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The perils of unpalatable periphyton: *Didymosphenia* and other mucilaginous stalked diatoms as food for tadpoles

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Proliferations of *Didymosphenia geminata* are becoming prevalent in rivers around the globe. In the Sierra Nevada of California, *Didymosphenia* and other taxa that produce mucopolysaccharide stalks (e.g., *Gomphoneis*, *Cymbella*) can dominate benthic environments, particularly in the altered hydrologic and thermal regimes downstream of dams. We compared the prevalence of stalked diatoms in paired reaches, one free-flowing and the other regulated, within two Sierran river systems, the American and Feather Rivers. In the regulated reaches, short-term power generation caused daily flow fluctuations and periphyton biovolume was dominated by either *Didymosphenia* (where hypolimnetic releases created cool summer temperatures) or other stalked diatom taxa (where temperatures were warm). Periphyton assemblages from the unregulated sites were significantly different from the regulated reaches based on biovolume, with *Gomphonema* being the genus at unregulated sites contributing to the dissimilarities after accounting for the stalked genera from the regulated reaches. We evaluated the consequences of mucopolysaccharides for a large-bodied grazer, tadpoles of the foothill yellow-legged frog (*Rana boylii*), in a factorial experiment manipulating diet and thermal regime. At 16.6°C mean daily temperature, tadpoles lost weight (72 h relative change of $-16.1 \pm 7.2\%$) when grazing on periphyton from a *Didymosphenia*-dominated site. At 19.9°C (similar to unregulated river conditions), tadpoles grazed *Didymosphenia* at a rate similar to tadpoles consuming higher protein control periphyton, but the former tadpoles did not grow (relative change of $4.3 \pm 5.4\%$ vs $30.7 \pm 3.4\%$ for control periphyton). When tadpoles were fed periphyton dominated by mucilaginous stalked diatoms other than *Didymosphenia*, tadpole weight loss was $21.0 \pm 9.2\%$ (cool) and $16.6 \pm 5.6\%$ (warm). The results illustrate that hydrologically or thermally mediated shifts in periphyton composition can have significant implications for the energy transferred to grazers.

Keywords: *dams,* Didymosphenia, Epithemia, *food quality, power-peaking,* Rana boylii, *stalked diatoms, tadpole*

Introduction

Dams provide hydropower, along with other services, but they imperil riverine species by altering two master variables, flow regime and water temperature (Richter et al. 1997, Bunn & Arthington 2002, Olden & Naiman 2010). Altered flow and temperature have direct effects on aquatic fauna by decoupling the seasonal predictability of flow fluctuation and warming from the timing of reproduction. As a result, organisms that evolved life history strategies to avoid flood-induced mortality by breeding during seasons when flows are stable, are especially vulnerable to loss of early life stages when discharge fluctuates seasonally (Lytle & Poff 2004). Deep releases of reservoir water can be several degrees cooler than historic pre-dam temperatures (Angilletta et al. 2008) and further disrupt the seasonal synchrony between the recruitment of native fauna and the timing of favourable thermal and flow conditions (Olden & Naiman 2010, Catenazzi & Kupferberg 2013). Of the different modifications to flow, daily fluctuations to generate hydroelectricity for periods of peak demand are some of the most extreme (Cushman 1985, Arthington 2012). Such flow modification results in the loss of shallow, relatively warm, slow-velocity habitat conditions needed by the early life stages of a diverse array of benthic species, such as the glochidia of mussels and juveniles ready to settle (Layzer et al. 1993, Hardison and Layzer 2001), larvae of amphibians (Kupferberg et al. 2012) and fish fry (Freeman et al. 2001, Young et al. 2011).

Artificial flow and thermal regimes indirectly affect consumers by changing primary productivity and shifting benthic algal assemblage composition (Power et al. 1996, Cross et al. 2013). In rivers with hydroelectric projects that are managed to follow peak energy demands, primary productivity in periodically exposed zones can be greatly reduced compared with continuously wetted areas (Blinn et al. 1998, Bergey et al. 2010). Periphyton that

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survive periodic exposure often include mucilaginous taxa, e.g., *Oscillatoria* Vaucher ex Gomont (Benenati et al. 1998), which are avoided by herbivores such as amphipods (Shannon et al. 1994). Another species reported to proliferate in association with flow regulation, especially at high-elevation sites with cool water temperatures, is the stalk-producing diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt (Kirkwood et al. 2009, Kumar et al. 2009, Kilroy & Bothwell 2012). Changes in the species composition of epilithic periphyton towards stalk producers, such as *Didymosphenia*, may have differential effects on grazers and other invertebrate dwellers within the mucilage. For example, the abundance of small taxa, such as chironomid midges (Gillis & Chalifour 2010, Kilroy et al. 2009, James et al. 2010) and aquatic worms (Larson & Carreiro 2008) can increase where *Didymosphenia* mats proliferate. Additionally, the abundance of these invertebrates has been shown to increase with mat depth (Kilroy et al. 2009, Whitton et al. 2009). At the same time, the abundance of larger invertebrates, such as larval Ephemeroptera, Plecoptera and Tricoptera, decrease in response to *Didymosphenia* proliferation (Gillis & Chalifour 2010, Kilroy et al. 2009, James et al. 2010). By contrast, little is known about the responses of larger grazers, such as aquatic vertebrates. In this study, we focus on the effects of *Didymosphenia* and other stalked diatoms on tadpoles in relation to conditions altered by dam operations, especially cooler temperatures and daily variation in flow.

In the foothills of the Sierra Nevada in California, tadpoles of *Rana boylii* Baird, the foothill yellow-legged frog, can be a locally abundant grazer. Tadpoles of this species, which is endemic to the rivers of California and southern Oregon, are efficient scrapers of periphyton (Kupferberg 1997a) and rely on diets rich in diatoms (especially *Epithemia* spp.) to enhance development and growth (Kupferberg 1997b). Foothill yellow-legged frogs have declined over the last 50 years and are now absent from more than half of their historically occupied range (Davidson et al. 2002, Lind 2005). They are more often absent downstream of large dams where extreme flow fluctuation in spring causes scouring and stranding of egg masses (Kupferberg et al. 2012). Furthermore, bedrock geology and snowmelt hydrology of rivers in the Sierra Nevada can create water chemistry (Rost et al. 2011) and thermal conditions favourable for proliferations of *Didymosphenia*. Our objective is to assess the indirect consequences of large dams for *R. boylii*, mediated through changes in periphyton food resources. We compare the periphyton assemblages in rivers with daily flow fluctuations to the periphyton in unregulated rivers. Specifically, we quantify the prevalence of *Didymosphenia*, other mucilaginous stalk-producing diatoms (e.g., *Cymbella* Agardh and *Gomphoneis* Cleve), and more protein-rich diatom taxa (e.g., *Epithemia* Kützing and *Rhopalodia* Müller which contain N_2-fixing spheroid bodies). To understand the effects of altered thermal regime, altered flow regime and concomitant changes in periphyton species composition on tadpole grazing, we manipulated diatom species consumed in the laboratory under temperature conditions simulating regulated and unregulated rivers.

Materials and methods

Study system

We worked in the American and Feather River basins in the western foothills of the Sierra Nevada mountains (California, USA), where steep gradient rivers are favourable for the development of hydroelectric projects (Table A1). In the Middle Fork American River (MF American, hereafter termed cold-peaking reach) the river stage fluctuates by an average of 0.55 m (Placer County Water Agency 2010) when daily pulses from a powerhouse are timed to follow power demands and provide whitewater boating opportunities (Fig. 1). In a short reach downstream of the Rock Creek Powerhouse in the North Fork Feather River

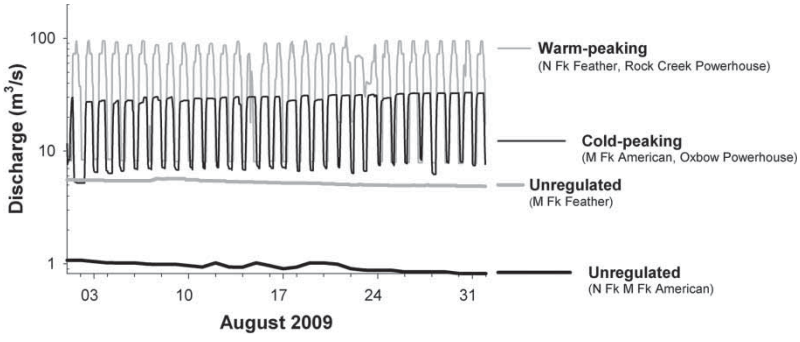

Fig. 1. August hydrographs for algal sampling river reaches. In a warm-peaking reach of the North Fork of the Feather River (N Fk Feather) flows fluctuate daily for power generation and summer daily mean water temperatures can reach 20°C (see Fig. 2). In a cold-peaking reach of the Middle Fork of the American River (M Fk American) flows fluctuate daily and summer daily mean temperatures reach 15–16°C. Middle Fork of the Feather River (M Fk Feather) and North Fork Middle Fork American (N Fk M Fk American) are both unregulated (i.e., free-flowing). The gauge on North N Fk M Fk American was discontinued by the United States Geological Survey, so daily means are plotted for the period of operation (1965–1985). Data from Placer County Water Agency 2010, Pacific Gas and Electric (unpublished), and the United States Geological Survey.

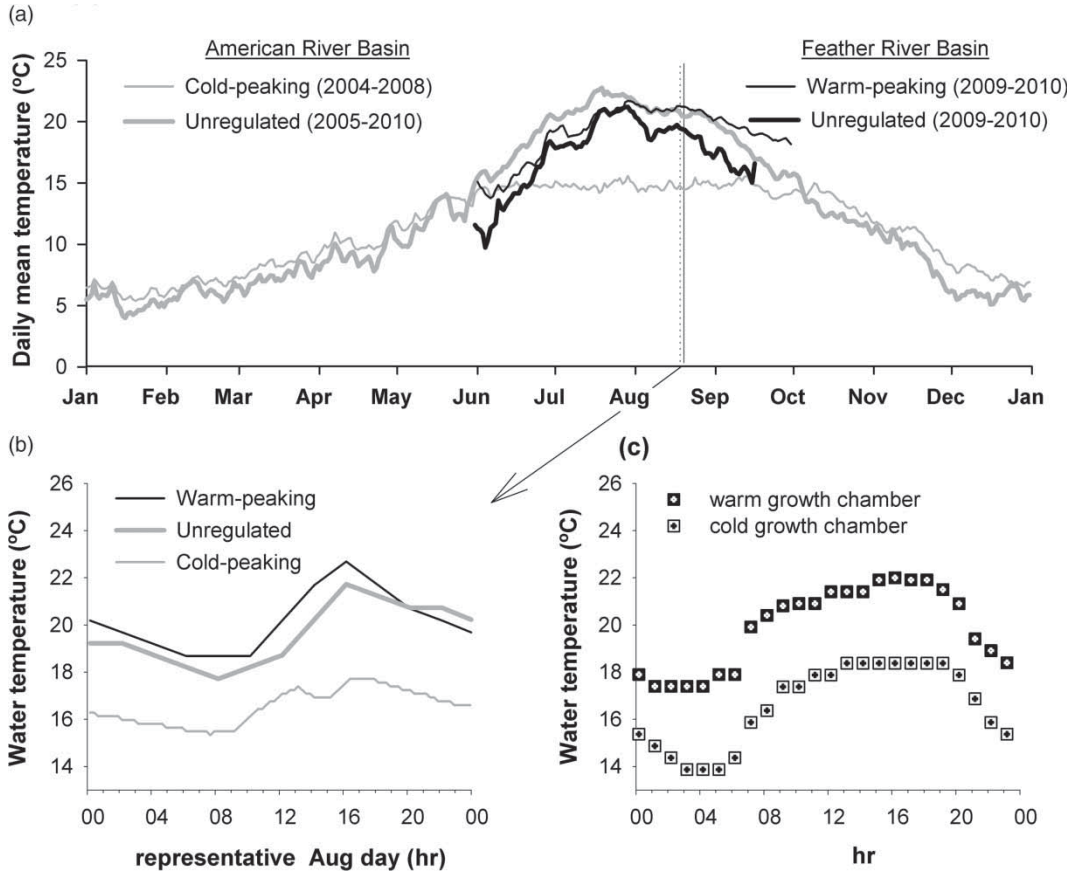

Fig. 2. Water temperatures in the four algal collection reaches of the American and Feather river systems (a); August daily variation in the cold-peaking Middle Fork American (b), the unregulated river reaches (b) and the aquaria in growth chambers (c). Data from Placer County Water Agency 2010, Pacific Gas and Electric (unpublished), the United States Geological Survey and thermisters placed by Kupferberg et al. (2011a).

(NF Feather, hereafter termed warm-peaking reach), power-peaking operations similarly create daily flow fluctuations. In two undammed rivers in these basins (the North Fork Middle Fork American River and the Middle Fork Feather River, hereafter termed unregulated reaches), summer flows are consistently low (Fig. 1). Water temperature data show that the thermal regime in the cold-peaking reach is 4–6°C cooler in summer compared with the other study reaches (Fig. 2).

Algae collection and analysis

During the late summers of 2009 and 2010 we sampled three sites in each of four study river reaches (Table A1). At each of the 12 sampling sites we scraped periphyton from three rocks in the varial zone (where water levels fluctuate in the two power-peaking reaches), and three in the 'thalweg' (fully wetted zone, all four river reaches). From an area delineated by a 23 × 34 mm frame, we removed periphyton using a toothbrush and suctioned with a pipette until no biofilm was macroscopically visible. In 2009, we topped up samples with river water to 40 mL and transported under refrigeration. We processed samples for 30 s on the low setting of an Osterizer® Blender before subsampling. Rock scrapes at the warm-peaking sites were pooled prior to analysis. We preserved 2–5 mL aliquots in ∼2% formalin for quantitative algal analysis. In 2010, we topped up samples with river water to 38 mL and added 2 mL of formalin (total volume = 40 mL). We removed the formaldehyde solution prior to microscope analysis by centrifuging samples at 4000 r.p.m. for 1 min (Fisher Accuspin Micro 17) and replaced the supernatant with deionized water twice. To determine algal density, cells were resuspended in distilled water and transferred to Palmer–Maloney counting chambers (Wildco Wildlife Supply, Buffalo, NY, USA). Algae were counted based on the lowest reasonable taxonomic unit (e.g., sp. 1). We counted a minimum of 300 cells/counting units at ×400 using Nikon Optiphot (Nikon Corporation, Japan) and Leica DM LS2 (Leica Microsystems, Germany) photomicroscopes. Where algal cells were sparse and a 300-cell count could not reasonably be achieved, we counted 100 fields of view. A whole cell was considered to be a

counting unit for all algae, except for filamentous cyanobacteria where one 10 μm length was considered to be counting unit. To determine the density of *Didymosphenia* more effectively, cells were also enumerated at ×100 from the entire area of a Palmer–Maloney counting chamber filled three separate times. Biovolume estimates were calculated from geometric shapes (Hillebrand et al. 1999) based on measurements taken at ×1000 magnification of 30 cells or counting units for the dominant taxa and five to ten cells for rare taxa.

For presentation purposes, we combined taxa into the following groups: *Didymosphenia*; other stalked diatoms – capable of producing mucilaginous stalks or tubes (*Encyonema* Kützing, *Cymbella*, *Gomphonema* Ehrenberg, *Gomphoneis*); diatoms with N_2-fixing endosymbionts – Rhopalodiaceae which contain cyanobacteria-derived spheroid bodies (*Epithemia* spp., *Rhopalodia gibba* (Ehrenberg) O. Müller); small diatoms – (e.g., *Achnanthidium minutissimum* (Kützing) Czarnecki); Synedra/Fragilaria (*Synedra* Ehrenberg & *Fragilaria* Lyngbye); Naviculoid/Nitzschioid diatoms – motile diatoms (*Navicula* Bory de Saint-Vincent & *Nitzschia* Hassall); other diatoms (*Cocconeis* Ehrenberg, *Cyclotella* (Kützing) Brébisson, *Diatoma* Candolle in Lamark & A.P. de Candolle, *Melosira* Agardh and *Rhoicosphenia* Grunow); Chlorophyta – green algae (*Cladophora* Kützing, *Desmodesmus* (Chodat) An, Friedl & Hegewald, *Oedogonium* Kützing ex Hirn, *Spirogyra* Link, *Stigeoclonium* Kützing, *Ulothrix* Kützing, and *Zygnema* Agardh); and Cyanobacteria (*Calothrix* Agardh ex Bornet & Flahault, *Nostoc* Vaucher ex Bornet & Flahault, *Tolypothrix* Kützing ex Bornet & Flahault and other filamentous cyanobacteria).

Based on the lowest taxonomic unit, we examined periphyton clustering between rivers and zones (varial and wetted) within rivers using non-metric multidimensional scaling (MDS) ordination of Bray–Curtis similarities from standardized, $\sqrt{}$-transformed abundance and biomass of taxa (Primer 6, v6.1.15; Primer-E Ltd., 2012). To ensure sufficient data for ordination, we excluded taxa that were only present in one or two samples. Points were examined based on factors of unregulated sites and the varial and wetted zones of cold- and warm-peaking sites. Analysis of similarity (ANOSIM) routines were derived from the similarity matrix to first determine if there were differences in periphyton between areas from the unregulated sites in the American and Feather River watersheds, and between the varial and wetted zones within each regulated site. Holm-Bonferroni corrections were applied to post hoc pairwise comparisons. Because there were no significant differences in periphyton between sites from different watersheds of the unregulated rivers and between the varial and wetted zones within a regulated site, data were pooled for subsequent analyses comparing sites. ANOSIM *R* values were explored (larger values indicating greater separation of algal assemblages). Similarity percentage analyses (SIMPER) were used to determine which taxa contributed the most to similarities within a site and differences between sites.

Food quality experiment

We assayed food quality in the power-peaking study reaches using a short-term two-factor tadpole rearing experiment (three levels of periphyton source × two levels of temperature). The assay duration was brief because previous rearing trials in unregulated rivers have documented that short-term growth responses to algal diet manipulation are consistent with longer term responses such as time to and size at metamorphosis (Kupferberg et al. 1994, Kupferberg 1997a, Catenazzi and Kupferberg 2013). We collected embryos from a single clutch of eggs on 16 June 2010 from the MF American (Latitude 39.007558°N, Longitude 120.731403°W), and reared the full sibling group at the UC Berkeley Richmond Field Station in 85 L tubs. We conducted the feeding experiment between 26 and 29 August 2010 when the pre-metamorphic tadpoles' hind limbs were still small, and toes not fully differentiated (median stage=34, Gosner 1960). During the trial, we housed tadpoles individually in aquaria (5 L capacity) placed in diurnal illumination incubators, Shel Lab Model LI 15: (5 replicates × 3 food treatments in the cold chamber) + (4 replicates × 3 food treatments in the warm chamber) = 27 total. The photoperiod was 14 h light, 10 h dark. Cool conditions (16.6°C daily mean) mimicked the study reach of a river receiving hypolimnetic releases from an upstream reservoir and warm conditions (19.9°C) mimicked unregulated conditions (Fig. 2). Each aerated aquarium included a periphyton-covered cobble (median diameter 130 – 160 mm) transported from the *Didymosphenia*-dominant cold-peaking reach, the warm-peaking reach where other stalked diatoms dominated, or a warm control site (Alameda Ck.) where *Epithemia* spp. and chlorophytes dominate and tadpoles are known to grow to relatively large sizes (Kupferberg et al. 2011b). In the warm treatment, *Didymosphenia* was kept at ~ 4°C above ambient conditions relative to where it was collected. To assess differences among the three periphyton treatments, we conducted one-way analyses of variance (ANOVAs) of ash-free dry mass (AFDM mg cm^{-2}) and per cent organic material (AFDM / total Dry Mass (DM)) scraped from a 23 × 34 mm area on a subset of cobbles used in the experiment ($n = 17$). We filtered samples onto pre-ashed, pre-weighed 4.7 cm GF/C filters, dried at 60°C for ≥ 48 h (Precision Scientific Economy Oven: CAT 51220135 45EM), then weighed to 0.0001 g (Mettler AT201 Scale), ashed for 1.5 h at 500°C (Fisher Isotemp® Muffle Furnace), maintained in an oven ≥ 48 h then weighed upon cooling. Because cell counts do not correlate with the amount of stalk material produced (Kilroy & Bothwell 2011), AFDM was considered a better estimate of stalk production. The UC Davis Analytical Laboratory (http://anlab.ucdavis.edu) conducted analyses of total nitrogen, % protein, % crude fat and % silica. The

quantity required (50 g dried) necessitated that we scrape entire cobbles at the end of the experiment and pool cobbles from each source.

The response variables were tadpole growth [proportional change in weight = $(g_{end}–g_{start})/g_{start}$] and an index of food consumption, egestion standardized by tadpole body size (mg fecal dry mass/mm body length/h). We used a two-way ANOVA to assess the main effects of periphyton and temperature, and the interaction between periphyton and temperature. We measured body length to the nearest 0.1 mm using dial calipers and weighed each tadpole to the nearest 0.1 g after blotting excess moisture and placing in a container of water on an OHaus® electronic balance. For each tadpole, we used pipettes to collect feces as it was produced (to prevent coprophagy) every five minutes over the course of one hour. We placed feces on pre-weighed filters, dried at 60°C for 48 h and weighed to the nearest 0.1 mg. We also preserved feces for microscopic analysis. To determine if the diatoms consumed by tadpoles were eaten in proportion to their relative abundance, we compared the % biovolume in feces to % biovolume in rock scrapings, for the most common groups comprising the fecal content using *T*-tests with Holm-Bonferroni corrections.

Results

Epilithic algal assemblages

Periphyton assemblages were similar between the unregulated reaches of the American and Feather River systems, and between the varial and wetted zones of each regulated reach (Table 1, $ANOSIM_{1+2}$). Cold-peaking sites clustered together, whereas warm-peaking and unregulated river sites were more variable in the MDS analysis (Fig. 3). Periphyton assemblages from the cold-peaking sites were significantly different from the unregulated and warm-peaking sites based on both abundance and biovolume (Table 1, $ANOSIM_{3+4}$). Periphyton assemblages from the unregulated sites were similar to the warm-peaking sites based on abundance, but were significantly different based on biovolume (Table 1, $ANOSIM_{3+4}$). The best discriminating genera for biovolume were *Gomphoneis*, which was characteristic of the warm-peaking sites, and *Gomphonema*,

Table 1. Posthoc comparisons from an analysis of similarity (ANOSIM) derived from the Bray–Curtis similarity of standardized, √-transformed abundance ($ANOSIM_{1+3}$) and biomass ($ANOSIM_{2+4}$) of periphyton. $ANOSIM_{1+2}$ examined similarities between unregulated sites from different watersheds and the varial and wetted zones within regulated sites. To account for multiple comparisons, the *p* values for the post hoc comparisons were assessed with Holm's sequential Bonferroni procedure.

			Abundance		Biovolume	
			$ANOSIM_1$		$ANOSIM_2$	
Site categorization			Global $R = 0.664$; $p = 0.001$		Global $R = 0.734$; $p = 0.001$	
			R	*p*	*R*	*p*
Unregulated 1	vs.	Unregulated 2	0.250	0.333	0.000	1.00
Cold-peaking-varial	vs.	Cold-peaking-wetted	−0.032	0.563	0.040	0.325
Warm-peaking-varial	vs.	Warm-peaking-wetted	−0.500	1.000	−0.500	1.00
			$ANOSIM_3$ Global $R = 0.806$; $p = 0.001$		$ANOSIM_4$ Global $R = 0.937$; $p = 0.001$	
			R	*p*	*R*	*p*
Unregulated	vs.	Cold-peaking	0.857	**0.001**	0.960	**0.002**
Unregulated	vs.	Warm-peaking	0.056	0.321	0.604	**0.029**
Warm-peaking	vs.	Cold-peaking	0.942	**0.003**	0.988	**0.002**

Note: Significant *p* values (post hoc) are given in bold.

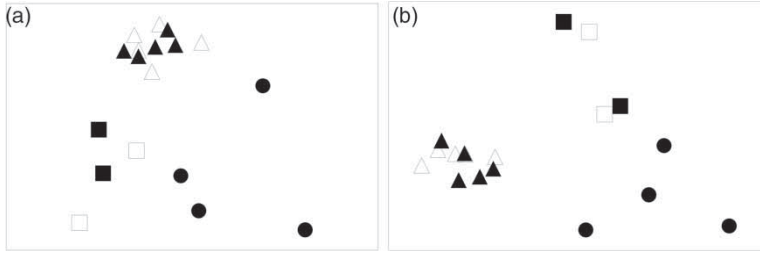

Fig. 3. A two-dimensional non-metric multidimensional scaling (MDS) ordination of Bray–Curtis similarities from standardized, √-transformed taxa abundance (a) and biomass (b) from the cold-peaking varial (△) and wetted (▲) zones, the warm-peaking varial (□) and wetted (■) zones, and the unregulated (●) sampling sites. Stress values = 0.12 for both figures.

Table 2. Results from the SIMPER routine. Taxon groupings contributing >10% to similarity or dissimilarity are included, unless all values were <10%, then the top two contributors were included.

		Abundance					
		Average similarity (%)			Average dissimilarity (%)		
		Unreg.	Cold-P	Warm-P	Unreg. vs Cold-P	Unreg. vs Warm-P	Warm-P vs Cold-P
	Total assemblage	37.0	64.4	49.0	68.1	62.2	63.7
Diatoms	*Ach. minutissimum*		29.8		12.9		16.8
	Epithemia sorex	10.6					
	Fragilaria spp.			9.8		7.2	10.8
	Gomphonema sp.	17.1					
	Other small diatoms	15.8	10.8				
Cyanobacteria	*Calothrix*	16.1			10.5	10.1	
	Cyanobacterial fil.	11.0		24.7			10.3
		Biovolume					
	Total assemblage	31.8	69.4	44.7	80.0	73.6	73.0
Diatoms	*Diatoma vulgaris*			11.2			
	Didymosphenia		52.8		25.3		27.8
	Epithemia sorex	15.2		11.5			
	Gomphoneis			9.6		7.8	
	Gomphonema sp.	47.0			9.8	9.3	
	Synedra sp. 2		12.8	14.6			

Notes: Unreg.: unregulated; P: peaking; *Ach.*: *Achnanthidium*.

Table 3. Nutritional analysis of pooled samples of periphyton and biomass density (ash free dry mass, AFDM) sub-sampled from individual cobbles used in feeding experiment.

Source site	% Total N	% Protein	% Crude fat	% Total Si	AFDM mg cm^{-2} mean (SE)	% Organic material mean (SE)
Control.	1.64	10.3	0.39	8.32	3.2 (1.1)	45.7 (4.1)^{b}
Cold-peaking	0.26	1.6	< 0.25	13.51	4.4 (0.7)	10.7 (0.89)^{c}
Warm-peaking	0.86	5.4	< 0.25	16.21	8.5 (0.6)^{a}	20.6 (1.5)^{d}

Notes: $^{a-d}$Superscripts denote significant ($p < 0.05$) differences among sources in post hoc multiple comparison *t*-tests (ANOVA of AFDM $F_{2,16} = 10.5$, $p = 0.002$; ANOVA on angular transformation of % organic material $F_{2,16} = 70.4$, $p < 0.001$).

which was characteristic of the unregulated sites (Table 2). Mats of stalked periphyton were generally 2–4 cm in height at the warm-peaking site and 2–3 cm high at the cold-peaking sites (personal observation). Periphyton from the warm-peaking sites had higher AFDM compared with that from the cold-peaking sites (Table 3).

Algal taxa present in the unregulated sites were generally also found in the regulated, warm-peaking sites but in different relative abundances (Fig. 4). In the unregulated sites, the diatoms *Epithemia sorex* Kützing, *Gomphonema* sp., and small diatoms, along with the cyanobacteria *Calothrix* and miscellaneous filaments dominated the taxonomic SIMPER results for periphyton abundance; for periphyton biovolume, *Gomphonema* sp. and *E. sorex* dominated (Table 2). By contrast, at the warm-peaking sites, cyanobacteria filaments dominated the SIMPER results for abundance and the diatoms: *Diatoma vulgaris* Bory, *E. sorex*, *Gomphoneis* and *Synedra*, for biovolume (Table 2). The diatoms with the N_2-fixing cyanobacterial endosymbionts were more common in the unregulated and warm-peaking sites than the cold-peaking sites (Fig. 4; Table 2).

Didymosphenia was only present in the cold-peaking sites and dominated the relative biovolume of periphyton in both the varial and wetted zones (Fig. 4b). *Didymosphenia* dominated the SIMPER results for the biovolume of periphyton, and was the best discriminating genus when comparing assemblages with the unregulated and warm-peaking sites (Table 2). *Achnanthidium minutissimum* dominated the SIMPER results for periphyton abundance and was the best discriminating species when comparing assemblages with the unregulated and warm-peaking sites (Table 2; Fig. 4a).

Tadpole feeding experiment

Algal content in the fecal pellets (Fig. 5) indicates that tadpoles generally consumed the periphyton available (Fig. 4).

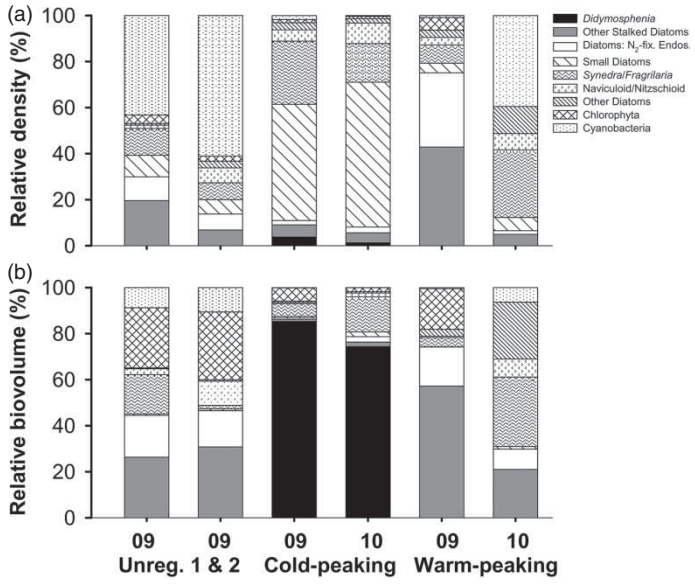

Fig. 4. Mean relative abundance (a) and relative biovolume (b) of epilithic algae from unregulated (Unreg.) sites from 2009 and from regulated sites from 2009 and 2010. Diatoms: N_2-fix; Endos.: Diatoms with N_2-fixing cyanobacterial endosymbionts; Unreg. 1: sites within the same river system as cold-peaking sites; Unreg. 2: sites within the same river system as warm-peaking sites.

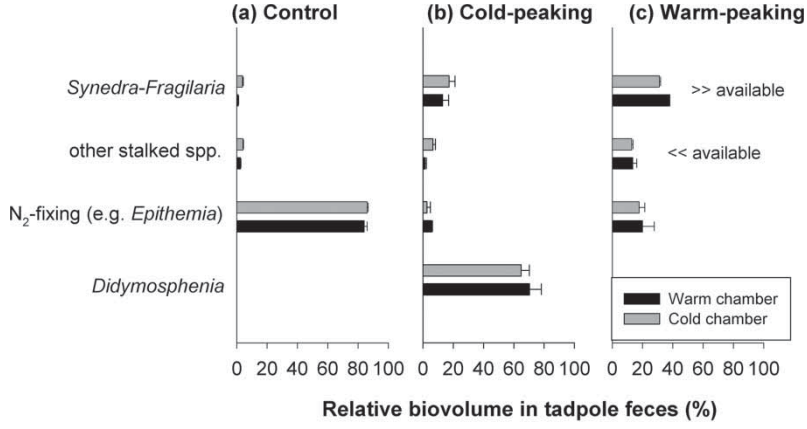

Fig. 5. Relative biovolume of the most common components of fecal algae collected from tadpoles in cold or warm treatments and fed periphyton from (a) control (Alameda Ck); (b) cold-peaking=MF American; and (c) warm-peaking=NF Feather River (where << and >> indicate diatom groups consumed out of proportion with availability). N_2-fixing: diatoms with N_2-fixing cyanobacterial endosymbionts.

Stalk material was observed undigested in fecal pellets from both power-peaking river reaches. Overall, diatom frustules found in the fecal pellets were empty (devoid of cytoplasm) or partially broken, indicating digestion, although dead diatom cells may also have been consumed during grazing. On the control diet, tadpoles mostly ate diatoms with N_2-fixing cyanobacterial endosymbionts, e.g., *Epithemia* spp. (Fig. 5). *Didymosphenia* dominated the algal biovolume (Fig. 5) in the fecal pellets of tadpoles fed periphyton from the cold-peaking reach of the river, but *A. minutissimum* was also present (Fig. A1), indicating that stalk material was consumed. When fed periphyton from the warm-peaking reach, the most common diatoms with respect to fecal biovolume were *Synedra* and *Fragilaria*, and these were significantly more abundant than in the rock scrapings ($T = -7.15$, $df = 5$, $p \ll 0.01$). Although stalked diatoms comprised one quarter of the rock scraping biovolume, relative fecal biovolumes were $13.6 \pm 2.5\%$ (warm) and $12.0 \pm 0.8\%$ (cold) ($T = 10.2$, $df = 5$, $p \ll 0.01$).

With respect to tadpole growth (Fig. 6a), the main effects of temperature and periphyton source were significant (temperature $F_{1,21} = 35.4$, $p < 0.001$; periphyton $F_{2,21} = 40.1$, $p < 0.001$). While consuming control periphyton, which was highest in protein (Table 3), tadpoles in the cool treatment maintained their weight (i.e., proportional change not significantly different from zero, $T = 0.14$, $p = 0.45$). When consuming *Didymosphenia*, which was low in protein (Table 3), tadpoles lost weight in the cool treatment. In the warm treatment, tadpoles eating *Didymosphenia* had a 72 h relative weight gain of $4.3 \pm 5.4\%$, compared with $30.7 \pm 3.4\%$ for tadpoles grazing control periphyton. Tadpoles lost weight regardless of temperature when consuming

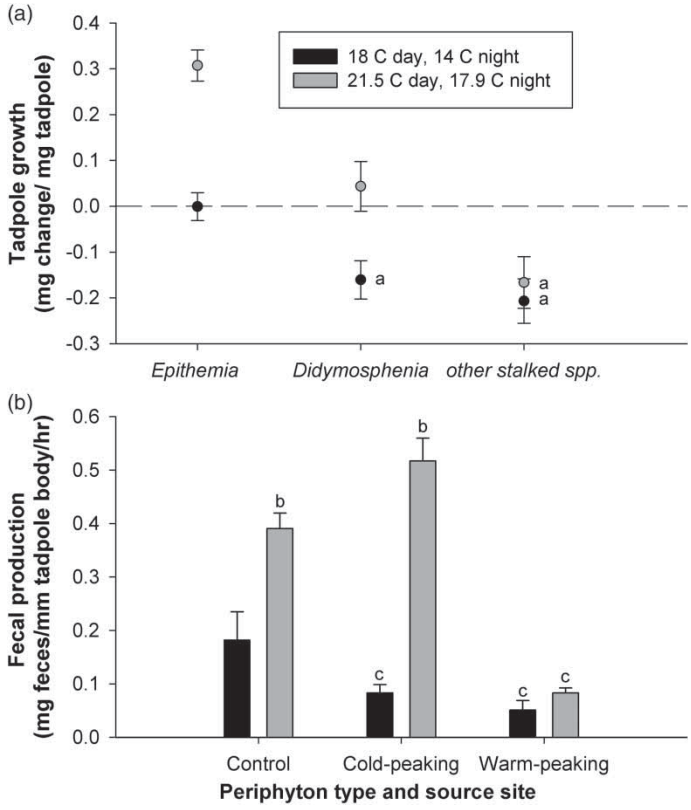

Fig. 6. Growth (a) and rate of fecal production (b) by tadpoles of *Rana boylii* fed diets rich in *Epithemia* from a warm unregulated river, *Didymosphenia* from a cold-peaking reach of a river, or other mucilaginous stalked diatoms from a warm-peaking reach. Lower case letters indicate treatments that were not significantly different from each other in post hoc multiple comparisons.

warm-peaking periphyton, which was dominated by stalked diatoms other than *Didymosphenia*, -21.0 ± 9.2 (cool treatment) and $-16.6 \pm 5.6\%$ (warm). This response contributed to a significant Periphyton Source × Temperature interaction ($F_{2,21} = 6.5, p = 0.006$).

Using production of feces as an index of consumption, periphyton source had a significant effect ($F_{2,21} = 24.7$, $p < 0.001$). Tadpoles ingested *Didymosphenia* within the warm treatment at a rate similar to tadpoles consuming control periphyton (Fig. 6b, post hoc Holm–Sidak comparison $p = 0.1$), and consumed two to three times more periphyton in the warm treatment than in the cold treatment ($F_{1,21} = 50.3, p < 0.001$). Periphyton from the warm power peaking site was not appreciably consumed regardless of temperature, as indicated by the significant interaction of periphyton source×temperature ($F_{2,21} = 9.3, p = 0.001$).

Discussion

At the sites downstream of power facilities where flow fluctuated daily (Fig. 1), the algal assemblages shifted to include a greater proportion of biovolume from stalk-producing diatom taxa and less from N_2-fixing taxa relative to assemblages at unregulated sites. Because of its large cell size, *Didymosphenia* dominated the biovolume of periphyton, whereas *A. minutissimum* that grows densely on *Didymosphenia* stalks (Kilroy et al. 2009, Whitton et al. 2009) was numerically most abundant. This result was observed in the MF American where both master variables, flow and temperature, were altered (Fig. 2, Table 2). However, mat depth and AFDM were low relative to reports from other rivers (discussed in Spaulding & Elwell 2007; Whitton et al. 2009), where phosphorus-poor conditions can promote substantial stalk production (Kirkwood et al. 2009, Kilroy & Bothwell 2011, 2012, Bothwell et al., 2014). By contrast, the warm-peaking site was dominated by other stalked genera, such as *Gomphoneis* (Fig. 4), and had greater AFDM cm^{-2} (Table 3) than the cold-peaking *Didymosphenia*-dominated site, despite having a smaller total biovolume of diatom cells (average of varial and wetted samples from warm-peaking site $2010 = 7.37 \times 10^7$ vs cold peaking $= 2.36 \times 10^9$ μm^3 cm^{-2}; Table A2). The combination of higher AFDM with lower cell biovolume may indicate that the other stalked diatoms produced more extracellular stalk material than *Didymosphenia.* The shift toward mucilaginous, stalk-producing diatoms occurred in both the varial zone, as well as in the wetted zone.

Although the stressors differ between the varial and wetted zones, mucilage may be adaptive in both zones and may explain the lack of significant differences in periphyton composition between the two zones. Changes in river stage regularly expose periphyton in the varial zone to drying,

whereas increased current velocity affects periphyton in the wetted zone. In the varial zone, mucilage may confer resistance to desiccation because it retains moisture, acts as reservoir for water, and lessens osmotic stress to cells during drying (Shepard 1987, Hoagland et al. 1993). Mucilage secretion may also be stimulated with periodic desiccation (Peterson 1987). Stalks may increase surface area, which has been shown to slow the rate of dehydration in marine algae (Dromgoole 1980). Alternatively, diatom cells may have tolerated episodic exposure downstream of the powerhouses because low flows occur between midnight and early morning, when demand for electricity is low and powerhouses are off-line. Bergey et al. (2010) found that reductions in chlorophyll *a* due to aerial exposure were less if exposure occurred at night rather than during the day. Circadian rhythms exhibited by algae, such as the timing of cell division, gene expression or other cellular processes (Suzuki & Johnson 2001) may further facilitate night-time survival under desiccating conditions. In the fully wetted zone, mucilaginous mats may resist dislodgment when velocity and shear stress increase (Biggs et al. 1998). Elevated current velocity has been shown to increase stalk length and mucilage production in diatom taxa such as *Gomphoneis herculeana* (Ehrenberg) Cleve (Biggs & Hickey 1994).

While mucilage serves many functions, including adhesion to substratum, motility, binding sediment and desiccation resistance (Hoagland et al. 1993), its deterrent effect on grazing (Power et al. 1988, Reynolds 2007) is relevant for understanding the trophic consequences of proliferations of stalked diatoms. The shifts toward mucilaginous diatoms with fluctuating flow had negative consequences for tadpole growth (Fig. 6). Microscopic examination of tadpole fecal pellets (Fig. 5) revealed that *Epithemia* dominated the diet in the high food quality control treatment. In this treatment, tadpoles gained body mass in warm temperatures and maintained body weight in cold temperatures. *Epithemia* cells are loosely associated with the periphyton matrix (not attached), and may be more readily consumed than other taxa (Steinman 1996, Furey et al. 2012). In the cold treatment, *Didymosphenia* mats remained intact. Tadpoles ingested little material and lost weight. In the warm treatment, *Didymosphenia* mats sloughed and tadpoles passed similar amounts of biomass through their guts as when fed the control diet. The warmer conditions may have caused *Didymosphenia* cells to detach from their stalks and the cells may have been more readily grazed. Broken, empty *Didymosphenia* frustules in feces indicate that cells were digested, but tadpoles did not gain weight. Our observation of *Didymosphenia* cells being digested is consistent with other investigations of interspecific variation in diatom digestibility associated with differences in cell size, shape and degree of silicification. Taxa with large surface areas, and presumably greater contact with digestive enzymes, appear to have low resistance to digestion (Underwood & Thomas 1990, Peterson & Boulton 1999, Peterson & Jones 2003). By contrast, the stalked diatom assemblage from the NF Feather appeared resistant to grazing regardless of temperature.

The result that cool water temperature exacerbated the effects of poor nutritional value on tadpole growth highlights the importance of considering food resources when assessing the effects of altered thermal and flow regimes. *Didymosphenia* and the other mucilaginous diatoms were relatively low in total nitrogen and per cent protein compared with the periphyton dominated by *Epithemia* spp. (Table 3). High levels of dietary protein enhance growth, development and survival, as demonstrated by tadpoles reared on algal diets naturally high in protein or artificial diets enhanced with animal protein (Steinwachser & Travis 1983; Pandian & Marian 1985; Martinez et al. 1993, Kupferberg 1997a). For algal grazing tadpoles specifically (Skelly & Golon 2003), and algivorous vertebrates generally (Wikelski et al. 1993, Clements et al. 2009), warm temperatures that are optimal for consumption rate, food conversion efficiency and growth determine individual fitness and species distribution. The benefits of operating at optimal temperatures may be greater in developing organisms, such as tadpoles, than in adults because temperature (Berven & Gill 1983, Harkey & Semlitsch 1988) and food quality (Kupferberg et al. 1994, Alvarez & Nicieza 2002) directly influence differentiation and time to metamorphosis. Given the rate of weight loss on diets of mucilaginous diatoms in the cool treatment, tadpoles would not have survived until metamorphosis. Like riverine fish, which depend on survival of early life stages for population viability (Strange et al. 1993, Humphries & Lake 2000), analyses conducted for *R. boylii* indicate that extirpation probabilities are sensitive to early life stage survival (Kupferberg et al. 2009). By integrating the impacts of local diatom assemblage composition and temperature, our simple experiment illustrates a mechanistic link from altered thermal and hydrologic regimes to concomitant changes in diatom assemblages and frog populations.

Although there is a growing appreciation that ecologically sustainable dam management should evolve to include considerations of both water quantity and its thermal quality (Olden & Naiman 2010), our results suggest that the food quality of periphyton can also be a central factor for maintaining functional food webs and biodiversity conservation. The documented association among hydropower projects, *Didymosphenia* abundance, and frequency of mat proliferations (Kirkwood et al. 2009) adds complexity to understanding how multiple stressors affect grazers. Given the impaired growth of tadpoles on diets from sites where mucilaginous stalk production was low to moderate, the trophic implications of increasing prevalence of *Didymosphenia* reported around the globe (Spaulding & Elwell 2007, Blanco & Ector 2009), could be stronger than we observed. Under conditions where periphyton mats produce abundant mucilage, food resource limitation for other large grazers, such as algivorous fish, could be a great concern.

Global change scenarios in which water temperature, river flow or nutrients would cause shifts in the species composition of algal assemblages or promote the production of mucilaginous stalk material could come at a cost to large grazers.

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Appendix

Table A1. Location of river reaches and diatom sampling sites and temperature monitoring locations in the Sierra Nevada mountains of California.

Temperature and Regulation Category*	Fork of River – Location (County)	Elevation (m)	Frog breeding	Algal collection (number of sites, year)	Temperature monitoring (number of sites, years)†	Sample type
Cold-peaking	Middle Fork American – downstream of Oxbow and Ralston powerhouses[3] (Placer/Eldorado)	165–175	Absent	3, 2009–10	2004–2008	Varial Zone Fully Wetted
Unregulated	North Fork of the Middle Fork American (Placer/Eldorado)	322–460	Present	3, 2010	3, 2009–10	Fully Wetted
Warm-peaking	North Fork Feather downstream of Rock Creek powerhouse‡(Plumas)	518	Absent	3, 2009–10	2009–10	Varial Zone Fully Wetted
Unregulated	Middle Fork Feather (Plumas)	462–593	Present	3, 2010	3, 2009–10	Fully Wetted

Notes: *Regulation status: peaking means flows fluctuate from very low to very high over a 24-h period for hydroelectric power generation; unregulated means no upstream powerhouses or diversions. †Temperature data sources: Pacific Gas and Electric for Rock Creek Reach, N. Fk. Feather; Placer County Water Agency (2010) for American River sites, and Kupferberg et al. (2011). ‡Rocks from the wetted zones of these sites were used as sources for the rearing experiment.

Table A2. Density (units cm^{-2}) and biovolume ($\mu m^3\ cm^{-2}$) parsed by diatom groups.

River	North Fork Middle Fork of the American	Middle Fork American				Middle Fork Feather	North Fork Feather			
Code	Unregulated	Cold peaking				Unregulated	Warm Peaking			
Year	2009	2009		2010		2009	2009		2010	
		Varial	Wetted	Varial	Wetted		Varial	Wetted	Varial	Wetted
Taxon group	AVG±SE	AVG±SE	AVG±SE	AVG±SE	AVG±SE	AVG±SE	***	***	***	***
Density (units cm^{-2})										
*Didymosphenia**		19 348±2143	1615±124	7549±2472	7847±3033					
*Didymosphenia***	0±0	32 273±8562	4596±0	22 835±10 662	16 655±8145	0±0	0	0	0	0
Other stalked diatoms	3783±688	39 066±9427	6051±689	75 419±47 646	51 574±28 033	1376±344	431 224	69 421	8994	11 115
Diatoms with N_2-fixing endosymbionts	1203±860	9921±7945	4060±1302	33 448±5608	23 708±14 904	1548±1204	93 105	194 650	4197	2084
Small diatoms	1376±344	266 749±39 518	107 159±45 269	924 969±392 876	710 382±306 824	1376±1032	0	32 669	11 392	11 810
Synedra/ Fragilaria	1204±1204	242 338±19 965	33 550±18 536	199 563±85 055	200 712±81 277	1720±1720	34 302	40 836	77 946	38 208
Naviculoid/ Nitzschioid	688±688	41 330±9715	6434±2757	146 175±85 536	119 274±54 630	1204±172	19 601	13 612	11 392	17 367
Other diatoms	344±344	22 517±10 662	5592±2834	20 744±4896	20 469±8617	688±688	24 501	9528	14 990	33 345
Chlorophyta	516±172	7369±5393	2604±460	12 704±7244	7604±3484	516±516	31 852	23 140	0	0
Cyanobacteria	20 462±20 119	3828±3828	7200±6587	643±643	1103±1103	11 349±1720	0	6806	62 357	98 646
Total	**24 590±19 259**	**665 390±100 278**	**177 246±77 057**	**14 36 500±617 753**	**1 151 479±498 864**	**19 776±3611**	**634 586**	**390 661**	**191 268**	**212 576**
Biovol. ($\mu m^3\ cm^{-2}$)										
*Didymosphenia**		$1.34\times10^8\pm2.01\times10^7$	$1.53\times10^8\pm1.18\times10^7$	$7.16\times10^8\pm2.34\times10^8$	$7.44\times10^8\pm2.87\times10^8$					
*Didymosphenia***	0±0	$3.05\times10^9\pm8.12\times10^8$	$4.3\times10^8\pm0$	$2.16\times10^9\pm1.01\times10^9$	$1.58\times10^9\pm7.72\times10^8$	0±0	0	0	0	0
Other stalked diatoms	3 337 826±769 099	14 346 977±10 069 132	2 911 734±1 802 430	35 551 811±18 142 762	25 228 195±11 361 905	1 791 791±30 936	824 577 659	635 163 232	15 310 720	14 848 535
Diatoms with N_2-fixing endosymbionts	2 558 463±2 075 788	139 238 29±11 150 618	5 697 734±1 827 575	46 496 674±7 831 532	32 378 051±20 066 468	1 959 155±1 582 921	130 673 691	283 160 067	8 921 117	4 465 583
Small diatoms	87 606±28 085	14 259 725±2 097 984	5 839 813±2 529 527	48 963 057±20 757 420	38 292 003±16 663 898	91 139±66 587	0	1 996 553	813 279	828 820
Synedra/ Fragilaria	2 455 350±2 455 350	138 601 878±57 878 550	40 100 117±17 382 046	187 808 772±70 922 579	295 378 884±150 043 878	261 411±261 411	59 396 118	43 722 839	34 586 063	12 308 853
Naviculoid/ Nitzschioid	298 017±298 017	17 256 762±8 166 402	3 164 892±1 413 087	48 271 473±28 428 109	41 678 311±19 249 302	506 927±171 286	8 492 811	5 368 751	5 207 875	5 921 964
Other diatoms	49 322±49 322	15 160 621±10 218 819	3 605 128±971 980	6 726 435±1 166 144	12 044 275±4 471 612	98 643±98 643	66 258 273	16 109 197	17 547 710	17 832 663
Chlorophyta	3 272 279±1 131 333	181 872 751±104 849 947	36 142 922±6 773 137	27 777 954±20 193 111	11 770 364±3 162 695	5 027 158±5 027 158	298 739 731	160 239 558	0	0
Cyanobacteria	1 040 318±1 023 123	3 867 447±677 163	379 156±348 517	32 162±32 162	110 269±110 269	567 446±85 977	6 125 344	5 036 394	3 687 448	5 105 984
Total	$1.31\times10^6\pm1.29\times10^6$	$3.47\times10^9\pm9.99\times10^8$	$5.35\times10^8\pm2.93\times10^7$	$2.57\times10^9\pm11.1\times10^9$	$2.04\times10^9\pm8.84\times10^8$	$1.03\times10^7\pm6.75\times10^6$	1.46×10^9	1.17×10^9	8.61×10^7	6.13×10^7

Note: *_Didymosphenia_ enumeration based on cells counted at ×100 from the entire area of a Palmer–Maloney counting chamber filled three separate times.
**_Didymosphenia_ enumeration based on field of view counts at ×400 conducted until a minimum of 300 algal counting units observed.
***Rock scrapes at these sites were pooled, so AVG not calculated but field variability in algal samples was captured.

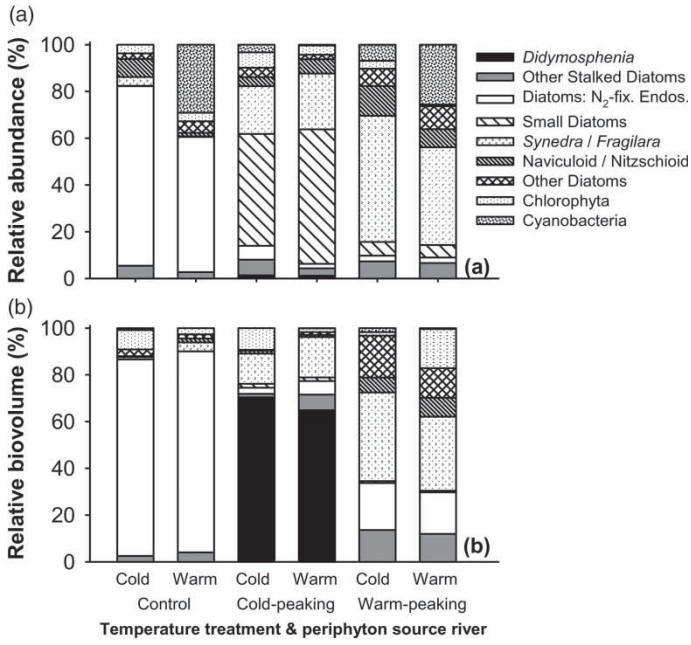

Fig. A1. Relative abundance (**a**) and relative biovolume (**b**) of fecal algae collected from tadpoles in cold or warm treatments and fed periphyton from different source rivers (control=Alameda Ck; cold-peaking=MF American; warm-peaking=NF Feather). Other stalked diat.=other stalked diatoms; Diatoms: N_2-fix. Endos.=Diatoms with N_2-fixing cyanobacterial endosymbionts; Small diatoms=*Achnanthidium minutissimum* and other small diatoms. See methods for additional details on algal groupings.