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Population density modifies the ecological impacts of invasive species

Jackson, M.C.[1,2*], Ruiz-Navarro A.[1,3] & Britton J.R.[1]

1. School of Applied Sciences, Bournemouth University, Poole, Dorset, UK
2. Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Gauteng, South Africa
3. Departamento de Zoología y Antropología Física, Universidad de Murcia, 30100 Murcia, Spain

***mjackson@zoology.up.ac.za**; +27739150170

Abstract

In assessments of ecological impact in invasion ecology, most studies compare un-invaded sites with highly invaded sites, representing the ‘worst-case scenario’, and so there is little information on how impact is modified by the population density of the invader. Here, we assess how ecological impact is modified by population density through the experimental development of density-impact curves for a model invasive fish. Using replicated mesocosms and the highly invasive *Pseudorasbora parva* as the model, we quantified how their population density influenced their diet composition and their impacts on invertebrate communities and ecosystem processes. The density-impact curves revealed both linear and non-linear density-impact relationships. The relationship between *P. parva* density and zooplankton body mass was represented by a low-threshold curve, where their impact was higher at low densities than predicted by a linear relationship. In contrast, whilst the relationship between density and zooplankton biomass and abundance was also non-linear, it was high-threshold, indicating a lower impact than a linear relationship would predict. Impacts on diversity and phytoplankton standing stock were linear and impacts on benthic invertebrate abundance and decomposition rates were represented by s-shaped curves. These relationships were underpinned by *P. parva* dietary analyses that revealed increasing reliance on zooplankton as density increased due to depletion of other resources. We caution against the common assumption that ecological impact increases linearly with invader density and suggest that increased understanding of the relationship between invader population density and ecological impact can avoid under-investment in the management of invaders that cause severe problems at low densities.

Introduction

Biological invasions have substantial adverse economic and environmental consequences worldwide (Pejchar and Mooney 2009; Pyšek and Richardson 2010; Vilá et al. 2011) and, therefore, research on their management receives considerable attention (e.g. Epanchin-Niell and Hastings 2010; Britton et al. 2011a; Januchowski-Hartley et al. 2011; Larson et al. 2011). Invasive species have been associated with declines in biodiversity and negative effects on ecosystem processes (e.g. Elgersma and Ehrenfeld 2011; Jackson et al. 2014). The impact of invaders, however, varies considerably among species and habitats, and is often context dependent (Larson et al. 2011; Kumschick et al. 2012).

It is commonly assumed that the effect of invasion increases proportionally as invader abundance increases (Yokomizo et al. 2009; Elgersma and Ehrenfeld 2011). However, impact studies tend to compare scenarios of high invader density (i.e. representing the ‘worst case scenario’) with those where the invader is absent. Consequently, there is little evidence to support this assumption, despite the fact that the abundance of a species can vary considerably across its invasive range (Hansen et al. 2013). Evidence suggests that ecological impacts can vary across invasion densities in a non-linear manner (Elgersma and Ehrenfeld 2011), with a recent study revealing that the impact of the ponto-caspian goby *Neogobius melanostomus* declines at higher densities due to increased intraspecific interactions (Kornis et al. 2014).

A limited number of studies have examined the density-dependent ecological impacts of invasive species and they detected both linear and non-linear relationships between population density and ecological impact (e.g. Li et al. 2007; Kulhanek et al. 2011; Thomsen et al. 2011 Green and Crowe 2014; Kornis et al. 2014; Wilkie et al. 2014). For example, Elgersma and

Ehrenfeld (2011) found that the cascading impacts of an invasive shrub (Japanese barberry, *Berberis thunbergii*) were linear on decomposition rates but non-linear on microbial community structure. Whilst animal invasions are generally less well studied when compared to plant invasions, especially in aquatic environments (Lowry et al. 2013), evidence suggests that invasive animals often have cascading impacts on lower trophic levels in lakes and rivers (e.g. Baxter et al. 2004; Ellis et al. 2011), with invasive fish altering primary productivity and decomposition rates (Zavaleta et al. 2001; Simon and Townsend 2003; Baxter et al. 2004). However, we are unaware of any studies that have examined how the population density of an invasive fish alters these cascading effects. Freshwater biodiversity is often considered proportionally the most at risk to environmental change on a global scale (Jenkins 2003) and, therefore, it is of paramount importance to deepen our understanding of relationships between aquatic invader densities and impacts. The population density of aquatic invasive animals is important in shaping not only their direct ecological impacts through their trophic links and competitive interactions, but also their indirect impacts by influencing the strength of trophic cascades. The strength of trophic cascades may be modified through influencing prey grazing pressure (Baum and Worm 2009), intraspecific competition and invader diet (Svanbäck and Bolnick 2007), or the feeding behaviour of the prey (Figueredo and Giani 2005).

Density-impact curves describe the relationship between the population density of an invader and its economic or ecological impact. Recently, they have been recognised as a valuable tool in the impact-assessment and management of invaders (Yokomizo et al. 2009), but they have rarely been applied. Yokomizo et al. (2009) described four potential relationships, including the linear relationship (Fig. 1) that is generally assumed by managers when data relating density and impact are absent. The occurrences of low-threshold and high-threshold curves are particularly important as they indicate that impact is either more or less severe than predicted by the linear relationship, respectively (Fig. 1). Finally, s-shaped curves occur when impact is more or less severe than predicted at low or high invasion densities and vice versa (e.g. Fig. 1; Yokomizo et al. 2009). Here, we adopt the approach of Yokomizo et al. (2009) with application to the ecological impact of an invasive fish. The shape of density-impact curves can depict which invaders have acute impacts at low densities and those whose populations have little ecological consequences other than at their highest population densities.

To develop the density-impact curves in this study, we use a mesocosm experiment to examine the density-dependent impacts of a model invasive fish. Although mesocosm experiments might lack the ‘realism’ of natural experiments or observations, they provide controlled environments where mechanistic relationships can be more easily quantified and scaled-up to represent larger-scale processes (Spivak et al. 2011). Further, mesocosm experiments have proved useful in numerous ecological impact studies on climate change (Stewart et al. 2013) and invasive species (e.g. Rudnick and Resh 2005; Ho et al. 2011; Jackson et al. 2014). The model species was the Southeast Asian fish topmouth gudgeon *Pseudorasbora parva* which is now present across much of Europe (Gozlan et al. 2010a). While studies have determined factors influencing their invasion success (Copp et al. 2007; Britton and Gozlan 2013; Jackson et al. 2013) and interactions with native fish (e.g. Beyer et al. 2007; Jackson and Britton 2013), their density dependent impacts are unknown. Our objectives were to (1) experimentally determine how *P. parva* population density modified (i) their diet composition, (ii) invertebrate community structure, and (iii) ecosystem processes; and (2) develop ecological relevant density-impact curves for the invasive fish. We hypothesise that the impact of the invader will increase linearly with population density, causing a shift in diet as preferred resources become depleted.

Materials and methods

In their native range, *P. parva* are present in both lentic and lotic systems. However, in much of their invasive range in Europe, their highest abundances tend to occur in relatively small lentic systems, with rivers used primarily for natural dispersal into waters downstream on the floodplain (Gozlan et al. 2010a). To represent these small lentic habitats, we used replicated outdoor pond mesocosms which were away from tree cover, located in Southern England, of 1000 L volume and 1.0 m^{-2} surface area. We chose to quantify *P. parva* impact in mesocosms as their relatively simple ecosystems enable accurate inferences to be drawn on impact without the additional complexity of including a broad range of native species that could represent experimental confounds. The experiment comprised four treatments; low, medium and high densities of *P. parva* (8, 24 and 48 individuals, respectively), and an un-invaded control. These fish numbers provided population densities within the range encountered in their invasive populations in Europe, including England where densities have been recorded to 65 m^{-2}, levels far in excess of native fishes (Britton et al. 2008, Britton et al. 2010b, Jackson and Britton 2013). Treatments were randomly assigned to mesocosms, all fish were female to avoid reproduction and maintain population density, and were of 48 to 68 mm starting fork length. Although *P. parva* may invade systems with other fish present, it was necessary for our study to use single populations in order to isolate their impacts and create density-impact curves.

The experiment commenced in July 2012 and ran for 120 days. We established the mesocosms 10 days prior to fish introduction with filling of water from an adjacent fishless pond with a zooplankton community dominated by copepods; 1000 L was added to each mesocosm to ensure that the zooplankton community was equally represented in each mesocosm. Each mesocosm was provided with clean gravel substrates, fish refuge (a 30cm length of 65 mm diameter drain pipe), a pond lily (*Nymphoides peltata*) and were seeded with equal aliquots of chironomid larvae and *Asellus aquaticus.* These species were chosen as: (i) they would mimic a simple pond food web commonly invaded by *P. parva*; (ii) Chironomid larvae, copepod species and *N. peltata* are commonly encountered in their invasive and native range: and (iii) *A. aquaticus* are encountered regularly in their diet in their invasive range (e.g. Rosecchi et al. 1993; Declerck et al. 2002). To quantify leaf litter breakdown rates, we attached a plastic mesh bag (5mm mesh size) containing a known mass (~1.5g) of dry leaf litter from a native tree (*Fagus sylvatica*) to the benthos of each mesocosm. The leaf litter decomposition rates were determined using their exponential decay rate coefficient (*k*; see Heiber and Gessner 2002) and calculated using the start and end dry weights of the packs.

At the end of the experimental period, we recaptured the *P. parva*, with an overall recapture rate of 86 %. All fish were over-anaesthetised (MS-222) before a dorsal muscle sample was taken from a maximum of eight fish (*n* = 3-8) from each mesocosm for carbon and nitrogen stable isotope analyses to determine diet composition. We also collected putative fish-food resources (algae, benthic invertebrates and zooplankton; *n* = 3-9 of each) from each mesocosm for stable isotope analyses. All samples were oven dried overnight at 60ºC before analysis at the Cornell Isotope Laboratory, New York, USA. The data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰) and were used to calculate the fish standard ellipse areas (SEA_c; Jackson et al. 2012) using the siar (Jackson et al. 2011) package in R (R Core Team

2013). These provide a bivariate estimate of a population's core isotopic niche and the subscript 'c' indicates that a small sample size correction was applied (Jackson et al. 2011).

On the final day of the experiment, we sieved 2L of water from each mesocosm through a 250μm sieve. Zooplankton densities and body sizes were determined using microscopy of the sieved water, with all individuals identified and counted to obtain abundance and diversity (Shannon's diversity index) estimates. Body mass estimates were derived using image analysis software (KLONK Image Measurement) where up to 20 randomly selected individuals per species were measured and their mass derived using length-weight regression equations (Bottrell et al. 1976). The total body-mass of all measured individuals of each species were used to estimate total zooplankton biomass. For the size spectra analysis, body mass is expressed in units of carbon (μg, C; assuming 40% of total weight is C; Reiss and Schmid-Araya 2008). To estimate macro-invertebrate abundance, we counted the number of Chironomids and *A. aquaticus* in each leaf litter bag as a standard measure.

As a proxy for primary productivity, we measured phytoplankton standing stock on the final day of the experiment by filtering a 0.5L water sample from each mesocosm through GF/C filters (Whatman®, Maidstone, UK) before adding 5ml of 90% acetone. After 24 hours the samples were centrifuged and the supernatant was used for spectrophotometry to determine chlorophyll-*a* concentration (see Jeffrey and Humphrey 1975; values expressed as mg ml^{-1}).

Data were $\log_{10}$ (x+1) transformed to meet the assumptions of parametric tests. We used analysis of variance to test for differences between treatments in all variables. When using multiple response variables per mesocosm (e.g. isotope data), mesocosm identity was included as a random factor, nested within treatment. If between-treatment effects were significant, post-hoc tests were performed (Tukey's HSD).

To develop the density-impact curves we related each transformed replicate response variable to invasive fish density using linear and non-linear (quadratic and cubic) models in the 'R' computing program (R Core Team 2013). Akaike's Information Criterion (corrected for small sample sizes; AIC_c) was used to select the model that best fit the data. The best fit model was then plotted as the density-impact curve for each response variable.

Results

Invasive fish diet composition

With increased population density, there was a significant dietary shift to resources with lower carbon isotope values ($F_{2,68} = 27.39$, $P < 0.001$; Fig. 2; *cf*. Supplementary Material Appendix 1). Since zooplankton had the lowest carbon signatures of the food resources, this suggests an increased reliance on this resource with increased density (Fig. 2). At low densities, *P. parva* isotope values were more closely associated with benthic invertebrates and algae (Fig. 2). There were no significant differences between the treatments in fish $\delta^{15}N$ ($F_{2,68} = 0.78$, $P = 0.48$) or trophic niche width (SEA_c; $F_{2,9} = 0.07$, $P = 0.93$).

Direct impacts

Overall, 2260 individual zooplankton were identified and 537 measured. Body mass ranged from 0.006 to 4.08 µg C in the un-invaded control compared to a reduced range of 1.48 to 3.70 in the high density treatments. Average zooplankton body mass was significantly higher in the medium and high density mesocosms compared to the control and low density mesocosms

($F_{3,531}$=14.02, P<0.001; Fig. 3A). The relationship between invasive population density and zooplankton body mass was represented by a low threshold curve (Fig. 4A; Table 1).

Zooplankton diversity was significantly reduced at medium and high invasion densities compared to the un-invaded control and, for the latter, compared to the low density invasion scenario ($F_{3,12}$ = 7.05, P = 0.005; Fig. 3B). The relationship between invasive population density and zooplankton diversity was linear (Fig. 4B; Table 1). Zooplankton abundance and biomass were reduced at high invasion densities compared to all other treatments (abundance: $F_{3,12}$ = 11.98, P = 0.001; biomass: $F_{3,12}$= 11.55, P = 0.001; Fig 3C, 3D). The relationships between invasive population density and zooplankton abundance and biomass were high threshold (Fig. 4C, 4D; Table 1).

Benthic macro-invertebrate abundance was significantly higher in the low density mesocosms compared to the medium and high invasion density mesocosms ($F_{3,12}$ = 17.83, P < 0.001; Fig. 3E). The relationship between invasive population density and macro-invertebrate abundance was represented by an s-shaped curve (Fig. 4E; Table 1).

Indirect impacts

Water chlorophyll-a concentrations, used as a measure of phytoplankton standing stock, were significantly higher in the high density treatment when compared to the low density treatment ($F_{3,12}$ = 11.89, P = 0.001; Fig. 3F). The relationship between invasive population density and phytoplankton standing stock was linear (Fig. 4F; Table 1). Leaf litter decomposition rates were significantly higher in the low density treatment compared to the high and medium density treatments ($F_{2,9}$ = 5.26, P = 0.03; Fig. 3G). The relationship between invasive population density and decomposition rates was represented by an s-shaped curve (Fig. 4G; Table 1).

Discussion

The ecological impacts of invasive species are complex and context-dependent (Thomson et al. 2011; Wilkie et al. 2014). Our study adds to a growing body of evidence that impact does not always increase proportionally with invader density (Thiele et al. 2010; Elgersma and Ehrenfeld 2011) and in the case of Staska et al. (2014), the relationship was counter-intuitive, with decreased impact at high density. In addition, our study illustrates that density-impact relationships are response-dependent, as each of the four response types described by Yokomizo et al. (2009) were identified for impacts caused by a single invasive species. This variation between different variables highlights the complexity of predicting the ecological consequences of biological invasions. Our data suggest that assuming linear density-impact relationships is likely to result in poorly directed management efforts that lack prioritisation according to realised, rather than perceived, impact (Yokomizo et al. 2009).

In contrast to the un-invaded control and low density populations, the medium and high invasive *P. parva* populations depleted the benthic invertebrate and zooplankton communities, resulting in two trophic cascades. Phytoplankton standing stock increased due to reduced grazing pressure and decomposition rates decreased due to a decline in shredding invertebrates. In contrast, low density invasive populations caused no statistically distinguishable impacts compared to the control. Similarly, evidence suggests that *P. parva* only have a detectable impact on native fish when present in high invasion densities (Britton et al. 2010b). However, this is independent of the negative consequences for the native fish caused by the rosette agent *Sphaerothecum destruens* for which *P. parva* is a healthy host (Andreou et al. 2012). In contrast, Kornis et al. (2014) found that the impact of the invasive round goby (*Neogobius melanostomus*) diminished at higher densities and attributed this to increased intraspecific interactions.

Nevertheless, where *S. destruens* is either not present or is assessed as causing negligible consequences, direct management intervention on *P. parva* may only be necessary when they are present at high densities when there are measurable impacts on ecosystem functioning. Cascading, indirect consequences for ecosystem functioning as a result of invasions are sometimes considered to be more important from a management prospective than direct impacts (Gozlan et al. 2010b) because they indicate that consequences of the invasion are spreading throughout the food web (e.g. Baxter et al. 2004).

Invasive species management often relies on the principle that the effect of invasion diminishes proportionally as abundance decreases (Yokomizo et al. 2009; Elgersma and Ehrenfeld 2011). For some aspects of ecological impact, our data supports this assumption: linear relationships were apparent between invasive fish density and zooplankton diversity and phytoplankton standing stock. The linear increase in phytoplankton standing stock with increasing fish density resulted from reduced grazing pressure caused by the truncated size range, and reduced biomass, abundance and diversity of zooplankton. This trophic cascade is already well documented in many native and invasive fish communities (e.g. Carpenter et al. 2001; Ellis et al. 2011). Native cyprinid fish are also managed on account of their ecological impacts on plankton through biomanipulation (e.g. Mehner et al. 2002; Hansson et al. 1998; Tátrai et al. 2009) and, therefore, density-impact curves for cyprinid fish might have resonance beyond invasive ecology. Indeed, the finding that *P. parva* were invoking cascading impacts in these mesocosm experiments is consistent with other mesocosm experiments that have shown similar cascading impacts of native fish (e.g. Havens 1993; Vakkilainen et al. 2004). Vakkilainen et al. (2004) found that native fish were more important in regulating zooplankton biomass, and subsequent cascades to phytoplankton, than nutrients. Additionally, there have been documented cases of common carp (*Cyprinus caprio;* Khan et al. 2003), rainbow trout (*Oncorhynchus mykiss*; Buria et al. 2010) and largemouth bass (*Micropterus salmoides*; Carpenter et al. 1987), all of which are invasive in parts of the world, causing similar cascading impacts to *P. parva* on phytoplankton or periphyton. However, a major difference between *P. parva* and native cyprinid fishes, in England at least, is the ability of *P. parva* to form very high population densities, sometimes even higher than those used in our high density treatment, and far in excess of those recorded naturally in native species such as roach (*Rutilus rutilus*; Britton et al. 2010b). Consequently, whilst the densities used in our experiment were applicable to *P. parva*, and most likely to other small, invasive fishes that can form similar population densities (Pinder and Gozlan 2003), they will be less relevant to native fishes.

Limited budgets mean that eradication is an unattainable goal to many managers dealing with invasive animals and instead, controlling abundance at relatively low densities is a common and cost-effective strategy (Simberloff 2009). Nonetheless, adopting this method when the density-impact relationship is non-linear may cause wasted management effort or avoidable impact (Yokomizo et al. 2009). Non-linear density-impact curves indicate that ecological impact is not directly proportional to invasion density and instead, the relationship between the two is context dependent, varying over the density gradient (Elgersma and Ehrenfeld 2011). Zooplankton body-size, abundance and biomass all had non-linear relationships with invader density. Similarly, non-linear relationships between impact and invasion density have been documented for locusts (*Robinia pseudoacacia*; Staska et al. 2014) and shrubs (Japanese barberry, *Berberis thunbergii;* Elgersma and Ehrenfeld 2011).

The mean body size of zooplankton increased as fish density increased, resulting from the loss of smaller cladoceran species. Whilst this was presumably as a result of preferential *P.*

parva predation, it is a counter-intuitive outcome given that a general increase in prey size is usually observed with increased *P. parva* body length (Gozlan et al. 2010a). Although speculative, this might relate to aspects of the biology of the larger zooplankton species to avoid predation, such as their morphology deterring *P. parva* predation, as observed in juvenile bluegill (*Lepomis macrochirus*) and some Daphnid species (Kolar and Wahl 1998). Nevertheless, the non-linear, low-threshold relationship between the density and zooplankton body size indicated that body-size was larger at lower densities than a linear relationship predicted, suggesting that even at low densities, *P. parva* invasions have acute implications for zooplankton size-structure. Community size-structure has an important role in maintaining food web structure (Yvon-Durocher et al. 2010) and ecosystem functioning (Dossena et al. 2012) and, therefore, the invasive fish have the potential to instigate whole ecosystem changes at low densities.

Low-threshold curves represent severe ecological impacts since the effect is apparent at low densities. In contrast, high-threshold curves, such as the relationship recorded between fish density and zooplankton total biomass and abundance, represent relatively minor ecological impacts as they only manifest at higher densities. This is also reflected in *P. parva* diet, since the reliance on zooplankton was only evident at medium and high densities, causing a cascading increase in phytoplankton. *Pseudorasbora parva* had lower carbon signatures at medium and high densities when compared to fish from the low density populations, indicating a greater reliance on zooplankton at higher densities due to the decline in other resources, including benthic invertebrates. Indeed, the distinct difference in the isotopic niche of low density populations reflects the less severe impact the invaders had on lower trophic levels and thus, resource availability, in the mesocosms.

The relationships between invasion density and benthic macro-invertebrate abundance and decomposition rates were both best represented by non-linear s-shaped curves. The presence of the invader in low densities appears to promote invertebrate abundance compared to the control (albeit not significantly), while medium and high invasion densities caused a disproportionate decline in abundance compared to that predicted by a linear relationship. This promotion of invertebrate abundance at low densities caused a cascading increase in decomposition rates. We speculate that invertebrate abundance may be promoted at low densities due to the advantage of increased nutrient input (from fish excretion; McIntyre et al. 2008) outweighing the disadvantage of predation risk. In lakes where native fish are present in sympatry with invasive *P. parva*, this effect may disappear because of additional predation by the native species on invertebrates. It was, however, necessary to exclude native fish from our study to isolate the impacts of *P. parva*. Interactions between *P. parva* and native fish may influence their density-dependent impacts and therefore further work is required in this regard. Despite this, our results suggest that density-impact relationships should be taken into account in management strategies to control the cascading impacts of invasive animals on lower trophic levels, especially since both non-linear and linear relationships were evident when examining the impact of a single species.

We suggest that linear approximations of density- impacts are frequently inaccurate and therefore caution against the common assumption that ecological impact increases linearly with invader density. Indeed, growing evidence indicates that the density-impact relationships of invasions are often non-linear (Yokomizo et al. 2009; Thiele et al. 2010; Elgersma and Ehrenfeld 2011; Staska et al. 2014), with five out of our seven measured response variables also being non-linear. Although our mesocosms represent artificial experimental conditions, this study further emphasises the value of understanding the impact of invasions across density gradients, especially given that management efforts are often only targeted at high-density populations. We suggest that the curves can be adopted for use within existing risk assessment and management frameworks to assist management decision-making (e.g. Britton et al. 2011b).

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Tables

Table 1. Best fit model selection for the relationship between invasion density and each ecological response variable. The best models were selected by the lowest AIC_c value (highlighted in italics).

Response	**Model**	**AICc**	**df**	**R-sq (adj)**
Zooplankton Body Size	Linear	-34.22	1,15	0.49
	Quadratic	*-35.29*	*2,14*	*0.59*
	Cubic	-31.04	3,13	0.56
Zooplankton Diversity	*Linear*	*-13.26*	*1,15*	*0.57*
	Quadratic	-12.89	2,14	0.58
	Cubic	-11.37	3,13	0.55
Zooplankton Abundance	Linear	61.56	1,15	0.71
	Quadratic	*58.01*	*2,14*	*0.7*
	Cubic	58.71	3,13	0.68
Zooplankton Biomass	Linear	71.05	1,15	0.7
	Quadratic	*67.28*	*2,14*	*0.7*
	Cubic	67.95	3,13	0.68
Macro-invertebrate Abundance	Linear	43.5	1,15	0.7
	Quadratic	40.2	2,14	0.68
	Cubic	*37.73*	*3,13*	*0.75*
Chlorophyll-a Concentration	*Linear*	*35.53*	*1,15*	*0.33*
	Quadratic	36.67	2,14	0.34
	Cubic	40.24	3,13	0.78
Decomposition Rates	Linear	-149.22	1,15	-0.002
	Quadratic	-150.72	2,14	-0.07
	Cubic	*-156.37*	*3,13*	*0.21*

Figure legends

Figure 1. The potential relationships between invasive population density and ecological impacts. Re-drawn from Yokomizo et al. (2009).

Figure 2. Isotopic bi-plot showing the standard ellipse area (core isotopic niche) for each mesocosm population of *P. parva* at low (dashed), medium (dark grey) and high densities (black). Filled ellipses show the core isotopic range of four resources across all treatments and mesocosms.

Figure 3. Zooplankton body mass (A); zooplankton diversity (B); zooplankton abundance (C); zooplankton biomass (D); benthic invertebrate abundance (E); pelagic chlorophyll-*a* concentration (F) and decomposition (G) in each treatment (U: un-invaded; L: low density; M: medium density; H: high density). The box plot shows the median, boxed by the interquartile range, and the bar charts show mean ± standard error. Treatments statistically indistinguishable from one another are grouped by lower-cased letters.

Figure 4. The invasion impact of each response variable across the *P. parva* densities used in each invaded treatment (8, 24 and 48 individuals). Zooplankton body mass (A); zooplankton diversity (B); zooplankton abundance (C); zooplankton biomass (D); benthic invertebrate abundance (E); pelagic chlorophyll-*a* concentration (F) and decomposition (G). All response values are transformed ($\log_{10}(x+1)$). Solid lines show the best fit relationship and represent the density-impact curve. Where the best fit was not linear, linear regressions are shown by the dotted lines for comparison. Note in graphs B, C, D, E and G the y-axis is inverted to allow comparison of curves across response variables.

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Appendices

Appendix 1: Data

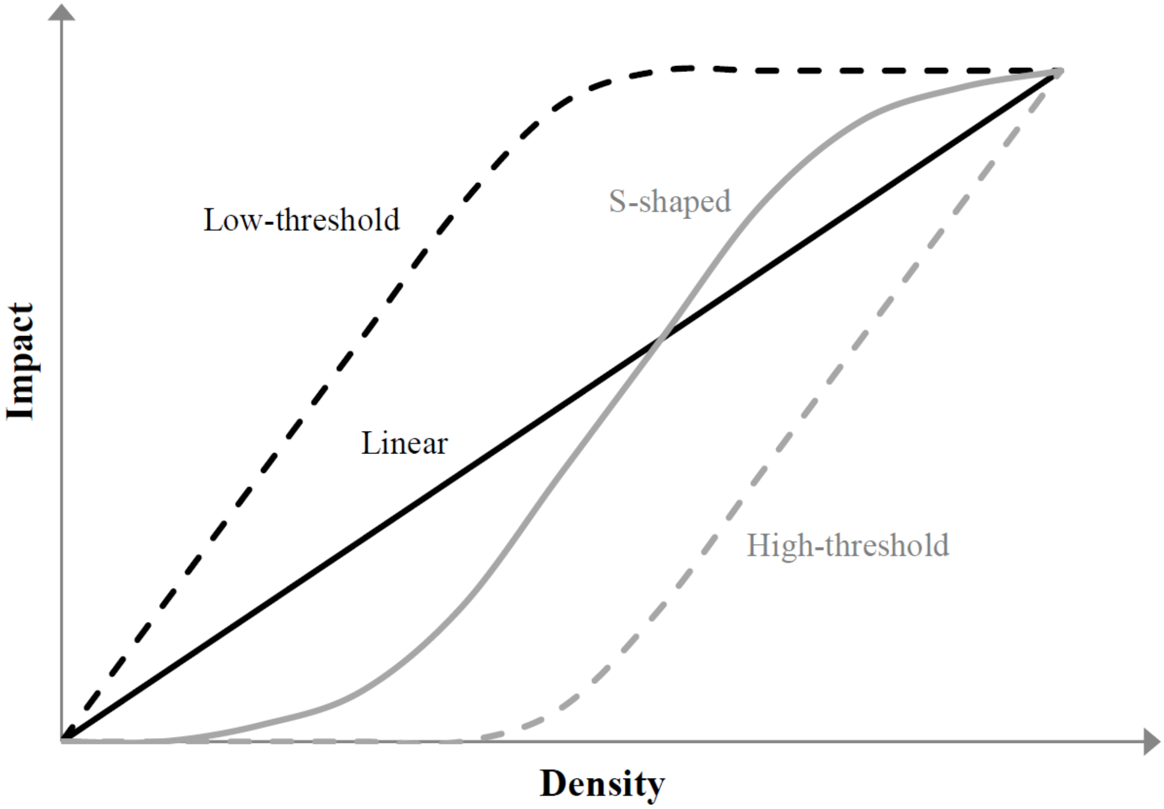
Table S1: Topmouth gudgeon isotope data

Table S2: Resource isotope data

Table S3: Zooplankton body size, abundance, biomass and richness

Table S4: Invertebrate abundance, phytoplankton standing stock and decomposition rates

Fig 1



Low-threshold
S-shaped
Linear
High-threshold
Impact
Density

Fig 2

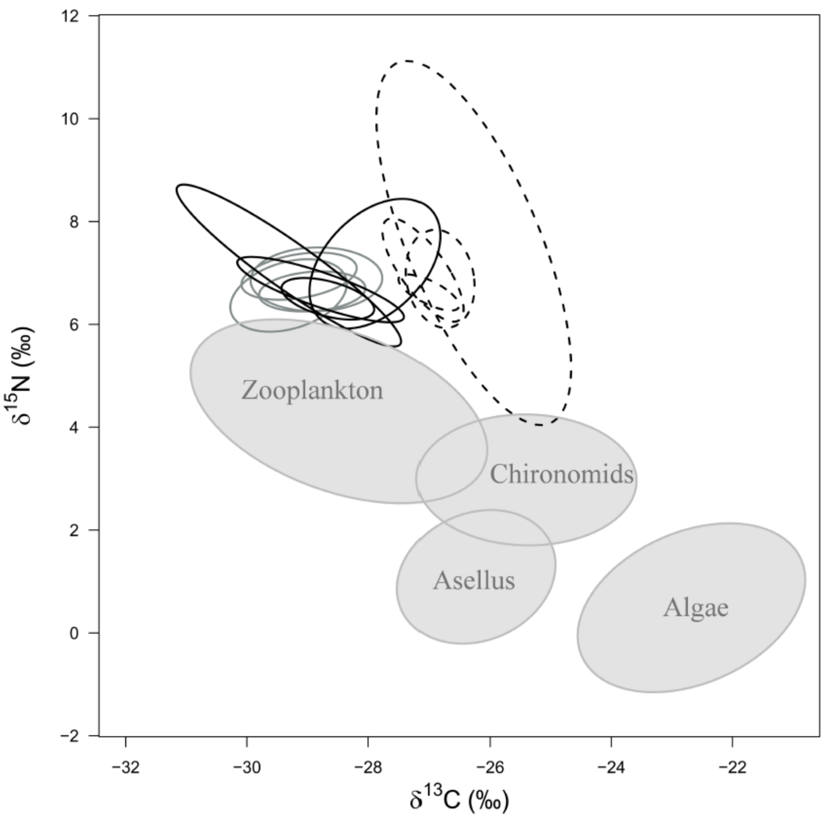

12
10
8
6
4
2
0
−2
−32
−30
−28
−26
−24
−22
δ^{15}N (‰)
δ^{13}C (‰)
Zooplankton
Chironomids
Asellus
Algae

Fig 3

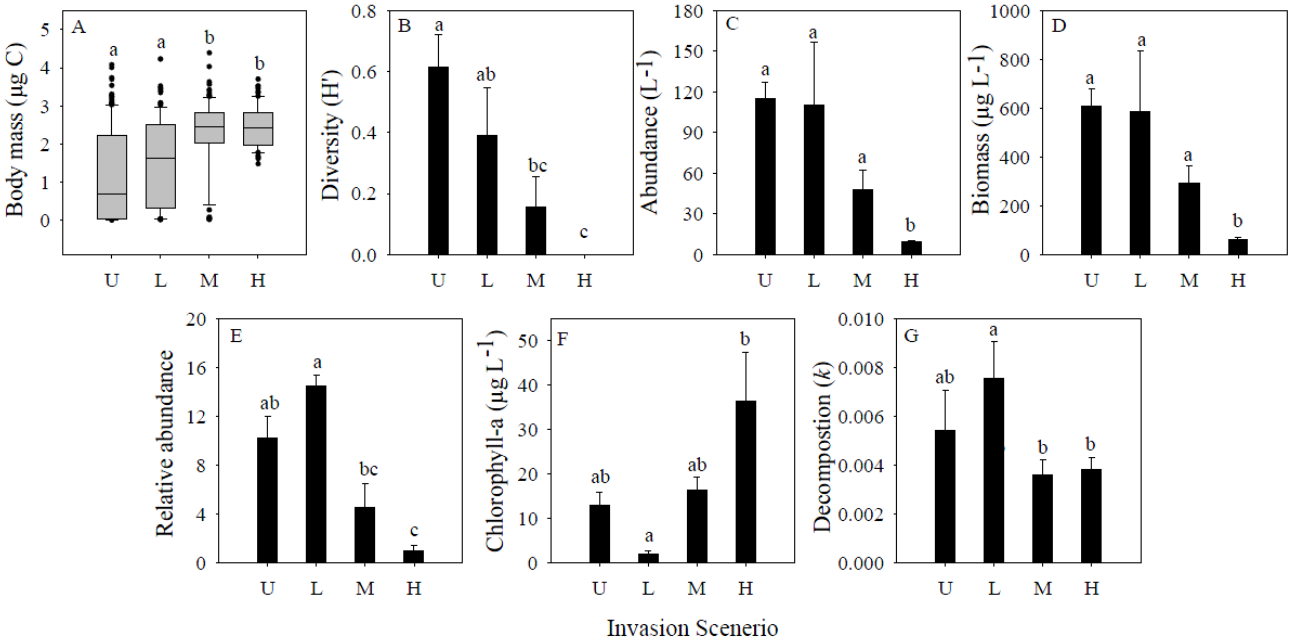
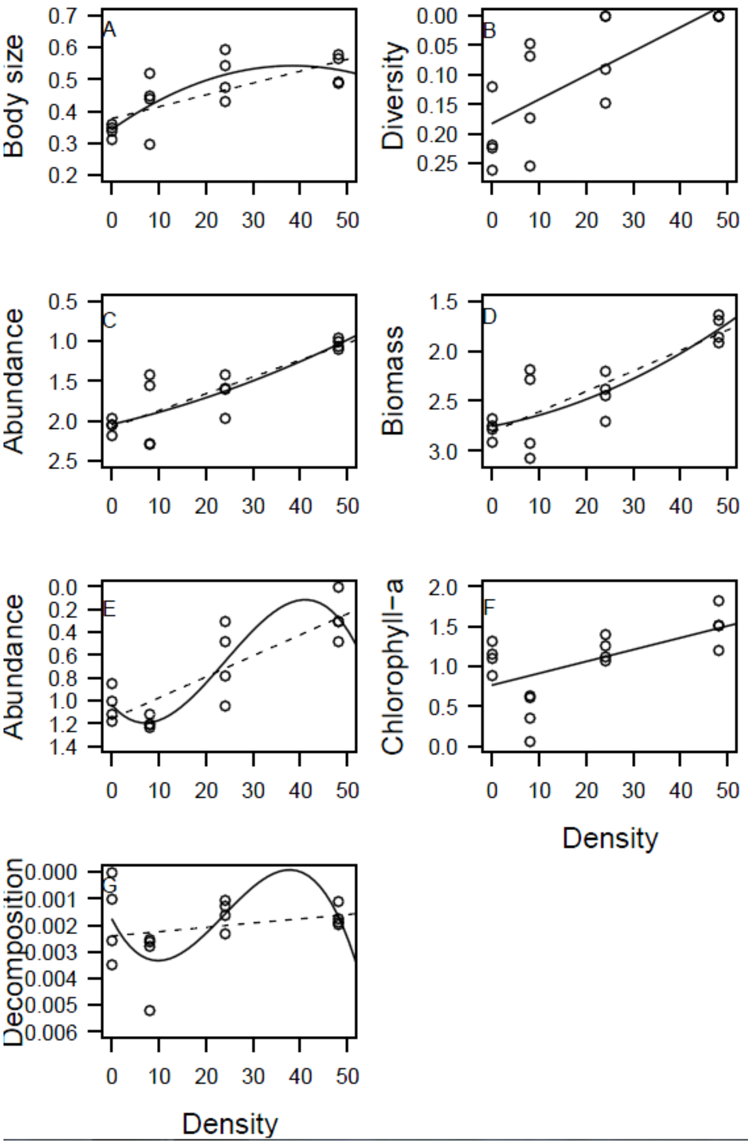

A
Body mass (µg C)
0
1
2
3
4
5
a
a
b
b
U
L
M
H
B
Diversity (H')
0.0
0.2
0.4
0.6
0.8
a
ab
bc
c
U
L
M
H
C
Abundance (L-1)
0
30
60
90
120
150
180
a
a
a
b
U
L
M
H
D
Biomass (µg L-1)
0
200
400
600
800
1000
a
a
a
b
U
L
M
H
E
Relative abundance
0
4
8
12
16
20
ab
a
bc
c
U
L
M
H
F
Chlorophyll-a (µg L-1)
0
10
20
30
40
50
ab
a
ab
b
U
L
M
H
G
Decompostion (k)
0.000
0.002
0.004
0.006
0.008
0.010
ab
a
b
b
U
L
M
H
Invasion Scenerio

Fig 4


A
Body size
0.7
0.6
0.5
0.4
0.3
0.2
B
Diversity
0.00
0.05
0.10
0.15
0.20
0.25
C
Abundance
0.5
1.0
1.5
2.0
2.5
D
Biomass
1.5
2.0
2.5
3.0
E
Abundance
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
F
Chlorophyll-a
2.0
1.5
1.0
0.5
0.0
G
Decomposition
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0
10
20
30
40
50
Density
Density