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Assessing the efficacy and ecology of biocontrol and biomanipulation for managing invasive pest fish

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Summary

1. Management of non-native species aims to prevent biological invasions using actions including control and containment of the potential invader. Biocontrol and biomanipulation strategies are used frequently to reduce population sizes of non-native species, and reduce their ecological impacts and dispersal rates.

2. Assessments of the efficacy of biocontrol and biomanipulation actions for managing non-native pest fish, and the ecological mechanisms involved, were studied here using lentic populations of the invasive fish *Pseudorasbora parva*. Biocontrol was through release of the indigenous piscivorous fish *Perca fluviatilis* and biomanipulation through intensive fish removals.

3. A combined biocontrol and removal programme was completed in an invaded pond over two reproductive seasons. Almost 10 000 *P. parva* were removed, with cumulative removal numbers significantly related to their decreased abundance (>60 to <0.1 m^{-2}). Ten adult *P. fluviatilis* were also released initially and reproduced each season. Analyses revealed *P. parva* contribution to *P. fluviatilis* diet was high initially, but decreased as *P. parva* abundance reduced. Individual contributions of the management actions to declined *P. parva* abundance were difficult to isolate.

4. The individual effects of biocontrol and removals on *P. parva* populations were then tested using a field trial in replicated pond mesocosms over three reproductive seasons. Replicates started with 1500 *P. parva*. The control (no interventions) revealed no significant temporal changes in *P. parva* abundances. In the removal

treatment, where over 17 000 *P. parva* were removed per replicate over the trial, abundance declined initially, but increased significantly after each reproductive season as remaining fish compensated through increased reproductive output. In the biocontrol, abundance declined and remained low; analyses revealed *P. parva* were an important dietary component of larger *P. fluviatilis*, with predation suppressing compensatory responses.

5. *Synthesis and applications.* Biocontrol and removals can significantly reduce abundances of lentic populations of small invasive fishes. Removals provide short-term population suppression, but high effort is needed to overcome compensatory responses. Biocontrol can provide longer-term suppression but could invoke unintended ecological consequences via 'stocking-up' food webs. Application of these results to decision-making frameworks should enable managers to make more objective decisions on risk-commensurate methodologies for controlling small invasive fishes.

Key-words: biocontrol, invasion, invasion management, non-native, stocking-up food webs; Perca fluviatilis; stable isotope analysis; *Pseudorasbora parva*.

Introduction

The effective prevention of biological invasions requires activities such as horizon scanning (Roy *et al*. 2014), import controls and screening (Lodge *et al*. 2006), auditing of regulated animal movements (Davies, Gozlan & Britton 2013) and the rapid detection of new introductions (Britton, Pegg & Gozlan 2011). If these activities fail to prevent a non-native species from being introduced, the species can colonize and disperse, initiating an invasion. Whilst eradication of new populations of non-native species might be the preferred option to prevent these invasions developing, eradication can be difficult and controversial (Myers, Savoie & Randen 1998; Simberloff 2002). Many methods are non-specific in their target species, such as chemical biocides that also result in mortalities of non-target species (Simberloff 2009). Biocide applications are also often inappropriate when the area of invasion has high conservation value, such as habitats containing protected species (Britton, Gozlan & Copp 2011).

Alternative approaches to managing populations of invasive species include control and containment programmes that aim to reduce population abundance and dispersal probabilities, and decrease ecological impacts on native biota (Britton *et al*. 2011). Although unlikely to achieve eradication (Manchester & Bullock 2000), these provide less controversial approaches that can limit the invasion's spatial extent (Allendorf & Lundquist 2003). This is important as river basins generally represent discrete biogeographic islands (Gozlan *et al*. 2010a); minimizing dispersal rates of non-native fish from ponds into river catchments can inhibit their invasion (Britton *et al*. 2011). Preventing these invasions either requires population extirpation by biocide, eliminating dispersal (Britton & Brazier 2006), or actions that reduce population abundance, minimizing dispersal, which also reduces impacts

on native species (Jackson, Ruiz-Navarro & Britton 2014). Although control and containment strategies are often used in attempts to control non-native fish populations, there is limited knowledge on the efficacy of their long-term applications and the ecological mechanisms involved, constraining the ability of managers to make objective decisions on their application (Britton, Gozlan & Copp, 2011).

Control techniques for managing invasive fish populations typically include their physical removal (biomanipulation) and enhancing populations of piscivorous fish to increase predation pressure (biocontrol) (Kolar & Lodge 2001; Lee 2001). The removal of individuals from non-native fish populations can be effective when applied to spatially limited, isolated populations (e.g. Knapp & Matthews 1998). Classical biocontrol programmes introduce a predator or pathogen from the native range of the invasive species to limit its population growth and has been used effectively for managing non-native plants (e.g. Gassman *et al*. 2006). However, the introduced predator may expand their prey range to non-target native species, leading to irreversible effects (Simberloff 2009). Consequently, for non-native fish, classical biocontrol is rarely feasible, with options limited to enhancing their predator populations using indigenous fish from the introduced range (Gozlan *et al*. 2010a).

The topmouth gudgeon *Pseudorasbora parva* (Temmink & Schlegel) is a highly invasive cyprinid fish species from Asia that has achieved pan-European distribution since its introduction in the 1960s (Gozlan *et al*. 2010b). Ecological consequences include modifications to food web structure (e.g. Britton, Davies & Harrod, 2010) and novel pathogen transmission (Andreou *et al*. 2012). In their invasive range, there is a desire to prevent their further spread and reduce their impacts (Britton, Gozlan & Copp 2011). Whilst this has been achieved in the UK through rotenone application to pond populations (Britton &

Brazier 2006), this is a non-species specific biocide whose application potentially incurs relatively high initial costs (Britton *et al*. 2011). In areas of the *P. parva* invasive range in Europe, its application is prohibited and so alternative management approaches are required. Consequently, *P. parva* is used here as the model invasive fish in wild and semi-controlled conditions to assess the efficacy and ecological mechanisms of biomanipualtion (by removals) and biocontrol (population enhancement of a facultative piscivorous fish) on their invasive populations. Objectives are to: (i) measure the effect on *P. parva* population abundance of a combined biomanipulation and biocontrol programme on a field site; (ii) determine the individual effects of biomanipulation and biocontrol measures on *P. parva* population abundance in a field trial using pond mesocosms; and (iii) assess the ecological mechanisms involved in the consequent reductions of the *P. parva* populations and their subsequent population responses. The originality and significance of the outputs are assessed in relation to the mechanisms and efficacy of the two methodologies, and their practical application to managing fish invasions.

Materials and methods

Field site

The field site was a 0.3 ha, shallow (< 1.5 m) pond in north-west England (53^{o}22’33’’N, 3^{o} 08’19’’W) where *P. parva* was detected in an initial survey in November 2005. Sampling commenced in April 2006 using a series of 25-m micro-mesh seine nets; population density estimates were derived from depletion estimates from successive deployments of the net in specific locations of the ponds (Cowx 1983). The presence of a very high *P. parva* density (Table 1) meant a biomanipulation programme (hereafter referred to as ‘removal’) was initiated to reduce their abundance by cropping (i.e. mass removal) at approximately 6-month

intervals for two years, covering two *P. parva* reproductive seasons, using the same sets of micromesh seine nets. The rationale for these time periods was the mature fish would be removed in the spring prior to their spawning and the young-of-the-year (YoY) produced by the remaining mature fish in the spawning season would be cropped in autumn. On each sampling occasion, depletion sampling was completed in advance to obtain the *P. parva* population estimate before the removal exercise was completed. The removals netted the pond until all major habitat areas had been netted at least once.

The effects of these removals on the *P. parva* population densities were reported in Britton, Davies & Brazier (2010). However, this management programme also incorporated the stocking of the native facultative piscivorous fish perch *Perca fluviatilis*, with the species also indigenous to the watershed. A total of 10 fish (210–325 mm) were released in April 2006. No obligate piscivorous fish were present in the pond and the other species were all of the family Cyprinidae. Initially, the efficacy of this aspect was not assessed, as it was not perceived to have contributed to the effectiveness of the removal programme. However, opportunities to test the contribution of *P. parva* to the diet of *P. fluviatilis* were available subsequently via scales for stable isotope analysis. The stable isotope data derived from fish scales significantly relate to those of dorsal muscle, which is used more generally, enabling their application in this manner (e.g. Grey *et al*. 2009). Thus, this assessed whether the *P. fluviatilis* were assisting the removals by consuming *P. parva* (as biocontrol). Stable isotope analyses reveal trophic linkages through the naturally occurring ratios of ^{15}N:^{14}N and ^{13}C:^{12}C (Grey 2006); carbon ratios reflect the consumer diet with typical enrichment of 0 to 1 ‰ and nitrogen ratios show greater enrichment of 2 to 4‰ from resource to consumer, indicating trophic position (Post 2002; McCutchan *et al*. 2003).

On each sampling occasion, between three and five scales were removed from a sub-sample of *P. parva* and from all sampled *P. fluviatilis*. During sampling of April 2007 and September 2007, macro-invertebrate samples had also been collected (n = 3 to 10 per resource). In the laboratory, the scales were prepared for analysis by taking material from only the very outer portions of scales, i.e. material produced through the most recent growth (Hutchinson & Trueman 2006). All scale and macro-invertebrate samples were oven dried to constant weight at 60ºC for 48 hours, before analysis at the Cornell Isotope Laboratory, New York, USA. Initial data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰). These data were then analysed in two ways. Firstly, data from each sampling occasion were tested for differences in $\delta^{13}C$ and $\delta^{15}N$ between *P. parva* and *P. fluviatilis* using a generalized linear model (GLM). The dependent variable was either $\delta^{13}C$ or $\delta^{15}N$ and the independent variable was the interaction of species and sampling date. Given the large size range of *P. fluviatilis* (approximately 40 to >300 mm), their data were split into different size classes ('small', <100 mm; 'large' >101 mm), as ontogenetic changes in gape size influences the body size of their prey fish (Dörner & Wagner *et al*. 2003). Differences in $\delta^{13}C$ or $\delta^{15}N$ of the fishes were determined using estimated marginal means and multiple pairwise comparisons with Bonferroni adjustment for multiple comparisons. Secondly, for data from April and October 2007 when the macro-invertebrate data were available as putative food resources, *P. fluviatilis* diet composition by size classes was estimated using Bayesian mixing models in the SIAR package in the R computing programme (Parnell *et al*. 2010; R Core Development Team 2013). Data for putative resources with similar isotope signatures were combined *a priori* to optimize model performance (Phillips, Newsome & Gregg 2005). Thus, they were pooled into: macro-invertebrates (*Gammarus pulex* and Chironomid larvae), 'small' *P. fluviatilis* (< 50 mm, to allow for cannibalism) and *P. parva*. To correct for isotopic fractionation between resources and consumers, 2.9 ‰ (±0.32 ‰) was used for $\delta^{15}N$

and 1.3 ‰ (±0.3 ‰) for $\delta^{13}C$ (McCutchan 2003). Outputs were the predicted contribution to diet of each resource.

Field trial

The field trial ran between February 2011 and October 2013, covering three *P. parva* reproductive seasons, and was completed on a disused aquaculture site in Southern England. It comprised of the following treatments, each replicated four times in identical pond mesocosms of approximately 200 m^{-2} where depths were to 2 m: control (no interventions), removal (involving cropping at 6-month intervals) and biocontrol (using released and indigenous *P. fluviatilis*). Prior to use, each pond was drained and dried in spring 2010 to ensure complete fish absence, followed by natural refilling. Measures to deter avian predators were then deployed, including anti-predator netting, before 1500 mature *P. parva* (fork lengths 40–70 mm and of approximately equal sex ratios) were introduced to each pond in June 2010 that were sampled randomly from 10 other ponds on the site.

These fish were left until the trial commenced in February 2011 when an initial sampling of all mesocosms was undertaken. This used rectangular fish traps comprising of a circle alloy frame of length 107 cm, width and height 27.5 cm, mesh diameter 2 mm and with funnel shaped holes (6.5-cm diameter) at either end to allow fish entry and capture. They were baited using fishmeal pellets (21-mm diameter) as these baited traps provide reliable *P. parva* catch per unit effort estimates (n fish h^{-1}; CPUE) (Britton Pegg & Gozlan 2011). Once the initial CPUE of each mesocosm had been determined, 20 *P. fluviatilis* of 100 to 140 mm were released into each biocontrol replicate, with each individual already tagged with passive integrated transponder (PIT) tags. The first *P. parva* removal event was also completed on all removal ponds, when traps were set in triplicate for two hours before lifting and removing all

fish. The removal concluded when the CPUE of the trapping reduced to levels <10 fish per trap per hour. Following these removals, all ponds were re-sampled in March 2011 to estimate CPUE once more.

Thereafter, until October 2013, the control and biocontrol ponds were left, other than sampling for CPUE each spring and autumn when a random sub-sample of 30 fish was removed per pond for subsequent analysis. For the removal ponds, sampling also occurred each spring and autumn until October 2013, but after each sampling event, a removal event was also completed, as described above In October 2013, the trial concluded by sampling and then draining each pond; for the biocontrol, all of the surviving *P. fluviatilis* and their progeny were collected, along with samples of *P. parva* and macro-invertebrates, including signal crayfish *Pacifastacus leniusculus*.

For the *P. parva* sub-samples, individuals were measured (fork-length, mm) and scales removed that were viewed on a projecting microscope (×30) and their ages estimated. For the samples of *P. fluviatilis* and *P. parva* collected from the biocontrol treatment mesocosms in October 2013, each fish was measured and samples of dorsal muscle removed and dried for stable isotope analysis (Perga & Gerdeaux 2009). The macro-invertebrate samples were treated as per those from the field site.

Field trial data analysis

CPUE per treatment over the trial was analysed using a GLM using the interaction of CPUE and sampling date as the dependent variable and treatment as the independent variable; outputs were the estimated marginal means of CPUE per treatment over time and the significance of their differences (pairwise comparisons with Bonferroni adjustment for

multiple comparisons). The *P. parva* age data were used to estimate the contribution (%) of young-of-the-year (YoY) fish to their population, with fish sampled in spring that were produced the previous summer still classed as YoY. These data were tested in a GLM as per CPUE. Significant differences in the *P. parva* YoY age and length data between treatments and over time were tested in a linear mixed model, with pond used as a random effect on the intercept to avoid inflating the residual degrees of freedom by using individual fish as true replicates. Differences in YoY age and lengths were determined using estimated marginal means and multiple comparison post-hoc analyses (general linear hypothesis test).

The stable isotope data for the biocontrol from October 2013 contained data for *P. fluviatilis* between 47 and 295 mm and could be split into three size ranges: small (< 100 mm; $n = 8$); medium (101–200 mm; $n = 13$) and large (>201 mm, $n = 5$). Initially, these data were used to determine the significance of differences between *P. parva* and the *P. fluviatilis* size classes in $\delta^{13}C$ and $\delta^{15}N$, with data were combined across replicates, as differences between the stable isotope data of the macro-invertebrates in each mesocosm were not significant (Mann Whitney U-test, $Z = 0.02$, $P > 0.05$ for *Asellus aquaticus* and Chironomid larvae). These data were used in a linear mixed model, with pond used as the random factor to avoid inflating residual degrees of freedom. Differences in $\delta^{13}C$ and $\delta^{15}N$ between the species and size classes were detected using multiple comparison post-hoc analyses (general linear hypothesis test). The diet composition of the perch size classes were then estimated from their putative food resources (*P. parva*, macro-invertebrates, *P. leniusculus* and smaller *P. fluviatilis*) using Bayesian mixing models, as per the Field site. All of the stable isotope data for *P. parva* and small *P. fluviatilis* were included in medium and large *P. fluviatilis* mixing models. For small *P. fluviatilis*, the only fish prey entered were < 50 mm.

Results

Field site

In the field site, *P. parva* population density estimates reduced from 63.1 to < 0.1 m^{-2} over the study period (see Table S1 in Supporting Information). The relationship between the cumulative number of *P. parva* removed and their subsequent population estimate was significant; abundance decreased as removal number increased (linear regression: $R^2 = 0.95$; $F_{1,3} = 53.17$, $P < 0.01$; Fig. 1a). Following the release of *P. fluviatilis* into the pond in spring 2006, they reproduced, with their progeny present in samples from April 2007 (Table 1, 2).

The stable isotope data of the *P. fluviatilis* size classes and *P. parva* varied between April 2006 and April 2008 (Table 1). The GLMs testing differences in $\delta^{13}C$ and $\delta^{15}N$ between *P. fluviatilis* and *P. parva* on each sampling occasion were significant ($\delta^{13}C$: Wald $\chi^2 = 275.48$, d.f. = 12, $P < 0.01$; $\delta^{15}N$: Wald $\chi^2 = 198.74$, d.f. = 12, $P < 0.01$). Excluding data from February 2006 (values for *P. fluviatilis* were from their original pond and not the field site), these data revealed significant higher values of $\delta^{15}N$ (to 4.24 ‰) in both size classes of *P. fluviatilis* than *P. parva* in samples to April 2007, but not thereafter (Table 2). For $\delta^{13}C$, there was a significant difference between the large *P. fluviatilis* size class and *P. parva* in April 2007 (mean difference 1.99 ‰) but not in any other sample (Table 2).

Stable isotope mixing models using data from April 2007 predicted the large *P. fluviatilis* were highly piscivorous, with mean *P. parva* contribution to their diet being 49% (Table 3). In October 2007, whilst the models predicted that these large perch were still mainly piscivorous, *P. parva* contribution reduced to a mean of 21%, with an increase in diet of small *P. fluviatilis* and macro-invertebrates (Table 3). The mixing models for small perch

296 revealed some piscivory of *P. parva* < 60 mm in April 2007 that declined to a very low level
297 by October 2007 (Table 3).
298
299 *Field trial*
300 The GLM testing CPUE from the Control, Removal and Biocontrol treatments revealed the
301 effect of the interaction of treatment and date was significant ($P < 0.01$), with estimated
302 marginal means and pairwise comparisons revealing no significant differences in CPUE in
303 the control over the trial, but with significant differences in the removal and biocontrol
304 treatments (Fig. 2). Comparison of CPUE in the removal versus the control on each sampling
305 occasion revealed significantly reduced *P. parva* CPUE from October 2011 to March 2012,
306 and in March 2013, but not in October 2012 and October 2013 when CPUE increased (Table
307 4; Fig. 2). Whilst the highest cumulative number of *P. parva* removed from a replicate in the
308 Removal treatment was over 18 500 fish, the relationship between the cumulative number of
309 *P. parva* removed and CPUE was not significant ($R^2 = 0.08$; $F_{1,5} = 0.04$, $P = 0.84$; Fig. 1b).
310 By contrast, there was a significant reduction in CPUE in the biocontrol compared to the
311 control from October 2011 that remained through to October 2013 (Table 4; Fig. 2).
312
313 The linear mixed effects model testing the proportion of YoY *P. parva* on each sampling
314 date in the control and treatments revealed the interaction of treatment and date was
315 significant ($P < 0.01$). Significant increases in the proportion of YoY were apparent in both
316 the Control and Removal treatment, but not in the Biocontrol treatment ($P < 0.01$; Fig. 3).
317 The linear mixed effects model testing the mean length of YoY on each sampling date from
318 the control and treatments revealed the effect of the interaction of treatment and date was also
319 significant ($P < 0.01$). Whilst there were no significant changes in mean lengths in the control

and biocontrol, significantly reduced YoY mean length was recorded in October 2012 and October 2013 in the Removal treatment (Fig. 3).

Following their release, *P. fluviatilis* reproduced in the biocontrol and so by the conclusion of the trial, there were three age classes present, age 0+ to 2+ years, plus a low number of tagged original fish (Table 5). The linear mixed effects model using stable isotope data from the biocontrol treatment from samples taken in October 2013 revealed that the effect of species/ size-class was significant for both $\delta^{13}C$ and $\delta^{15}N$, with significant differences apparent in $\delta^{15}N$ between *P. parva* and medium and large *P. fluviatilis*, and between all *P. fluviatilis* size classes (Table 5). Stable isotope mixing models indicated all *P. fluviatilis* size classes predated upon *P. parva*, with the contribution to diet increasing as mean body size increased (Table 5c).

Discussion

The results of the field site and trial indicated that invasive *P. parva* pond population abundances can be significantly reduced using removals and biocontrol. Given the considerable presence of other small, invasive pest fishes in lentic environments around the world, such as *Gambusia* species (e.g. Pyke 2008), Trinidadian guppy *Poecilia reticulata* Peters (Deacon, Ramnarine & Magurran 2011) and minnow *Phoxinus phoxinus* (Linnaeus) (Museth *et al*. 2007), these outputs have high application to the management of non-native fishes generally. It should be noted, however, that population extirpations were not achieved by these methods. If the management aim is extirpation then chemical biocide application remains the most effective method to achieve this (Britton, Gozlan & Copp 2011).

Here, the use of removals to suppress *P. parva* populations was effective initially, with rapid and significant reductions in population sizes. There was limited success thereafter as populations compensated for losses by increasing their reproductive output. Other studies using removals to manage invasive fish populations have also shown some effectiveness in suppressing populations of target species. For example, removals of invasive brook trout *Salvelinus fontinalis* by gill netting in California, USA, were effective in reducing abundances in small lakes (Knapp and Matthews 1998). Although trout below 110 mm were less susceptible to capture, the method provided some population control when biocide application was not possible for conservation reasons (Knapp and Matthews 1998; Knapp *et al*. 2007). Other operations have been less successful due to compensatory responses in the target species. The population suppression of invasive *P. fluviatilis* in New Zealand resulted in increased juvenile abundances as the cannibalistic adults were removed only after they had spawned (Ludgate and Closs 2003). The application of trapping and electric fishing to controlling black bullhead *Ameiurus melas* was relatively effective in a French lake as no compensatory responses were recorded (Cucherousset *et al*. 2006). In contrast, compensatory responses were detected in *A. melas* populations elsewhere following mass removals (Hanson *et al*. 1983). Thus, where the management aim is suppression of invasive fish populations then removals can provide an effective short-term measure. Its long-term effectiveness is, however, reduced substantially if the remaining fish exhibit compensatory responses, such as increased survival, growth and fecundity (Wydoski & Wiley 1999). Correspondingly, long-term population suppression using removals is likely to require sustained management efforts, potentially accruing high resource costs (Britton *et al*. 2011).

The use of fish as biocontrol agents has generally been applied to managing insects such as mosquito *Aedes aegypti* (Martínez-Ibarra *et al*. 2002), particularly using *Gambusia* species

(Pyke 2008). Wild fish populations, particularly of European eel *Anguilla anguilla*, are also recognized as strong resistors of invasions of non-native crayfishes (e.g. Musseau *et al*. 2015). However, there are no reported large-scale programmes of bio-control that have successfully utilized piscivorous fish to suppress the invasion of a non-native fish (Britton, Gozlan and Copp 2011). The outcome of this study suggest it has considerable potential for suppressing populations of small, invasive fishes, such as *P. parva* and *Gambusia* spp., particularly in lentic environments. Despite its action being less immediate than for removals it has the potential benefit of negligible long-term management costs.

Managers pursuing the implementation of this form of biocontrol face practical and ethical challenges. Primarily, they must consider the predatory species used, as although the release of piscivorous fish into invaded ponds can suppress invasive populations, it might also result in the undesirable consequences of 'stocking-up' food webs (Eby *et al*. 2006). This is where the stocked fish either increase the species richness of top predators or replace other ones. This can result in additional predation pressure on native fish communities, increasing top-down effects (Eby *et al*. 2006). Releasing a native piscivorous fish is arguably more ethical than introducing a non-native one, given the reported impacts on native fish communities by non-native piscivorous fish released for sport angling, such as *Cichla* species (Britton & Orsi 2012). A recent study found native pike *Esox lucius*, an obligate piscivore, was effective at suppressing *P. parva* populations in Belgium (Lemmens *et al*. 2014). However, the potential of *E. lucius* to grow to relatively large sizes (>10 kg), allied to their relatively large gape size (Nilsson & Brönmark 2000), means their potential prey species cover a substantially wider size range than *P. fluviatilis* (Dörner & Wagner 2003). This increases their risk of invoking undesirable cascading consequences in native prey fish populations. Correspondingly, in practical and ethical decisions over whether native predator enhancement is appropriate for

suppressing invasive fish populations, managers must firstly consider the potential risk of altering food-web structure and causing ecosystem-level effects. This risk should then be balanced against the ecological risk of the target species and their invasion probability if their populations are left uncontrolled.

The field study used the biocontrol and removals in combination, whereas the field trial used them individually. This meant that the field trial identified the mechanisms involved in the actions of each method in isolation, but it could not assess their efficacy in combination. A final treatment involving the two methods was not completed due to logistical constraints. Considering the outputs of the field study and field trial together suggests that their effects were either additive or synergistic. Removals of mature *P. parva* prior to their spawning season reduced their reproductive effort, biocontrol minimized their compensatory responses through increased predation pressure, and removals at the end of the reproductive season reduced their recruitment. Where managers are only able to use one of these methods then consideration is between using removals that achieve short-term population suppression with the likelihood of long-term effort to maintain this, versus the longer-term suppression achieved by biocontrol but that potentially incurs negative cascading effects in the ecosystem.

In conclusion, the study revealed biocontrol and removals provide effective methods for suppressing populations of lentic *P. parva* populations. As *P. parva* represent a strong model of small, invasive fish more generally (Gozlan *et al*. 2010b), the results are highly applicable to the management of small, invasive fishes in other systems and regions. In particular, these results can be applied to informing decision-making processes for invasive fishes. For example, where the management objective is extirpation of the target population then these methods are unlikely to be effective. If the objective is reducing their population abundance

and controlling their dispersal, then both methods could be effective when applied individually, with the method applied dependent on the timeframe of the objective, the resources available and the risk of incurring ecological consequences via stocking-up food webs. If the methods are used in combination, there is high potential that the population of the target species will be reduced to very low levels of abundance.

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Data accessibility:

Stable isotope data, and fish length and catch per unit effort data: Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.tv47p.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1: Population estimates of *Pseudorasbora parva* at the field site and the number and weight of *P. parva* removed.

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Table 1. Numbers of analysed fish, mean lengths and length range (mm) of *Perca fluviatilis* and *Pseudorasbora parva* from the field site. 'Large' *P. fluviatilis* were >101 mm, 'small' were ≤100mm

Date	Species	n	Mean length (mm)	Length range (mm)
Apr-06	Large *P. fluviatilis*	6	276 ± 35	235–323
	P. parva	6	40 ± 7	33–54
Sept-06	Large *P. fluviatilis*	10	147 ± 26	112–214
	P. parva	6	55 ± 22	41–98
Apr-07	Large *P. fluviatilis*	5	196 ± 94	132–359
	Small *P. fluviatilis*	6	55 ± 8	49–70
	P. parva	16	56 ± 15	38–95
Sept -07	Large *P. fluviatilis*	6	266 ± 60	206–352
	Small *P. fluviatilis*	9	80 ± 8	68–90
	P. parva	15	60 ± 23	23–93
Apr -08	Large *P. fluviatilis*	2	239 ± 171	118–360
	Small *P. fluviatilis*	8	90 ± 8	76–99
	P. parva	10	55 ± 16	25–77

Table 2. Mean adjusted $\delta^{13}C$ and $\delta^{15}N$ for *Perca fluviatilis* in (a) 'small' and (b) large size classes and *Pseudorasbora parva*, and their mean difference and significance according to pairwise comparisons (with Bonferroni adjustment for multiple comparisons) by sampling date at the field site. *Difference significant at $P < 0.05$; ** $P < 0.01$

(a)	'Small' *P. fluviatilis*		*P. parva*		Mean difference	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
April 2007	-30.34 ± 0.57	16.83 ± 0.45	-26.46 ± 0.38	13.71 ± 0.30	3.88**	3.11**
Sept 2007	-28.83 ± 0.45	14.57 ± 0.36	-29.35 ± 0.38	15.41 ± 0.30	0.52	0.84
Apr 2008	-27.54 ± 0.47	16.16 ± 0.37	-27.55 ± 0.46	15.02 ± 0.36	0.01	1.14

(b)	'Large' *P. fluviatilis*		*P. parva*		Mean difference	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Feb 2006	-23.29 ± 0.87	12.05 ± 0.56	-28.12 ± 0.59	15.03 ± 0.47	4.83**	2.98
Sept 2006	-26.46 ± 0.45	16.87 ± 0.36	-25.88 ± 0.57	13.89 ± 0.45	0.57	2.97**
April 2007	-28.45 ± 0.70	17.95 ± 0.34	-26.46 ± 0.38	13.71 ± 0.30	1.99*	4.24**
Sept 2007	-29.62 ± 0.84	17.42 ± 0.66	-29.35 ± 0.38	15.41 ± 0.30	0.27	2.00
Apr 2008	-27.41 ± 1.10	16.06 ± 0.85	-27.55 ± 0.46	15.02 ± 0.36	0.14	2.39

Table 3. Predicted mean proportions (%) and 95% confidence limits from Bayesian mixing models of putative food resources to the diet of (a) 'small' and (b) 'large' *Perca fluviatilis* in the field site

(a)	*Pseudorasbora parva* (< 50 mm)	*Perca fluviatilis* (< 50 mm)	Macro-invertebrates
April 2007	36 (1–64)	n/a	64 (36–99)
Sept 2007	13 (0–44)	n/a	87 (56–100)

(b)	*Pseudorasbora parva*	*Perca fluviatilis* (< 50 mm)	Macro-invertebrates
April 2007	49 (24–73)	22 (1–41)	29 (3–53)
Sept 2007	21 (0–50)	45 (4–86)	35 (0–67)

Table 4. Mean differences in the catch per unit effort (CPUE) of *Pseudorasbora parva* in the control and treatments by sampling date in the field trial. * $P < 0.01$

	Control - Removal	Control - Biocontrol	Removal - Biocontrol
Feb 2011	-8.8	-12.4	3.7
Mar 2011	29.7*	8.4	38.1*
Oct 2011	47.2*	51.0*	3.8
Mar 2012	40.3*	38.2*	2.2
Oct 2012	7.8	30.3*	-22.4*
Mar 2013	47.8*	45.0*	-2.83
Oct 2013	5.7	40.3*	-34.6*

Table 5. (a) Information on the fish analysed from the biocontrol treatment sampled at the conclusion of the trial; (b) Pairwise comparisons of $\delta^{13}C$ and $\delta^{15}N$ of *Pseudorasbora parva* and the three size classes of *P. fluviatilis*; *P < 0.01; (c) predicted mean proportions (%) and 95% confidence limits of putative food resources to the diet of *Perca fluviatilis* from the field trial

(a)

Species	n	Mean length (mm)	Length range (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
P. parva	10	50 ± 11	33–72	-29.53 ± 0.39	5.92 ± 0.15
Small *P. fluviatilis*	8	64 ± 11	47–90	-26.45 ± 0.44	5.92 ± 0.17
Medium *P. fluviatilis*	13	147 ± 24	105–181	-28.55 ± 0.34	7.68 ± 0.13
Large *P. fluviatilis*	5	282 ± 14	261–295	-27.79 ± 0.55	9.60 ± 0.21

(b)

Comparison	$\delta^{13}C$	$\delta^{15}N$
P. parva vs. Small *P. fluviatilis*	3.08 ± 0.59*	0.01 ± 0.23
P. parva vs. Medium *P. fluviatilis*	0.97 ± 0.52	1.76 ± 0.20*
P. parva vs. Large *P. fluviatilis*	1.73 ± 0.68	3.67 ± 0.26*
Small *P. fluviatilis* vs. Medium *P. fluviatilis*	2.10 ± 0.56*	1.77 ± 0.22*
Small *P. fluviatilis* vs. Large *P. fluviatilis*	1.34 ± 0.71	3.68 ± 0.28*
Medium *P. fluviatilis* vs. Large *P. fluviatilis*	0.76 ± 0.65	1.92 ± 0.25*

(c)

	Perca fluviatilis size class		
	Small	Medium	Large
Pseudorasbora parva	20 (0–48)	27 (0–46)	34 (7–60)
Perca fluviatilus (< 110 mm)	–	5 (0–15)	15 (0–33)
Pacifastacus leniusculus	36 (0–71)	22 (0–44)	29 (1–54)
Macro-invertebrates	44 (2–86)	47 (0–60)	21 (0–42)

Figure captions

Figure 1. Relationship of catch per unit effort (CPUE) and cumulative number of *Pseudorasbora parva* removed from (a) the field site; and (b) from the removal treatment in the field trial. The solid line denotes significant relationships between variables (linear regression) and error bars represent standard error.

Figure 2. Mean relative abundance estimates between February 2011 and October 2013 in the field trial for the control, removal and biocontrol. Error bars represent standard error. *P < 0.01 for catch per unit effort (CPUE) on that date and initial CPUE (February 2011).

Figure 3. Mean proportion of *Pseudorasbora parva* young-of-the-year (YoY; filled circle) in October of each year and March the following year (i.e. at age 1), and their mean length of (open circle) in the field trial for the control, removal and biocontrol. *P < 0.05, **P < 0.01 for proportion between that date and the initial estimate in February 2011.

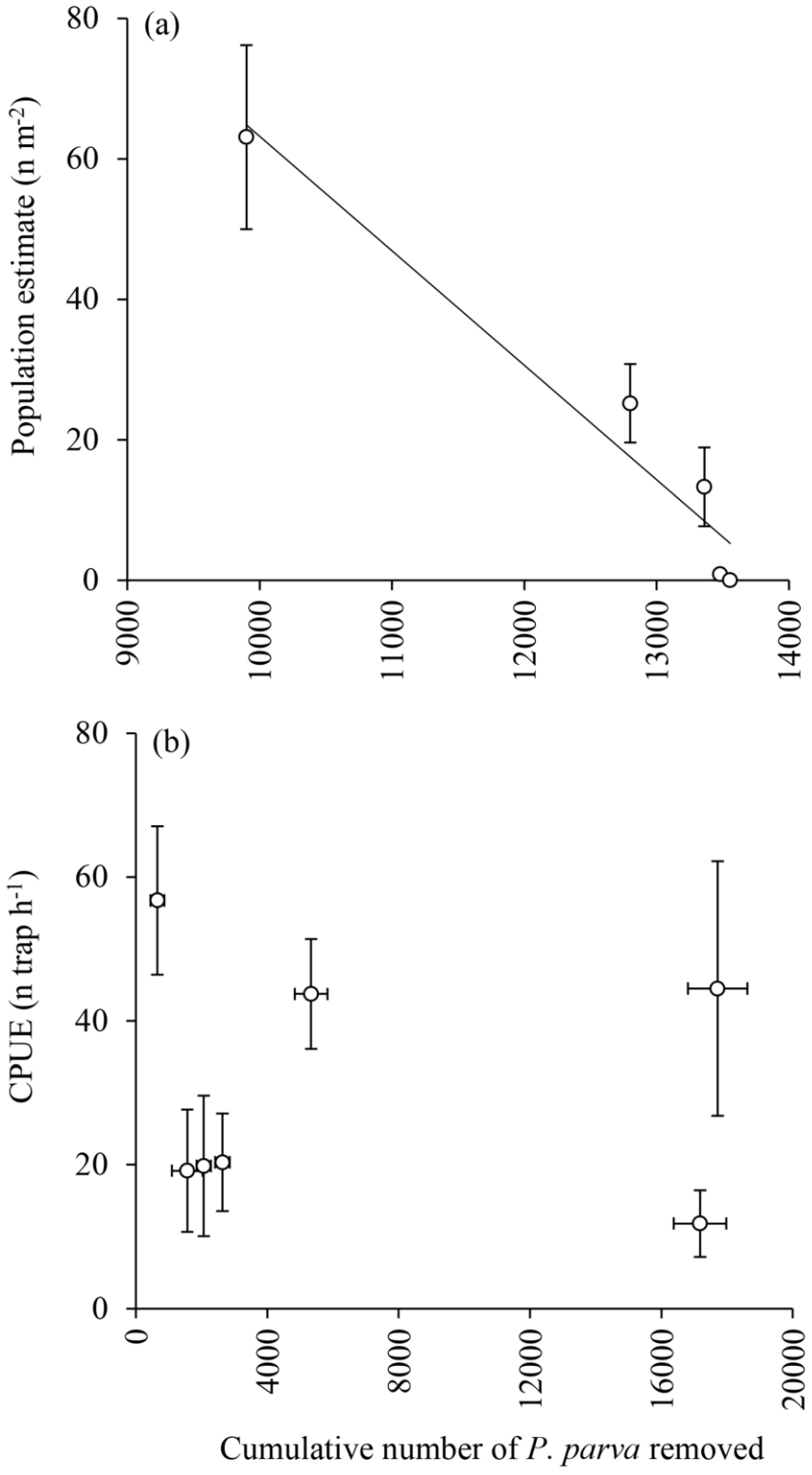
(a)
Population estimate (n m-2)
80
60
40
20
0
9000
10000
11000
12000
13000
14000
(b)
CPUE (n trap h-1)
80
60
40
20
0
0
4000
8000
12000
16000
20000
Cumulative number of P. parva removed

Figure 1.

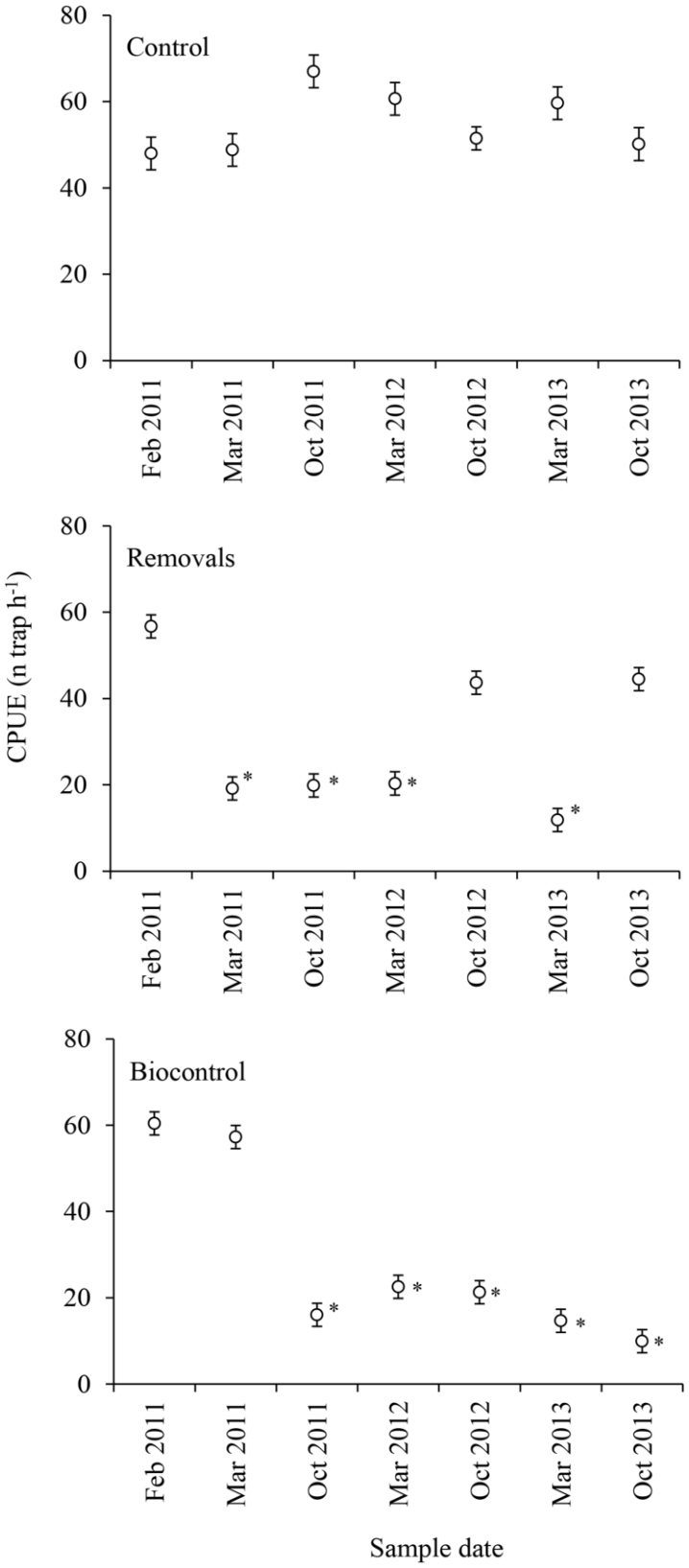
Control
Removals
Biocontrol
CPUE (n trap h-1)
Sample date
Feb 2011
Mar 2011
Oct 2011
Mar 2012
Oct 2012
Mar 2013
Oct 2013
0
20
40
60
80
*

Figure 2.

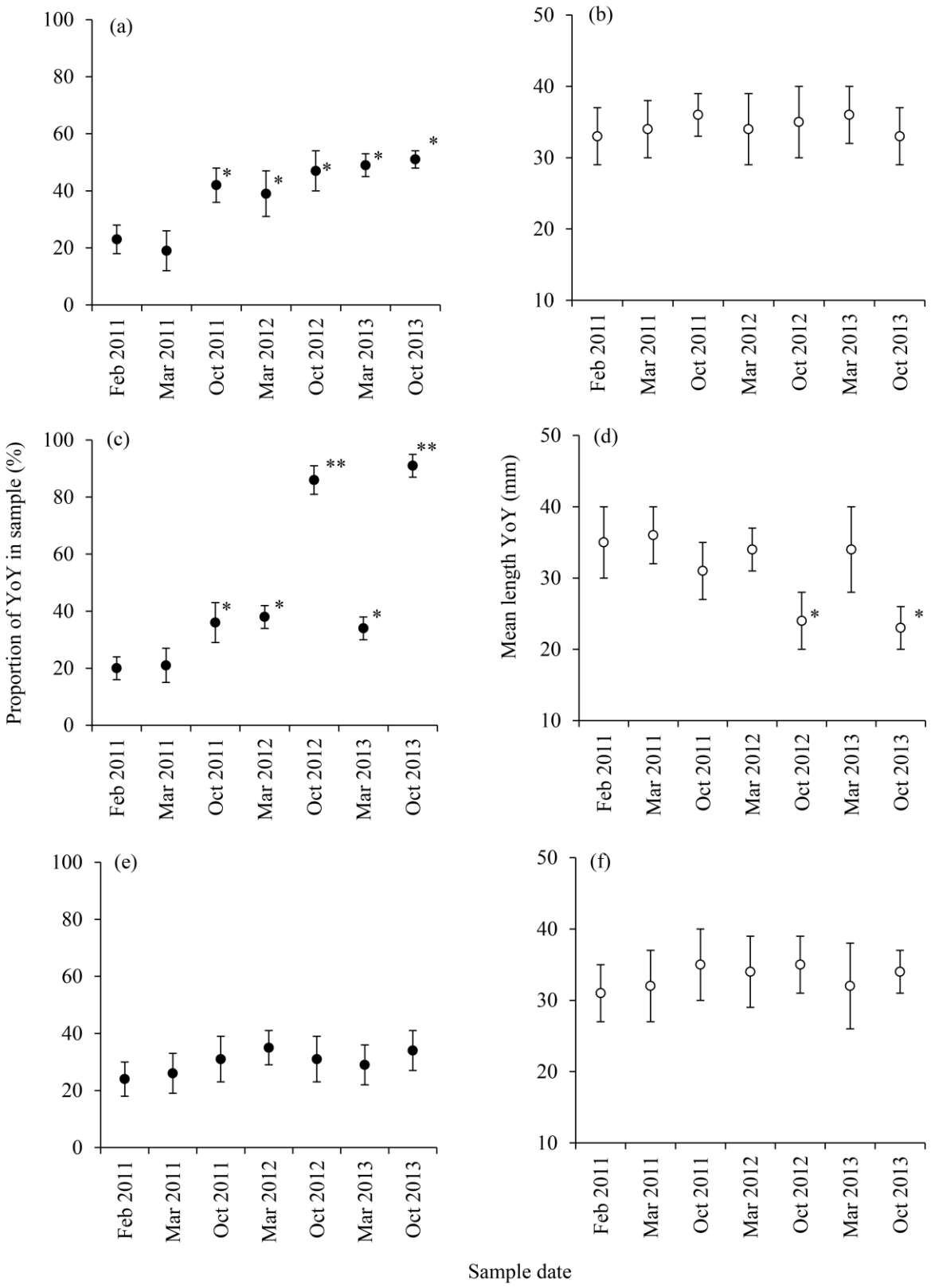
(a)
(b)
(c)
(d)
(e)
(f)
Proportion of YoY in sample (%)
Mean length YoY (mm)
Feb 2011
Mar 2011
Oct 2011
Mar 2012
Oct 2012
Mar 2013
Oct 2013
Sample date

Figure 3.