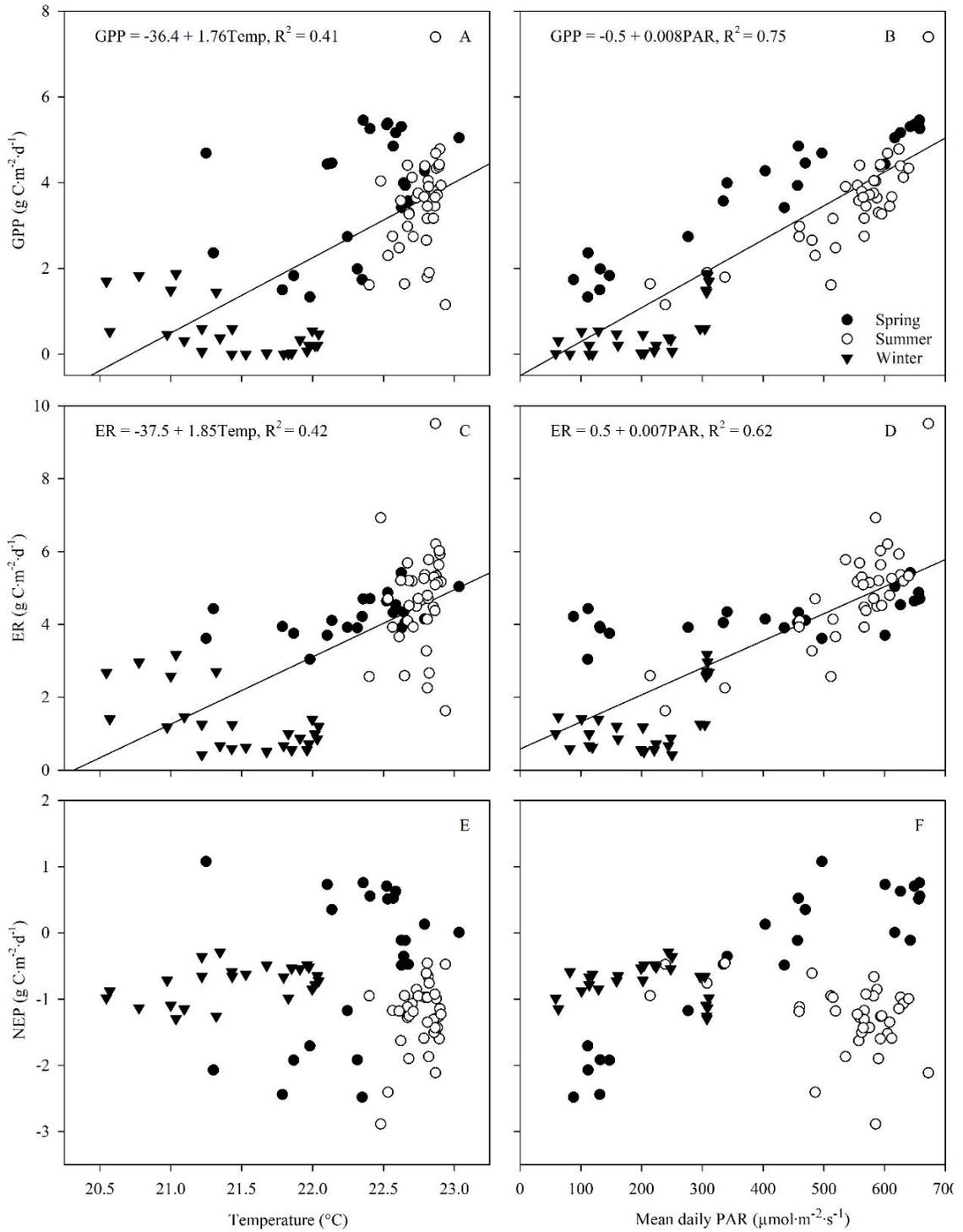


Figure 5. Metabolic outputs linear models; relationships between water temperature (temp) and (a) gross primary production (GPP), (b) ecosystem respiration (ER), and (c) net ecosystem production and mean daily photosynthetically active radiation (PAR) and (a) GPP, (b) ER, and (c) NEP. Relationships with significant correlation denoted with lines.

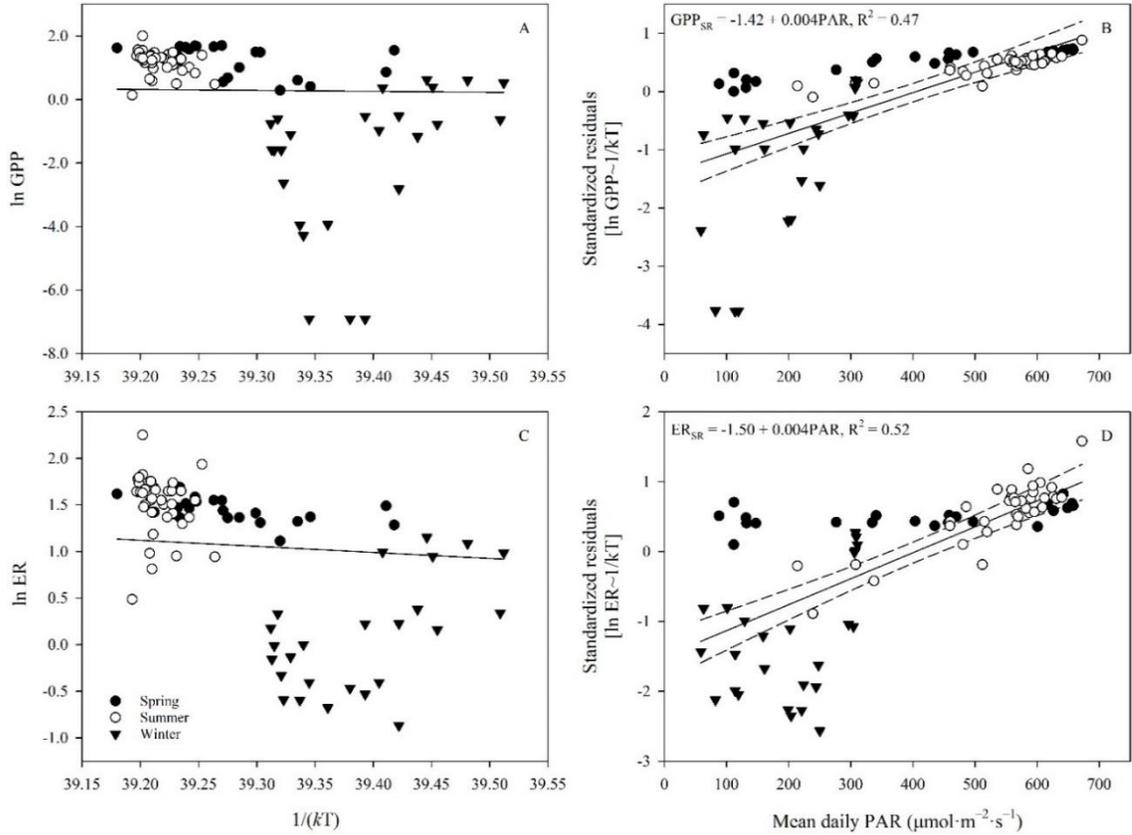


Figure 6. Arrhenius and standardized residual plots; Arrhenius plots of the natural log of (a) gross primary production (GPP) and (c) ecosystem respiration. Lines are plots of least-square fit equations with slopes constrained to the activation energies predicted for GPP (i.e., activation energy for RuBisCO carboxylation ~ 0.3 eV) and ER (i.e., aerobic catabolism ~ 0.65 eV), respectively. Standardized residuals from the Arrhenius plots of (b) GPP and (d) ER vs. photosynthetically active radiation (PAR).

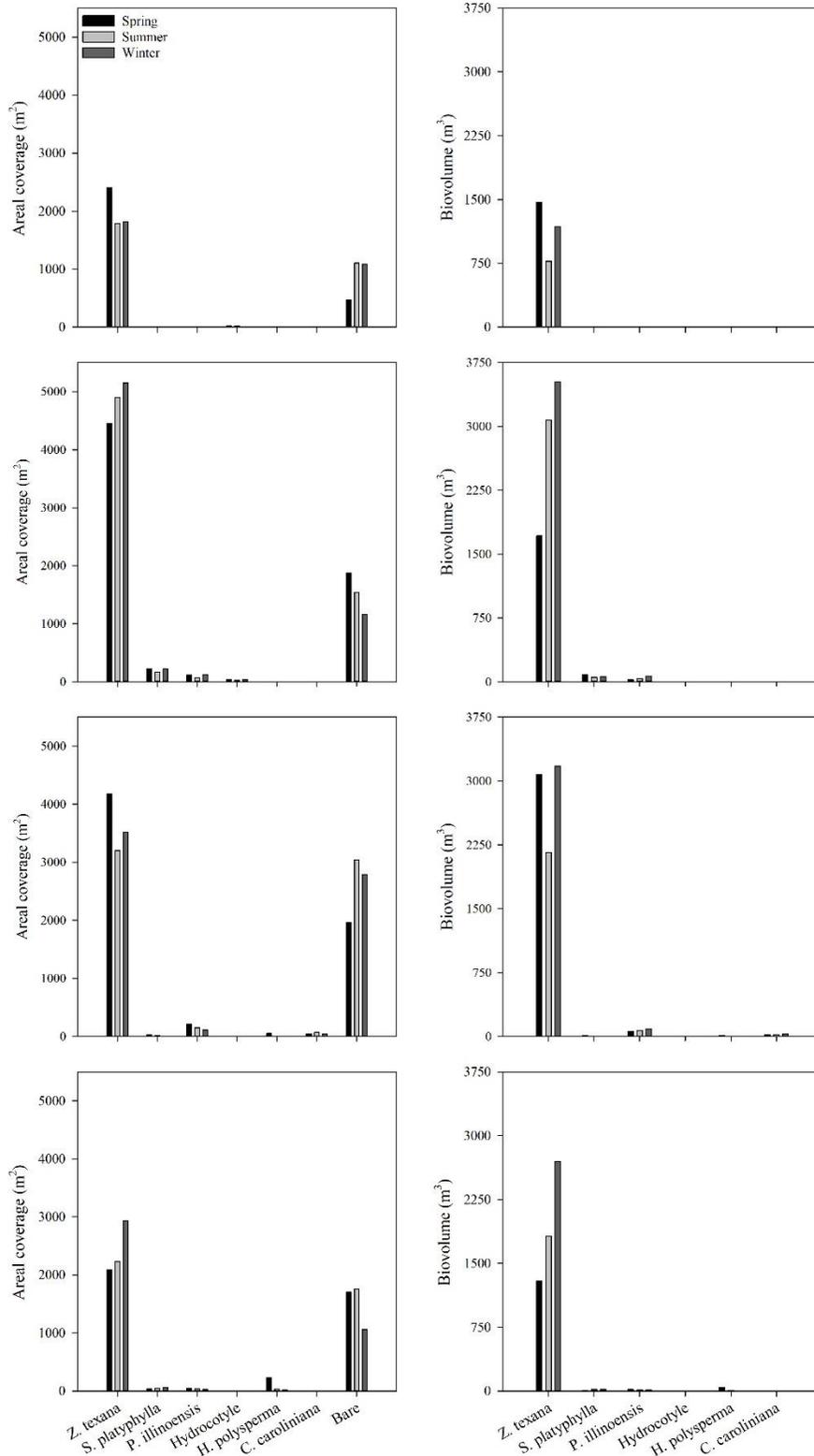


Figure 7. Macrophyte areal coverage and biovolume; areal coverage and estimated biovolume of macrophyte species and/or bare substrate for reach A (a,b), reach B (c,d), reach C (e,f), and reach D (g,h).

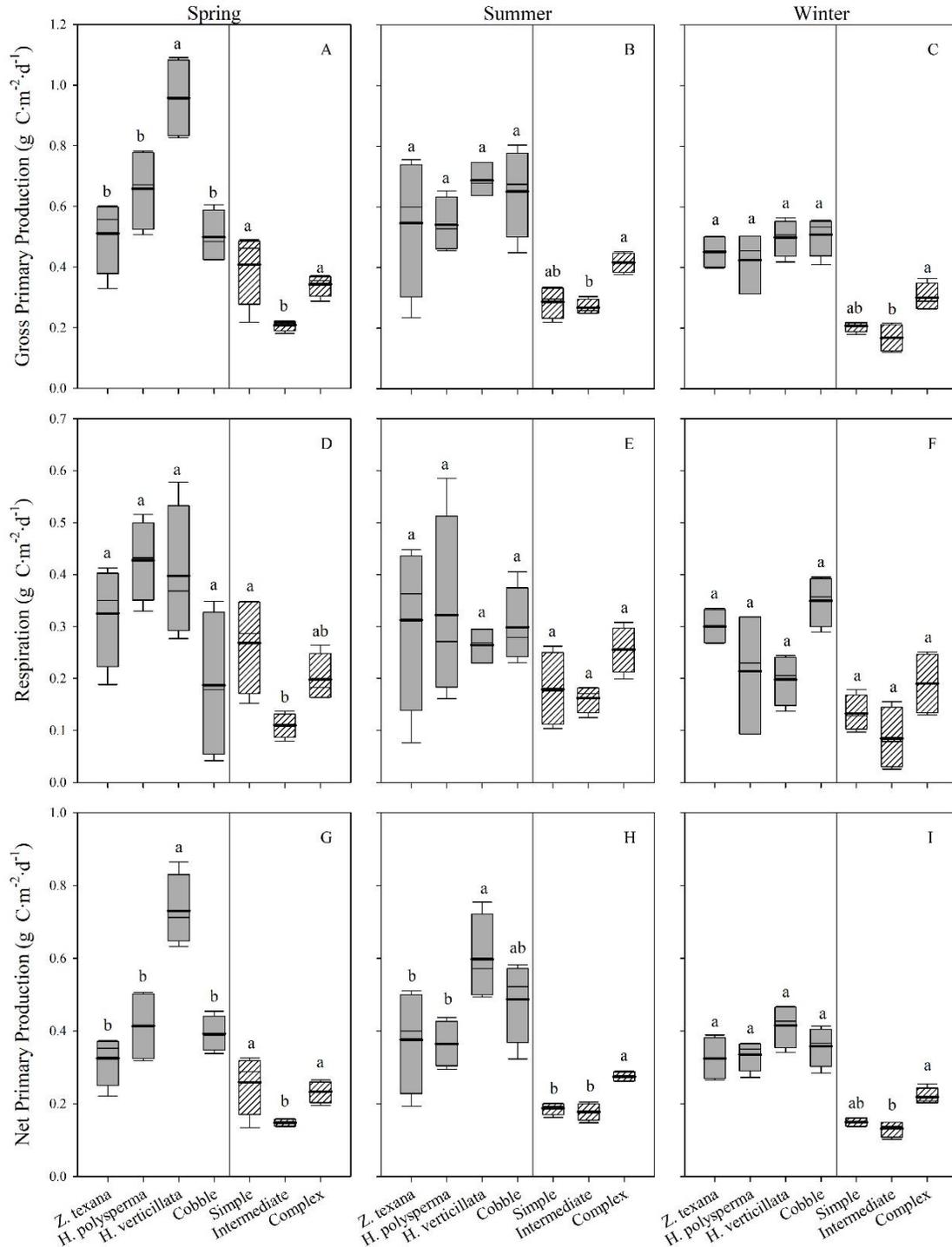


Figure 8. Autotrophic metabolism; box and whiskers plots of (a,b,c) gross primary production, (d,e,f) respiration, and (g,h,i) net primary production. Gray boxes represent 25th and 75th percentiles with the thin and thick horizontal lines representing the median and mean, respectively. Whiskers are 1.5x the interquartile range and outliers are represented as outsized points. Letters indicate significant differences ($p < 0.05$) between metabolic variables across species/cobble or analogue morphotype.

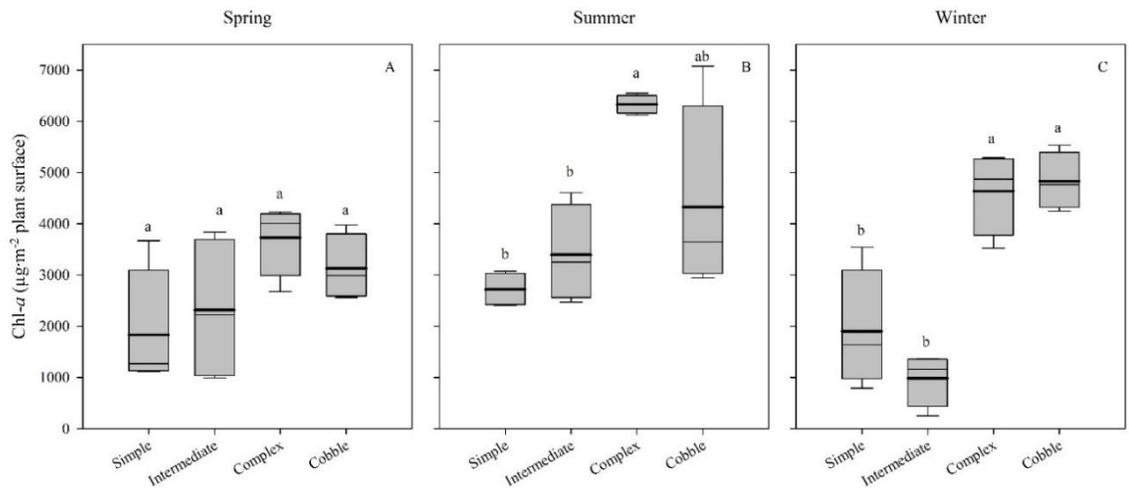


Figure 9. Chlorophyll *a* areal biomass; chlorophyll *a* (Chl-*a*) biomass for analogues and cobbles for (a) spring, (b) summer, and (c) winter. Gray boxes represent 25th and 75th percentiles with the thin and thick horizontal lines representing the median and mean, respectively. Whiskers are 1.5x the interquartile range and outliers are represented as outsized points. Letters indicate significant differences ($p < 0.05$) between chl-*a* areal biomass and analogues or cobble within a season.

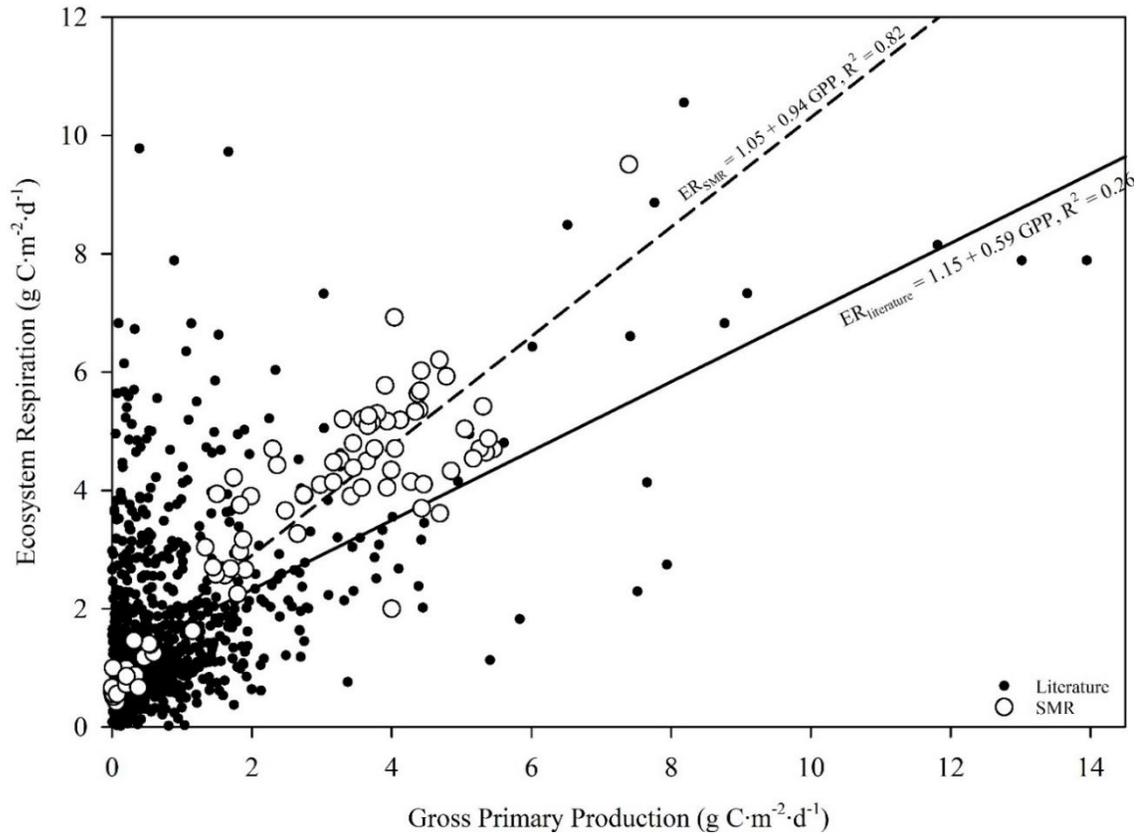


Figure 10. Ecosystem respiration/gross primary production comparison to literature; ecosystem respiration (ER) vs. gross primary production (GPP) for the San Marcos River (SMR) and pooled estimates of ER and GPP from the literature (Appling et al. 2018).

Table 1. Seasonal environmental and metabolic means and ANOVA output; seasonal means (± 1 SE) of mean daily water temperature, photosynthetically active radiation (PAR), discharge, and K_{600} . Seasonal means (± 1 SE) of mean daily gross primary production (GPP), ecosystem respiration (ER), net ecosystem production (NEP) and GPP/ER. Letters indicate significant differences within each variable across seasons.

Variable	Season			<i>F</i> -ratio	<i>p</i> value
	Spring	Summer	Winter		
Days	23	38	25	-	-
Temperature ($^{\circ}\text{C}$)	$22.32 \pm 0.09^{\text{b}}$	$22.76 \pm 0.02^{\text{a}}$	$21.46 \pm 0.10^{\text{c}}$	$101.40_{2,83}$	<0.0001
PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	$413.2 \pm 43.7^{\text{b}}$	$524.6 \pm 21.0^{\text{a}}$	$201.4 \pm 17.2^{\text{c}}$	$37.03_{2,83}$	<0.0001
Discharge ($\text{L}\cdot\text{s}^{-1}$)	$3352 \pm 82^{\text{c}}$	$5833 \pm 25^{\text{b}}$	$6373 \pm 56^{\text{a}}$	$856.70_{2,83}$	<0.0001
K_{600} ($\text{m}\cdot\text{d}^{-1}$)	$7.9 \pm 0.2^{\text{ab}}$	$9.4 \pm 0.5^{\text{a}}$	$3.3 \pm 0.4^{\text{b}}$	$49.42_{2,82}$	<0.0001
GPP ($\text{g C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	$3.83 \pm 0.30^{\text{a}}$	$3.47 \pm 0.19^{\text{a}}$	$0.53 \pm 0.12^{\text{b}}$	$68.84_{2,82}$	<0.0001
ER ($\text{g C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	$4.23 \pm 0.11^{\text{a}}$	$4.74 \pm 0.23^{\text{a}}$	$1.29 \pm 0.17^{\text{b}}$	$81.25_{2,82}$	<0.0001
NEP ($\text{g C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	$-0.40 \pm 0.24^{\text{a}}$	$-1.27 \pm 0.08^{\text{b}}$	$-0.71 \pm 0.08^{\text{a}}$	$11.34_{2,82}$	<0.0001
GPP/ER	$0.89 \pm 0.06^{\text{a}}$	$0.73 \pm 0.01^{\text{b}}$	$0.31 \pm 0.04^{\text{c}}$	$57.20_{2,82}$	<0.0001

Table 2. Macrophyte areal coverage and biovolume; areal coverage and biovolume for all observed macrophyte species and bare substrate for each reach (A, B, C, D) for each season.

Reach/Species	Areal Coverage (m ²)			Biovolume (m ³)		
	Spring	Summer	Winter	Spring	Summer	Winter
A						
<i>Z. texana</i>	2409.5	1784.7	1814.1	1471.4	775.9	1180.7
<i>P. illinoensis</i>	8.6	0.0	0.0	2.2	0.0	0.0
<i>Hydrocotyle</i> sp.	26.4	20.3	11.1	0.6	1.5	0.5
Bare	467.2	1106.7	1086.5			
B						
<i>Z. texana</i>	4449.5	4899.0	5150.4	1714.4	3074.7	3521.6
<i>S. platyphylla</i>	227.9	166.2	225.0	82.0	52.4	60.8
<i>P. illinoensis</i>	118.1	73.6	126.5	23.6	36.1	68.3
<i>Hydrocotyle</i> sp.	45.1	33.4	44.9	1.8	0.8	1.4
Bare	1872.8	1541.2	1166.6			
C						
<i>Z. texana</i>	4180.5	3203.0	3517.3	3076.0	2157.8	3174.4
<i>S. platyphylla</i>	25.0	9.5	4.2	7.9	3.2	1.2
<i>P. illinoensis</i>	206.1	147.4	105.6	53.6	67.8	87.5
<i>H. polysperma</i>	49.1	0.0	0.0	12.7	0.0	0.0
<i>C. caroliniana</i>	42.0	64.5	47.6	19.3	20.7	29.2
Bare	1963.3	3041.6	2791.3			
D						
<i>Z. texana</i>	2084.6	2227.0	2936.3	1290.4	1819.8	2694.1
<i>S. platyphylla</i>	38.5	50.7	60.7	7.9	17.8	18.5
<i>P. illinoensis</i>	45.0	38.9	25.6	17.7	10.0	13.6
<i>H. polysperma</i>	231.6	35.4	20.7	40.4	7.7	4.5
Bare	1706.2	1753.9	1062.6			

Table 3. Macrophyte and analogue ANOVA output; results from a two-way ANOVA for macrophytes/cobble and analogues, respectively, with gross primary production (GPP), respiration (R), and net primary production (NPP) as response variables and season (spring, summer, and winter), type (macrophyte species/cobble or structural analogue), and the interaction of season x type as factors.

Variable	Macrophytes						Analogues					
	GPP		R		NPP		GPP		R		NPP	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Season	9.234 _{2,34}	0.0006	1.395 _{2,34}	0.2617	7.538 _{2,34}	0.0018	12.336 _{2,27}	0.0002	4.578 _{2,27}	0.0194	7.544 _{2,27}	0.0025
Type	7.106 _{3,34}	0.0007	0.490 _{3,34}	0.6919	18.328 _{3,34}	<0.0001	19.472 _{2,27}	<0.0001	9.660 _{2,27}	0.0007	20.561 _{2,27}	<0.0001
Season x Type	3.564 _{6,34}	0.0076	2.603 _{6,34}	0.0348	3.162 _{6,34}	0.0135	4.536 _{4,27}	0.0062	2.540 _{4,27}	0.0629	3.934 _{4,27}	0.0121

Table 4. Chlorophyll *a* ANOVA output; results from a two-way ANOVA for analogues/cobble with chlorophyll *a* as the response variable and season (spring, summer, and winter), type (structural analogue and cobble), and the interaction of season x type as factors.

Variable	<i>F</i>	<i>p</i>
Season	9.277 _{2,36}	0.0005
Type	23.052 _{3,36}	<0.0001
Season x Type	2.631 _{6,36}	0.0322

Table 5. San Marcos River and literature metabolic means and ANOVA output; grand means (± 1 SE) of mean daily gross primary production (GPP), ecosystem respiration (ER), net ecosystem production (NEP), and GPP/ER for the San Marcos River (SMR) and from literature (Appling et al. 2018). Letters indicate significant differences for each metabolic variable between systems.

Variable	System		<i>F</i> -ratio	<i>p</i> value
	SMR	Literature		
Days	85	847	-	-
GPP (g C·m ⁻² ·d ⁻¹)	2.70 \pm 0.19 ^a	0.86 \pm 0.05 ^b	134.40 _{1,930}	<0.0001
ER (g C·m ⁻² ·d ⁻¹)	3.59 \pm 0.20 ^a	1.66 \pm 0.05 ^b	116.30 _{1,930}	<0.0001
NEP (g C·m ⁻² ·d ⁻¹)	-0.87 \pm 0.09 ^a	-0.80 \pm 0.05 ^a	0.30 _{1,930}	0.58
GPP/ER	0.65 \pm 0.03 ^a	0.74 \pm 0.07 ^a	0.14 _{1,930}	0.71

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