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Effect of mosquitofish (*Gambusia affinis*) and sestonic food abundance on the invertebrate community within a constructed treatment wetland

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SUMMARY

1. The effect of mosquitofish (*Gambusia affinis*) predation on the invertebrate community in a hypereutrophic constructed treatment wetland in southern California was investigated at two nutrient levels that influenced sestonic food abundance.
2. *Gambusia affinis* and insect predators in the wetland had a significant impact on larval mosquito density in the wetland irrespective of nutrient level. At the end of the 5-month study, cladoceran abundance in predator exclusion enclosures was 2–3 orders of magnitude greater than in the treatments that allowed access by planktivores. Chironomids were the most abundant insect group collected in emergence traps, and midge production from the high nutrient location of the wetland was greater than from the low nutrient location, but was not affected significantly by *G. affinis*. The presence of abundant alternative prey in this highly enriched wetland may have weakened the predation impact of *G. affinis* on mosquitoes.
3. The abundances of six invertebrate groups in dipper samples and of four insect groups in emergence trap collections were analysed using a multivariate distance-based linear model. Fish treatment and location interactions with sampling date explained significant amounts of the variation in the abundance of invertebrate groups.
4. Multivariate multiple regression analysis showed that chlorophyll-*a* concentration explained a large portion of the variability in non-predatory insect and zooplankton abundance at the high nutrient location, whereas bacterial density explained a large portion of the variability in the abundances of these taxa at the low nutrient location. Predatory insects were not directly coupled to the bottom-up influence of bacterial abundance and chlorophyll-*a*.

Keywords: aquatic insects, community structure, constructed wetland, trophic interactions

Introduction

Treatment of nutrient-enriched water produced by human or animal sources, such as human wastewater or dairy operations, is needed in many regions of the world, especially the arid southwestern U.S. (Cole, 1998). In addition to treatment of nutrient-enriched wastewater, constructed wetlands can provide secondary benefits such as wildlife habitat or recreation, and may act as a venue for public education on issues related to water resources and wildlife conservation (U.S.E.P.A., 2000). However, along with these benefits come some undesirable costs, such as uncertainty regarding the long-term sustainability of the constructed wetland ecosystem (Sartoris *et al.*, 2000) and the possibility of disease in humans and animals

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caused by the production of insects serving as vectors for pathogens (Walton *et al*., 1998), including mosquitoes that may act as a vector for West Nile Virus.

Mosquitofish (*Gambusia* spp.), and especially *G. affinis* (Baird and Girard), have been used for mosquito biological control for nearly 90 years and are effective when used in 'appropriate environments' and within a range of specific parameters that allow the *G. affinis* to be an effective larvivore. The effect of predatory fish on the community ecology of mosquito habitats has a large literature (Bence, 1988; Castleberry & Cech, 1990; references in Swanson, Cech & Piedrahita, 1996). The presence of *G. affinis* in mosquito habitats can significantly alter aquatic invertebrate community structure (Hurlbert, Zedler & Fairbanks, 1972) because *G. affinis* are opportunistic omnivores that eat a variety of aquatic invertebrates. Although *G. affinis*–mosquito interactions in nutrient-enriched water have been studied (Mian, Mulla & Wilson, 1986), there are no published studies examining the effect of *G. affinis* upon aquatic insect community structure in nutrient-enriched constructed wetland systems.

Arguments involving aquatic community regulation primarily focus on top-down (Brett & Goldman, 1997) or bottom-up (Power, 1992) forces acting to determine community structure. Such perspectives are neither contradictory nor mutually exclusive when applied to aquatic systems (Hunter & Price, 1992). Both concepts can be invoked to explain variation in the distribution and abundance of aquatic insects and their foods. While 'bottom-up' and 'top-down' forces can act on communities simultaneously, variation at different levels of the food chain, or in abiotic factors, can influence the relative strengths of 'bottom-up' and 'top-down' forces (Oksanen *et al*., 1981; Liebold, 1989). However, more recent arguments involving aquatic community regulation suggest that intermediate-consumer identity (Kneitel, 2007) and controphic species (Stav, Blaustein & Margalit, 2005) may also play a large role in population dynamics. With a few notable exceptions, there are few studies focused on the relative roles of different ecological forces in determining population change and community structure of wetlands, and the large literature addressing the effect of resource gradients on food chains has ignored hypereutrophic constructed wetland systems (Batzer & Wissinger, 1996). The negative impact of *G. affinis* and the positive impact of abundant sestonic food can act simultaneously to influence the spatial and temporal population dynamics of aquatic invertebrates in wetlands. This study examines the impact of *G. affinis* and algal and bacterial food abundance on invertebrate abundance in a constructed treatment wetland.

Methods

Study site

This study was carried out in a 9.9 ha multipurpose demonstration constructed treatment wetland at Eastern Municipal Water District's (EMWD) Hemet-San Jacinto Regional Wastewater Reclamation Facility (HSJRWRF) in San Jacinto, California (33°48'N, 117°1'W; 454 m ASL). During the experiment, the wetland received 4200–7900 m^3 day^{-1} of secondary-treated municipal effluent from a conventional wastewater treatment facility. The nominal operating depth of the five inlet and two outlet marshes was 0.55 m and of the central pond was 1.9 m. Daily total inflow volume varied according to the operational requirements of the HSJRWRF; hydraulic residence time was 9–14 days (Sartoris *et al*., 2000).

The shallow zones of the wetland comprised 50% of the surface area and contained two bulrush species. California bulrush [*Schoenoplectus* (=*Scirpus*) *californicus* (C.A. Meyer)] and hardstem bulrush [*S. acutus* G.H.E. Muhlenberg ex J. Bigelow] were found in about 75% and 25% of the shallow marshes respectively (Sartoris *et al*., 2000). The vegetation had been planted on 1.2- or 2.4-m centres in autumn 1994 and dried and burned in 1998.

Nutrient levels were high and a substantial nitrogen gradient existed across the wetland during this study. The mean concentration (±1 SD, $n = 23$) of total nitrogen (17.5 ± 3.8 mg L^{-1}) in the inflow water was higher than in the outflow water (10.4 ± 3.0 mg L^{-1}; EMWD, unpubl. data). The mean phosphorus concentration in the inflow and outflow water was similar (total phosphorus: inflow, 3.1 ± 1.1 mg L^{-1}; outflow, 2.6 ± 0.7 mg L^{-1}). Sartoris *et al*. (2000) found that the total nitrogen concentration near the five influent weirs in the inlet wetlands did not differ appreciably and was lower in the outlet marsh that we used (Outlet Marsh A) than in the adjacent outlet marsh because of short-circuiting of water flow from an inlet marsh into the latter marsh.

Experimental design

Four treatments were used to study the effects of *G. affinis* predation on the aquatic insect community in the wetland. The four treatments were *G. affinis* exclosure (no fish: NF), enclosures with *G. affinis* (F), an enclosure control with screen on three sides (3S), and ambient wetland conditions (no enclosure: open, OP). Four blocks of treatments were set near one of the five inflows into the wetland (Inlet Marsh 2) and in one of the two polishing marshes (Outlet Marsh A) of the wetland (see Peck, 2004) to represent respectively the upper and lower ends of the nutrient gradient across the wetland. All enclosures were located at the emergent vegetation-open water interface.

Each enclosure had a water surface area of 0.5 m^2 and was constructed of amber Lumite screen (mesh aperture = 0.53 mm; BioQuip Corp., Rancho Dominguez, CA, U.S.A.) mounted on a polyvinyl chloride pipe (PVC pipe) frame. Enclosures were initially covered with screen on all four sides and the bottom when placed into the wetland, but were open at the top and left open throughout the experiment. Enclosures were attached to wooden stakes driven 0.3 m into the substratum. After the enclosure was secured in the substrate, the bottom screen was cut away to allow free passage of benthic organisms and emergent aquatic macrophytes from underground rhizomes. Emergent macrophytes quickly colonized the enclosures and approximately 50% of the water surface of each enclosure contained bulrush by the end of the study. Aquatic organisms quickly colonized the screened sides of the enclosures and the screened sides were scrubbed after each sampling period with a stiff brush to remove attached organisms to allow free passage of water, nutrients and microplankton into the enclosures.

Stocking rates of enclosures with *G. affinis* (F treatment) were determined by dragging a net (cross-sectional area = 0.5 m^2, aperture size = 0.5 mm diameter) near the emergent macrophyte-open water interface of each block within Inlet Marsh 2 and Outlet Marsh A of the wetland. Approximately 1 m^3 of water was sampled adjacent to each block, and the number of *G. affinis* captured was used as an estimate of ambient *G. affinis* density for stocking.

Initial stocking rates of enclosures with *G. affinis* were three males per enclosure on 9 May 2001. On 11 June, mean survivorship of the stocked *G. affinis* was 66%. On 25 June, *G. affinis* density in the wetland increased and 12 mixed-sex *G. affinis* were added to all F enclosures. On 18 July, 7 mixed-sex *G. affinis* were added to the existing populations in each F enclosure. No fish were added after 18 July.

Invertebrate abundance

Aquatic insect sampling commenced 19 days after installation of enclosures into the wetland and was carried out at 3-week intervals (first interval was 4 weeks) from May through September (18 May, 15 June, 6 July, 27 July, 17 August and 7 September). Immature mosquitoes and other aquatic fauna were sampled within each enclosure, or in the wetland (OP), by combining three 330-mL dipper samples into one sample using a filtering cup (mesh aperture size = 0.1 mm). Samples were placed on ice, transported to the laboratory within 3 h and preserved after arrival in the laboratory in 95% ethanol.

Emerging mosquitoes and other insects were sampled using 0.25 m^2 emergence traps (Walton, Workman & Keiper, 1999). Emergence traps were placed into enclosures or the wetland 5 days prior to each sampling date, then removed for 14 days after the collection jars were retrieved. Removing the emergence trap between sampling dates allowed oviposition by insects onto the entire water surface area of enclosures before the emergence traps were deployed for the next sample. Collection jars containing insects were taken back to the laboratory and frozen (−10 °C) until enumeration. There were no emergence trap data for 17 August because argentine ants (*Linepithema humile* Mayr) infested the laboratory and consumed the insect collections as the samples were being thawed before enumeration. All aquatic invertebrate samples were counted at 25–50× using a dissecting microscope.

Invertebrates from dipper samples were placed into six groups: (i) mosquitoes (*Culex tarsalis* Coquillett); (ii) non-predatory insects: Ephemeroptera, Chironomidae, Corixidae, adult Hydrophilidae and Ephydridae; (iii) predatory insects: Odonata, Ceratopogonidae, Coleoptera (larval and adult Dytiscidae, larval Hydrophilidae), Notonectidae, Belostomatidae, Mesoveliidae and Veliidae; (iv) Cladocera; (v) Copepoda and (vi) Ostracoda. Aquatic insects from emergence traps were placed into four groups: (i) mosquitoes; (ii) Chironomidae; (iii) non-predatory Diptera (as larvae;

e.g. Ephydridae, Psychodidae and Sciaridae) and (iv) predatory Diptera (as larvae; e.g. Sciomyzidae, Tabanidae, Muscidae, Empididae and Dolichopodidae).

Phytoplankton biomass

Water samples were taken near (within 1 m) each of the four blocks in each location within the wetland on each sampling date. A 1-L dark brown polyethylene bottle was submerged and filled about 5 cm below the water in an undisturbed area near the emergent macrophyte-open water interface. A 5-mL sub-sample of water for quantification of bacterial abundance was placed into a clear plastic (20 mL) Nalgene® bottle (Nalgene, Rochester, NY, U.S.A.), preserved with 1 mL of 25% glutaraldehyde in the field and, along with the water sample in the brown bottle, placed on ice for transport to the laboratory. At the laboratory under subdued light, subsamples (300 mL) of water were filtered (Supor-200, diameter 47 mm, pore size = 0.45 μm; Gelman Science, Ann Arbor, MI, U.S.A.) under low vacuum to extract phytoplankton. Filters were frozen in foil envelopes overnight and pigments extracted for 24 h at 4 °C in 90% alkaline acetone. Following centrifugation at 2012 *g* for 5 min, aliquots of the supernatant were analysed for chlorophyll-*a* content using a Biospec-1601 UV-Visible spectrum spectrophotometer (Shimadzu Scientific, Columbia, MD, U.S.A.) following methods in Wetzel & Likens (1991).

Bacterial abundance

Preserved bacterial samples were kept refrigerated (4 °C) until staining with 60 μL of a 1 μM solution of a DNA fluorochromatic dye (Hoechst 33324 DNA dye) in 1% dimethylsulfoxide (Paul, 1982). Under darkened conditions, known volumes of stained samples were filtered across black filters (pore size = 0.22 μm, 25 mm diameter; Poretics Corp., Livermore, CA, U.S.A.) and slide mounted as per the methods of Velji & Albright (1993). Slides were frozen immediately and stored at −10 °C until bacterial enumeration.

Fluorescently stained bacteria were counted using a Leica DM RB compound microscope (Leica microsystems, Bannockburn, IL, U.S.A.) fitted with a 100×/1.25 oil immersion objective and a UV filter set (exciter BP 340 nm, dichroic mirror RKP 400 nm, barrier filter 425 nm). Images of stained bacteria were captured with a low light digital video camera (model DEI-470; Optronics Engineering, Goleta, CA, U.S.A.) and processed for automated counting on a computer running IMAGE PRO PLUS (version 3.0; Media Cybernetics, Silver Spring, MD, U.S.A.) and Microsoft Excel (version 97). Ten fields per slide were counted using randomly assigned Cartesian coordinates. Bacterial densities were computed by method 9216B described in APHA (1995).

G. affinis abundance

Gambusia affinis abundance outside of enclosures was measured on 22 June, 6 July, 20 July, 31 July, 17 August, 31 August and 26 September using Gee® minnow traps (Cuba Specialty Co., Fillmore, NY, U.S.A.) lined with fibreglass window screen (1.5 × 1.1 mm mesh opening). Two pieces of dry dog food and a float, to maintain a portion of the trap above the water surface, were placed into each trap. On each sampling date, traps were deployed in the morning (*c.* 09:00 hours) within Inlet Marsh 2 (n = 6) and Outlet Marsh A (n = 6) of the wetland and collected the following morning (*c.* 09:00 hours). *Gambusia affinis* within traps were counted and released.

Data analysis

Bacterial abundance, chlorophyll-*a* concentration and *G. affinis* trap catch data were $\log_e(x + 1)$-transformed and then tested for variance homogeneity with the F_{max} test. To evaluate treatment effects on each of the three variables across locations and over time, a repeated measures (RM)-ANOVA [general linear model option (GLM)] in SYSTAT (version 9.01; SPSS, 1999) was run as a one-way MANOVA with location as a between-subjects independent variable and the abundance of each taxon on the sampling dates as dependent variables. Transformation failed to homogenize variances of chlorophyll-*a* biomass; a Kruskal–Wallis test for differences of location in ranked concentration data was performed. Median abundances between locations and over time were compared using a multiple pair-wise comparison of ranks (Siegel & Castellan, 1988).

To homogenize variances, mosquito abundance was $\log_e(x + 1)$-transformed and averaged over time and over blocks to create eight treatment groups. An ANOVA was run on the collapsed data set (GLM in SYSTAT, version 9.01; SPSS, 1999). ANOVA interactions

were not significant for immature mosquitoes in dipper samples, and marginal means were calculated across locations and compared using a step-wise procedure (Welsch, 1977). The aformentioned methods failed to homogenize variances of emergent mosquito abundance; a Kruskal–Wallis test for differences of location in ranked adult mosquito abundance data was run. Median emergent mosquito abundances between treatments were compared using a multiple pair-wise comparison of ranks.

A distance-based multivariate analysis for linear models, NPMANOVA (DISTLM version 5; Anderson, 2004), was used to test for multivariate treatment effects. Data were examined for significant differences in multivariate dispersions before NPMANOVA using the deviations from Gower's mediancentre and permutation of least-absolute-deviation (LAD) residuals. Multivariate equality of dispersion among groups was tested using MEDIAN5 (M.J. Anderson, University of Auckland, NZ, U.S.A., unpubl. software) with $\log_e(x + 1)$-transformed abundances, Euclidean distance, and 9999 permutations. NPMANOVA permutational tests for the multivariate null hypothesis of no relationship between the matrix of response variables [the abundances of mosquitoes, insect functional feeding groups (non-predatory and predaceous insects) and other aquatic invertebrate taxa] and the 8-term experimental design matrix were carried out using the following options within the DISTLM software: $\log_e(x + 1)$-transformation of abundance data, Euclidean distance, no standardization, unrestricted permutation of raw data and 9999 permutations for each NPMANOVA term tested. The MS denominator of all NPMANOVA F ratios was the residual MS, except the location factor that had a denominator of MS of blocks nested within location. Further, the number of permutable units for blocks nested within location was 8 (four blocks per location), not 192 as used for all other terms tested. A permutational analogue of parametric P-values (the permutation P) was used in the statistical tests and, because the location term had a limited number of permutable units, Monte Carlo P-values for the asymptotic distribution of the pseudo-F statistic under permutation are reported.

To investigate the relationship between bacterial abundance, chlorophyll-*a* and *G. affinis* trap catches, and abundances of aquatic insects and zooplankton, multivariate multiple regression analysis (MMRA) with forward selection of environmental variables (Anderson, 2003) was used. Before MMRA, variables in each of the eight subsets were checked for equality of multivariate dispersions using MEDIAN5 as indicated previously. The marginal tests and conditional tests of MMRA are reported. Eight subsets of the total data set were investigated after partitioning the data by location, then by enclosure treatment. Thus each subset consisted of 24 observations (four blocks across six dates for dipper samples). The P-values for the F_{pseudo} test were obtained by permutation (Anderson, 2003). The $\log_e(x + 1)$-transformed variates, Euclidean distances, and 9999 permutations were used for each MMRA. Because NF and F treatments were closed to the wetland *G. affinis* population, only bacterial abundance and chlorophyll-*a* biomass were used as independent variables for those treatments. The 3S and OP treatments were analysed with all three variables.

Detrended correspondence analysis indicated that the lengths of the first two ordination axes were each <2 SD; therefore, linear ordination methods (CANOCO, version 4.5; ter Braak & Smilauer, 2002) were used to examine changes in the invertebrate community structure in dipper samples. Redundancy analysis (RDA) was carried out using nominal environmental variables (treatment, position in the wetland, date, presence versus absence of fish). Five dates were used for the analysis (18 May, 15 June, 6 July, 17 August and 7 September).

Results

Invertebrate abundance

Larval mosquito abundance decreased in all treatments in both locations during the experiment (Fig. 1). Stepwise comparison of marginal means showed that there were significantly more larval mosquitoes in enclosures without fish, intermediate numbers in enclosures with fish, and lowest numbers in the three-sided and no enclosure treatments which did not differ significantly (Table 1). A strong enrichment effect was observed on only two dates: a four- to sixfold enhancement of larval mosquitoes in NF and F enclosures in the inlet marsh in mid-June and in the NF enclosure in mid-August as compared to the abundances in same treatments in the outlet marsh.

Adult mosquito production from NF and F treatments was higher than from the 3S and OP treatments

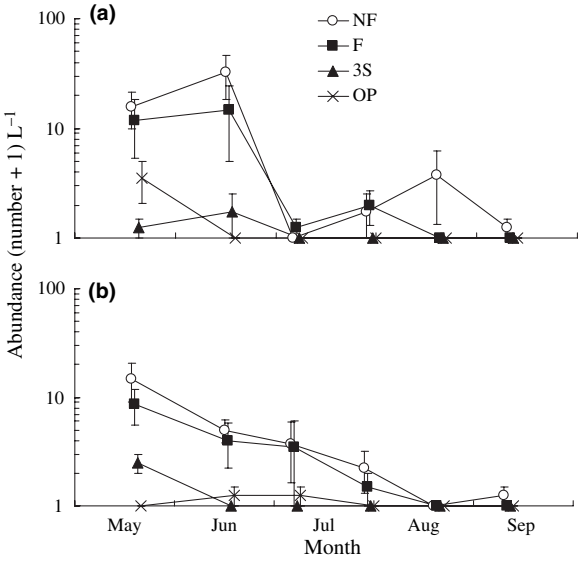

Fig. 1 Larval mosquito abundance (mean ± 1 SE, n = 4) in dipper samples from treatments in Inlet Marsh 2 (a) and Outlet Marsh A (b). Treatments: NF, enclosures without *Gambusia affinis*; F, enclosures with *G. affinis*; 3S, enclosure control (screen on three sides); OP, wetland control (open: no enclosure). Data points at each sampling date are offset horizontally to facilitate illustration.

during May and June (Fig. 2). Thereafter, mosquito production from the two full enclosure treatments (NF, F) declined to the low levels observed in treatments (3S, OP) that provided access to predators in the wetland. The pattern of mosquito emergence from the 3S and OP treatments remained relatively stable across the study. The trends for mosquito emergence were consistent among treatments regardless of position in the wetland.

Adult mosquito production from enclosures in the inlet marsh was significantly greater than from enclosures in outlet marsh (Kruskal–Wallis test: $H_{adj.} = 21.63$, $\chi^2_7 = 14.07$, $P < 0.005$). Mosquito production from the full enclosure treatments (N and NF) in the inlet marsh was two to five times greater than from the outlet marsh during late spring–early summer (Fig. 2). Mosquito production from NF and F enclosures was not statistically different within location and was greater than from the 3S and OP treatments (Table 1: pair-wise comparisons of ranked medians). Treatments that allowed *G. affinis* and macroinvertebrate predators unobstructed access to prey (3S and OP) had statistically equal numbers of adult mosquitoes emerging even though no mosquito production was observed from the inlet OP treatment.

Table 1 *Culex* spp. larval abundance and adult production among four fish treatments in a constructed wetland in southern California

Treatment	
(a) Number (composite dipper sample)$^{-1}$ (95% CI)	
NF	2.78^{a} (2.23–3.46)*
F	1.92^{b} (1.27–2.92)
3S	1.12^{c} (1.03–1.21)
OP	1.11^{c} (0.99–1.24)
(b) Number emerging 0.25 m^{-2} 5 day^{-1} (range)	
Inlet	
NF	4.40^{a} (3.22)†
F	3.65^{ab} (2.93)
3S	0.07^{cd} (0.15)
OP	0.00^{d}
Outlet	
NF	2.07^{b} (5.15)
F	2.03^{b} (3.00)
3S	0.07^{cd} (0.32)
OP	0.29^{c} (0.43)

Treatments: NF, enclosures without *G. affinis*; F, enclosures with *G. affinis*; 3S, enclosure control (screen on three sides); OP, wetland control (open: no enclosure).
*Backtransformed means followed by the same letter do not differ significantly by step-wise comparison using the Welsch step-up procedure (α = 0.05).
†Backtransformed medians followed by the same letter do not differ significantly by multiple pair-wise comparison of ranks (α = 0.05). No mosquitoes emerged in the inlet OP treatment.

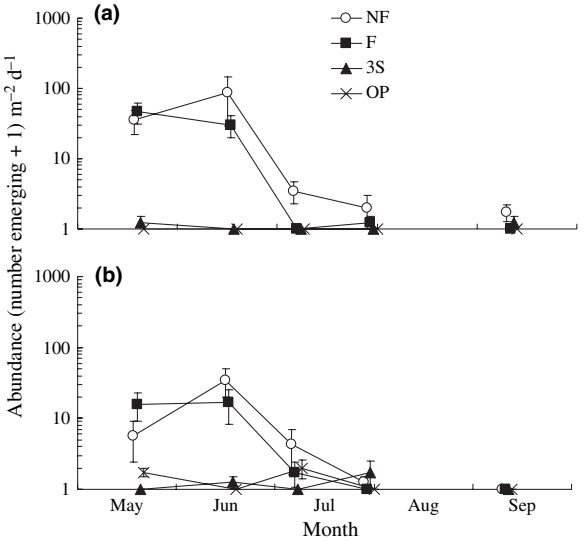

Fig. 2 Mean (±1 SE, n = 4) adult mosquito abundance in inlet (a) and outlet emergence traps (b). Refer to Fig. 1 for description of treatments. Data points at each sampling date are offset horizontally to facilitate illustration.

Trends in abundance for non-mosquito invertebrate taxa depended on sampling method, location within the wetland, enclosure treatment and sampling date. Non-predatory aquatic insect abundance (from dipper samples) was <10 individuals L^{-1} on most dates, with a notable increase in abundance in the inlet marsh late in the summer (data not shown: see Peck, 2004). Predatory aquatic insect abundance was <10 individuals L^{-1} throughout the sampling period, although there was a gradual increase in the inlet marsh NF treatment and insect predator abundance did not differ appreciably among treatments. Non-predatory and predatory insects were rare (<1 individual m^{-2} day^{-1}) in emergence traps throughout the study and showed little temporal change in abundance.

Fish and insect predator (predator from here on) treatments had a strong effect on cladoceran abundance and no statistically significant effect on the abundance of the two other zooplankton groups. Cladoceran abundance in the NF and F treatments was greater than in the 3S and OP treatments, especially late in the summer (Fig. 3). The general temporal trend was a decrease in cladoceran abundance over time, except for inlet marsh NF treatment. Copepod abundance ranged between 10 and 1000 individuals L^{-1}, with the outlet marsh having the highest abundances in late summer, but was similar among the treatments (see Peck, 2004). Ostracod abundance ranged from 10 to 5000 individuals L^{-1}, with both locations showing increasing abundance over time. Predator treatments showed a small effect on ostracod abundance, but most treatments clustered tightly for each sampling date for both locations (data not shown: see Peck, 2004).

Adult chironomid production ranged from <10 to >200 individuals m^{-2} day^{-1}, and was greater in the inlet marsh than in the outlet marsh. Chironomid abundance increased during late summer in the inlet marsh, but decreased in the enclosure treatments in the outlet marsh. Fish and insect predators had little effect on chironomid production, as most midge production from the four treatments was similar on most sampling dates.

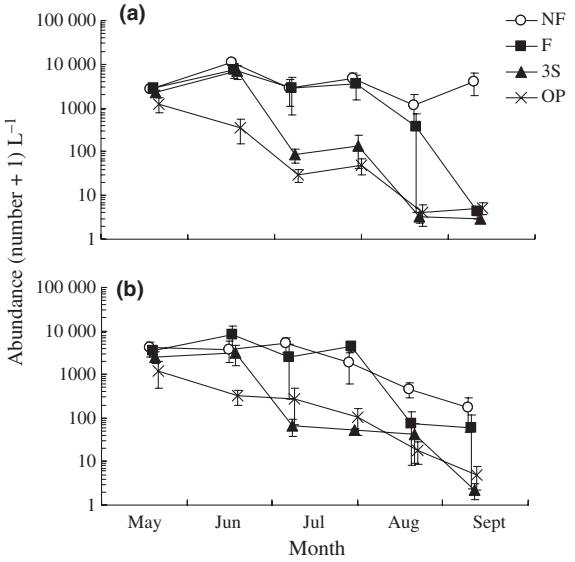

Fig. 3 Mean (±1 SE, $n = 4$) abundance of cladocerans in dipper samples from inlet (a) and outlet (b) treatments. Refer to Fig. 1 for description of treatments. Data points at each sampling date are offset horizontally to facilitate illustration.

Phytoplankton biomass

Chlorophyll-*a* concentration in the water column of the inlet marsh increased 100-fold between May and September, whereas chlorophyll-*a* concentration in the outlet marsh increased rapidly between May and June to *c.* 10-times that in the inflow marsh, decreased during August, and then increased again during September (Fig. 4a). Phytoplankton biomass was significantly different depending on location and sampling date (Kruskal–Wallis test: $H_{adj.} = 40.22$, $\chi^2_{(0.05,\ 7)} = 14.07$, $P < 0.005$). Separation of rank medians using multiple pair-wise comparisons showed that phytoplankton biomass in the inlet marsh compared to the outlet marsh was equal on 18 May and 27 July, less on 15 June and 6 July, and greater on 17 August and 7 September.

Bacterial abundance

The general trend over the study was of an order of magnitude increase in water column bacterial abundance, with the outlet marsh having slightly greater bacterial densities than the inlet marsh (Fig. 4b). RM-ANOVA of bacterial density showed a significant main effect of location (Table 2). MANOVA of bacterial density showed a significant effect of sampling date, but the location × date interaction was marginally significant. The significant *P*-values associated with Pillai's trace suggest that the composite of sampling dates is different for the two locations,

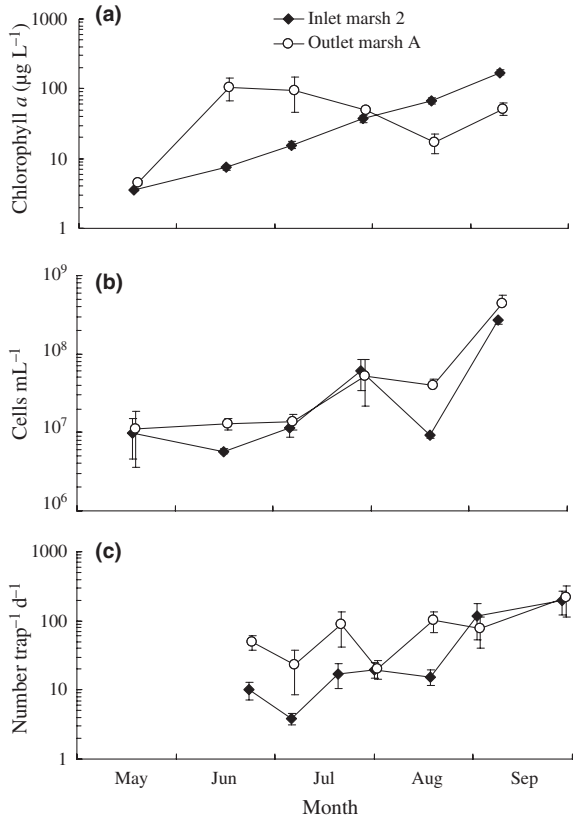

Fig. 4 Mean (±1 SD, $n = 4$) chlorophyll-*a* concentration (a), bacterial abundance (b), and *Gambusia affinis* abundance in minnow trap collections (c) in the water column of Inlet Marsh 2 and Outlet Marsh A in the HSJRWRF demonstration wetland, San Jacinto, CA. Data points at each sampling date are offset horizontally to facilitate illustration.

Table 2 Repeated measures ANOVA of bacterial density and *Gambusia affinis* minnow trap catch data

Subject	Source	MS	d.f.	Statistic	Value	*P*-value
Bacteria	Between-subjects					
	L	2.571	1	*F*	31.603	0.001
	Error	0.081	6			
	Within-subjects					
	D		5, 2*	Pillai's trace	0.999	0.002
	L × *D*		5, 2	Pillai's trace	0.971	0.071
G. affinis	Between-subjects					
	L	8.011	1	*F*	4.88	0.052
	Error	1.870	10			
	Within-subjects					
	D		6, 5	Pillai's trace	0.927	0.010
	L × *D*		6, 5	Pillai's trace	0.892	0.026

L, location; *D*, date.
*Hypothesis d.f., error d.f.

but that there was no significant interaction between the composite of sampling dates and location (L × D).

G. affinis abundance

Compared with bacterial abundance and chlorophyll-*a*, *G. affinis* abundance patterns had greater variability regardless of location and sampling date. The RM-ANOVA showed a marginally significant location effect and MANOVA tests showed a significant effect of sampling date and sampling date–location interaction (Table 2). The general trend was of increasing *G. affinis* abundance from early to late summer, with the inlet marsh *G. affinis* trap catches showing a 10-fold increase, while trap catches in the outlet marsh showed approximately a sixfold increase. *Gambusia affinis* trap catches in the inlet marsh were lower than the outlet marsh in early summer but became more equal in late summer. Both locations showed early summer decreases then increasing *G. affinis* abundance over time, although there was a second decrease for *G. affinis* density in the outlet marsh (Fig. 4c).

Community structure

Predator treatment and location had a significant effect on community structure of aquatic insects and zooplankton, but this effect was conditional upon sampling date. NPMANOVA of the six invertebrate groups in dipper samples showed significant main effects of predator treatment (Trt) and time (*D*), and significant Trt × *D* and location (*L*) × *D* interactions (Table 3). Multivariate dispersion among groups was equal for dipper samples ($F_{\text{med.}} = 0.932$, LAD-$P = 0.305$) and for emergence trap collections ($F_{\text{med.}} = 0.795$, LAD-$P = 0.620$).

NPMANOVA of the four invertebrate groups in emergence trap collections showed significant main effects of location, fish treatment, time and blocks nested within location [Blk(*L*)], and significant Trt × *D* and *L* × *D* interactions (Table 3). Thus, predation by fish and insects, location and block within a location had a significant effect on community structure of emerging aquatic insects, but differences in emerging insects among fish treatments and between locations were again conditional upon sampling date.

Table 3 DISTLM results for mosquitoes, non-predatory insects, predatory insects, cladocerans, copepods and ostracods from dipper samples and mosquitoes, chironomids, non-predatory and predatory insects from emergence samples

Source	Sample	d.f.	MS	Pseudo-*F*	Permutation *P*	Monte Carlo *P*
L	Dip	1, 6	28.223	2.261	0.088	0.118
	Emr	1, 6	95.698	10.755	0.084	0.0014
Trt	Dip	3, 138	115.639	14.432	0.0001	0.0001
	Emr	3, 118	22.351	10.033	0.0001	0.0001
D	Dip	5, 138	244.038	30.456	0.0001	0.0001
	Emr	4, 118	16.939	7.603	0.0001	0.0001
Blk(*L*)	Dip	6, 138	12.482	1.558	0.083	0.080
	Emr	6, 118	8.898	3.994	0.0001	0.0001
L × Trt	Dip	3, 138	9.039	1.128	0.327	0.325
	Emr	3, 118	4.127	1.852	0.085	0.083
Trt × *D*	Dip	15, 138	13.269	1.656	0.010	0.011
	Emr	12, 118	6.021	2.703	0.0002	0.0001
L × *D*	Dip	5, 138	45.093	5.628	0.0001	0.0001
	Emr	4, 118	11.222	5.037	0.0001	0.0001
L × Trt × *D*	Dip	15, 138	5.036	0.629	0.968	0.965
	Emr	12, 118	1.122	0.504	0.986	0.982
Error	Dip	138	8.013			
	Emr	118	2.228			

Dip, dipper samples; Emr, emergence samples; *L*, location within wetland; Trt, treatment; *D*, sample date; Blk(*L*), blocks nested within location.

The amount of variation explained by bacterial abundance, chlorophyll-*a* and *G. affinis* density on the six-group aquatic (dipper sample) assemblages depended on location within the wetland, enclosure treatment and regression model (Table 4). Multivariate dispersions among all eight treatment groupings for aquatic assemblages were equal (see Peck, 2004). For MMRA marginal test results of all treatment groups within the inlet marsh, chlorophyll-*a* explained a moderate amount of variation, with R^2 values ranging from 19% to 50% (Table 4). Bacteria and *G. affinis* abundances had lower and about equal explanatory ability: R^2 ranged from 9% to 19% for bacteria, and 10–19% for *G. affinis*. For conditional test results of all treatment groups within the water column of the inlet location, adding bacterial abundance to the model with chlorophyll-*a* increased explanatory strength slightly (1–2%), while adding bacteria and *G. affinis* increased explanatory strength by *c.* 7%.

Bacterial abundance explained a moderate amount of the variation in aquatic invertebrate abundance in the outlet location. For marginal test results of all treatment groups within the outlet marsh, bacteria abundance had the strongest explanatory ability, with R^2 values ranging from 22% to 34% (Table 4). Chlorophyll-*a* had comparatively less explanatory ability for aquatic invertebrate abundance, with R^2 values ranging from 1% to 18%, while *G. affinis* trap catches had weak explanatory ability, with R^2 values ranging from 5% to 6%. For conditional test results of all dip treatment groups within the outlet marsh, adding chlorophyll-*a* biomass to the model with bacteria abundance increased R^2 values considerably (2–19%), while adding *G. affinis* abundance to the model with chlorophyll-*a* and bacteria increased R^2 values by only *c.* 2–3%.

The importance of variation among sampling dates and the treatments is illustrated in the ordination biplot (Fig. 5). The first two axes of the ordination accounted for about 50% of the total variance in the invertebrate abundances, with the first axis explaining 44% of the variation in abundances of the wetland invertebrates in dipper samples. Mosquitoes and cladocerans that were abundant in fishless enclosures during the early sampling dates were inversely associated with notonectids, belostomatids, surface-dwelling hemipterans, and treatments in the wetland (3S and OP) where insect and fish predators were present. Ephemeroptera numbers in the wetland treatment (OP) were enhanced relative to the other three treatments, especially on the third sampling date (July), and were inversely related to the abundance of immature dragonflies and damselflies. Ostracods

Table 4 MMRA of 6 invertebrate groups (mosquitoes, non-predatory aquatic insects, predatory aquatic insects, cladocerans, copepods and ostracods) in dipper samples using DISTLM-*forward*

			Test									
			Marginal				Conditional					
Location	Treatment	EV	SS(trace)	F_{pseudo}	*P*-value	R^2	SS(trace)	F_{pseudo}	*P*-value	R^2	R_c^2	Correlation
Inlet	NF	Chlor	65.73	5.09	0.002	0.19	65.73	5.09	0.002	0.19	0.19	0.74 bc
		Bact	31.66	2.19	0.076	0.09	8.97	0.68	0.609	0.03	0.21	
	F	Chlor	197.67	14.03	<0.001	0.39	197.68	14.03	<0.001	0.39	0.39	
		Bact	76.05	3.88	0.034	0.15	6.88	0.48	0.739	0.01	0.40	
	3S	Chlor	142.86	18.18	<0.001	0.50	142.86	18.18	<0.001	0.50	0.50	0.77 cb
		Bact	53.78	4.20	0.025	0.19	11.25	1.47	0.213	0.04	0.54	0.78 bg
		Gam	53.24	4.15	0.024	0.19	8.72	1.15	0.327	0.03	0.57	0.66 cg
	OP	Chlor	64.34	8.78	<0.001	0.33	64.34	8.78	<0.001	0.33	0.33	
		Bact	17.77	1.79	0.150	0.09	10.50	1.47	0.212	0.05	0.38	
		Gam	18.74	1.90	0.138	0.10	2.80	0.38	0.811	0.01	0.40	
Outlet	NF	Bact	61.69	6.20	<0.001	0.22	61.69	6.20	<0.001	0.22	0.22	0.19 bc
		Chlor	3.91	0.31	0.89	0.01	4.23	0.41	0.842	0.02	0.23	
	F	Bact	137.71	9.41	<0.001	0.30	137.71	9.41	<0.001	0.30	0.30	
		Chlor	6.64	0.32	0.794	0.01	11.59	0.78	0.509	0.03	0.32	
	3S	Bact	86.98	8.35	<0.001	0.34	86.98	8.35	<0.001	0.32	0.32	−0.19 cb
		Chlor	49.73	3.98	0.018	0.18	33.44	3.69	0.013	0.12	0.44	0.25 bg
		Gam	14.28	0.99	0.383	0.05	5.20	0.56	0.654	0.02	0.46	−0.07 cg
	OP	Bact	57.21	5.83	<0.001	0.24	57.21	5.83	<0.001	0.24	0.24	
		Chlor	48.77	4.74	0.004	0.21	43.64	5.58	0.003	0.19	0.43	
		Gam	14.14	1.16	0.325	0.06	7.85	1.00	0.396	0.03	0.46	

EV, environmental variable; NF, enclosures without fish; F, enclosures with fish; 3S, enclosure control; OP, wetland control; Chlor, chlorophyll-*a* biomass; Bact, bacteria abundance; Gam, *G. affinis* in minnow trap collections; R_c^2, cumulative coefficient of determination. Pairwise correlation: b, bacteria; c, chlorophyll-*a*; g, *G. affinis*.

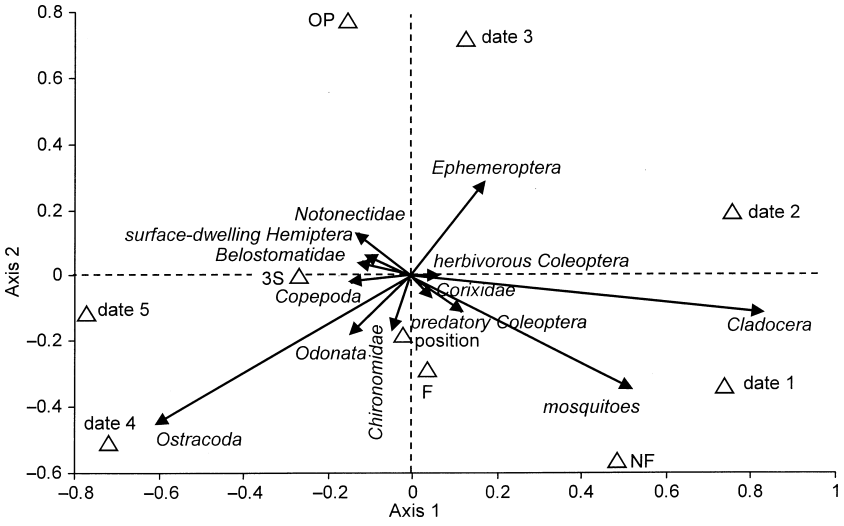

Fig. 5 Ordination (RDA) diagram illustrating the variation in the abundances of invertebrate taxa in four treatments in a hypereutrophic wetland. The nominal explanatory variables are indicated as centroids (Δ) in the ordination diagram.

dominated the invertebrate communities in the four treatments late in the experiment and ostracod abundance was not strongly associated with the abundances of cladocerans and larval mosquitoes. Position (i.e. inlet marsh versus outlet marsh) in this hypereutrophic wetland did not have a strong effect on invertebrate community composition although the abundances of the ostracods, cladocerans and immature mosquitoes were weakly positively associated with treatments in the inlet marshes.

Discussion

The spatial and temporal patterns of abundance of the aquatic invertebrate community in this wetland were influenced by natural enemies and nutrient loading. The greater abundances of larval mosquitoes and cladocerans in enclosures that excluded fish and many insect predators (NF) as compared to the treatments (3S and OP) with predator access indicate a significant top-down impact of the assemblage of predators found in the wetland on the two controphic species. The greater abundances of larval mosquitoes and cladocerans in the NF enclosures in the inlet marsh compared to the outlet marsh indicate that bottom-up forces were also important determinants of invertebrate abundance in the constructed treatment wetland. However, the timing of bottom-up effects differed for the two taxa. As compared to the abundances in the outlet enclosures, the abundance of larval mosquitoes in enclosures in the inlet marsh was enhanced mostly early in the study and cladoceran abundance was comparatively enhanced in the inlet enclosures from July through September. Abundant zooplankton throughout the experiment may be interpreted as evidence of an abundance of food resources that are derived directly from sewage and from the nutrients provided by the wastewater.

The combined predatory effect of *G. affinis* and macroinvertebrate predators on larval mosquito abundance is reflected in the greater numbers of larval mosquitoes observed in dipper samples from the NF enclosure treatments compared to the other three treatments, but the similarity of mosquito numbers in the F and NF treatments indicates that the contribution of *G. affinis* to total predation on mosquitoes may be weak under hypereutrophic conditions. The similar trends for larval mosquito abundance in the F and NF enclosures (except for late August in the inlet marsh) was unexpected and might have been caused by several factors. First, the initial *G. affinis* stocking rate into the F enclosures might have been too low to be representative of *G. affinis* populations in the wetland.

Secondly, high cladoceran abundance in the F enclosures might have reduced *G. affinis* predation on the immature mosquitoes. This effect would have been stronger in the spring when the number of fish in the F enclosures was less than later in the year. Cladoceran populations in the 3S treatments in both locations declined nearly two orders of magnitude between late June and mid July. During this period, cladoceran abundance in the inlet marsh OP treatments declined by an order of magnitude and declined only slightly in the outlet marsh OP treatment; yet, cladoceran abundance in the NF and F enclosures did not decline markedly. Juvenile mosquitofish feed preferentially on prey smaller than mosquito larvae (Bence, 1988; Swanson *et al.*, 1996). Mosquitofish populations increased during this period and juvenile mosquitofish might have been concentrating their feeding on the comparatively smaller sized cladocerans in the wetland.

Thirdly, the comparatively high mosquito abundance in the F treatment also may be attributed to predation by insects on mosquito larvae in 3S and OP treatments. There was a marked difference in larval mosquito abundance between F enclosures versus the treatments (3S and OP) that permitted access of *G. affinis* and insect predators in the wetland, especially during the spring. Larval beetles, notonectids, odonate nymphs and other predaceous insects were rare in the NF and F treatments and their large, late instars were excluded by the mesh; thus their predatory effects are stronger in the 3S and OP treatments. Ordination analyses indicated that aquatic hemipterans (i.e. notonectids) and fish were associated negatively with both larval mosquitoes and cladocerans. Walton & Workman (1998) found that mortality rates of mosquito larvae were directly related to macroinvertebrate predator abundance in dipper samples from experimental wetlands receiving secondary-treated municipal effluent.

Last, seasonal trends in mosquito abundance and a decrease in mosquito oviposition into enclosures might have contributed to the general decline in larval and adult abundances for the NF and F treatments over the experimental sampling period. *Culex tarsalis* host-seeking populations are most abundant at the site between May and early July and decline during the hot summer conditions (Walton *et al.*, 1998). Larval mosquito populations in the NF and F enclosures in the outlet marsh declined continuously during the experiment and were similar to those in the 3S and OP treatments after mid-August. Immature mosquito abundance in the NF and F enclosures in the inlet marsh was more variable than in enclosures in the outlet marsh, but was also lower during the summer than during the spring. The

equality of larval mosquito abundance in the 3S and OP treatments suggests that the presence of the enclosure was not affecting predator ability in reducing larval and adult mosquito abundances.

Top-down and bottom-up forces affected the invertebrate community of the hypereutrophic constructed treatment wetland; however, the significant interactions between enclosure treatment, location and date emphasize the complex interrelationship between invertebrate predator density, invertebrate food density and time. Previous studies in mesocosms suggest that invertebrate foods and invertebrate predators do not act as independent regulators but have effects that can only be predicted from an understanding of their combined impacts (Drenner *et al.*, 1990; Lancaster & Drenner, 1990), and complex temporal fluctuations in aquatic invertebrate communities have been observed previously in mesocosms (Walters & Legner, 1980) and in wetland research cells receiving secondary-treated municipal effluent (Walton & Workman, 1998). Of the invertebrate groups examined, only abundances of mosquitoes and cladocerans were affected by enclosure treatment, NF and F being markedly greater than 3S and OP depending on sampling date. Decreased immature mosquito abundance in dipper samples was reflected in lower levels of adult mosquito production. These results suggest that the NF and F treatments offered a refuge from predation occurring in the wetland.

The influence of abundance of food (bacterial abundance and chlorophyll-*a* concentration) and of *G. affinis* on invertebrate abundances depended on taxonomic group, location within the wetland and enclosure treatment. Predatory insects were not directly coupled to the bottom-up influence of bacterial abundance and chlorophyll-*a*, but their abundances may have been impacted by *G. affinis* predation upon early instars of the predatory insects. Abundance of non-predatory insect and zooplankton taxa collected in dipper samples was strongly associated with chlorophyll-*a* in the inlet marsh, but with bacterial density in the outlet marsh. The lower spatio-temporal variability in chlorophyll-*a* in the inlet marsh compared to the outlet marsh may be the primary reason for the relatively high R^2 values for invertebrate abundances. In contrast to this finding, the relatively higher concentration of bacteria in the outlet marsh and the less predictable chlorophyll-*a* trends explain the patterns of partitioning of variation in the MMRA analysis. The associations of *G. affinis* with aquatic invertebrate abundances were weaker than for planktonic foods, and under some circumstances this can indicate a stronger bottom-up regulation of community structure.

The amount of unexplained variation in our models suggests that unmeasured independent variables play a role in the community regulation of this wetland. Blooms of toxic algae (Marten, 1986) or bacteria (Murty, Srinivas & Sekar, 1994), variation in total suspended solids in the water column (Batzer & Wissinger, 1996) and the biochemical quality of sestonic foods (Muller-Navarra *et al.*, 2000) may have a profound spatio-temporal influence on invertebrate community structure. Analysis of the forces determining community structure in hypereutrophic wetlands requires a working model that incorporates heterogeneity (Hunter & Price, 1992) and thereby recognizes differences among species within a trophic level, differences in species interactions in a changing environment and changes in population quality with population density.

Interrelationships between and among taxa were complex, and were dictated by variation in the spatio-temporal patterns of invertebrate sestonic foods, predator density and enclosure treatment type. A full understanding of how community patterns emerge from a complex network of interacting species and abiotic processes requires simultaneous study of multiple community components and examination of all relevant interactions (Polis & Winemiller, 1996; DeWitt & Langerhans, 2003). The present study investigated the effect of predator exclusion and its relationship to food type and food abundance on aquatic invertebrate community structure. The variable ability of *G. affinis* to suppress mosquito abundance has been shown to be a function of differences in environmental conditions (Blaustein & Chase, 2007). Environmental variables may have reduced the potential of *G. affinis* as a biological control agent of larval mosquitoes within this highly enriched aquatic habitat.

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