

Effects of an invasive crayfish on trophic relationships in north-temperate lake food webs

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SUMMARY

1. The introduction of invasive species is one of the main threats to global biodiversity, ecosystem structure and ecosystem processes. In freshwaters, invasive crayfish alter macroinvertebrate community structure and destroy macrophyte beds. There is limited knowledge on how such invasive species-driven changes affect consumers at higher trophic levels.
2. In this study, we explore how the invasive rusty crayfish *Orconectes rusticus*, a benthic omnivore, affects benthic macroinvertebrates, as well as the broader consequences for ecosystem-level trophic flows in terms of fish benthivory and trophic position (TP). We expected crayfish to decrease abundance of benthic macroinvertebrates, making most fish species less reliant on benthic resources. We expected crayfish specialists (e.g. *Lepomis* sp. and *Micropterus* sp.) to increase their benthic dependence.
3. In 10 northern Wisconsin lakes, we measured rusty crayfish relative abundance (catch per unit effort, CPUE), macroinvertebrate abundance, and C and N stable isotope ratios of 11 littoral fish species. We used stable isotope data and mixing models to characterise the trophic pathways supporting each fish species, and related trophic structure to crayfish relative abundance, fish body size and abiotic predictors using hierarchical Bayesian models.
4. Benthic invertebrate abundance was negatively correlated with rusty crayfish relative abundance. Fish benthivory increased with crayfish CPUE for all 11 fish species; posterior probabilities of a positive effect were >95%. TP also increased slightly with crayfish CPUE for some species, particularly smallmouth bass, largemouth bass, rock bass and Johnny darter. Moreover, both fish body size and lake abiotic variables explained variation in TP, while their effects on benthivory were small.
5. Rusty crayfish abundance explained relatively little of the overall variation in fish benthivory and TP. Although rusty crayfish appear to have strong effects on abundances of benthic macroinvertebrates, energy flow pathways and trophic niches of lentic fishes were not strongly influenced by invasive rusty crayfish.

Keywords: hierarchical Bayesian model, invasive species, lake food web, stable isotopes, trophic niche

Introduction

Ecosystems worldwide are experiencing unprecedented rates of species extinctions and introductions. The result is

rapidly changing biotic composition and, more specifically, biotic homogenisation (Rahel, 2002). Freshwater ecosystems have the highest rate of species endangerment and extinction (Ricciardi & Rasmussen, 1999), and are also

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particularly subject to the effects of species invasions because of their isolation and insularity (Sala *et al.*, 2000; Lodge, 2001; Dudgeon *et al.*, 2006). The rate and magnitude of biotic change are increasingly well documented; though, our understanding of the implications of biotic change for the structure, functioning and ecosystem services provided to humans remains limited (Dobson *et al.*, 2006; Byrnes, Reynolds & Stachowicz, 2007; Pejchar & Mooney, 2009).

Efforts to understand biotic change have traditionally focused on easily measured descriptors of ecosystems: biodiversity, community composition and species abundances. In contrast, trophic flows and the networks of interactions among species that maintain the ecosystem (*sensu* McCann, 2007) have been notably difficult to measure and have thus been mostly neglected. One approach for understanding the effect of biotic change on food-web structure is to examine food webs across gradients of impact (e.g. land use change: Tylianakis, Tschamntke & Lewis, 2007 & pH: Layer *et al.*, 2010). The handful of studies that have examined effects of species invasions on trophic flows or food-web structure have used stable isotopes, typically carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), as ecological tracers across gradients of impacts (Vander Zanden, Casselman & Rasmussen, 1999; Gorokhova *et al.*, 2005; Rennie, Sprules & Johnson, 2009).

Crayfishes are large-bodied benthic omnivores that often play an important structuring role in lotic and lentic ecosystems (Lodge *et al.*, 1994; Nyström *et al.*, 2001). However, food-web studies of crayfishes have generally used small-scale experiments to examine their effect on the abundance of other taxa and trophic levels (Lodge *et al.*, 1994; Nyström *et al.*, 2001; Dorn & Wojdak, 2004). There is growing concern about the spread and impact of invasive crayfishes worldwide (Lodge *et al.*, 2000). Thus, to advance the science and management of invasive crayfish, there is need for interpretation of patterns that occur on the landscape level (Carpenter, 1998). Although negative impacts on macrophytes and benthic invertebrates are well documented (Wilson *et al.*, 2004; McCarthy *et al.*, 2006), the omnivorous habits of most crayfishes often produce complex and unpredictable food-web effects that deviate from simple predictions of cascading trophic interactions (Dorn & Wojdak, 2004).

Rusty crayfish are native to the Ohio River Valley of North America, are spreading rapidly (Olden *et al.*, 2006) and have become a highly problematic invader in north-temperate lakes (Lodge *et al.*, 1998). The reduction of macrophytes in lakes invaded by rusty crayfish has led to declines in macrophyte-reliant fishes such as *Lepomis* sp. Notably, *Lepomis* sp. feeds on young crayfish, such that

their decline may further reinforce crayfish dominance in invaded systems (Roth *et al.*, 2007).

This study examines the food-web consequences of rusty crayfish (*Orconectes rusticus*, Girard) invasion in North American north-temperate lakes. We examined lakes along a gradient of rusty crayfish relative abundance and considered how invasive species-driven changes in benthic invertebrate abundance correspond to fish trophic position (TP) and benthivory. Our approach extends the traditional approach of examining food-web effects by linking changes to the trophic pathways that support higher trophic levels. We expect negative effects of rusty crayfish on benthic invertebrate abundances. The implications of this for the trophic niche of fishes are more difficult to predict. There are two alternative outcomes: (i) the predicted reduction in benthic prey could lead to reduced fish reliance on benthic prey but no or small changes in TP and (ii) rusty crayfish 'repackage' benthic production into larger portions, thereby facilitating the transfer of benthic production to higher trophic levels, and also increasing TP because crayfish add an extra trophic level. However, the effect may be species dependent, whereby fishes that specialise on crayfish (e.g. *Lepomis* sp. and *Micropterus* sp.) would be more benthic in crayfish-dominated lakes, while fishes not capable of feeding on crayfish because of gape limitation would shift towards pelagic prey because there are fewer benthic resources in crayfish-dominated lakes. Thus, for the food web as a whole, rusty crayfish-dominated lakes could exhibit increased segregation between pelagic and benthic trophic pathways.

Methods

Between 15 and 28 July 2002, we sampled 10 lakes in the Northern Highlands Lake District, Wisconsin (Vilas County) for crayfish, benthic macroinvertebrates and fish. We selected lakes based on data from other research projects (e.g. Hrabik *et al.*, 2005; Magnuson, Kratz & Benson, 2006) for which crayfish abundance and fish community data were available. To avoid distribution bias, we chose only lakes with pH (mean \pm 1 SD, 7.4 ± 0.4) and calcium concentrations ($9.9 \pm 1.1 \text{ mg L}^{-1}$) well within the limits required by rusty crayfish for survival and reproduction (c. 2.5 mg L^{-1} for Ca) (Capelli & Magnuson, 1983). Lakes varied in physicochemical attributes, but there were no relationships with crayfish catch per unit effort (CPUE) (Table 1). At each lake, we randomly chose one sand site and one cobble site, located away from cottages, swimming beaches and boat launches, to reduce anthropogenic disturbances (e.g., wading, boat scars and macrophyte removal).

Table 1 Abiotic lake data, rusty crayfish catch per unit effort (CPUE) and retained axes (eigenvalues >1) from the performed principal component analysis. Factor loadings >0.5 are in shown bold

Lake	Big St				Wild rice	White		Big			PC 1	PC 2
	Germain	Lost	Palmer	Tenderfoot		sand	Island	lake	Trout	Boulder		
Lake area (km ²)	6.66	2.24	2.63	1.58	1.52	3.05	3.6	3.44	15.66	2.16	0.844	0.313
Mean depth (m)	6.4	3.4	2.4	4.4	3.4	10.1	4	6.1	14.9	3.7	0.938	0.067
Secchi depth (m)	2.4	2	1.4	2.2	2.7	5.4	2.5	3.1	6.1	1.7	0.869	-0.051
Chlorophyll- <i>a</i> (mg m ⁻³)	1.9	8.5	11.4	8.9	7.5	6.8	10.9	8.4	33	11.1	-0.771	-0.191
DOC (mg L ⁻¹)	3.7	4	7.9	12.9	5.2	3.7	4.9	6.1	3	7.1	-0.816	0.194
Tot phosphorous (mg m ⁻³)	28.3	14	24	21.4	21.2	8.8	37.3	31.6	16.9	23.8	-0.383	0.851
Conductivity (µS cm ⁻¹)	91	75	81	100	92.7	74	95	130.8	98	82.5	0.060	0.903
Rusty crayfish relative abundance (CPUE)	0	0	0	0	1.1	8.9	15.8	22.3	65.8	70.4	-	-
Eigenvalue											3.758	1.719
Variance explained (%)											53.679	24.554

Benthic invertebrates

To characterise the littoral benthic invertebrate community in each lake, invertebrates were collected using an underwater airlift sampler within a 0.25-m² quadrat (see the study by Butkas, Vadeboncoeur & Vander Zanden, 2011, for details about the airlift). This method samples the overall macroinvertebrate community (both epi- and in-faunal species). Samples were collected at a depth of 1 m in triplicate for both sand and cobble habitat in a 500-µm mesh bag at the top of the airlift. Samples were transported on ice and hand-sorted within 4 h of collection. Picked specimens were fixed in 70% ethanol and identified to genus. For statistical analysis, we pooled macroinvertebrate numbers into broad taxonomic groups, namely Tricoptera, Ephemeroptera, Diptera, Amphipoda, Isopoda and Mollusca, because of large among-lake variability in presence at the genus level.

We estimated crayfish relative abundance with 15 modified baited minnow traps per lake. Traps were set in sand and cobble habitat and retrieved after 24 h. Rusty crayfish occupy both sand and cobble habitat (K. Wilson, unpubl. data) and will move across habitats in the pursuit of baited traps (Byron & Wilson, 2001). Males enter traps more frequently than females, and thus our CPUE data reflect male relative abundance rather than population relative abundance. Data are expressed as the mean whole-lake catch per unit effort (CPUE; crayfish trap⁻¹ day⁻¹).

Stable isotope analysis

Fishes were collected for stable isotope analysis using overnight fyke net sets, seine nets and electroshocking. Dorsal muscle tissue was taken from larger fishes using a

5-mm biopsy punch. Wounds were doused with Betadine, an antibacterial solution, and then sealed using medical superglue before the fish was returned to the water. Smaller fish (generally ≤100 mm in length) were sacrificed, placed on ice, and the dorsal tissue was dissected in the laboratory. We collected tissue samples from up to five individuals each of up to 11 commonly occurring fish species from each lake: black crappie (*Pomoxis nigromaculatus*, Lesueur), bluegill (*Lepomis macrochirus*, Rafinesque), bluntnose minnow (*Pimephales notatus*, Rafinesque), Johnny darter (*Etheostoma nigrum*, Rafinesque), largemouth bass (*Micropterus salmoides*, Lacepède), logperch (*Percina caprodes*, Rafinesque), pumpkinseed sunfish (*L. gibbosus*, L.), rock bass (*Ambloplites rupestris*, Rafinesque), smallmouth bass (*Micropterus dolomieu*, Lacepède), walleye (*Sander vitreus*, Mitchill) and yellow perch (*Perca flavescens*, Mitchill).

Benthic macroinvertebrates for stable isotope analysis were collected by hand, using the airlift or with hand held nets. At least three amphipod specimens and five bivalves were collected per lake as littoral and pelagic baseline indicators, respectively. We also sampled Gastropoda, Ephemeroptera, Chironomidae and Odonata, but all groups were not found in all lakes. We collected additional crayfish by hand in lakes with low crayfish trap catches.

Fish samples were dried for 24–48 h at 60 °C, ground to a fine powder and 1 ± 0.2 mg of dry powder was put into tin capsules. Samples were analysed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.), by the University of California, Davis stable isotope facility. Twenty per cent of the samples were run in duplicate; the mean standard error of replicates was 0.11‰ for N and 0.097‰ for C.

Benthivory and TP calculations

We estimated benthivory, defined as the estimated reliance on littoral benthic resources, of each individual fish using $\delta^{13}\text{C}$ data and a two-source mixing model (Vander Zanden & Rasmussen, 2001):

$$\text{Benthivory} = \frac{(\delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{mussels}})}{(\delta^{13}\text{C}_{\text{amphipods}} - \delta^{13}\text{C}_{\text{mussels}})} \quad (1)$$

Littoral amphipods (whole) and unionid mussels (foot tissue) from depths ≤ 1.5 m were used as benthic and pelagic end members, respectively. We assumed no trophic fractionation for C, since estimates of carbon trophic fractionation broadly overlap with zero (Post, 2002; McCutchan *et al.*, 2003), and using a different value does not change the overall conclusions, only the actual benthivory estimates.

Trophic position of each individual fish was calculated as:

$$\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - [\delta^{15}\text{N}_{\text{amphipod}} \times \text{benthivory} + \delta^{15}\text{N}_{\text{unionid}} \times (1 - \text{benthivory})]) / 3.4 + 2, \quad (2)$$

where the contribution of unionid mussel and amphipod $\delta^{15}\text{N}$ was weighted by the estimated benthivory for each individual fish. Unionids and amphipods were assumed to have a TP of 2.0. We assumed $\delta^{15}\text{N}$ trophic fractionation to be 3.4‰ based on the published literature (Vander Zanden & Rasmussen, 2001; Post, 2002).

Statistical analyses

To summarise differences in abiotic factors among lakes, we performed a principal component analysis (PCA) with a correlation matrix. All variables (Table 1) were log-transformed, centred on their mean and normalised prior to analyses (Quinn & Keough, 2002). We present the non-rotated solution because rotating the PC axis did not improve interpretation. PC axis one explained 53% of the variation in abiotic factors among lakes and was associated with lake size, chlorophyll-*a* concentration and DOC concentration (Table 1). PC 1, hereafter referred to as Lake Index, was used in the following analysis to account for these abiotic among-lake differences.

We used two multivariate analyses of covariance (MANCOVA) to separate the effects of abiotic factors and crayfish CPUE on benthic macroinvertebrate densities for the six taxa that occurred in all lakes. In the first analysis,

we summarised the effect of Lake Index (covariate) on macroinvertebrate densities (dependent variables, $\log(x + 1)$ transformed), while controlling for sampled habitat (fixed block factor) by saving the residuals. In the second MANCOVA, we used the saved residuals as dependent variables and tested the effect of rusty crayfish CPUE ($\log(x + 1)$ -transformed) (covariate). The MANCOVA considers potential autocorrelation among dependent variables, and significant MANCOVA factors were further evaluated with univariate between-subject ANCOVAs (Quinn & Keough, 2002).

We were interested in determining whether variation in rusty crayfish density is associated with changes in trophic niche (benthivory or TP) for common fish species in our study lakes. In examining any such relationship, we wanted to control for fish length and lake effects (Lake Index), both of which could also influence trophic niche. Furthermore, we assumed that crayfish, fish length and lake effects all might vary among species. We therefore used multilevel models to describe the variation in benthivory and TP among individuals in the data set. Specifically, the predicted benthivory or TP of individual *i* from species *j* in lake *k*, as a linear function of the predictors, was:

$$\eta_i = \beta_{0,j[i]} + \beta_{1,j[i]}L_i + \beta_{2,j[i]}C_{k[i]} + \beta_{3,j[i]}P_{k[i]} \quad (3)$$

where L_i is the length of individual *i*, $\ln(x + 1)$ transformed and centred on the mean, $C_{k[i]}$ is the crayfish CPUE in lake *k* that contains individual *i*, $\ln(x + 1)$ transformed and centred on the mean, and $P_{k[i]}$ is the Lake Index in lake *k* that contains individual *i*. The four parameters $\beta_{0,j[i]}$, $\beta_{1,j[i]}$, $\beta_{2,j[i]}$ and $\beta_{3,j[i]}$, collectively β_j , represent the intercept, length effect, crayfish effect and Lake Index effect for species *j* that contains individual *i*. The β_j for the 11 fish species follow a multivariate normal distribution,

$$\beta_i \sim N(\mathbf{M}_\beta, \Sigma_\beta) \quad (4)$$

where \mathbf{M}_β is a vector containing the mean value for each of the four parameters and Σ_β is the corresponding variance-covariance matrix, which we modelled using the scaled inverse-Wishart distribution to account for correlations among the parameters (Gelman & Hill, 2007). Because observations of benthivory are constrained between 0 and 1, we modelled them using the beta distribution with a link function relating the linear predictor η_i to the parameters of the beta distribution:

$$y_i \sim \beta(\varphi\mu_i, \varphi(1 - \mu_i))$$

$$\mu_i = \frac{1}{(1 + \exp(-\eta_i))}$$
(5)

Similarly, because observations of TP as defined earlier are constrained to be ≥ 2 , we modelled them using the gamma distribution, $\text{tpb } 3$

$$z_i \sim \gamma(\varphi\mu_i, \varphi)$$

$$\mu_i = \exp(-\eta_i) + 2$$
(6)

We put uninformative priors on the hyperparameters of eqns 3, 4 and 5, and fit the models for benthivory and TP using the WinBUGS software (Lunn *et al.*, 2000), keeping 1000 samples from the posterior of each parameter after burning in and thinning each of three Markov chains.

We report parameter estimates from the linear portion of the model (eqn 3), which describe the change in the linear predictor η per unit change in the transformed predictor. These parameter estimates are the clearest way to indicate whether a given effect is 'significant' in the model. However, because of the nonlinear relationships between η and benthivory or TP (eqns 5 and 6), and to the transformations of the predictor variables, these parameter estimates are not directly interpretable as a change in benthivory or TP per unit change in the predictor. For TP in particular, a negative parameter estimate from eqn 3 actually indicates a positive effect on TP, because of the inverse relationship in eqn 6. Therefore, to put the parameter estimates in context, in some cases, we also describe the change in benthivory or TP that corresponds to a given parameter estimate.

Results

Lake Index

The PCA retained two axes that explained 53.7 and 24.6% of the variation in abiotic factors among lakes. PC1 (Lake Index) described a gradient in lake DOC concentration, chlorophyll-*a* concentration and lake size, while PC2 described nutrient status (total phosphorous) and conductivity (Table 1). Chlorophyll-*a* and DOC concentration were negatively correlated with PC1, whereas lake size and Secchi depth had positive correlations with PC1. Thus, lakes with negative values on PC1 were smaller, with low Secchi depth, higher chlorophyll-*a* and DOC

concentrations than lakes with positive values on the PC1 axis.

Macroinvertebrate community

Increasing densities of rusty crayfish were associated with a reduction in macroinvertebrate species richness from 53 to 20, and a decrease in macroinvertebrate densities from 2100 to 176 m^{-2} (partial correlation, correcting for Lake Index, log species richness versus log CPUE; $r = -0.862$, $P = 0.003$, d.f. = 10; log invertebrate density versus log CPUE; $r = -0.837$, $P = 0.005$, d.f. = 7). Smaller lakes with higher chlorophyll-*a* and DOC concentrations had higher densities of macroinvertebrates than larger, less productive lakes; that is, there was a significant effect of Lake Index (Wilks' $\lambda = 0.764$, $F_{52.0,6.0} = 2.681$, $P = 0.024$). There were also differences in invertebrate densities between habitats (MANCOVA, Wilks' $\lambda = 0.463$, $F_{52.0,6.0} = 10.06$, $P < 0.001$), with higher densities in sandy habitat. Residuals from the MANCOVA using Lake Index were subsequently used to examine the effects of crayfish CPUE. MANCOVA revealed that there was an effect of crayfish CPUE on the six invertebrate taxa that occurred in all lakes (Wilks' $\lambda = 0.381$, $F_{52.0,6.0} = 14.109$, $P < 0.001$) (Fig. 1), but there was no difference in densities between habitats (Wilks' $\lambda = 1$, $F_{52.0,6.0} = 0$, $P = 1.0$). Individual between-subject ANCOVAs revealed that densities of Trichoptera ($F_{1,58} = 21.292$, $P < 0.001$), Amphipoda ($F_{1,58} = 9.754$, $P = 0.003$), Isopoda ($F_{1,58} = 5.858$, $P = 0.020$) and Mollusca ($F_{1,58} = 49.644$, $P < 0.001$) decreased with increasing crayfish CPUE, whereas there were no effects on Diptera ($F_{1,58} = 0.391$, $P = 0.538$) and Ephemeroptera ($F_{1,58} = 0.534$, $P = 0.472$) (Fig. 1).

Fish benthivory

Fish benthivory varied substantially both among and within species. The model provided unbiased predictions of benthivory and explained 36% of the observed variation. Among species, there were large differences in mean benthivory, small differences in the effects of fish length and Lake Index on benthivory and very little difference in the effect of rusty crayfish relative abundance on benthivory (Fig. 2). For fish of average length in lakes with intermediate Lake Index and crayfish relative abundance, the mean predicted proportional benthivory ranged from *c.* 0.40 for walleye to *c.* 0.90 for Johnny darter and log perch (Fig. 2a). In general, fish length had little effect on benthivory over the size range that we considered (Fig. 2b). Yellow perch were a

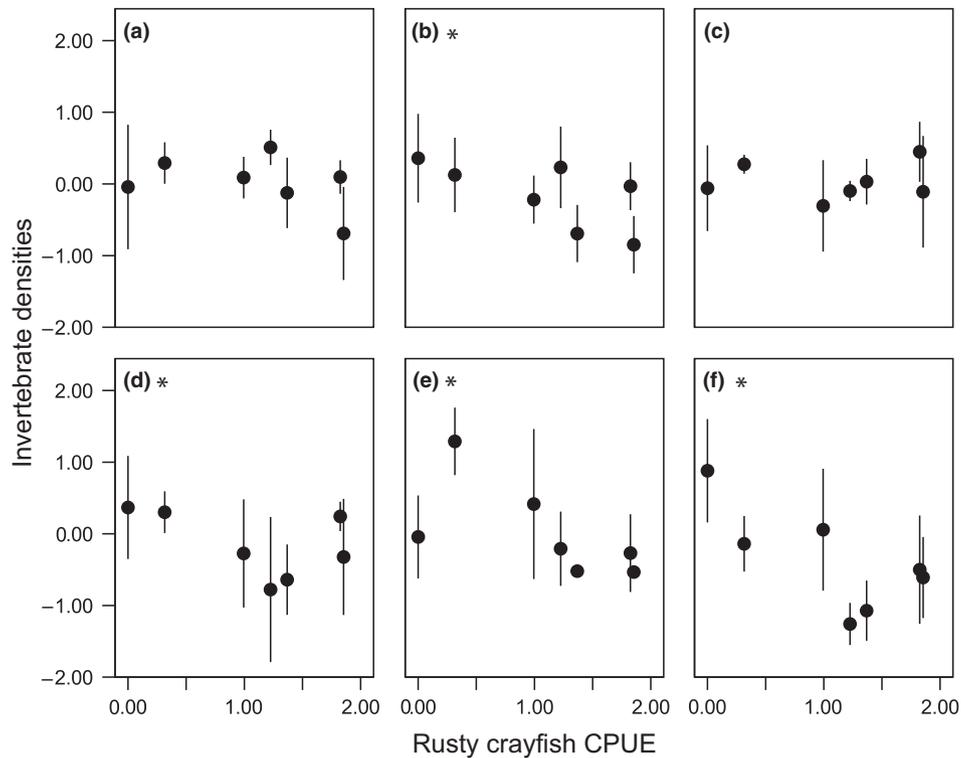


Fig. 1 The effect of increasing rusty crayfish densities (CPUE, $\log(x + 1)$) on benthic macroinvertebrate densities ($\log(x + 1)$), with variation caused by abiotic factors (Lake Index) removed (see text for details), (a) Ephemeroptera, (b) Trichoptera, (c) Diptera, (d) Amphipoda, (e) Isopoda and (f) Mollusca. Since habitat was not significant in the second analysis, density data were pooled by lake. Error bars denote ± 1 SD. Significant effects are indicated by *.

notable exception to this pattern; the predicted proportional benthivory for perch in an average lake was 0.40 at 50 mm length, but 0.71 for individuals at 150 mm length. There was also some evidence for a positive effect of fish length on benthivory for largemouth bass and log perch.

Benthivory was positively associated with rusty crayfish relative abundance for most of the species that we considered (Figs 2c & 3). Estimated 95% Bayesian credible intervals (95% CIs) for the crayfish effect were bounded above zero for all species except for bluegill and yellow perch, for which the 95% CIs narrowly overlapped zero (Fig. 2c). The posterior probability of a positive relationship between benthivory and crayfish CPUE was more than 95% for all 11 species (Fig. 3). Across all 11 species, mean estimates of crayfish effects were clustered fairly tightly around 0.12. This corresponds to an increase in proportional benthivory associated with crayfish CPUE of roughly 0.03 for an average fish in an average lake (Fig. 3).

Benthivory was lower in lakes with high Lake Indexes (larger lakes, with low chlorophyll-*a* concentrations) (Fig. 2d). This effect was strongest for yellow

perch and largemouth bass; for the other species, 95% Bayesian credible intervals for the Lake Index effect overlapped zero. The mean estimate of this effect was 0.21, corresponding to a decrease in proportional benthivory of -0.05 for a change in Lake Index from -0.5 to 0.5 (i.e. from a small lake high in chl-*a* to a large, clear lake).

Fish TP

As with benthivory, TP varied substantially among and within species. Model predictions were unbiased and explained 62% of the observed variation in TP. Among species, there were large differences in mean TP and smaller differences in the effects of fish length, crayfish relative abundance and Lake Index on TP (Fig. 4). For fish of average length in lakes with intermediate Lake Indexes and intermediate crayfish relative abundances, the mean predicted TP ranged from 3.16 for bluntnose minnow to 3.90 for walleye (Fig. 4a). There was a strong positive effect of length on TP for all species (Fig. 4b); the mean estimate of this effect was -0.24 , which corresponds to an increase in TP of 0.37 trophic levels

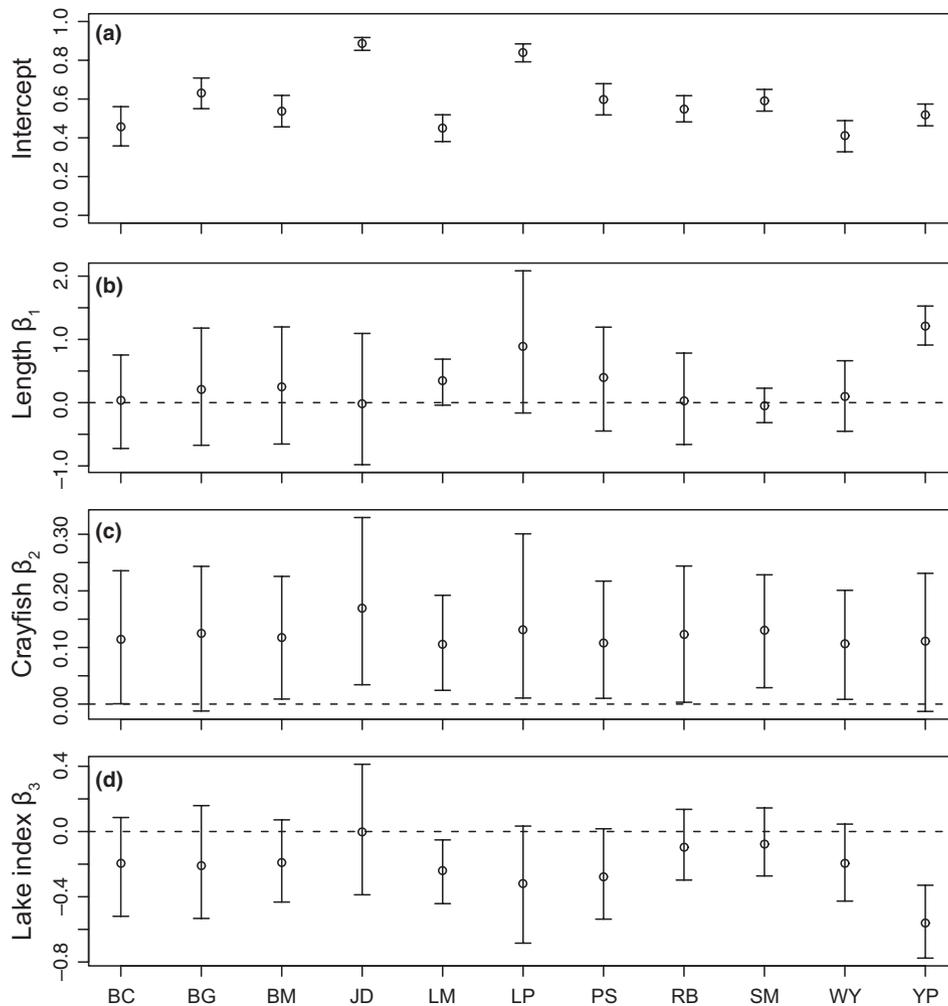


Fig. 2 Species-specific parameter estimates (means and 95% Bayesian credible intervals) for the model fit to benthivory data. (a) mean benthivory (proportions) of an individual with length equal to the mean observed length for that species; (b) effect of length on benthivory; (c) effect of rusty crayfish relative abundance on benthivory; (d) effect of Lake Index on benthivory. Note that the mean benthivory values (panel a) have been converted to the units of the data but that other parameters cannot be directly interpreted as changes in benthivory per unit change in predictor (see text). BC, black crappie; BG, bluegill; BM, bluntnose minnow; JD, Johnny darter; LM, largemouth bass; LP, log perch; PS, pumpkinseed sunfish; RB, rock bass; SM, smallmouth bass; WY, walleye and YP, yellow perch.

for an average fish growing from 50 to 150 mm in an average lake.

Variation in crayfish relative abundance had weak effects on TP. Point estimates of these parameters were negative (indicating a positive effect on TP) for all species except pumpkinseed. The posterior probability of a positive relationship between TP and rusty crayfish CPUE was more than 95% for six species (Fig. 5), but 95% CIs were bounded away from zero only for Johnny darter and smallmouth bass (Fig. 4c). Even for these species, a change in transformed crayfish relative abundance from -0.5 to 0.5 (corresponding to a change in rusty crayfish CPUE from 2.5 to 8.5) corresponded to an increase in TP of <0.08 trophic levels for an average-

length fish in a lake with an intermediate Lake Index (Fig. 5). TP was negatively related to Lake Index for most species, and the effect was most pronounced for yellow perch (Figs 4d & 5). The mean estimate of this effect was 0.077, corresponding to a decrease in TP of 0.11 trophic levels for a change in the Lake Index from -0.5 to 0.5 (i.e. from a small lake high in chl-*a* to a large, clear lake).

Discussion

Species invasions are a major threat to global biodiversity and often affect ecosystem structure and functioning. Hence, there are concerns that invasive species alter

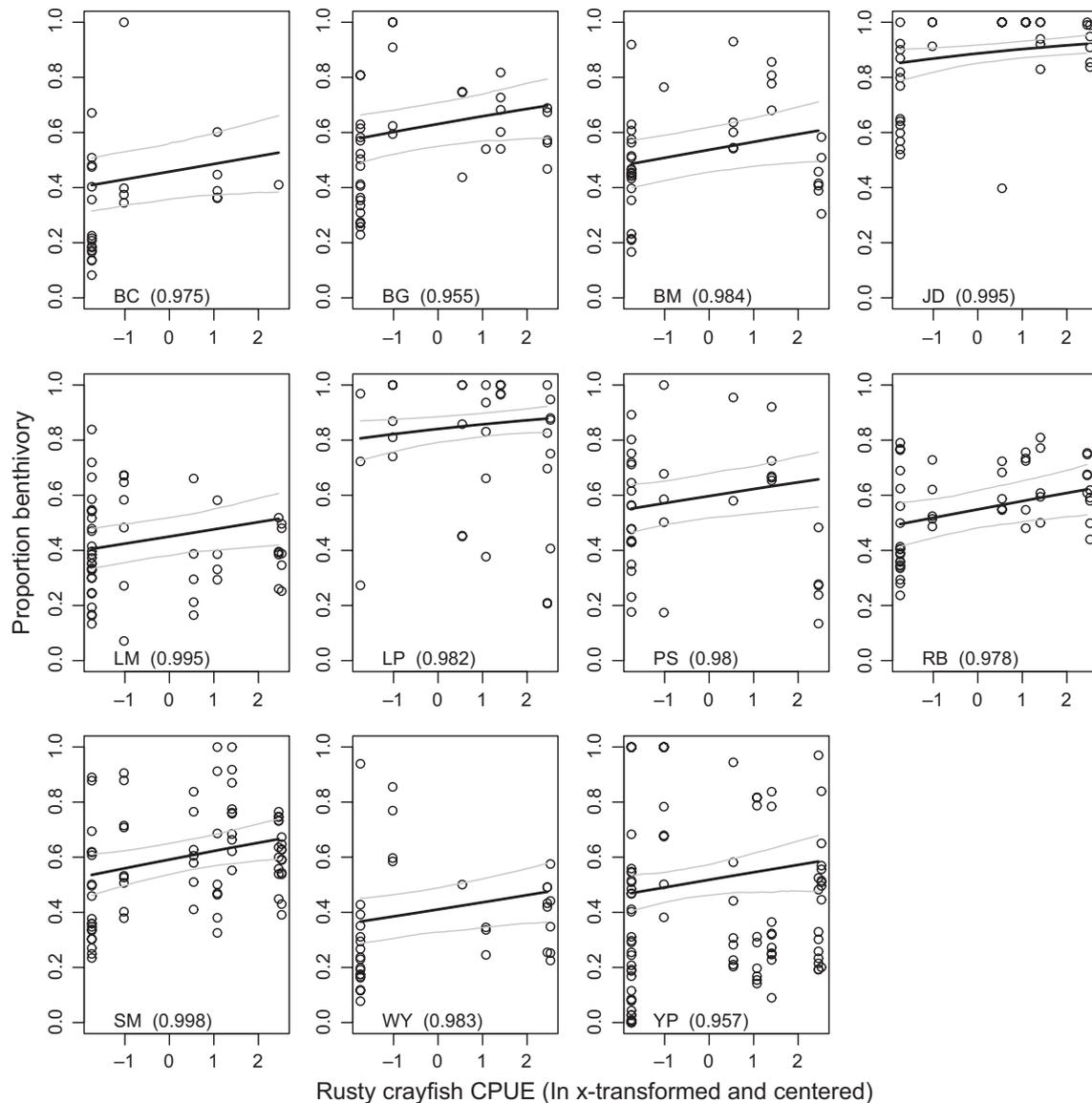


Fig. 3 Relationship between rusty crayfish relative abundance and benthivory for 11 fish species. Points show the observed data; lines show the predicted benthivory (mean and 95% Bayesian credible interval) at a given crayfish relative abundance for an average-length individual of the species in a lake with an average Lake Index. Numbers in brackets are the posterior probabilities of a positive slope for each species. Species abbreviations are the same as in Fig. 2.

both energy pathways in food webs and connectedness between habitats (Schindler & Scheuerell, 2002). Both Rennie *et al.* (2009) and Vander Zanden *et al.* (1999) found dramatic effects on food-web structure when comparing food webs before and after invasion. Potential trophic alterations in invaded lakes could make them less resilient to other anthropogenic changes (Strayer, 2010).

Here, we found a strong negative relationship between the abundance of invasive rusty crayfish and benthic macroinvertebrates, in terms of both species richness and

abundance. In contrast, effects on fish trophic niche variables were more subtle. Increasing crayfish CPUE had a small but significantly positive correlation with benthivory for all fish species. The Lake Index, which summarises a suite of abiotic factors, also had minor effects on overall fish benthivory. There was a tendency for fish to have a higher TP in lakes with high crayfish CPUE, while smaller lakes with high chlorophyll-*a* concentrations tended to have fish with higher TP. Thus, benthic pathways appear to be fairly robust to crayfish relative abundance.

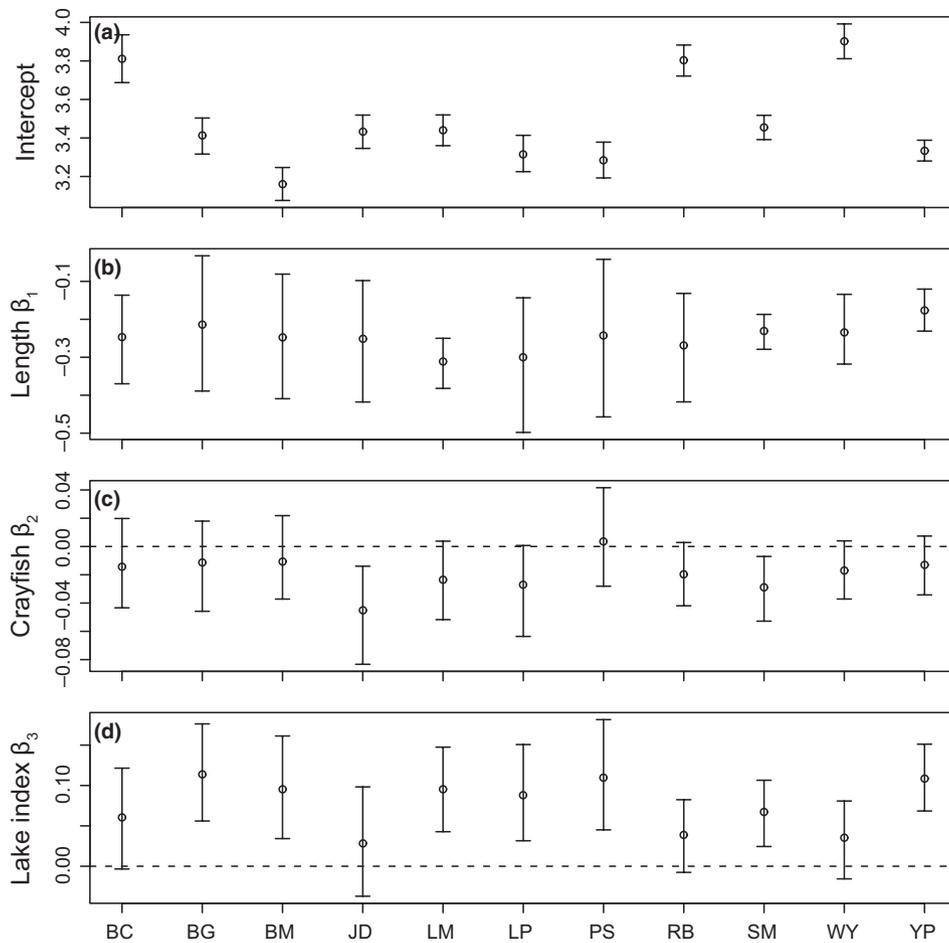


Fig. 4 Species-specific parameter estimates (means and 95% Bayesian credible intervals) for the model fit to trophic position (TP) data. (a) mean TP of an individual with length equal to the mean observed length for that species; (b) effect of length on TP; (c) effect of rusty crayfish relative abundance on TP; (d) effect of Lake Index on TP. Note that the mean TP values (panel a) have been converted to the units of the data but that other parameters can not be directly interpreted as changes in TP per unit change in predictor; in particular, negative estimates of the length, crayfish and productivity parameters indicate positive effects on TP (see text). Species abbreviations are the same as in Fig. 2.

Macroinvertebrate community

With increasing densities of invasive rusty crayfish, overall benthic macroinvertebrate density and species richness declined, as did four of the most commonly occurring taxa. The abiotic factors summarised in the Lake Index were also correlated with macroinvertebrate abundances, but the effect of crayfish density persisted even when variance explained by the Lake Index was removed. Our results suggest that rusty crayfish are a top-down regulating factor on macroinvertebrate communities in these invaded lakes. Although we cannot infer causality from our comparative results, our findings are consistent with other studies. For example, McCarthy *et al.* (2006) showed negative effects of crayfish densities on several macroinvertebrate taxa in a combined meta-analysis of experimental results and a time series approach. This

reduction in benthic prey resources could affect the TP of higher trophic levels. It could also affect grazing and detrital processes in the littoral zone since macroinvertebrates are not only prey for higher consumers, but contribute to ecosystem processes in the littoral zone (Covich, Palmer & Crowl, 1999).

Fish benthivory

Fish benthivory increased with crayfish relative abundance for nine of 11 species. One possible explanation is that crayfish serve as a food source for most of the fish species, thereby compensating for the decrease in other zoobenthic prey. The increase in fish benthivory with increasing crayfish relative abundance further emphasises fish reliance on littoral benthic food sources, as has been previously suggested (Schindler & Scheuerell, 2002; Van-

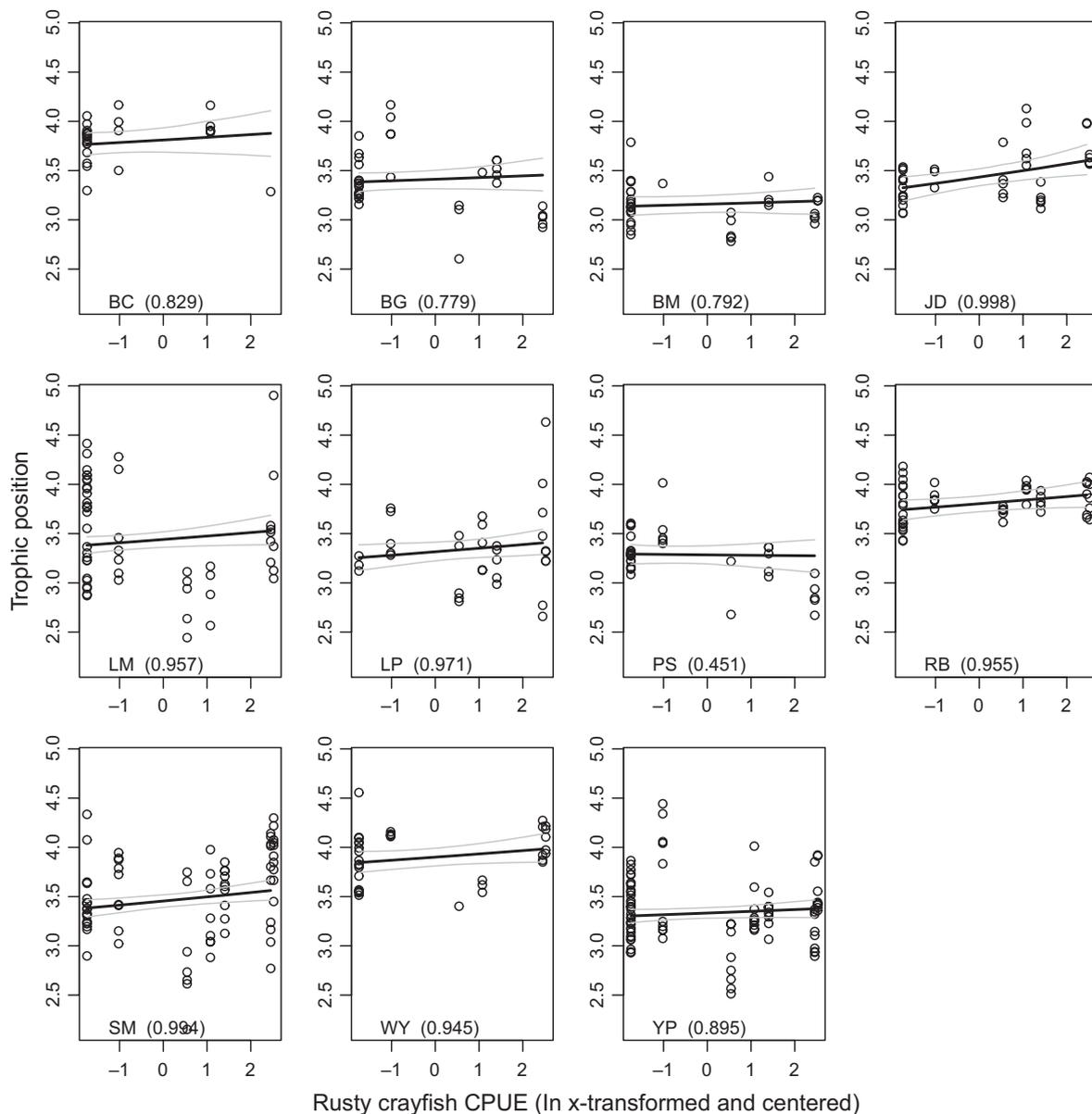


Fig. 5 Relationship between rusty crayfish CPUE and trophic position (TP) for 11 fish species. Points show the observed data; lines show the predicted TP (mean and 95% Bayesian credible interval) at a given crayfish relative abundance for an average-length individual of the species in a lake with average Lake Index. Numbers in brackets are the posterior probabilities of a positive slope for each species. Species abbreviations are the same as in Fig. 2.

der Zanden & Vadeboncoeur, 2002; Karlsson & Byström, 2005). Contrary to our expectation, there were no fish species that decreased in benthivory with increasing crayfish relative abundance. This suggests that fish generally feed on benthic and pelagic resources in similar proportions, irrespective of rusty crayfish abundance. We predicted that larger fish species would be more benthivorous in crayfish-dominated lakes because of direct consumption of crayfish. The results supported this prediction, with the greatest increases in benthivory for

smallmouth bass and largemouth bass. However, small-mouthed fish species like Johnny darter, bluegill and pumpkinseed also increased their benthic reliance. A previous study that compiled diet data for most of the fish species included in this study showed widespread crayfish reliance (Vander Zanden, Cabana & Rasmussen, 1997), including the smaller fish species. However, small-mouthed fish species can probably only consume small, juvenile crayfish that are present for a limited time of the year. Thus, direct consumption of crayfish is

perhaps only part of the explanation for the overall increase in benthivory.

Our analysis does not enable us to measure change in fish benthivory following crayfish invasion, and isotope data were not collected for lakes prior to rusty crayfish invasions. Thus, the observed increase in fish benthivory with increasing crayfish relative abundance could result from a spurious correlation with unmeasured lake-specific features. We found minor effects of the Lake Index on benthivory for most species, whereby fish were less benthic in large lakes with low chlorophyll-*a* concentrations. Even though the Lake Index explains some of the among-lake variation in abiotic factors (Table 1), it does not include size and structure of the littoral zone, which can influence crayfish densities and TP (Nyström *et al.*, 2006; Stenroth *et al.*, 2008). Other studies have found productivity in the littoral zone of small, clear water lakes to be a driver of fish reliance on benthic resources (Karlsson & Byström, 2005). It may be that conditions that allow for high crayfish densities also favour high fish benthic reliance and that the relationship is not causal in nature.

Fish TP

The addition of crayfish to the food web increases the number of consumer steps, which could increase TP of consumers of crayfish. There was a positive effect of crayfish CPUE on TP of smallmouth bass and Johnny darters, and there was a high probability of a positive relationship for six of the 11 fish species. Rusty crayfish have a higher TP than other benthic macroinvertebrates (Roth, Hein & Vander Zanden, 2006), as do other crayfish species (Parkyn, Collier & Hicks, 2001; Olsson *et al.*, 2008). The increase in TP was most pronounced for fish species that also increased their benthivory, suggesting that it is a result of direct consumption of crayfish by both large and small fish species, which is supported by compiled dietary data (Vander Zanden *et al.*, 1997). As for benthivory, there could be between-lake differences that we have not considered that also affect TP. The Lake Index had a larger impact on fish TP than did crayfish CPUE. Fish in large lakes with low chlorophyll-*a* concentrations tended to have lower TP; this was most pronounced for yellow perch. Although rusty crayfish have well-documented effects on macrophytes, benthic macroinvertebrates and certain littoral fish species (Wilson *et al.*, 2004; Willis & Magnuson, 2006; Roth *et al.*, 2007), the effects on the trophic pathways supporting fishes were subtle. For individual lakes, we might be underestimating the effects of

crayfish densities on trophic pathways since there could be among-lake differences that we have not considered. A within-lake time series approach might find stronger effects on trophic pathways over time than we have detected using space as a substitute.

Several invasive species have dramatically affected both structure and functioning of their new ecosystem (Snyder & Evans, 2006). The zebra mussel (*Dreissena polymorpha*, Pallas), for example, has changed carbon flow in lakes, with respect to both routes and amounts, while at the same time promoting periphyton and macroinvertebrates and shifting food webs towards benthic pathways (Jaeger Miehls *et al.*, 2009; Higgins & Vander Zanden, 2010). Moreover, rainbow trout (*Onchorhynchus mykiss*, Walbaum) in streams in Japan have affected both the stream and the surrounding terrestrial ecosystem by altering feeding habits of the native Dolly Varden charr (*Salvelinus malma*, Walbaum). Rainbow trout introduction reduced instream macroinvertebrate abundances and increased periphyton biomass. In addition, insect emergence decreased, thereby decreasing the biomass of terrestrial spiders (Baxter *et al.*, 2004). The relatively weak link between decreased abundance of benthic resources and alterations of food-web structure in our study may be the result of crayfish serving as an alternate resource in the same habitat. Crayfish also feed on algae and detritus, thereby maintaining functioning of the littoral zone and perhaps even enhancing detritus processing because of their larger body size (Parkyn *et al.*, 2001; Usio & Townsend, 2002; Olsson *et al.*, 2008).

Studies to date have found dramatic negative impacts of rusty crayfish invasions on fishes such as *Lepomis* sp. in northern Wisconsin lakes (e.g. Wilson *et al.*, 2004; Roth *et al.*, 2007). Our cross-lakes comparison suggests that energy flow pathways in these lakes are relatively robust to the effects of invasive rusty crayfish and the associated reduction in benthic macroinvertebrates. Several underlying factors could explain this. Even though rusty crayfish appear to decrease the abundance of other benthic macroinvertebrates, rusty crayfish also serve to 'repackage' this energy in the form of larger prey. Crayfish are not a novel prey item for fishes, since these lakes contain a native congener, the virile crayfish (*Orconectes virilis*, Hagen). Resident fishes do consume both juvenile and adult crayfish; though, the extent to which they are reliant is not well known. Finally, our study did not consider the effect of rusty crayfish abundance on fish abundance. It is possible that crayfish-driven reductions in benthic macroinvertebrates produced a decline in the abundance of fish species that are reliant on this resource. Thus, it would be important to consider not only shifts in

trophic pathways supporting fishes, but also effects on fish abundances (Walters & Post, 2008).

Our results support the notion that fish populations tend to be rather catholic with regard to resource use and that they tend to be reliant on a mix of benthic and pelagic resources, as has been suggested in previous studies (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002). In this study, densities of invasive rusty crayfish had relatively minor effects on fish benthivory and TP. Although we cannot infer causality, the results suggest that the broad-scale energy flow pathways in lakes tend to be robust to the rather dramatic effects of invasive rusty crayfish on benthic macroinvertebrate communities. The contrast between invasive species effects on benthic macroinvertebrate population abundances and energy flow pathways is notable, highlighting the importance of incorporating an ecosystem approach. This study is a novel approach to understanding rusty effects in real food webs. Ultimately, we will need both controlled experiments and landscape-level surveys to understand what goes on in real ecosystems (Carpenter, 1998). Further studies should explicitly consider how perturbations such as species invasions affect energy flow pathways and if possible by using stable isotope data prior to invasion from archived samples, fish scales and invertebrate macrofossils.

Acknowledgments

We acknowledge logistic and field support from Trout Lake Station, the National Science Foundation North Temperate Lakes Long-Term Ecological Research (NTL-TER) site, and the Wisconsin Department of Natural Resources. Benthic macroinvertebrate identification was performed by Kurt Schmude (University of Wisconsin – Superior). E.N. was funded with a post doctoral scholarship from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

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(Manuscript accepted 9 August 2011)