The effect of different nutrient concentrations on the growth rate and nitrogen storage of watercress (Nasturtium officinale R. Br.)

PRIMARY RESEARCH PAPER

The effect of different nutrient concentrations on the growth rate and nitrogen storage of watercress (*Nasturtium officinale* R. Br.)

Barbara Fernandez-Going · Thomas Even · Juliet Simpson

Abstract The mechanisms that allow broadly distributed aquatic plants to inhabit variable resource environments are unclear, yet understanding these mechanisms is important because broad environmental tolerance is often linked to invasiveness in terrestrial and aquatic plants. In an experimental stream, we examined the effects of different nutrient concentrations on the growth rate, biomass, and foliar nutrient concentrations of a cosmopolitan and potentially invasive aquatic plant, *Nasturtium officinale* (R. Br.). *Nasturtium* seedlings were grown under six nutrient treatment levels ranging from 0.64 μm N:0.09 μm P to 1531 μm N:204.13 μm P, for 8 weeks. Absolute and relative growth rates, and biomass of seedlings increased along a gradient of increasing nutrient concentrations but the effect of nutrient concentration was dependent on growing time.

Seedling biomass varied among nutrient treatments in weeks 4 through 8 of the experiment, but did not differ in week 2. By week 8, the two highest nutrient treatments had greater biomass than the two lowest nutrient treatments. Foliar nitrogen concentration increased, whereas carbon concentration and C:N ratios decreased in response to increasing nutrients. *Nasturtium* grows slowly in nutrient-poor conditions but rapidly increases its growth, biomass accrual, and nitrogen storage as conditions become nutrient-rich. The response of *Nasturtium* to enhanced nutrient conditions may indicate how aquatic nuisance species successfully invade and dominate plant communities in streams, where resources often vary both temporally and spatially.

Keywords *Nasturtium officinale* · Nutrients · Relative growth rates · Biomass · Foliar nitrogen

Handling editor: Katya E. Kovalenko

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Introduction

The distribution and abundance of species are determined by a suite of biotic (e.g. competition, mutualism, facilitation, etc.) and abiotic factors (e.g. light and nutrient availability, substrate quality, etc.), which are often patchy in space and time (Brown, 1984; Hutchings et al., 2003). In many environments, including freshwater rivers, nutrients are often patchy in distribution, largely due to variation in local

geology, current and historical land-use, flash events and variable watershed inputs, and the composition and abundance of riparian and in-stream vegetation (Pringle et al., 1988; Dent & Grimm, 1999). Small-scale spatial heterogeneity in factors such as nutrient availability within stream ecosystems can generate species composition patterns in which some species specialize on low-resource patches, one or a few species dominate high-resource patches and species richness is greatest in intermediate-resource patches (Riis & Sand-Jensen, 2001; Santamaría, 2002; Bornette & Puijalon, 2011). In addition, community dominance and composition patterns shift along nutrient gradients as species intolerant of nutrient-rich conditions are replaced by more tolerant species, or as growth form shifts from submerged aquatic plants to replacement by emergent or floating species as nutrient availability increases over time (Riis & Sand-Jensen, 2001).

Despite variation in resource availability, among and within stream systems, many aquatic plants are broadly distributed and show relatively low taxonomic differentiation as well as low within- and between-population genetic variation (reviewed in Santamaría, 2002). Recent work indicates that stream physical and chemical characteristics can predict species richness, but not species composition due to the presence of ubiquitous species that are tolerant to a wide range of environmental conditions (Makkay et al., 2008). The growth and biomass production of these types of common species may vary with nutrient availability; yet few studies have specifically examined the response of common aquatic plants to nutrient gradients. Mony et al. (2007) found that one common European species, *Ranunculus peltatus*, was able to adjust its relative growth rate by increasing phosphorus uptake and storage along a gradient of phosphorus availability, suggesting that physiological plasticity is one potential mechanism explaining a widespread distribution. Physiological adjustments allow a species to respond rapidly to changing environmental conditions without a costly investment in new tissue (Perez et al., 1994). Aquatic plants may also respond to this gradient by altering biomass allocation patterns, such as allocating more biomass to roots at the low nutrient end of the gradient, or by altering growth rates, such as increasing growth in response to high nutrient concentrations.

Nutrient enrichment of freshwater streams, largely due to inputs from agriculture runoff and point-source pollution, often results in explosive growth of nuisance algae and aquatic plants, which can affect the diversity and structure of stream biota and impact ecosystem function (Vitousek et al., 1997; Riis & Sand-Jensen, 2001). Characteristics that promote the successful establishment and spread of nuisance or invasive aquatic plants include rapid growth and high biomass accrual, clonal, or vegetative reproduction, and tolerance of a wide variety of environmental conditions (Zedler & Kercher, 2004; Hastwell et al., 2008). In both terrestrial and aquatic systems, invasive species respond differently to resource availability than non-invasive species with invasive species often being able to exploit resources more quickly than their native counterparts. For example, using congener pairs, Burns (2004) found that the relative growth rates of invasive species did not differ from their non-invasive congeners under low nutrient conditions, but that invasive species had significantly faster growth rates under high nutrient conditions. Further, Daehler (2003) showed that invaders tend to be phenologically more plastic than natives and outperformed co-occurring natives under conditions of increased resource availability. These studies suggest that there may be critical resource levels that determine the success and impact of invasive or nuisance species.

Nasturtium officinale (syn. *Rorippa nasturtium-aquaticum,* hereafter *Nasturtium*) is a creeping emergent macrophyte native to Europe that has since become naturalized in the United States (Howard & Lyon, 1952; Les & Mehrhoff, 1999). *Nasturtium* has been reported as potentially invasive in some parts of its naturalized range (United States Department of Agriculture, http://plants.usda.gov/java/profile?symbol=NAOF), sharing certain characteristics with nuisance species, such as rapid vegetative growth that can continue through above-freezing winters, and the ability to propagate via fragmentation (Thommen & Westlake, 1981; Nichols & Shaw, 1986; Rejmankova, 1992). In Europe, *Nasturtium* is associated with nutrient-rich waters (Robach et al., 1996; Schorer et al., 2000), and there is evidence that it may respond positively to very high concentrations of nutrients, particularly nitrogen (Crisp, 1970; Bennett, 1986). Vincent & Downes (1980) observed that *Nasturtium* can remove high concentrations of nitrate from a New Zealand stream and showed that the leaves of this

species are high in nitrate reductase, suggesting the capacity to assimilate large quantities of nitrogen, thereby maximizing growth in high nitrogen environments. In contrast, phosphorus uptake is lower in *Nasturtium* and remains constant throughout the growth period (Bennett, 1986). Although *Nasturtium* is a common community member of North American streams, particularly those in urban areas, it is unclear how *Nasturtium* responds to the range of nutrient concentrations typical of these systems and whether plasticity in its response to variable nutrient concentrations may account for both its cosmopolitan distribution and its potential to become invasive in some parts of its naturalized range.

Nasturtium commonly occurs in urban streams where it has been observed to achieve biomasses of up to 200 g m^{-2}, and can dominate the aquatic plant assemblage (J. Simpson, unpublished data). It can also be found at lower density but as a common community member in relatively pristine and oligotrophic streams (personal observation). Little is known about how *Nasturtium* responds to enhanced nutrient loads beyond a general pattern of increased growth, and no studies to date have examined *Nasturtium's* growth and nutrient uptake responses to the large gradient of nitrogen and phosphorus concentrations typical of urban streams, which may account for the abundance patterns observed for *Nasturtium* in the field. In a mesocosm study, we grew *Nasturtium* seedlings under six different nutrient concentrations based on field conditions typical of urban streams, ranging from 0.64 μm N:0.09 μm P to 1531 μm N:204.13 μm P (constant N:P), for 8 weeks and measured their absolute and relative growth rate and biomass accrual. In addition, we examined *Nasturtium's* nutrient uptake patterns and assessed its physiological response to the nutrient concentrations by measuring the foliar carbon, nitrogen, and carbon to nitrogen (C:N) ratios of seedlings. Based on field observations and the literature, we predicted that *Nasturtium's* growth rates and biomass would respond positively to increasing nutrient concentrations but that the dynamics of its growth response under different levels of nutrients would vary based on nutrient availability. Further, we predicted that carbon content would decrease and that percent nitrogen content would increase, thereby decreasing the C:N ratio as seedlings were exposed to higher nutrient concentrations and continued to accumulate biomass.

Materials and methods

Nasturtium seeds for the experiment were collected from wild stock from seven streams in Santa Barbara and Ventura counties (Franklin, Mission, Rattlesnake, Refugio, San Antonio, San Jose, and San Pedro Creeks), California, USA. Seeds were collected from mature fruits in the early stages of dehiscence from 4 to 6 plants at each stream, pooled and stored in the dark at 4°C until the experiment began. Seedlings were germinated within a flat of sterile sand, in full sunlight, 1 month prior to the initiation of the experiment to reduce variation in germination and initial growth. Planting was randomized to mitigate for potential differences in physiological responses of plants grown from seed stock collected from different streams. Seedlings were watered every 2–3 days, or as needed, with deionized water (DI). Seedlings of equal size (approximately 2 cm tall) were transplanted to 100 cm^3 plastic pots of sterile sand at an initial density of four per pot (to allow for potential mortality). However, all seedlings survived transplanting and after 1 week seedlings were randomly thinned to two per pot. Plants did not flower during the experiment.

Nasturtium seedlings were grown for 8 weeks in fall conditions (Sept–Nov; 10–12 h day length) under a gradient of six nutrient levels in a recirculating stream system constructed and housed in an outdoor area of the University of California Santa Barbara campus green house (34.41 N, 119.71 W). The stream system consisted of six 132.5 L reservoirs (Rubbermaid™ Roughneck storage containers) and twelve 11.4 L channels (Sterilite™ plastic containers). Stream flow, current velocity, and dissolved oxygen levels were standardized and maintained with six small Beckett® fountain pumps, which delivered deionized water from reservoirs into the channels via 1.90 cm (inner diameter) Tygon™ tubing. Each reservoir held one pump, which was split with a Y-valve to supply each of two channels. The tubing was attached to the interior of each channel with a tube clamp so that the outflow was positioned less than 5 cm from the bottom of the channel. A 2.5-cm deep layer of pre-scrubbed river rock was placed in each channel to support five pots of seedlings (12 channels, 60 pots, and 120 seedlings). Pots were placed on top of rocks to prevent them from becoming root bound and to raise the seedlings above the water level. Nearly all seedling leaves remained above the water level

throughout the experiment. The water level in each channel was maintained at a depth of 3.5 cm, which was 2.5 cm below the rims of the seedling pots. Water from the channels was then returned to the reservoirs via 1.27 cm diameter outflows.

Six nutrient concentrations were created in experimental channels by using a dilute Hoagland's solution (Hoagland & Arnon, 1950) in combination with increasing concentrations of ammonium nitrate (N) and potassium phosphate (P). Widely used in plant experiments, Hoagland's solution is a balanced nutrient solution consisting of macronutrients (N, P, K, Mg, and Fe) and micronutrients (trace metals, etc.) (Vojtíšková et al., 2004). Nutrient concentration values were selected from data sets provided by the Santa Barbara Coastal Ecosystem LTER and approximated a natural range of urban stream nutrient conditions, from relatively pristine and oligotrophic streams where *Nasturtium* is found at lower density, to eutrophic streams where it can dominate the aquatic plant assemblage. High and low nutrient treatment levels were chosen to bracket high and low nutrient concentrations recorded for streams in Santa Barbara and Ventura counties and intermediate levels were selected to achieve an exponential increase in nutrient concentrations from the lowest to highest value. Six N and P concentrations, with two replicate channels each, were assigned as nutrient treatments T1–T6 (Table 1). Full strength Hoagland's solution was diluted to 4.25×10^{-5} to reach a 0.638 μm N/0.09 μm P concentration and was used as nutrient treatment 1; this solution also served as the base medium for treatments 2–6. Ammonium nitrate and potassium phosphate were added in a molar N:P ratio of 7.5 N:1 P to treatments 2–6 to achieve the appropriate nutrient concentrations (Table 1).

Nutrient concentrations were maintained throughout the duration of the experiment (8 weeks) by draining the system every 4 days, rinsing it with deionized water, and then replenishing it with fresh deionized water and assigned nutrient loads. Standard maintenance every 2 weeks prevented the growth of nuisance algae. Pots were removed and the channels, reservoirs and rocks were flushed with hot tap water and then scrubbed and rinsed with deionized water. In addition, any epibenthic algae growing in the pots or on the seedlings were manually removed by gentle brushing of substrate surfaces weekly. Because the experiment was conducted at the start of the rainy season, a canopy was built over the apparatus using an aluminum frame and was covered with 0.6 mm clear polyethylene sheeting to prevent rainwater from potentially diluting the nutrient concentrations. The canopy extended 1.5 m beyond the experimental system on all sides and nutrient treatments were randomized by location. Light levels were measured at 30 cm above the channels, and outside the canopy as a reference, at 1200 h on a clear, cloudless day with a Li-250 (Li-Cor, NE, USA) light meter at the start of the experiment. The presence of the canopy reduced ambient solar irradiance by 20%, from 1078.06 ± 33.43 to 861.9 ± 8.07 μE m^{-2} s^{-1}, with all channels subjected to the same light regime.

Water samples were collected from the reservoirs over a 3-day period in the second, fifth, and eighth week of the experiment to measure whether there was a depletion of nutrients between water changes. For each sampling period, a water sample was taken 4 h after a water change and then for two consecutive days at 1200 h. Water was filtered through a Gelman (Type A/E) 25 mm glass fiber filter and then frozen at $-10°C$ until analysis (at the end of the experiment). Water was analyzed at the Marine Science Analytical Laboratory (Santa Barbara, CA) for phosphate, nitrite + nitrate, and ammonia concentrations. A regression analysis of nutrient concentrations in water samples against target concentrations showed that the desired concentrations were maintained over the course of the experiment ($P < 0.0001$, $R^2 = 0.92$ for nitrogen and $P < 0.0001$, $R^2 = 0.92$ for phosphorus, $n = 6$).

We calculated both absolute and relative growth rates. To determine growth rates, plants were sampled every 2 weeks for the first 5 weeks, and then once a

Table 1 Gradient of nutrient concentration treatments in experimental channels

Treatment	N (μM)	P (μM)	NH$_4$NO$_3$ addition (μM N)	KH$_2$PO$_4$ addition (μM P)
T1	0.64	0.09	0.00	0.00
T2	3.41	0.45	2.77	0.37
T3	18.24	2.43	17.60	2.35
T4	97.49	13.00	96.85	12.91
T5	521.20	69.49	520.50	69.41
T6	1531.00	204.10	1530.00	204.05

Range of nutrient concentrations was determined from Santa Barbara Coastal Ecosystem LTER nutrient profiles of Santa Barbara/Ventura County coastal streams

week for the last 3 weeks for a total of five sampling dates (weeks 2, 4, 6, 7, 8). At each sampling date, one pot was randomly selected and seedlings were removed from each channel, oven dried at 60°C for 3 days (to constant weight), and measured for dry mass. Absolute growth rates were calculated as the difference in biomass over time (e.g., (Week 4 biomass − Week 2 biomass)/number of days). Relative growth rates were calculated using the formula:

$$RGR = Ln(W_t/W_o)/(t_1 - t_2)$$

where W_o and W_t are dry weights at the beginning and end of a sampling period of t days (as described in Mitchell & Tur, 1975). Absolute and relative growth rate determination began in week 4 because calculations required biomass data from the first sampling period (week 2). Leaves collected from plants during weeks 7 and 8 were dried in plastic vials, ground to powder and analyzed for carbon, nitrogen, and carbon to nitrogen ratio analyses (Marine Science Institute Analytical Laboratory, Santa Barbara, California).

The environmental conditions of the system were monitored every 3 h, from 0900 to 1800 h, during the last 2 days of the experiment, for a total of six sampling points. Discharge, water temperature, and soil temperature in the first 3 cm of sand were measured for all channels. Discharge of each channel was determined by measuring the volume of water exiting each channel for 10 s. There were no significant differences among channels for either temperature or discharge, which averaged 18.3 ± 0.1°C for soil and 17.0 ± 0.1°C for water, and 47.2 ± 0.5 ml s^{-1} for discharge (approximate velocity of 23.4 ± 0.02 cm s^{-1}). Water temperature, conductivity, dissolved oxygen, and pH was measured for all reservoirs. There were no significant differences among reservoirs for dissolved oxygen, temperature, and pH, which averaged 9.4 ± 0.1 mg l^{-1}, 17.5 ± 0.1°C, and 6.3 ± 0.2, respectively. Conductivity increased exponentially with increasing nutrient concentrations ($F_{29,35} = 2043.57, P < 0.0001$, ANOVA); treatments 1, 2, and 3 were all below 20 µS cm^{-1}, while treatment 5 averaged 57.8 ± 1.5 µS cm^{-1} and treatment 6 averaged 157.5 ± 2.7 µS cm^{-1}. Conductivity, dissolved oxygen, and temperature were taken with an YSI 85 Multi-function Meter. A Corning 320 pH meter was used to measure pH. Air temperature was measured with a standard mercury thermometer and ranged from 12 to 20°C over the course of the experiment.

Statistical analysis

Two-factor analysis of variance (ANOVA) was used to examine the effect of nutrient concentrations on the growth of *Nasturtium* over time. Nutrient treatment (ordinal variable) and time (continuous variable) were the independent variables and the absolute growth rate, relative growth rate, and biomass were the continuous dependent variables. Data were tested for normality and homoscedacity using Levene's test. Biomass values were log transformed to meet normality assumptions. Significant interactions between nutrient treatment and time were further evaluated using one-factor ANOVA by week, with log nutrient concentration (ordinal variable) as the independent variable, and absolute growth rate, relative growth rate, and biomass as the response variables. A Bonferroni-adjusted alpha level of $P = 0.01$ was used as the critical value for one-factor ANOVAs. Tukey–Kramer HSD post hoc tests for multiple comparisons were then conducted to determine differences among nutrient treatments.

Preliminary analyses suggested that the nutrient treatments grouped into two broad categories. Thus, we used discriminant analysis to determine how meaningful our nutrient treatment assignments were. This analysis checks for misclassifications in predetermined groups and is often used as a follow-up analysis to further describe groups (McCune & Grace, 2002). We corrected for differences in initial biomass by fitting a linear regression model to biomass with interaction between RGR and time as the predictor variables to determine if the differences between nutrient treatment groups were greater than the differences within these groups. Estimated biomass values were then calculated using the equation: RGR × time + intercept, using the intercept from the previous analysis. Estimated biomass was then used as the response variable in one-factor ANOVAs where nutrient treatment and group (three highest nutrient treatments vs three lowest nutrient treatments) were the independent variables. Estimated biomass values were log transformed to meet parametric assumptions.

Finally, one-factor ANOVAs were conducted to determine how different nutrient concentrations affected foliar carbon, nitrogen and C:N ratios in the final week of the study. Analyses were conducted on untransformed data as all variables met parametric

assumptions. All statistical analyses were performed with JMP 8.0 statistical package (SAS Institute 2008).

Results

Absolute growth rates of *Nasturtium* seedlings (Fig. 1) increased along a gradient of increasing nutrient concentrations but the effect of nutrient concentration was dependent on growing time (AGR, nutrients: $F_{5,36} = 9.07$, $P < 0.0001$; time: $F_{1,36} = 0.03$, $P = 0.87$; interaction: $F_{5,36} = 2.75$, $P = 0.03$). With the exception of the highest nutrient treatment (T6), in which relative growth rate was initially high and then slightly declined through time, relative growth rates of *Nasturtium* seedlings (Fig. 2) in treatments T1–T5 also increased along a gradient of increasing nutrient concentrations but the effect of nutrient concentration was dependent on growing time (RGR, nutrients: $F_{5,36} = 9.07$, $P < 0.0001$; time: $F_{1,36} = 0.03$, $P = 0.87$; interaction: $F_{5,36} = 2.75$, $P = 0.03$). Absolute growth rates of seedlings differed among nutrient treatments in weeks 4 and 6, but not in week 8 of the experiment due to variability (one-factor ANOVA, Table 2). Relative growth rates varied among nutrient treatments during week 4 of the experiment but there were no differences among treatments in weeks 6 through 8 (one-factor ANOVA, Table 2). In week 4, relative growth rates were greater in the three highest treatments (T4–T6) than in the three lowest treatments (T1–T3) (one-factor ANOVA, Table 2).

Similarly, seedling biomass increased along a gradient of increasing nutrient concentrations, but the effect of nutrient concentration was depending on growing time (Fig. 3; nutrients: $F_{5,48} = 60.7$, $P < 0.0001$, time: $F_{1,48} = 4.64$, $P = 0.036$, interaction: $F_{5,48} = 13.2$, $P < 0.0001$). Seedling biomass varied among nutrient treatments in weeks 4, 6, 7, and 8 of the experiment, but did not differ in week 2 (Table 2). In week 4, biomass was greater in the three highest nutrient treatments than in the two lowest nutrient treatments and in weeks 6 and 7 biomass was greater in the two highest nutrient treatments than in the three lowest nutrient treatments. Final total biomass in week 8 was greater in the two highest nutrient treatments than in the two lowest nutrient treatments (one-factor ANOVA, Fig. 3b).

Discriminant analysis indicated that nutrient treatments were more efficiently grouped into two groups
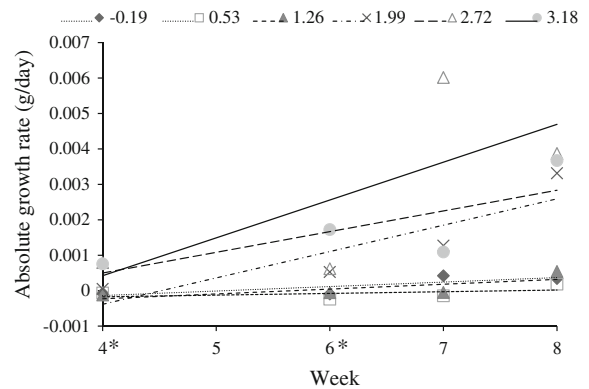

Fig. 1 Absolute growth rates over time of *Nasturtium officinale* seedlings as a function of nutrient concentrations (Log_{10} μm N). Regression equations: −0.19 (T1): $y = 6.57\text{e}^{-5}x − 0.0002$, $r^2 = 0.16$, $P = 0.33$, $\text{SD}_m = 0.001$, $\text{SD}_c = 0.002$; 0.53 (T2): $y = 0.0001x − 0.0006$, $r^2 = 0.55$, $P = 0.03$, $\text{SD}_m = 0.0006$, $\text{SD}_c = 0.0001$; 1.26 (T3): $y = 0.0003x − 0.002$, $r^2 = 0.28$, $P = 0.18$, $\text{SD}_m = 0.004$, $\text{SD}_c = 0.0006$; 1.99 (T4): $y = 0.0007x − 0.003$, $r^2 = 0.34$, $P = 0.13$, $\text{SD}_m = 0.008$, $\text{SD}_c = 0.01$; 2.72 (T5): $y = 0.002x − 0.007$, $r^2 = 0.47$, $P = 0.06$, $\text{SD}_m = 0.06$, $\text{SD}_c = 0.002$; 3.18 (T6): $y = 0.0009x − 0.003$, $r^2 = 0.57$, $P = 0.03$, $\text{SD}_m = 0.006$, $\text{SD}_c = 0.0008$. Legend represents (Log_{10} μm N) for each treatment T1–T6. *Asterisks* indicate weeks in which there were significant differences among treatments (one-factor ANOVA)
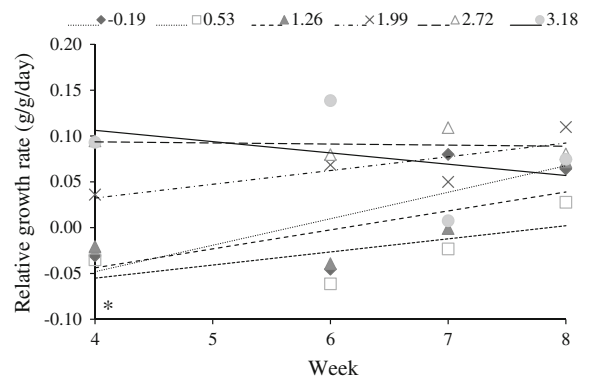

Fig. 2 Relative growth rates over time of *Nasturtium officinale* seedlings as a function of nutrient concentrations (Log_{10} μm N). Regression equations: −0.19 (T1): $y = 0.017x − 0.091$, $r^2 = 0.23$, $P = 0.45$, $\text{SD}_m = 0.23$, $\text{SD}_c = 0.04$; 0.53 (T2): $y = 0.034x − 0.19$, $r^2 = 0.51$, $P = 0.20$, $\text{SD}_m = 0.25$, $\text{SD}_c = 0.04$; 1.26 (T3): $y = 0.040x − 0.20$, $r^2 = 0.52$, $P = 0.07$, $\text{SD}_m = 0.28$, $\text{SD}_c = 0.04$; 1.99 (T4): $y = 0.0074x + 0.021$, $r^2 = 0.08$, $P = 0.55$, $\text{SD}_m = 0.18$, $\text{SD}_c = 0.03$; 2.72 (T5): $y = −0.0019x + 0.094$, $r^2 = 0.012$, $P = 0.84$, $\text{SD}_m = 0.13$, $\text{SD}_c = 0.02$; 3.18 (T6): $y = −0.0098x + 0.13$, $r^2 = 0.82$, $P = 0.50$, $\text{SD}_m = 0.23$, $\text{SD}_c = 0.04$. Legend represents (Log_{10} μm N) for each treatment T1–T6. *Asterisks* indicate weeks in which there were significant differences among treatments (one-factor ANOVA)

Table 2 Results of one-factor ANOVAs by week to determine the effects of nutrients on the absolute and relative growth rates, and biomass of *Nasturtium* seedlings grown for 8 weeks under a gradient of increasing nutrient concentrations

Response variable	Week 2	Week 4	Week 6	Week 7	Week 8
Absolute growth rate (g/day)	N/A	$F_{5,11} = 6.06$	$F_{5,11} = 5.44$	$F_{5,11} = 2.78$	$F_{5,11} = 3.39$
		$P = 0.02$	$P = 0.03$	$P = 0.12$	$P = 0.09$
Relative growth rate g/g/day)	N/A	$F_{5,11} = 26.3$	$F_{5,11} = 1.65$	$F_{5,11} = 0.63$	$F_{5,11} = 0.52$
		$P = 0.0005$	$P = 0.28$	$P = 0.68$	$P = 0.76$
Biomass (g)	$F_{5,11} = 0.48$	$F_{5,11} = 30.28$	$F_{5,11} = 14.35$	$F_{5,11} = 21.12$	$F_{5,11} = 23.27$
	$P = 0.78$	$P = 0.0003$	$P = 0.003$	$P = 0.001$	$P = 0.0007$

Biomass was log transformed prior to analysis. A Bonferroni-adjusted alpha level of $P = 0.01$ was used as the critical value for one-factor ANOVAs
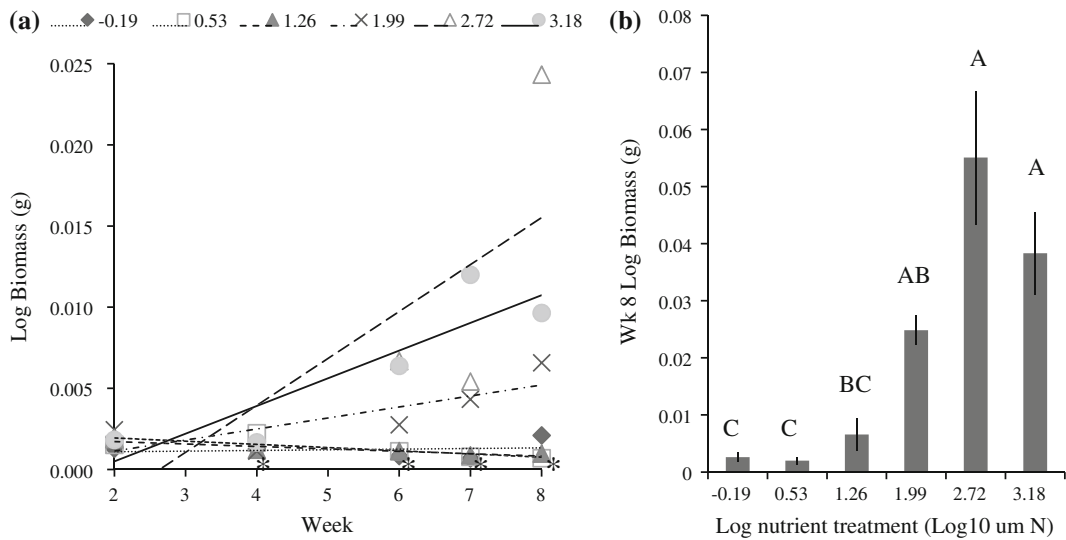

Fig. 3 Total biomass **a** over time, and **b** after 8 weeks, of *Nasturtium* seedlings as a function of nutrient concentrations (Log$_{10}$ μm N). **a** Regression equations: −0.19 (T1): $y = 0.14x + 0.49$, $r^2 = 0.44$, $P = 0.17$, SD$_m$ = 0.44, SD$_c$ = 0.07; 0.53 (T2): $y = −0.0050x + 1.063$, $r^2 = 0.0005$, $P = 0.79$, SD$_m$ = 0.57, SD$_c$ = 0.10; 1.26 (T3): $y = 0.23x − 0.11$, $r^2 = 0.38$, $P = 0.17$, SD$_m$ = 0.74, SD$_c$ = 0.13; 1.99 (T4): $y = 0.50x − 0.68$, $r^2 = 0.86$, $P = 0.0004$, SD$_m$ = 0.42,

SD$_c$ = 0.07; 2.72 (T5): $y = 0.65x − 1.092$, $r^2 = 0.96$, $P < 0.0001$, SD$_m$ = 0.21, SD$_c$ = 0.04; 3.18 (T6): $y = 0.56x − 0.57$, $r^2 = 0.89$, $P = 0.004$, SD$_m$ = 0.51, SD$_c$ = 0.09. Legend represents (Log$_{10}$ μm N) for each treatment T1–T6. *Asterisks* indicate weeks in which there were significant differences among treatments (one-factor ANOVA). **b** Treatments labeled with the *same letters* are not significantly different at $P \leq 0.05$ (Tukey–Kramer HSD)

(T1–T3 and T4–T6) (percent misclassified 66.6%). Estimated biomass (measured biomass corrected for initial growth) was similar across individual nutrient treatments ($F_{5,48} = 1.39$, $P = 0.24$). However, estimated biomass was higher in the three highest nutrient treatments (T4–T6 nutrient group) than the three lowest nutrient treatments (T1–T3 nutrient group) ($F_{1,48} = 5.58$, $P = 0.022$). Initial plant height in week one ranged from 16 to 28 mm, and by week 8, plant height ranged from 30 to 150 mm (weekly biomass means and standard deviations are given in Appendix Table 1—Supplementary Material).

Foliar nitrogen content of seedlings increased along the gradient of nutrient concentrations (one-factor ANOVA: $F_{5,11} = 6.90$, $P = 0.04$, Fig. 4), ranging from 0.72% in the lowest treatment to 3.65% in the highest treatment (a 400% increase in foliar nitrogen compared to a 2391% increase in nitrogen in the water). Foliar carbon content decreased with increasing nutrients (one-factor ANOVA: $F_{5,11} = 21.2$, $P = 0.006$, Fig. 4) and, consequently, the carbon to nitrogen ratio decreased along a gradient of increasing nutrient concentrations in the highest treatment (one-factor ANOVA: $F_{5,11} = 44.4$, $P = 0.001$, Fig. 4).
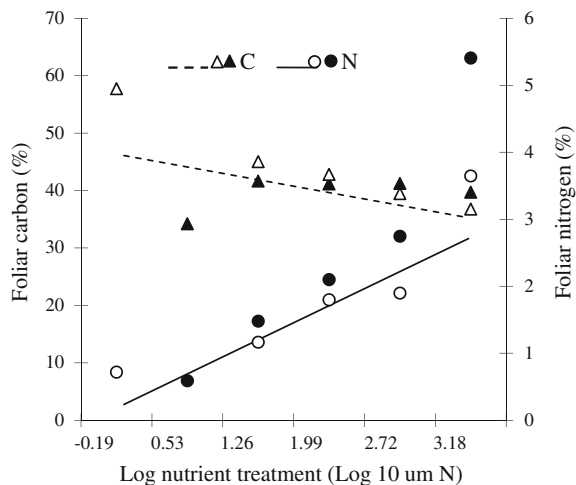
Fig. 4 Foliar carbon and nitrogen concentrations of *Nasturtium* seedlings as a function of nutrient concentrations. Leaf samples from week 7 (*closed symbols*) and week 8 (*open symbols*) were pooled for statistical analysis. Regression equations: Carbon: $y = -0.82x + 46.52$, $r^2 = 0.16$, $P < 0.05$; Nitrogen: $y = 0.79x - 0.96$, $r^2 = 0.88$, $P < 0.05$

Discussion

Nasturtium officinale is tolerant of a wide range of nutrient concentrations found in freshwater streams, and is often a dominant community member in streams with high nutrient loads (Robach et al., 1996; Schorer et al., 2000). *Nasturtium* survived and grew, albeit slowly at very low nutrient concentrations, across a gradient of nutrient concentrations, representing a range of natural conditions found in urban streams. *Nasturtium* responds to high nutrient concentrations with rapid growth and the accrual of biomass. In this study, seedlings exposed to the three highest nutrient levels had on average 1.5-fold greater relative growth rates than seedlings exposed to the three lowest nutrient levels, suggesting that Nasturtium has a broad tolerance to variable resource environments but that it accumulates biomass more quickly at intermediate to high nutrient concentrations.

Nasturtium, growth rates are greatest when nitrogen and phosphorus are readily abundant. For example, *Nasturtium* growth rates ranged from 0.0388 to 0.0414 g day^{-1} in nutrient-rich chalk streams (Thommen & Westlake, 1981) and approached 0.0569 g day^{-1} in a New Zealand stream where nitrate values typically approach 1 mg l^{-1} and phosphate values approach 0.0915 mg l^{-1} (Howard-Williams et al., 1982). These

reported values approximate our mid to high nutrient concentrations (T3–T6) where *Nasturtium* growth rates are consistent with those reported in the field by Howard-Williams et al. (1982), but are nearly three times lower than growth rates reported in optimal laboratory conditions at 20°C (0.1500 g day^{-1}) when fertilized with a 744 μm N:316 μm P (2.35 N:P) nutrient solution, in which nitrate was the primary source of nitrogen (Grime & Hunt, 1975). Previous work has shown that watercress biomass increases with photoperiod and light availability (Engelen-Eigles et al., 2006; Going et al., 2008). The discrepancy in growth rates reported for Grime and Hunt's (1975) laboratory experiments and our mesocosm experiment may be attributable to a longer day length in the laboratory study (18 h), compared to our study in which natural day length ranged from 10 to 12 h over the duration of the experiment. Nonetheless, the pattern of rapid, vigorous growth of *Nasturtium* in response to an increase in nutrient concentration is consistent among studies.

Nasturtium's relative and absolute growth rates increased for all treatments in response to increasing nutrient concentrations but relative growth rates were greater in high nutrient treatments relative to low nutrient treatments through week 4 of the experiment and then converged, whereas absolute growth rates displayed considerable variation among treatments. It is possible that the low sample size for each growth rate measurement may have masked our ability to detect differences earlier or later in the experiment. The growth rates observed for *Nasturtium* at the termination of our experiment (0.056–0.0149 g day^{-1}) approach or exceed the rates of other aquatic species, including some nuisance species, such as *Salvinia molesta* (17.16% per day, Mitchell & Tur, 1975) and *Elodea canadensis* (0.045 g day^{-1}, Madsen & Baattrup-Pedersen, 1995). The convergence of *Nasturtium* relative growth rates under both low and high nutrient levels over time suggests a maximum rate for growth, and more importantly, it also shows that under high nutrient levels peak rate is reached more quickly than under low nutrient levels. The ability of *Nasturtium* to grow in nutrient-limited conditions and also respond rapidly to elevated nutrient concentrations may be advantageous in urban streams where resources may be both temporally and spatially heterogeneous. For example, in urban streams anthropogenic sources of nutrients via runoff from agriculture and urban development may increase with seasonal rains resulting in high nutrient

concentrations but only for a limited time (Walsh et al., 2005). Nutrient inputs may also be greatest and hence nutrient concentrations highest near anthropogenic point sources and decrease in other regions of the river farther away from these sources (Walsh et al., 2005).

Nasturtium biomass increased along a gradient of nutrient concentrations, with seedlings attaining the greatest biomass in the three highest nutrient treatments. The production of *Nasturtium* biomass observed in this study is consistent with that reported for several other species of nuisance aquatic plants grown under high nutrient concentrations, including *Eichhornia crassipes* and *S. molesta* (Mitchell & Tur, 1975; Reddy et al., 1989; Crossley et al., 2002; Xie et al., 2004). For example, *E. crassipes* biomass increased along a gradient from 0.5 to 5 mg l^{-1} N (approximately 357.1 to 3571 μm N) after which biomass did not respond to further increases in nitrogen (Reddy et al., 1989), which suggests that maximum growth rates were achieved at nitrogen concentrations of less than 3571 μm N. Similarly, *Nasturtium* seedlings in our study did not respond to further increases in nutrient concentrations beyond 97.49 μm N:13.00 μm P, suggesting a critical nutrient range between 18.24 and 97.49 μm N at which plant growth is either optimized or becomes limited by another resource.

Previous work has shown that aquatic plants may experience a shift from nutrient limitation to light or oxygen limitation and a subsequent reduction in growth when grown under high nutrient concentrations (Perez et al., 1994). Since *Nasturtium* has been reported to have higher maximum growth rates in laboratory studies (Grime & Hunt, 1975), it seems likely further growth in our study was limited by another resource, such as day length or water temperature. Reductions in the growth rates of aquatic vegetation have also been attributed to the potential toxic effects of high nutrient loads (Best, 1980; Santamaría et al., 1994; Vojtíšková et al., 2004; Boedeltje et al., 2005). For example, *Ruppia drepanensis* showed a reduction of growth and development with increasing nitrogen concentrations (54.4 mg N to 228 mg per cup sediment), which was attributed to the toxic effects of high ammonium concentrations in the interstitial water (0.5–40 mg l^{-1}) near the root zone (Santamaría et al., 1994). In our study, ammonium concentrations for the two highest nutrient treatments

were 262 ± 13.2 and 860 ± 11.6 μm NH_4, respectively, which are outside the range of nutrient conditions normally encountered by the plants used in this experiment. Nearly all seedlings in the two highest nutrient treatments showed signs of ammonium toxicity (personal observation), which Britto & Kronzucker (2002) define as the display of chlorotic leaves, discoloration, and leaf senescence. Although shifts in resource limitation (i.e., a shift from nutrient to light or other resource limitation) or chemical toxicity may ultimately be is responsible for slower growth in the highest nutrient treatments over time, initial, rapid growth rates allow *Nasturtium* to attain greater biomass under high nutrient concentrations.

Nasturtium's foliar nitrogen concentration responded strongly to nutrient availability, increasing more than fivefold from the lowest to the highest nutrient treatments. Foliar nitrogen content at the upper end of the gradient is within the range of previously reported values from laboratory grown *Nasturtium* (5.61% N, Kerfoot et al., 1998; Kopsell et al., 2007) and from *Nasturtium* occurring in the field when NH_4 concentrations were below those found in our three lowest treatments (3.39% N, Howard-Williams et al., 1982, 3.80–6.86% N; Newman et al., 1992; 5.91% N, Kerfoot et al., 1998), suggesting that *Nasturtium* may be capable of luxury uptake (i.e., uptake that does not affect yield). *Nasturtium* leaves have a high concentration of nitrate reductase, which plays a role in the rate of nitrate uptake, and thus have both a high nitrogen content and storage capacity (Vincent & Downes, 1980). Nitrogen concentrations in *Nasturtium* leaves approach or exceed 4.00% N which is higher than other common aquatic plants that typically range from 1.70 to 3.10% N of dry weight (Duarte, 1992). Our findings support these results and indicate that *Nasturtium* can continue nitrogen uptake even at very high concentrations, which may be advantageous in urban streams, where nutrient concentrations can often be high but temporally and spatially variable (Walsh et al., 2005).

Investigations into the effects of nutrient loading on lotic plant communities have generally shown that an increase in nutrient levels results in an alteration in community structure as diversity is reduced and as community composition shifts, due to replacement of species unable to tolerate high nutrient levels by species well adapted to more nutrient-rich environments (Smith et al., 1999; Schorer et al., 2000; Tracy et al., 2003). However, some species are known to

persist along large resource gradients and may attain nuisance status in high-resource conditions. For example, *Myriophyllum aquaticum* achieved its greatest growth at nitrogen concentrations of 1.8 mg l^{-1} (approximately 128 μm l^{-1}), which is a concentration typically of some eutrophic streams (Nichols & Shaw, 1986). *Nasturtium* occurs across a wide range of nutrient availability and it can be abundant in both nutrient-limited and nutrient-rich streams and patches, suggesting that this species has adaptive mechanisms to tolerate both types of resource environments. Our work shows that *Nasturtium* responds to a gradient of naturally occurring nitrogen and phosphorus concentrations, by growing slowly and gradually in nutrient-poor conditions and by rapidly increasing its growth and biomass accrual as conditions become nutrient-rich.

In the oligotrophic environment characteristic of many lotic systems, *Nasturtium* is often present but does not dominate the community. Under these nutrient-poor conditions, *Nasturtium*'s slow growth and biomass accrual may reduce the impact of this species on native species diversity and ecosystem function. However, our work also shows that the growth, biomass accrual and nitrogen storage of *Nasturtium* rapidly increases as nutrient levels rise and, under these eutrophic conditions, can approach or exceed the growth rates of known invasive aquatic species (Crossley et al., 2002; Nichols & Shaw, 1986; Wersal & Madsen, 2011). In this mesocosm study, nitrogen to phosphorus ratios were held constant for logistical purposes, but they are likely to vary widely in streams, which may result in nutrient limitation and decreased biomass production. For example, excess phosphorus, relative to nitrogen, can inhibit nitrate uptake and reduce yield (Reddy et al., 1990). However, the potential for vigorous growth demonstrated by *Nasturtium* may explain its dominance in streams with excessive but temporally and spatially variable nutrient inputs, and its spread should be monitored closely in streams meeting these conditions in order to reduce ecological impacts. As *Nasturtium* continues to expand its range, the results of this study could prove useful for modeling its growth response under different nutrient regimes.

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