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Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities

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SUMMARY

1. The effects of omnivorous exotic species on native communities are often difficult to predict because of the broad diets and behavioural flexibility of the omnivore, and the diverse abiotic and biotic characteristics of invaded systems. We investigated experimentally the effects of a gradient of density of the introduced, omnivorous red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) on two stream communities in southern California, U.S.A.

2. The Ventura River is a clear, flowing stream with a cobble substratum, with abundant algae but low densities of large invertebrates, small herbivores and snails. The Santa Ynez River at the time of the study consisted of a series of drying pools underlain by sand, with abundant charophytes, large predatory invertebrates and herbivores, including snails.

3. In the Ventura River, periphyton biomass and inorganic sediment decreased with increasing crayfish abundance, but in the Santa Ynez River, periphyton and sediment were unrelated to crayfish densities.

4. In the Ventura River, the biomass and density of all benthic invertebrates combined, chironomids, micropredators, the meiofauna (chydorid cladocerans, copepods and ostracods), and specific predatory and herbivorous taxa, as well as taxon richness, were negatively related to crayfish density. In the Santa Ynez River, the biomass and average body size of benthic invertebrates, predatory invertebrates, herbivores and chironomids, but not total invertebrate density or taxon richness, were negatively related to crayfish density.

5. Fewer large predatory invertebrates and snails (*Physella gyrina*) in both streams, and baetid mayflies in the Ventura River, were visible at night in channels where crayfish were abundant. Snails responded to crayfish by moving above the water line in the Santa Ynez River, but not in the Ventura River.

6. We suggest that the same omnivore had different effects on these neighbouring streams because of crayfish predation on large invertebrates in the Santa Ynez River and the scarcity of such prey in the Ventura River, leading to increased crayfish grazing on periphyton, and reductions in periphyton-associated invertebrates, in the Ventura River.

Keywords: crayfish, indirect effects, invertebrates, omnivory, periphyton

Introduction

Exotic species have had significant effects on native species and ecosystems through predation, competition and the alteration of habitat conditions; however, prediction of the impacts of exotic species is difficult because of wide variation in the characteristics of invaders and invaded ecosystems (Levine *et al.*, 2003; Lake & Leishman, 2004; Moyle & Marchetti, 2006; Gerhardt & Collinge, 2007). Predictions are particularly difficult when the invader is an omnivore, which is able to survive in a variety of environments and respond behaviourally and demographically to changes in the environment and resources (Stenroth & Nyström, 2003; Dorn & Wojdak, 2004; Vandermeer, 2006; Thompson *et al.*, 2007).

Large omnivorous decapods, such as crayfish, occur in streams, rivers, lakes and ponds in many parts of the world and feed on detritus, algae, plants, invertebrates (including other decapods) and vertebrates, often dominating invertebrate production and acting as keystone

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species in food webs (Parkyn, Collier & Hicks, 2001; Usio & Townsend, 2002; Stenroth & Nyström, 2003; Nyström, 2005; Usio *et al.*, 2009). In Europe and North America, non-indigenous crayfishes have eliminated or reduced native crayfish, amphibians, invertebrates and aquatic vegetation in lakes and streams, sometimes displacing fish and invertebrates that use these resources (Feminella & Resh, 1989; Gamradt, Kats & Anzalone, 1997; Covich, Palmer & Crowl, 1999; Stelzer & Lamberti, 1999; Lodge, 2000). The net outcome of direct and indirect effects of crayfish on other species depends on the strength of species interactions. For example, crayfish often reduce the abundance of grazing snails, releasing algae from snail grazing; however, crayfish also directly consume algae, so the net effects of crayfish on algae will depend on the relative magnitude of direct and indirect effects (Lodge *et al.*, 1994). Similar considerations apply when examining the role of crayfish as ecosystem engineers, where crayfish indirectly affect invertebrates or algae by altering sedimentation and substratum characteristics (Statzner, Peltret & Tomanova, 2003; Usio *et al.*, 2009). Further, changes in the behaviour and distribution of aquatic consumers induced by crayfish may affect the predators, competitors or resources of these consumers (Lima & Dill, 1990; Klose, 2011).

The red swamp crayfish (*Procambarus clarkii* Girard) was introduced into southern California sometime between 1924 and 1954 and is considered a large, prolific, aggressive and adaptable species (Holmes, 1924; Huner & Barr, 1994). Owing to its broad environmental tolerances, *P. clarkii* has successfully invaded a wide range of habitats in California, including commercial rice fields, irrigation canals, freshwater marshes, streams, rivers, lakes and ponds (Sommer & Goldman, 1983; Feminella & Resh, 1989; Gamradt *et al.*, 1997). Although recent studies have shown that crayfish play an important role in benthic food webs (Covich *et al.*, 1999; Usio & Townsend, 2002; Nyström, 2005), no attempts have been made to evaluate crayfish effects on different stream communities within the same geographical area. Many earlier studies of the effects of crayfish on lower trophic levels were conducted in the laboratory or small cages in a single system, and the indirect and direct effects of the same omnivorous predator on the complex food webs of disparate systems are less well known. Because streams vary greatly in their environmental and biological characteristics, it is possible to compare the effects of *P. clarkii* on stream communities that differ in a variety of such characteristics.

We used the same methods to compare the effects of the same crayfish species on two California streams, the Santa Ynez and the Ventura Rivers, which differed in their physical and biological characteristics. At the time of our study, the Santa Ynez River consisted of stagnant pools with high densities of large invertebrates, such as predators and snails, whereas the Ventura River consisted primarily of flowing riffles and runs with abundant algae, but few large invertebrates. We address the following questions: (i) Does *P. clarkii* have different impacts on the abundance of algae, invertebrates and sediment in the two streams? (ii) What are the relative strengths of direct and indirect effects of the red swamp crayfish on the different stream food webs and what drives the effect of crayfish on different systems? Based on the relative availabilities of different food types (e.g. invertebrates versus algae), we hypothesised that crayfish would reduce the large, dominant herbivores (particularly snails) in the Santa Ynez River, resulting in an increase in periphyton, but that crayfish would mainly consume periphyton in the Ventura River, leading to decreases in algae, sediment and other invertebrate grazers (Weber & Lodge, 1990; Hart, 1992; Lodge *et al.*, 1994; Creed & Reed, 2004). Because crayfish are omnivorous, behaviourally flexible and may be sensitive to many abiotic and biotic factors which differ in these two streams, we tested our hypotheses by manipulating the density of *P. clarkii* experimentally.

Methods

Study sites and invasion history

Studies were conducted in the Santa Ynez and Ventura Rivers, in southern California, U.S.A. Historical sources of the populations of *P. clarkii* used here are unknown, although it was introduced from its native habitats in north-eastern Mexico and the southern U.S.A. into southern California streams as early as the 1920s (Holmes, 1924). It is likely that crayfish were introduced into both systems at about the same time, given their proximity and early use by anglers. Neither stream has any native decapods.

Field work was conducted in a fifth-order section (width 5–6.5 m) of the Santa Ynez River in Santa Barbara County, California, U.S.A. (34°32.37′N, 119°51.50′W; altitude, 231 m), in June 2002. The section, 2 km upstream of Cachuma Reservoir, flows westward through a narrow channel between mountains. The stream is slightly alkaline and oligotrophic (Table 1) and has an open riparian canopy and streamside vegetation dominated by willow (*Salix* spp. L), western sycamore (*Plantanus racemosa* Nutt) and coast live oak (*Quercus agrifolia* Née). At the time of the study, average water depth was 0.4 m in riffles and

Table 1 Means (with, in a few cases, associated standard errors) of selected physical, chemical and biological data for the Santa Ynez and Ventura Rivers. Also included for each site are the number of fish collected in minnow traps and the species of fish observed

Variables	Santa Ynez River	Ventura River
Water temperature (°C)	21.2 ± 1.3	21.7 ± 1.1
Current speed (m s^{-1})	0.04 ± 0.01	0.16 ± 0.01
Open canopy (%)	100	71
Conductivity (μS cm^{-1})	1292	915
Dissolved oxygen (mg L^{-1})	14.1	10.8
pH	7.9	8.0
NH$_4^+$ (μM)	4.7	0.4
NO$_3^-$ (μM)	0.1	34.5
SRP (μM)	2.2	1.6
Crayfish density (No. m^{-2})[*]	2	4
Crayfish length (cm)	8.9 ± 0.10	9.1 ± 0.07
Fish density (No. per 20 minnow traps per night)[†]		
Cottus asper Richardson	8	2
Gasterosteus aculeatus L.	–	14
Lepomis macrochirus Rafinesque	1	–
Oncorhynchus mykiss Walbaum	1	–
Fish observed[‡]		
Ameiurus sp. Rafinesque	X	
Cottus asper Richardson	X	X
Gasterosteus aculeatus L.		X
Gila orcutti Eigenmann and Eigenmann	X	X
Lepomis macrochirus Rafinesque	X	
Micropterus dolomieui Lacepède	X	
Micropterus salmoides Lacepède	X	
Oncorhynchus mykiss Walbaum	X	X

[*]Crayfish abundance was estimated by visual counts of crayfish at night on two occasions in each of the streams by scanning known flagged areas of the stream bottom and turning over large rocks and cobbles in these areas.
[†]Fish species present at each site were determined using 20 baited minnow traps (each 42 cm L × 23 cm D, with 4-cm-D funnel) set out overnight.
[‡]Direct observations of fish in the clear waters of each stream.

0.7 m in pools, current velocity ranged from 0 to 12 cm s^{-1}, and water temperatures ranged from 17 to 22 °C; however, this section of the river was drying up and consisted primarily of a series of discrete, stagnant pools. The macroalga *Chara* (L.), and microalgae (primarily diatoms) covered the bed, which consisted of silt, sand and gravel with a few small cobbles, and the benthos was composed primarily of snails (*Physella gyrina* Say), worms (oligochaetes and nematodes), crustaceans [*Hyalella azteca* (Saussure), ostracods] and predatory invertebrates [hemipterans, including the Family Corixidae: *Graptocorixa* (Hungerford) and Family Naucoridae: *Ambrysus* (Stål), and damselflies, Family Coenagrionidae: *Enallagma* (Charpentier)], with oligochaetes and *H. azteca* dominating invertebrate biomass. At this site, mean crayfish density

was 2 m^{-2} and seven fish species were observed or collected (Table 1).

On the Ventura River, the study site was located in a third-order section (width 6–8 m) in Ventura County, California, U.S.A. (34°34.438′N, 119°29.913′W; altitude, 61 m). The Ventura River is alkaline and mesotrophic, with a mostly open canopy, and riparian vegetation dominated by giant reed (*Arundo donax* L.), western sycamore and willow (Table 1). Current velocity and water temperature at the time of the study in July 2003 ranged from 4 to 25 cm s^{-1} and 18–22.5 °C, respectively, and mean depth was 0.3 m in riffles and ranged from 1 to 4 m in pools. The substratum consisted largely of sand and gravel in pools, medium to large cobbles in riffles, and the dominant macroalga was *Cladophora* sp. (Kützing), and dominant microalgae were diatoms. The benthos was dominated by mayflies (*Tricorythodes* Ulmer), dipterans (Chironomidae: Chironominae, Orthocladiinae), worms (oligochaetes), crustaceans (*H. azteca*) and microcrustaceans (ostracods, chydorid cladocerans and copepods). Mean crayfish density was 4 m^{-2}, and four fish species were observed or collected (Table 1). We observed crayfish throughout the study reach and in all areas of experimental channels where they were introduced.

Experimental design

We conducted field experiments using the same channel set-up in both streams. Twelve parallel stream channels constructed from polyvinyl chloride (PVC) pipe cut in half longitudinally were placed in an unshaded stream reach with the top edges raised 2–3 cm above the surface of the water. Two (2 m L × 0.31 m W × 0.15 m H) stream channels were mounted onto each of six 1.2 m × 2.4 m plywood sheets coated with Varathane, a non-toxic water sealant. The ends of each channel were fitted with coarse-mesh plastic screens (0.7 cm width), which were cleaned daily, allowing continuous water flow and colonisation by invertebrates while preventing the escape or entry of crayfish. Natural substrata from the streambed were elutriated 10 times to remove invertebrates and detritus, mixed well and added to each stream channel to a depth of 2.5 cm. The substratum in the Santa Ynez River was mainly silt and sand with a few cobbles ranging from 2 to 10 cm in circumference, whereas in the Ventura River, the substratum in channels contained little silt and sand and was composed of equal numbers of small, medium and large cobbles (mean circumferences 21, 36 and 58 cm, respectively). We then collected invertebrates and detritus from an area of streambed equal to the area of all of the experimental channels combined, then thoroughly mixed

this water sample (20 L) in a large container and distributed equal aliquots to each stream channel, ensuring that the initial abundance of invertebrates and detritus was similar among channels. In addition, colonisation by invertebrates and algae into channels was allowed for 1 week prior to the start of the experiment.

Square unglazed ceramic tiles (2.3 cm on each side × 0.5 cm thick) were leached in distilled water for 2 weeks, then transferred to the stream bed for 4 weeks for periphyton colonisation and growth. Fifty of these conditioned tiles were placed on the bottom of each stream channel at equally spaced intervals at the beginning of the experiment. Because tiles may turn over or become covered in sediment, more tiles were placed on the bottom of channels than were collected.

At each site, channels were stocked with crayfish at one of six densities (0, 1, 2, 3, 4 and 6 crayfish per channel, two replicates per treatment). This density gradient covered the range of local *P. clarkii* densities found within each study reach. All individual crayfish were of similar length and mass [8.9 ± 0.1 and 9.3 ± 0.2 cm TL and 23.0 ± 0.8 and 26.2 ± 1.1 g wet mass (mean ± 1 SE)] in the Santa Ynez and Ventura Rivers, respectively, and survived for the duration of the experiment; however, two individuals missing a claw were immediately replaced. Four open-ended PVC cylinders, each 20 cm long × 8 cm in diameter, were placed on the bottom of each stream channel at equally spaced intervals to provide shelter for crayfish. Channel tops were covered with thin, transparent netting (0.4 cm mesh) and secured with removable metal clips to exclude avian and mammalian predators and to prevent crayfish and snails from escaping. Light (PAR) was measured below the surface of the water within each stream channel using a Li-Cor 250 light meter, both with and without the netting in place, to estimate the amount of light reduction. The netting reduced irradiance by 4.5% within each stream channel.

Experimental sampling

Both experiments ran for 14 days. Ten days after the start of the experiments, night visual surveys were performed within each stream channel to determine the number of benthic invertebrates visible [i.e. epibenthic invertebrates on the substratum surface and on the walls of stream channels and exposed surfaces of crayfish shelters, which could be seen and counted with the naked eye, including the number of snails (*P. gyrina*) floating at the water surface, and below and above the water line of each channel]. One hour after sunset, two individuals independently counted all invertebrates visible within the entire stream channel using a flashlight or headlamp and these individual counts were averaged for each channel.

Channel substrata and ceramic tiles were sampled prior to the addition of crayfish to determine initial invertebrate densities and periphyton biomass [estimated from ash-free dry mass (AFDM) and chlorophyll *a* concentrations], using a 0.041-m^2 Surber sampler (mesh size, 250 μm) for invertebrates (one sample per channel) and ceramic tiles for periphyton (4 removed per channel). At the end of the experiment, four Surber samples were taken from each channel, pooled into one composite sample and then preserved in the field in 75% ethanol, with rose bengal added to aid in the separation of invertebrates from particulate matter. Four ceramic tiles were collected from each channel for periphyton determinations at the end of the experiment, placed in black plastic canisters and put on ice and then frozen on return to the laboratory.

Laboratory methods

Invertebrates were identified to the lowest practical taxonomic level (generally family or genus for insects and molluscs) and counted using a dissecting microscope at 12× magnification. Average individual dry mass for each taxon within each channel was determined by averaging the lengths of 10 individuals collected from each channel and measured to the nearest 0.01 mm using digital callipers, then calculating dry mass using published length–mass relationships (Meyer, 1989). Invertebrate taxa were assigned to functional feeding group or size (i.e. meiofauna) categories, including small herbivores/detritivores, predatory invertebrates, micropredators, meiofauna and filter-feeders, using designations in Merritt & Cummins (1996) and Thorpe & Covich (2001) (Supporting Information, Appendix S1 & S2). Owing to their broad range of feeding modes and diets, Chironominae (here Chironominae and Orthocladiinae combined) were excluded from functional feeding group categorisations; however, the Tanypodinae were treated separately as micropredators. All chironomids (Chironominae, Orthocladiinae and Tanypodinae) were included in total invertebrate density and biomass calculations, and Chironominae/Orthocladiinae and Tanypodinae densities and biomasses were examined as separate response variables.

Periphyton samples were obtained by scrubbing ceramic tiles with a plastic brush, rinsing with distilled water into a graduated cylinder, diluting the resulting slurry to a known volume and then mixing thoroughly and splitting into two equal aliquots for AFDM and chlorophyll *a* determinations. Each aliquot was filtered onto an A/E

glass fibre filter and then stored at −20 °C in the dark until later analysis (filters for AFDM were pre-combusted for 30 min at 450 °C and then pre-weighed). For chlorophyll *a* analysis, filters were extracted in the dark at 4 °C in 90% acetone for 24 h and chlorophyll *a* concentrations corrected for phaeophytin were measured by fluorometry (Turner Designs 10-AU) (Sartory & Grobbelaar, 1986). For AFDM analysis, filters were dried at 60 °C for 24 h and weighed to the nearest 0.1 mg, and then filters were reweighed following combustion in a muffle furnace (Thermolyne 48000) at 500 °C for 2 h and drying at 60 °C for 24 h. AFDM was determined as the difference between initial and final dry mass following ignition, and inorganic sediment concentrations were measured as the final dry mass (Steinman & Lamberti, 1996).

Data analysis

We used linear and polynomial least squares regression analyses to assess the effects of a gradient of crayfish density on periphyton biomass, inorganic sediment and the density, biomass, Simpson's diversity, evenness (Pielou, 1966), taxon richness and body size of total benthic invertebrates, individual invertebrate taxa and invertebrate functional feeding groups. Rarefaction techniques were applied to taxon richness data because the number of taxa collected will depend on sample size; consequently, we examined the number of species collected at a standardised number of individuals (300) using resampling methods (Gotelli & Entsminger, 2009). Prior to all analyses, normality was tested using the Kolmogorov–Smirnov test (Zar, 1996) and residuals were plotted and examined. If assumptions of normality and homogeneity of variances could not be met, then the dependent variable was log-transformed. When discrete or continuous data included zero values, one or the smallest detectable unit for that response variable was added to all values for that variable prior to transformation (Quinn & Keough, 2002). All statistical analyses were performed using JMP Version 6.0.0 for WindowsTM (SAS Institute, Inc. Cary, NC, U.S.A.).

Results

Periphyton (diatoms) and inorganic sediment

Mean abundance of periphyton (mg m^{-2}) and inorganic sediment (mg m^{-2}) was greater in the Ventura River than in the Santa Ynez River across all stream channels (chl *a* t-test: $t = 20.4$, $P < 0.0001$; AFDM t-test: $t = −9.38$, $P < 0.0001$, and inorganic sediment t-test: $t = −5.58$,

$P = 0.0002$, respectively, d.f. = 22 in all cases) (Fig. 1). There were no significant relationships between periphyton biomass or inorganic sediment and crayfish density in the Santa Ynez River, although periphyton and inorganic sediment declined with increasing crayfish abundance in the Ventura River (Fig. 1).

Night visual surveys

The numbers of snails and predatory invertebrates (i.e. Hemiptera, Corixidae: *Graptocorixa* and Odonata, Coenagrionidae: *Enallagma*) in the Santa Ynez River, and snails, predatory invertebrates (i.e. *Graptocorixa*) and baetid mayflies in the Ventura River, exposed on the substratum in channels at night, declined significantly with increasing crayfish density (Fig. 2). The proportion of *P. gyrina* populations occurring above the water line increased with increasing crayfish abundance in the Santa Ynez River, but not in the Ventura River (Fig. 2). Snail density was very low in the Ventura River, however, and was two orders of magnitude less than that in the Santa Ynez River (snail density t-test: $t = −3.66$, d.f. = 22, $P = 0.004$) (Fig. 2).

Invertebrate responses

Invertebrate biomass and density across treatments in the Santa Ynez River were dominated by Oligochaeta (40% of biomass, 30% of total density), *H. azteca* (34, 18%), Ostracoda (9, 29%), *P. gyrina* (6, 1%) and Nematoda (3, 18%). Small herbivores comprised 85.5%, predatory invertebrates 4.2%, micropredators 0.3%, meiofauna 9.4% and Chironominae/Orthocladiinae 0.6% of total invertebrate biomass and 68.4, 0.2, 0.6, 30.5 and 0.3%, respectively, of total invertebrate density in the Santa Ynez River (Appendix S1 & S2). In the Ventura River, invertebrate biomass and density were dominated by *H. azteca* (33% of total biomass, 8% of total density), Chironominae/Orthocladiinae (32, 14%), Oligochaeta (9, 5%), Chydoridae (7, 22%), *Tricorythodes* (5, 2%) and Ostracoda (5, 38%). Small herbivores comprised 50%, predatory invertebrates 2%, micropredators 2%, meiofauna 13%, filter-feeders 0.3% and Chironominae/Orthocladiinae 32% of total invertebrate biomass and 19, 1, 0.8, 66, 0.02 and 14%, respectively, of total invertebrate density in the Ventura River (Appendix S1 & S2).

Rarefied taxon richness was marginally higher in the Ventura River (26 taxa) than in the Santa Ynez River (18), and the total number of taxa collected and rarefied taxon richness declined, and evenness increased, with crayfish density in the Ventura River, but not in the Santa Ynez River (Fig. 3). Crayfish reduced 38% (10 of 26) of the taxa
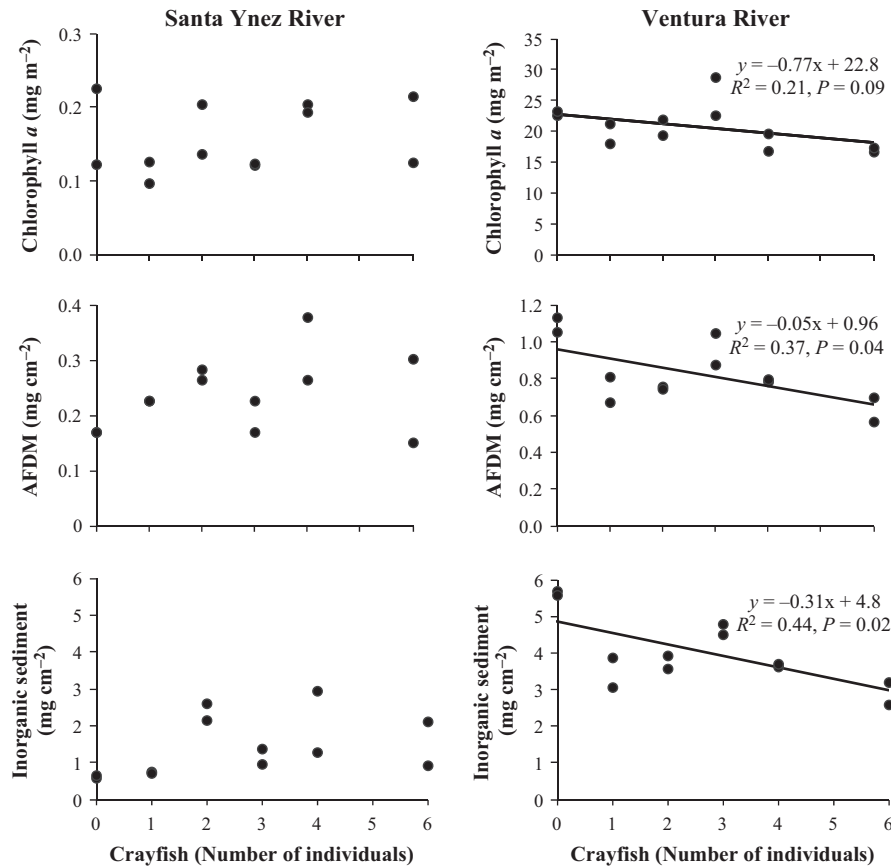
Fig. 1 Relationships between chlorophyll *a* concentration, ash free dry mass, and inorganic sediment versus crayfish density in the Santa Ynez River (left column), and Ventura River (right column). Linear regression equations, fitted lines, coefficients of determination (R^2) and overall *P*-values are shown on each graph with a significant relationship.

in the Ventura River (i.e. *Helisoma*, *Graptocorixa*, Chironominae/Orthocladiinae, Tanypodinae, *Tricorythodes*, *Hydroptila*, Nematoda, *Caloparyphus*, Ostracoda and Cyclopoida), most of which were associated with high abundance of periphyton or sediment (except for *Helisoma* and *Graptocorixa*, these taxa were related to AFDM [Pearson's $r = +0.37$ to $+0.44$, $P < 0.04$) and inorganic sediment (Pearson's $r = +0.35$ to $+0.49$, $P < 0.05$)].

Mean total invertebrate biomass and density across treatments were not different between the Santa Ynez and Ventura Rivers (3160 ± 521 and 2758 ± 363 mg m^{-2} and 29618 ± 3133 and 38564 ± 5515 m^{-2} mean ± 1 SE; *t*-test: $t = 0.75$, d.f. $= 22$, $P = 0.47$, *t*-test: $t = -1.33$, d.f. $= 11$, $P = 0.21$, respectively), but total benthic invertebrate biomass at both sites, and total invertebrate density in the Ventura River, declined with increasing crayfish density (Figs 4 & 5). The biomass of herbivores/detritivores, particularly snails and amphipods, and the density of elmid beetle (*Optioservus*) larvae declined with increasing crayfish density in the Santa Ynez River, but not in the Ventura River. Chironominae/Orthocladiinae density in

the Ventura River, and biomass at both sites, declined with increasing crayfish abundance, as did the densities of *Tricorythodes*, *Hydroptila* and *Helisoma* in the Ventura River. Meiofaunal (primarily ostracods, copepods and chydorid cladocerans) biomass and density decreased significantly with increasing crayfish density in the Ventura River, but not in the Santa Ynez River (Figs 4 & 5).

Although the biomasses of total invertebrates, herbivores and Chironominae/Orthocladiinae declined with increasing crayfish density in the Santa Ynez River, the densities of these groups were not significantly related to crayfish density. This difference could be attributed to the effect of crayfish on the size structure and mean individual biomass of invertebrates, because the average body size of all invertebrate taxa, including herbivorous, micropredatory (*Probezzia* sp., Tanypodinae) and predatory invertebrates (*Graptocorixa*, *Enallagma*), decreased with increasing crayfish density in the Santa Ynez River (Fig. 6). In contrast, total invertebrate density declined with increasing crayfish density in the Ventura River, and the effect of crayfish on total invertebrate biomass was
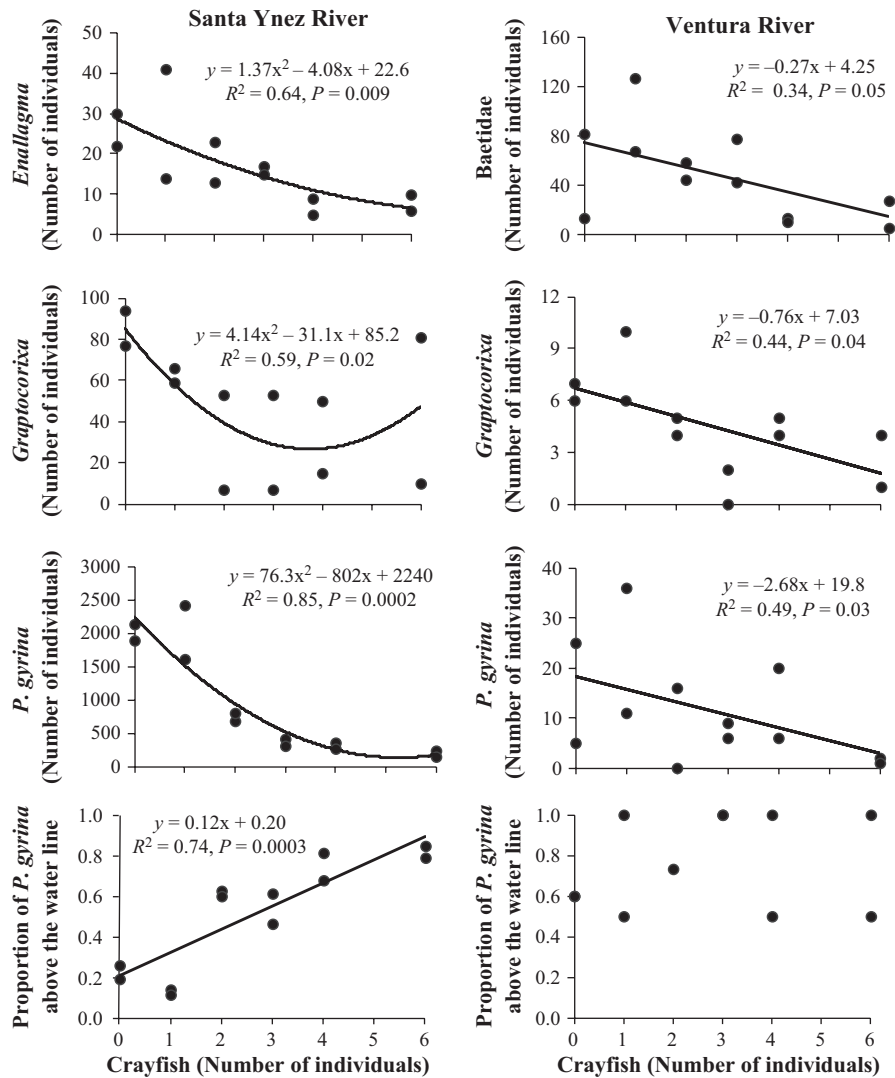
Fig. 2 Relationship between the number of individuals of selected taxa (*Enallagma*, Baetidae, *Graptocorixa* and *Physella gyrina*) observed on the substratum in channels at night and crayfish abundance, and the relationships between the proportion of *P. gyrina* populations observed above the water line at night and crayfish abundance, in the Santa Ynez River (left column) and the Ventura River (right column). Best fit lines and regression equations, coefficients of determination (R^2) and overall *P*-values are shown on graphs showing significant relationships.

enhanced by reductions in the body size of Chironominae/Orthocladiinae (the second most abundant invertebrate group in the Ventura River) induced by crayfish (Fig. 6). Chironomids were smaller in the Ventura River than in the Santa Ynez River (mean length = 7.8 ± 1.3 mm in the Santa Ynez River and 6.0 ± 0.1 mm in the Ventura River; chironomid length *t*-test: $t = -3.12$, d.f. = 12, $P = 0.005$).

The biomass, density and mean body size of predatory invertebrates (i.e. *Enallagma*, *Graptocorixa*, *Probezzia* sp.), as well as naucorid beetle (*Ambrysus*) density, declined with increasing crayfish density in the Santa Ynez River. In the Ventura River, biomass and densities of two specific predatory taxa, *Graptocorixa* and tanypod midges, de-

creased with increasing *P. clarkii* density (Fig. 6; Appendix S1 & S2). The biomass of caddisflies, snails, amphipods and flatworms in the Ventura River, nematodes in the Santa Ynez River and mayflies, naucorids, sphaerid clams, oligochaetes and mites in both systems showed no relationships with crayfish density.

Discussion

The broad geographical distribution and high abundance of *P. clarkii* worldwide, and its complex effects on benthic invertebrates, periphyton, macrophytes, detritus and inorganic sediment, underscore the potential importance of this invasive species in altering the structure and function
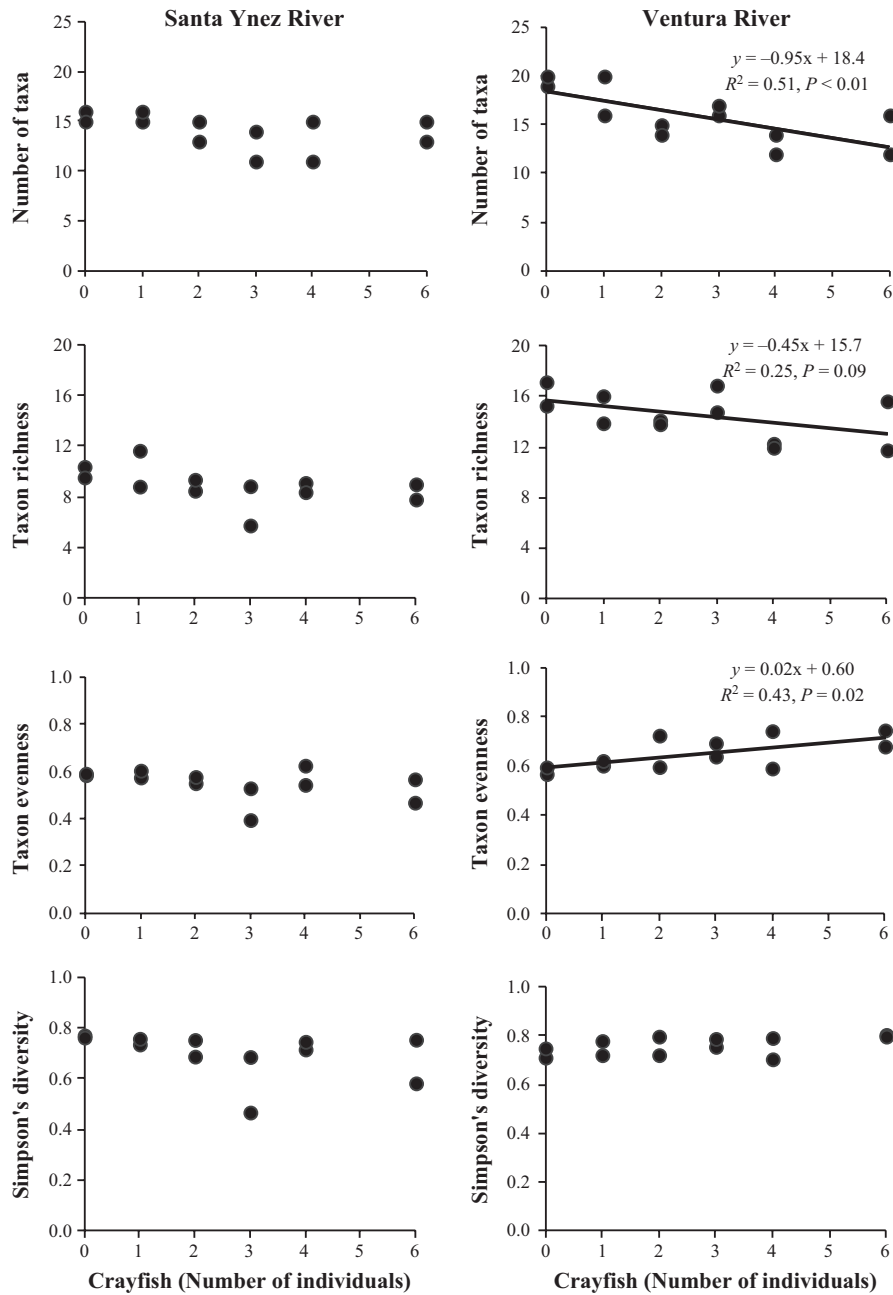
Fig. 3 Relationships between the number of taxa collected, rarefied taxon richness, evenness, Simpson's diversity, and crayfish abundance in the Santa Ynez River (left column) and Ventura River (right column). Lines and equations for the best fit linear or polynomial regression models, coefficients of determination (R^2), and overall *P*-values are shown on the graphs showing significant relationships.

of freshwater communities (Rudnick & Resh, 2005; Gherardi & Acquistapace, 2007). We showed that this large omnivore had different effects on periphyton biomass, amounts of inorganic sediment, invertebrate taxonomic richness and evenness, and benthic invertebrate biomass, density and community structure in nearby streams dominated by pools (Santa Ynez River) or riffles (Ventura River). Our results suggest that crayfish effects on stream communities will be influenced by environmental and biological factors, such as flow regime, habitat complexity, the taxonomic composition of the recipient invertebrate assemblage and the abundance of individual taxa. Crayfish densities were two times lower, *Physella* abundance an order of magnitude higher, and AFDM and chlorophyll *a* between one and two orders of magnitude lower in the Santa Ynez River than in the Ventura River, suggesting that crayfish impacts on these communities depended on the availability of different food types.
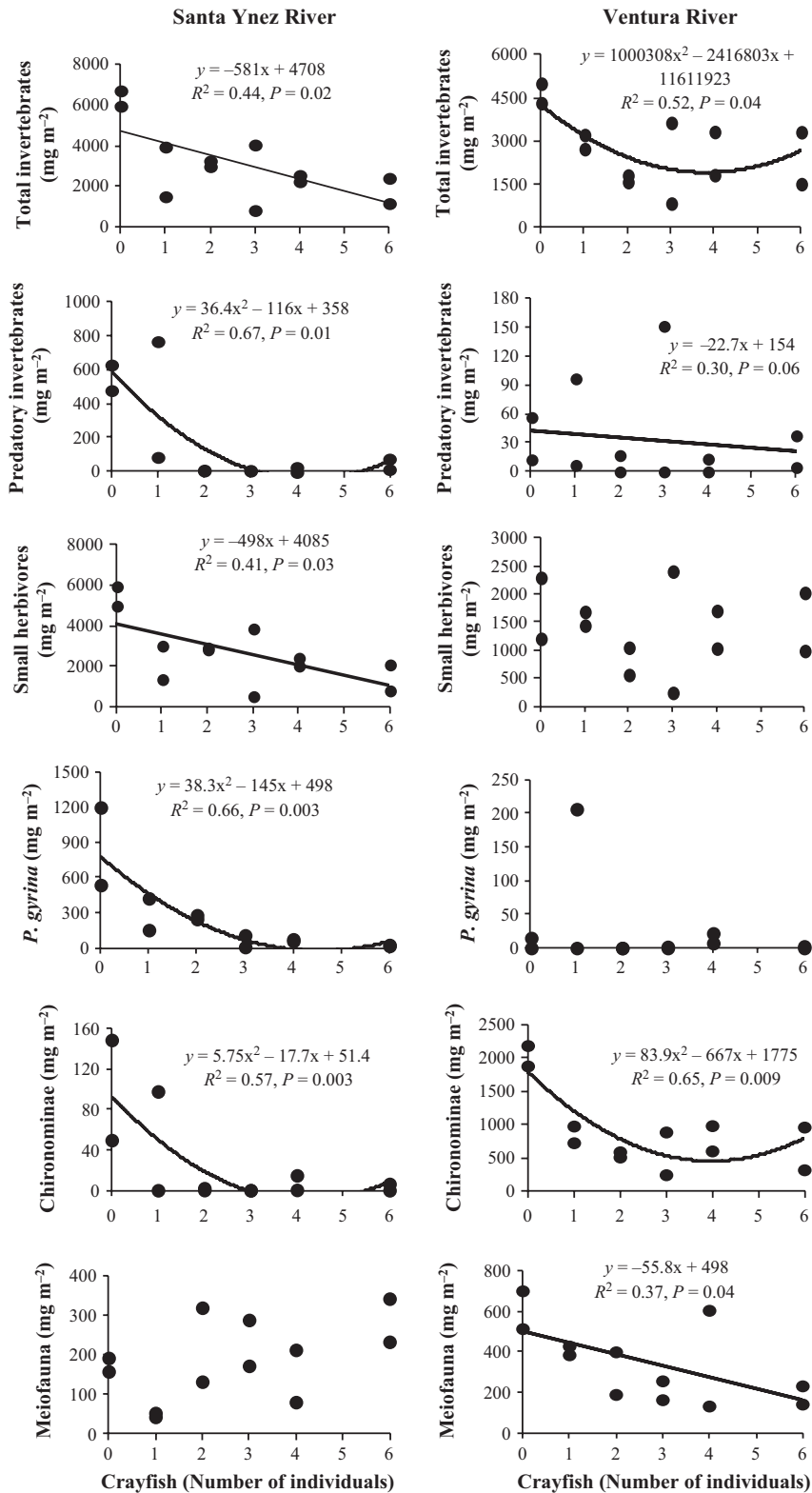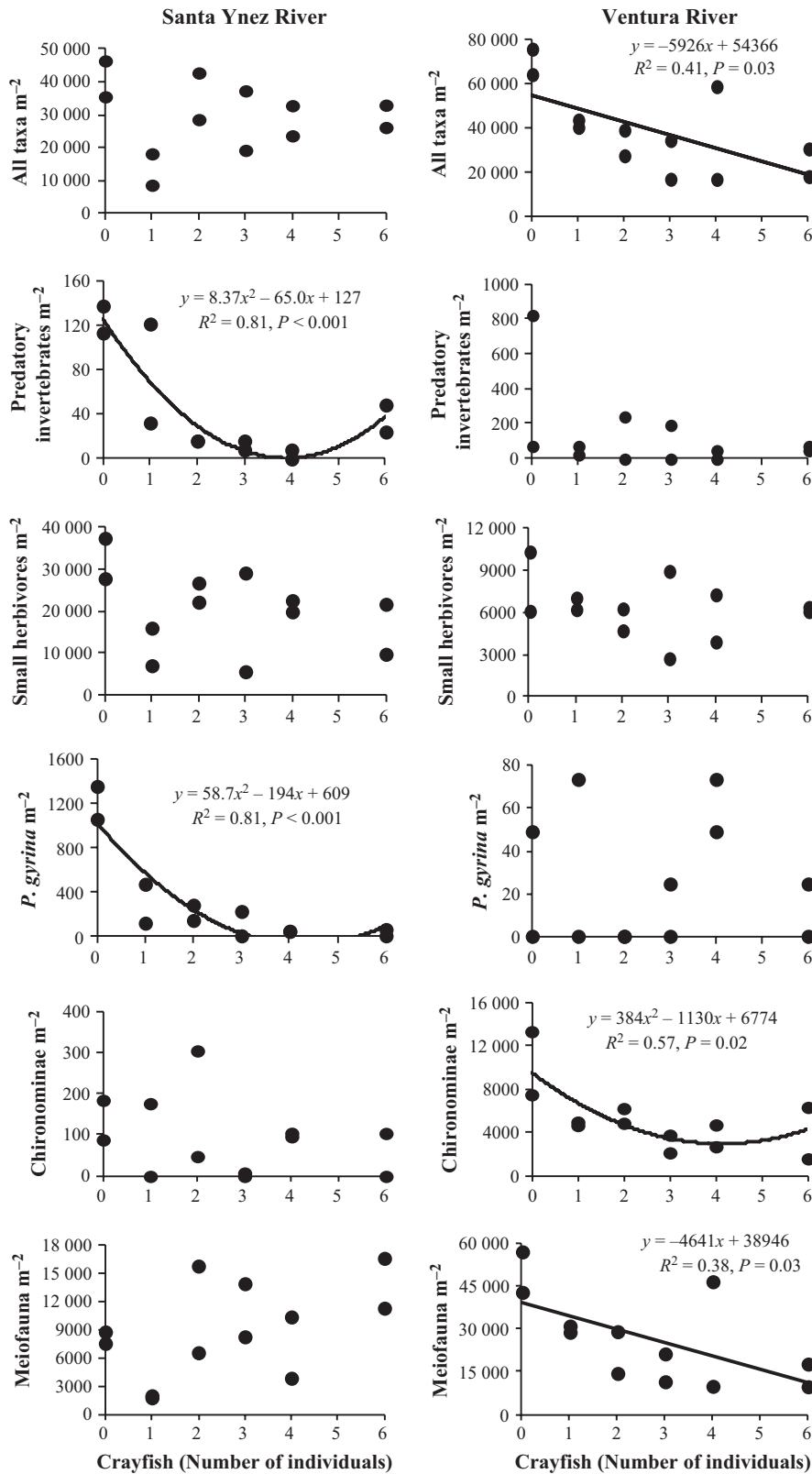
Santa Ynez River   **Ventura River**

Fig. 4 Relationships between the biomass of different feeding or size groups [predatory invertebrates, small herbivores (herbivores/detriti-vores), physid snails (*Physella gyrina*), *Chironominae (=Chironominae + Orthocladiinae), meiofauna] and crayfish abundance in the Santa Ynez River (left) and Ventura River (right). Lines and equations for the best fit linear or polynomial regression models, coefficients of determination (R^2), and overall *P*-values are shown on the graphs showing significant relationships.

Santa Ynez River **Ventura River**

Fig. 5 Relationships between the density of different feeding or size groups [predatory invertebrates, small herbivores (herbivores/detritivores), physid snails (*Physella gyrina*), *Chironominae (=Chironominae + Orthocladiinae), meiofauna] and crayfish abundance in the Santa Ynez River (left) and Ventura River (right). Lines and equations for the best fit linear or polynomial regression models, coefficients of determination (R^2), and overall *P*-values are shown on the graphs showing significant relationships.
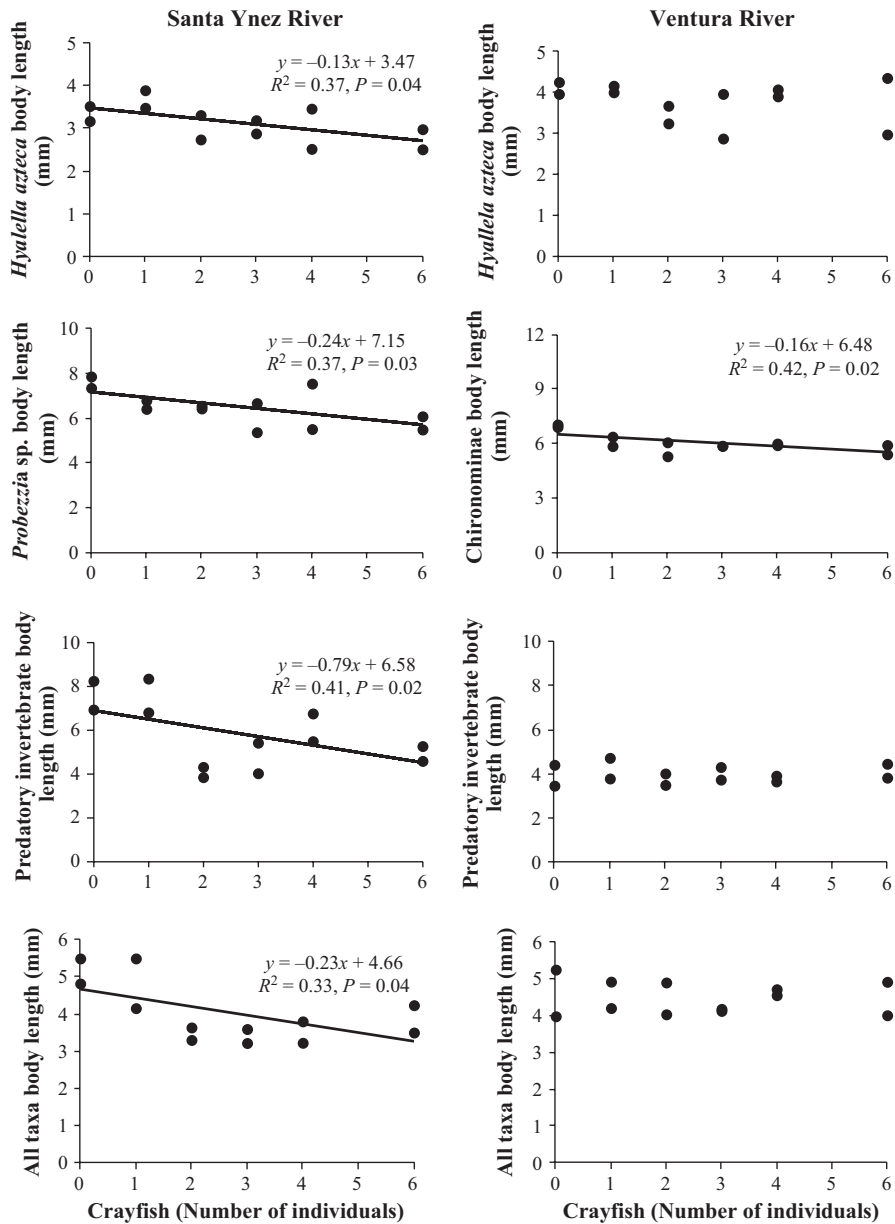
Fig. 6 Relationships between the mean body length of *Hyalella azteca*, *Probezzia* sp. (Santa Ynez River), Chironominae (=Chironominae + Orthocladiinae) (Ventura River), predatory invertebrates and all taxa versus crayfish abundance in the Santa Ynez River (left column), and Ventura River (right column). Best fit regression equations, coefficients of determination (R^2), and overall *P*-values are shown on graphs with significant relationships.

Based on the literature, the known food habits of crayfish and the availability of different food types, we predicted that crayfish would reduce snails in the Santa Ynez River, causing an increase in periphyton biomass, whereas crayfish would primarily reduce periphyton in the Ventura River, causing associated declines in sediment and periphyton-associated invertebrates. The degree to which these expectations were met was variable. In the Santa Ynez River, the biomass of predatory invertebrates, chironomids and herbivores (particularly snails) was

negatively related to crayfish density, whereas, contrary to our prediction, that of periphyton and meiofauna was unaffected by crayfish. Crayfish effects on benthic microalgal biomass reported in the literature have ranged from positive to negative to negligible (Creed, 1994; Nyström, Brönmark & Granéli, 1999; Stelzer & Lamberti, 1999).

Although predation by crayfish on grazers (i.e. snails) often results in indirect positive effects on periphyton biomass (Luttenton, Horgan & Lodge, 1998), crayfish may also indirectly positively affect microalgal biomass by

removing senescent cells and detritus allowing the viable algal understorey to increase (Charlebois & Lamberti, 1996). We hypothesise that we did not observe an indirect positive effect of crayfish on microalgae in the Santa Ynez River because of the combined effects of indirect and direct pathways of interaction, with indirect positive effects on algae being ameliorated by direct crayfish consumption of algae and predatory invertebrates, the latter perhaps leading to a trophic cascade (Charlebois & Lamberti, 1996; Nyström, Brönmark & Granéli, 1996; Stelzer & Lamberti, 1999). Our results were consistent with those of Charlebois & Lamberti (1996), who reported that crayfish (*Orconectes rusticus* Girard) reduced the abundance of most common macroinvertebrate taxa but had weak effects on periphyton (mostly diatoms). In the Ventura River, in contrast, there were negative relationships between periphyton, specific predatory invertebrates (*Graptocorixa*, Tanypodinae), chironomids, and meiofaunal biomass, and crayfish density, but no discernible effects of crayfish on other herbivores or filter-feeders. Carbon stable isotope analysis indicated that microalgae were a dominant food source for snails (*Lymnaea stagnalis* Linnaeus) but a minor food source of crayfish in a Swedish system (Nyström *et al.*, 1999). However, Nyström *et al.*'s (1999) algal community was dominated by adnate, tightly attached green microalgae (e.g. *Coleochaete* sp. Brebisson), whereas the periphyton of the Ventura River was comprised of many less tightly attached diatoms [e.g. there were substantial numbers of *Melosira varians* C. Agardh., *Navicula* sp. Bory and *Synedra ulna* (Nitzsch) Ehrenb.], suggesting that direct crayfish grazing could have reduced benthic algal biomass at this site (Schofield *et al.*, 2008). Further, simultaneous reductions in both sediment and algae indicated that crayfish may have been sweeping or scraping the substratum surface to obtain food.

Crayfish reduced total invertebrate density, the number of taxa collected and rarefied richness in the Ventura River and total invertebrate biomass in both systems. The negative effect of *P. clarkii* on the number of taxa in the Ventura River appears to be one of the few documented instances of a negative effect of invasive crayfish on the diversity of native invertebrates (also see Stenroth & Nyström, 2003). *Procambarus clarkii* reduced 10 of 26 taxa in the Ventura River, most of which were associated with high levels of periphyton or sediment, suggesting that declines in the abundance and diversity of these groups were mediated through reductions in periphyton and sediment caused by crayfish (Helms & Creed, 2005). In contrast, crayfish mainly reduced abundant, large, slow-moving prey, such as snails and odonates, in the Santa Ynez River and did not eliminate any taxa, resulting in no

relationship between taxon richness and crayfish density in that system. Crayfish reduced invertebrate biomass, including the biomass of 44 and 39% of all taxa in the Santa Ynez and Ventura Rivers, respectively, agreeing with the results of other studies (Stelzer & Lamberti, 1999; Nyström *et al.*, 2001; Stenroth & Nyström, 2003; Usio *et al.*, 2006). Despite general relationships between invertebrate biomass and crayfish density, there were clear differences in the responses of specific invertebrate taxa and trophic groups to *P. clarkii* between these systems, indicating the difficulties associated with predicting general community responses to crayfish introductions.

As postulated, the different effects of crayfish on these two systems could be attributed to the food preferences of crayfish and to differences in community composition. In general, crayfish prefer relatively large, slow-moving taxa, such as snails and odonates, which were abundant in the Santa Ynez River but rare in the Ventura River (Usio & Townsend, 2002; Stenroth & Nyström, 2003; Dorn & Wojdak, 2004; Nyström, 2005; Usio *et al.*, 2006, 2009). Crayfish had strong negative effects on the biomass, body sizes and/or density of large predatory invertebrates (odonates, naucorids and corixids), herbivores (snails and amphipods) and large chironomids (mean length in 0 crayfish treatment = 9–12 mm) in the Santa Ynez River, agreeing with previous studies (Stelzer & Lamberti, 1999; Nyström *et al.*, 2001; Helms & Creed, 2005; Dickey & McCarthy, 2007). Although the mechanisms causing these reductions are uncertain, it is probable that crayfish reduced sedentary or slow-moving prey through direct consumption; however, it is possible that mobile prey (e.g. amphipods, corixids and naucorids) avoided or fled from channels with high crayfish activity (Englund, Cooper & Sarnelle, 2001; Usio *et al.*, 2009). Although particular predatory and herbivorous taxa (e.g. *Graptocorixa*, *Tricorythodes*, *Hydroptila*, Chironominae/Orthocladiinae, Tanypodinae and small snails) were negatively affected by crayfish in the Ventura River, these taxa were much less abundant or smaller than the dominant predators or herbivores in the Santa Ynez River. For example, the dominant invertebrate predators in the Ventura River, the Tanypodinae midges, were rare in the Santa Ynez River and much smaller than the dominant invertebrate predators in the Santa Ynez system (hemipterans and odonates). Previous studies have reported positive, negative or no crayfish effects on the abundance of many of these taxa, and it appears that responses to crayfish are somewhat species specific, depending on the behavioural and size characteristics of prey (Hart, 1992; Creed & Reed, 2004; Olsson *et al.*, 2008).

In both of our systems, crayfish reduced disproportionately the densities of the largest individuals of dominant

taxa. In addition, because the densities of many small mayfly, caddisfly, chironomid and meiofaunal taxa are positively related to algal abundance, crayfish may have reduced these taxa indirectly by reducing algal biomass (Dudley, Cooper & Hemphill, 1986; Hart, 1992; Creed & Reed, 2004). In summary, we postulate that crayfish had the greatest effects on large, epibenthic prey, such as snails, amphipods, predatory invertebrates and large chironomids in the Santa Ynez River, whereas crayfish in the Ventura River fed primarily on abundant periphyton and periphyton-associated taxa because of the scarcity of large, epibenthic prey in this system, probably leading to reductions in both (Fig. 7).

Crayfish are active nocturnal predators, and there was a strong agreement between the results of night-time surveys and benthic sampling (Cukerzis, 1988). There was also a strong positive relationship between the proportion of *P. gyrina* populations above the water line at night and crayfish density in the Santa Ynez River, but not in the Ventura River. These results largely agree with other studies demonstrating that actively foraging crayfish reduce the abundance of conspicuous invertebrates and alter prey behaviour, such as inducing snails to leave the water (Stelzer & Lamberti, 1999; Turner, Turner & Lappi, 2006; Dickey & McCarthy, 2007; Klose, 2011). Although such changes in the distribution of snails in response to crayfish might influence periphyton abundance, no net effects of crayfish on periphyton were observed in the Santa Ynez River.

In conclusion, crayfish primarily reduced large consumers in the Santa Ynez River and the abundance of periphyton and sediment in the Ventura River (with indirect or direct effects on small invertebrates associated with periphyton), indicating that crayfish were largely secondary or tertiary consumers in the Santa Ynez River and primary consumers in the Ventura River. Because streams in Mediterranean climates have long periods of low or non-existent flows, and because many climate change models forecast increased droughts in these regions (e.g. Null, Viers & Mount, 2010), our results suggest that the effects of this exotic omnivore may vary with climate change as indicated by the contrasting effects of crayfish on drying (Santa Ynez River) versus free-flowing (Ventura River) systems.
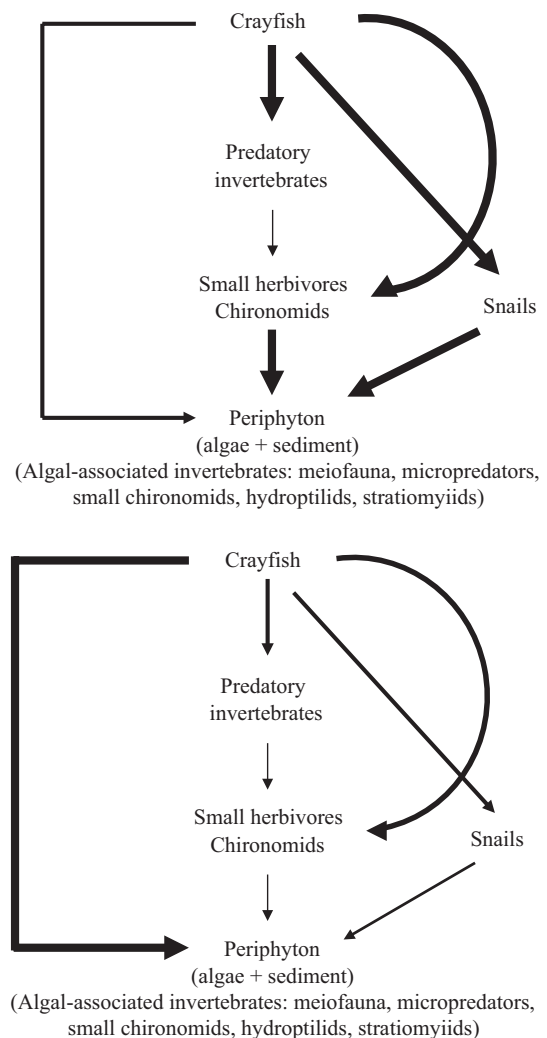
Acknowledgments

Fig. 7 Postulated pathways of interaction in the Santa Ynez River (top) and Ventura River (bottom), California, explaining observed results (pathways are based on the results of experimental manipulations of crayfish abundance and other food web components (Klose, 2007), as well as literature results). Arrow thickness indicates the strength of direct effects and point from consumers to resources.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Mean (±SE) absolute (mg dry mass m^{-2}) and relative ((mg dry mass per taxon mg^{-1} dry mass across all taxa) × 100) biomass of benthic taxa across all experimental treatments in the Santa Ynez River and Ventura River (14 days after crayfish were added).

Appendix S2. Mean (±SE) absolute (number of individuals m^{-2}) and relative ((number of individuals per taxon/number of individuals across all taxa) × 100) abundance of benthic taxa across all experimental enclosures in the Santa Ynez River and Ventura River (14 days after crayfish were added).

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