

Do trophic subsidies affect the outcome of introductions of a non-native freshwater fish?

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SUMMARY

1. Understanding how environmental variables and human disturbances influence the outcomes of introductions of non-native freshwater fish is integral to their risk management. This can be complex in freshwater ecosystems that receive subsidies that increase food availability, as these may influence the outcome of introductions through promoting the survival, reproduction and establishment of the introduced propagules through increasing their access to food resources.
2. We determined how natural and/or artificial trophic subsidies affected the reproduction and establishment of the introduced topmouth gudgeon (*Pseudorasbora parva*) in replicated pond mesocosms. The mesocosms all started with eight mature fish and were run for 100 days during their reproductive season. The subsidies consisted of natural terrestrial prey and/or fishmeal pellets (a common trophic subsidy that can be significant in systems that are used as sport fisheries or for aquaculture).
3. After 100 days, fish in the natural subsidy ponds showed minimal growth and very low reproductive output. Analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicated that their progeny, 0+ fish produced in the ponds, exploited the terrestrial prey. By contrast, in ponds where pellets were added, mineral nutrient availability and primary production were significantly increased, and the mature fish fed mainly on the aquatic resources. The increased productivity of the ponds significantly increased fish growth and fitness, resulting in high numbers of 0+ individuals that did feed on the pellets.
4. Thus, subsidies that can increase both primary production and food resources (such as pelletised fishmeal) can significantly influence the ability of colonists to establish a population rapidly. Management efforts to minimise the risk of introductions should thus consider the role of these types of allochthonous subsidies.

Keywords: aquaculture, fisheries, invasive species, *Pseudorasbora parva*, terrestrial prey subsidies

Introduction

Understanding how environmental variables and human disturbance influence the outcome of introductions of non-native species is important for the management of ecological invasions (Kolar & Lodge, 2002; Gozlan *et al.*, 2010). The management of invasions relies on assessing risks, based on knowledge of the drivers of introductions (e.g. Pysek *et al.*, 2010) and of the factors that facilitate establishment and success (Lockwood, Cassey & Blackburn, 2005). The latter include plasticity in both life-history traits (Sakai *et al.*, 2001) and resource use (Griffen *et al.*, 2012). The charac-

teristics of the recipient ecosystem are also important (Lonsdale, 1999; Catford *et al.*, 2012), with its “invasibility” determined by both abiotic and biotic properties (Lonsdale, 1999; Catford *et al.*, 2012; Li & Stevens, 2012), including species richness (Elton, 1958), predation pressure (Britton, 2012) and habitat heterogeneity (Davies *et al.*, 2005; Fridley *et al.*, 2007). Access to adequate resources, such as refugia, mineral nutrients and food, might also be important for the survival, reproduction and establishment of the introduced species (Davies *et al.*, 2005; Li & Stevens, 2012), although its role in affecting the outcomes of introductions of non-native fauna is uncertain.

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Invasion theory suggests that species-rich communities resist invasion because resource competition is high (Elton, 1958). Conversely, when resources are plentiful, the probability of establishment may be increased (Davis, Grime & Thompson, 2000). In habitats where autochthonous resources are limiting, then food subsidies from donor habitats are important in maintaining consumer populations (Jones *et al.*, 1998; Marczak, Thompson & Richardson, 2007). Thus, allochthonous subsidies may be important in providing introduced, non-native consumers with access to sufficient resources for their survival, reproduction and establishment. Trophic subsidies can be natural, such as terrestrial prey (Kawaguchi, Taniguchi & Nakano, 2003; Baxter, Fausch & Saunders, 2005; Marczak *et al.*, 2007). However, human activities that supplement food supply may also be important. Whilst the role of such anthropogenic subsidies in determining the success of invasions by non-native fauna has not been tested, they do significantly influence the outcome of non-native plant introductions through increasing nutrient availability (Davis *et al.*, 2000; Alston & Richardson, 2006; Besaw *et al.*, 2011).

Freshwater ecosystems are often highly disturbed (Jackson & Grey, 2013) and those that are used for angling and aquaculture often receive substantial trophic subsidies. These are often dominated by pelletised fishmeal feeds ("pellets"), with the global annual use of fishmeal in aquaculture estimated at 3.7 million tonnes or more (Tacon & Metian, 2008). These feeds are also increasingly used as "groundbait" by freshwater recreational anglers. The pellets can alter food-web structure and ecosystem processes (Fernandez-Jover *et al.*, 2011) and provide high quality food for resident native fishes (Arlinghaus & Niesar, 2005; Fernandez-Jover *et al.*, 2011). However, their role in affecting the outcome of non-native fish introductions has not been tested. Here, we test experimentally the independent and interactive effects of natural and pellet-based trophic subsidies on the establishment of an introduced non-native fish in pond mesocosms. We predicted that the natural and pellet subsidies would significantly alter the productivity of the ponds and the populations of fish, resulting in greater reproduction, faster body growth and higher population densities.

Methods

Model species

The topmouth gudgeon *Pseudorasbora parva* was selected as the "model" species, as this small (<10 cm) cyprinid

has proved highly invasive in Europe since its introduction from East Asia via aquaculture in the 1960s. *Pseudorasbora parva* demonstrates great plasticity in its habitat and food preferences; it occupies both lentic and lotic waters and is an omnivorous planktivore (Gozlan *et al.*, 2010). Invasive populations of topmouth gudgeon are present over a range of densities across habitats (Gozlan *et al.*, 2010), with higher densities in ponds used for recreational angling and aquaculture (Britton, Davies & Brazier, 2010a).

Experimental set-up

The experiment comprised four treatments with two fixed factors: natural terrestrial inputs (two levels: present and absent) and pellet-based subsidies (two levels: present and absent). Hereafter, the treatments are referred to as "Pellet" (pellets added and terrestrial inputs blocked), "Natural" (no pellets but with terrestrial inputs), "Both" (pellets added and with terrestrial inputs) and "None" (no pellets and terrestrial inputs blocked). Each treatment was replicated four times in 1000 L fibreglass mesocosms (dimensions: 1 × 1 × 1 m) that were situated in the open air, on grass and close to tree cover (within 15 m). One month before the start of the experiment, the mesocosms were filled with water from a nearby fishless pond and provided with a gravel (*c.* 6 mm diameter) substrata (1.5 cm depth), fish refuge structures (two open-ended circular plastic tubes of 15 cm length and 6 cm diameter) and a native pond lily (*Nymphoides peltata*; uniform wet mass were 10 ± 1 g). They were then seeded with Chironomidae, *Asellus aquaticus* and *Gammarus pulex* (20 individuals of each).

The experiment ran for 100 days between April and July 2012 when water temperatures were recorded between 7.5 and 18.8 °C (mean ± SE: 15.2 ± 0.3 °C). Terrestrial material was prevented from entering the mesocosms by covering them with 1 mm mesh that prevented invertebrates and detritus from entering the water but did not affect light (Baxter *et al.*, 2004). Pellets (1.5 mm diameter) were released daily into the relevant mesocosms via an automated feeder over a 3-h period between 07:00 and 10:00 hours. They were designed to release sufficient pellets to provide each fish with a mass of food 0.5% day⁻¹ of its starting body mass (mean mass 2.5 g), rather than the 1.5% necessary to satisfy the daily food requirement (Britton *et al.*, 2011b). This equated to 0.15 g day⁻¹, equivalent to 56 ± 4 pellets being released into each mesocosm. As the pellets comprised *c.* 1% phosphorus, there was an approximate input of

0.15 mg L⁻¹ of total phosphorus into each of these ponds over the 100 days. In addition, this feeding rate was considered close to that occurring in lakes used for catch-and-release recreational angling in the U.K., where substantial amounts of pellets are used as an attractant for various cyprinid species throughout the summer (J. R. Britton, pers. obs.). In the Natural and Both treatments, terrestrial prey and detritus were allowed to enter the mesocosms naturally, with no manipulation. The replicates for each treatment were randomly assigned to the mesocosms. Each mesocosm was also covered with 20 mm nylon mesh to prevent access by avian predators.

A mix of four male and four female mature *P. parva* (50–80 mm, males being larger) was then released into each mesocosm just before the start of their mating season (April). Males were identified from females by body colour. Across the ponds, there were no significant differences in *P. parva* starting length (ANOVA: $F_{1,14} = 1.14$, $P > 0.05$). Prior to their release, each fish was anaesthetised (MS-222) and measured (fork length, nearest mm) and fin clipped, allowing its identification at the conclusion of the experiment.

Experimental sampling protocol

At fortnightly intervals, dissolved oxygen concentration and temperature were measured in each mesocosm, using a hand-held meter (YSI-85), and two water samples (200 mL) were also taken from each mesocosm for subsequent chemical analysis in the laboratory. The first water sample was used to measure nitrate concentration following Doane & Horwath (2003) and Miranda, Espey & Wink (2001); phosphorus concentration following Murphy & Riley (1986); and ammonia concentration following the National Environmental Methods Index (NEMI, 2013). The second water sample was filtered through GF/C filters (Whatman®, Maidstone, U.K.) before adding 5 mL methanol. The samples were left overnight and the supernatant used for spectrophotometry. The concentration of chlorophyll-*a* was then quantified after Wellburn (1994).

On days 29, 60 and 91, the quantity of natural prey falling into the relevant mesocosms was estimated by direct counts taken over 1 h at 09.00, 12.00 and 15.00 h, with samples also taken for identification and stable isotope analysis. On day 100, each mesocosm was emptied to enable recovery of all adult *P. parva* and collection of all the offspring (hereafter referred to as 0+ fish). An average of 79% ± 0.04 of adult fish was recovered across all mesocosms. Adult *P. parva* were killed using

an overdose of anaesthetic (MS-222) before each was identified, remeasured, its total and gonad mass recorded, and a proportion of muscle tissue removed for subsequent stable isotope analyses. The 0+ fish were also euthanised before counting (*n*) and measuring (fork length). The three largest individual 0+ fish from each mesocosm were then prepared for use in stable isotope analysis.

Stable isotope analysis

The naturally occurring ratios of ¹⁵N:¹⁴N and ¹³C:¹²C were measured (Grey, 2006). Carbon ratios reflect the carbon in the consumer's diet, with typical enrichment of 0–1‰, whereas the nitrogen ratios are enriched by 2–4‰ from resource to consumer (i.e. indicate trophic position) (Post, 2002; McCutchan *et al.*, 2003). In order for the isotopic signature of the fish to reflect its diet under experimental conditions, sufficient time is needed for isotopic turnover in the muscle tissue; 100 days is sufficient for isotopic turnover in *P. parva* based published data on turnover rates in fish (*cf.* McIntyre & Flecker, 2006) at the same temperature of this study (7.5–18.8 °C; e.g. Bosley *et al.*, 2002). In addition to the analysis of the fish tissues, samples of three putative resources (algae, Chironomidae and zooplankton) were collected from each mesocosm on day 100, to enable their relative importance to *P. parva* diet to be assessed. Samples of the terrestrial material taken from the surface of the uncovered mesocosms and of the pellets were also analysed. All samples for stable isotope analysis were dried at 60 °C for 48 h before being processed at the Cornell Stable Isotope Laboratory, Ithaca, U.S.A. Carbon and nitrogen stable isotope ratios are expressed relative to conventional standards as δ¹³C and δ¹⁵N, respectively.

Data analysis

The growth of *P. parva* over the experiment was assessed using two metrics: *incremental fork length* (IL) and *condition* (K). Incremental fork length was calculated for each individual as $(L_{t+1} - L_t)/t$, where L_t and L_{t+1} were the starting and finishing fork lengths, respectively, and t was the number of days between L_t and L_{t+1} . *Condition* was calculated for each individual as $100 \times W/L^3$, where W was weight – gonad weight (g) and L was fork length (mm). Regarding fitness, as this could not be measured directly for each individual, then we used the total number of 0+ fish present in each mesocosm as a measure of fitness across all the mature fish per replicate.

We used two-tailed ANOVAS to test for differences between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the natural terrestrial prey (adult Calliphoridae, Tipulidae) and the pellets, and for a significant effect of the presence of natural and pellet subsidies on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the putative resources (algae, zooplankton and chironomids) from each mesocosm. The Student–Newman–Keuls procedure enabled *post hoc* comparisons among levels of significant terms.

We used two-tailed permutational multivariate analysis of variance (PERMANOVA; Anderson, Gorley & Clarke, 2008) to test for effects of the experimental manipulations (pellet subsidy = present or absent; natural subsidy = present or absent) on the temperature, dissolved oxygen and chlorophyll-*a* concentrations over the course of the experiment using a repeated measures design in the PERMANOVA+ add-in to PRIMER[®] version 6.1 (PRIMER-E Ltd, Plymouth, U.K.). This statistical design tests for any independent and combined effects of each subsidy instead of testing for differences between the four treatments. PERMANOVA was also used to test for effects of the manipulations on a) the growth and condition of the individual adult fish (starting fork length and sex were initially incorporated as random factors and had no effect; therefore, we removed them to maximise the power of the subsequent analysis); b) the number of 0+ in each pond; and c) on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of individual adult and 0+ fish. These analyses were carried out with 9999 permutations of the residuals under a reduced model (McArdle & Anderson, 2001; Anderson *et al.*, 2008) and were based on Euclidean distance matrices calculated from log ($X + 1$)-transformed data (except for the stable isotope data, where raw values were used). Mesocosm number was incorporated as an additional random factor in all tests (except where a repeated measures design was used) and had no effect; therefore, data were pooled from each treatment for further analysis of stable isotope data.

We used five quantitative population metrics derived from stable isotope data to reveal the key aspects of the food resource base of adult and 0+ fish from each treatment. Three metrics give information on the isotopic niche of a population; *nitrogen range* (NR_b) and *carbon range* (CR_b) provide a univariate indication of the total nitrogen and carbon range exploited (Layman *et al.*, 2007; Jackson *et al.*, 2012), and *standard ellipse area* (SEA_c) is a bivariate estimate of the core isotopic niche (Jackson *et al.*, 2011). The metric *standard deviation of mean distance to centroid* (SDCD_b) was used as a measure of population trophic diversity, and *standard deviation of nearest neighbour distance* (SDNND_b) can be used to infer population

trophic evenness (Layman *et al.*, 2007). The subscript “*b*” indicates that the metrics were bootstrapped ($n = 10\,000$) based on the minimum sample size in the data set ($n = 6$) to allow comparison among populations where sample size varied (Jackson *et al.*, 2012). The subscript “*c*” indicates that a small sample size correction was used to increase accuracy (Jackson *et al.*, 2011). All metrics and correlations were calculated using the R statistical computing package (R Development Core Team, 2011), see Jackson *et al.* (2011) for detailed methodology of the metrics and Layman *et al.* (2007) for original descriptions of the community-level metrics the calculations were based on. Finally, we also calculated SEA_c separately for the adult fish from each mesocosm, to determine whether there was a correlation between niche width and fish production (measured as average IL, K and 0+ fish density).

Results

Natural prey subsidies

In the ponds receiving natural prey, the main terrestrial species present were of the dipteran families Calliphoridae, Tipulidae and Acrididae, and of the order Arachnida. The mean number of terrestrial items detected on the surface of ponds was $10.2 \pm 4.3 \text{ h}^{-1}$ over the three sampling occasions. Differences in the total number of prey entering mesocosms were not significant on any sampling occasion (ANOVA: day 29: $F_{1,14} = 1.21$, $P > 0.05$; day 60: $F_{1,14} = 0.92$, $P > 0.05$; day 91: $F_{1,14} = 1.42$, $P > 0.05$). However, the number of prey entering the ponds was significantly higher at 15.00 than at 9.00 and 12.00 (ANOVA: day 29: $F_{2,13} = 4.47$, $P < 0.04$; day 60: $F_{2,13} = 4.12$, $P < 0.04$; day 91: $F_{2,13} = 4.99$, $P < 0.04$).

Water chemistry and nutrient status

The consequences of the two trophic subsidies related primarily to the influence of the pellets on the chemical and nutrient status of the mesocosms, with significantly decreased concentrations of dissolved oxygen and significantly higher concentrations of orthophosphate and chlorophyll-*a* (PERMANOVA: $n = 128$, Pseudo- $F_{1,12} = 5.57$, $P = 0.014$; $n = 136$, Pseudo- $F_{1,12} = 16.05$, $P < 0.001$; $n = 136$, Pseudo- $F_{1,12} = 36.55$, $P < 0.001$, respectively; Fig. 1). The only significant consequence detected in the mesocosms with natural subsidies was an increase in chlorophyll-*a* concentrations (PERMANOVA: $n = 136$, Pseudo- $F_{1,12} = 3.82$, $P < 0.03$; Fig 1c). This was, however, probably due to the high concentrations in the “Both”

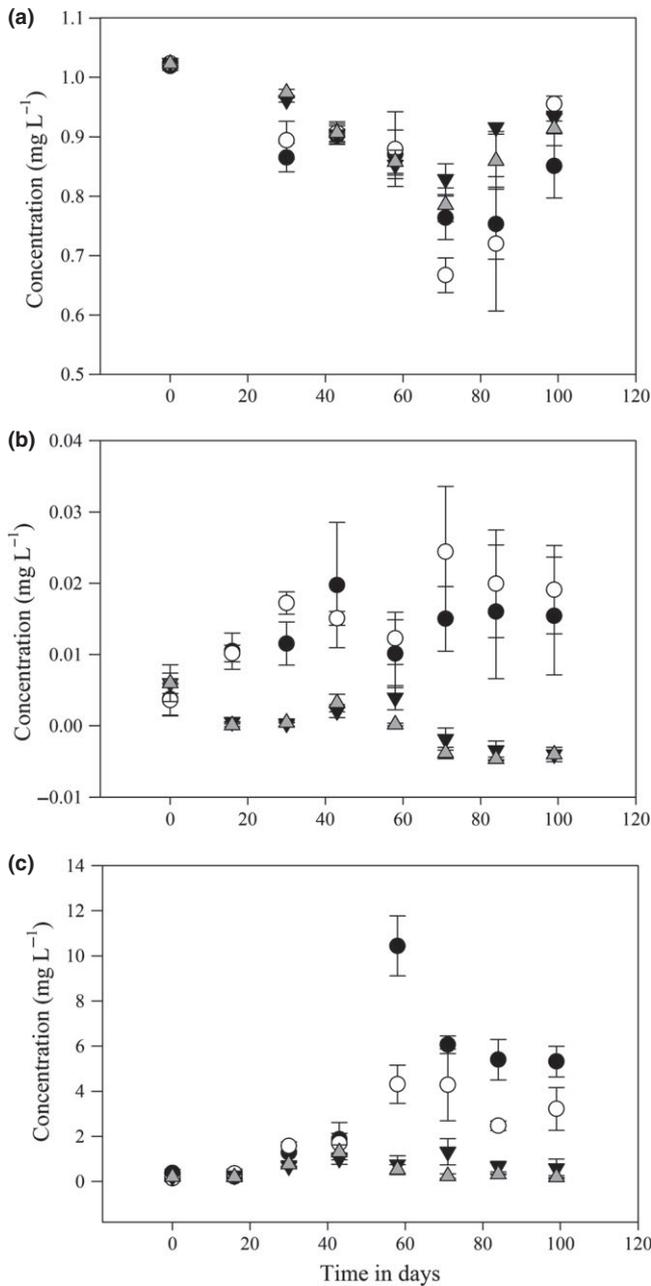


Fig. 1 Fluctuations in (a) dissolved oxygen, (b) phosphate concentration and (c) chlorophyll-*a* over 100 days in the four experimental treatments; pellet (white circle), both (black circle), natural (black downwards triangle) and none (grey upward triangle). Data points represent mean ± standard error (*n* = 4).

treatment compared with the pellet treatment (Fig. 1c). There was no interactive effect of pellets or natural subsidies on any variable and no effect of either subsidy on ammonia or nitrate concentration in the water column over the course of the experiment. There were also no significant differences in water temperatures between the treatments (PERMANOVA *n* = 136, Pseudo-*F*_{1,12} = 0.91, *P* = 0.61).

Trophic effects

The δ¹³C of terrestrial prey and the pellets differed significantly, with the pellets having relatively high values (ANOVA; *n* = 6, *F*_{1,7} = 18.60, *P* = 0.004; Table 1). The δ¹⁵N signature of algae was high in the treatments with pellets (ANOVA; *n* = 31, *F*_{1,27} = 9.10, *P* = 0.006), as was that of zooplankton (ANOVA; *n* = 31, *F*_{1,27} = 12.59, *P* = 0.001) and chironomids (ANOVA; *n* = 32, *F*_{1,28} = 3.62, *P* < 0.001; Table 1). These high δ¹³C and δ¹⁵N values were also observed in the adult fish in ponds to which pellets were added (Table 1; Fig. 2a). There was a significant and independent effect of pellets on the δ¹³C of adult fish (PERMANOVA; *n* = 100, Pseudo-*F*_{1,96} = 27.07, *P* < 0.001) and δ¹⁵N (PERMANOVA; *n* = 100, Pseudo-*F*_{1,96} = 128.8, *P* < 0.001) but no effect of natural terrestrial prey on either.

For 0+ fish, the pellets also had a significant and independent effect on their δ¹⁵N, resulting in higher values (PERMANOVA: *n* = 39, Pseudo-*F*_{1,35} = 132.1, *P* < 0.001), but the natural subsidy did not. In contrast to the adult fish, there was a significant and independent effect of the natural subsidy on the δ¹³C of 0+ fish, resulting in lower values (PERMANOVA; *n* = 39, Pseudo-*F*_{1,35} = 6.63, *P* = 0.031), but no effect of the pellet subsidy (Fig. 2b).

Table 1 The stable isotope values (mean ± SE) of the resources (*n* = 7–8 for all algae, zooplankton and chironomid samples) and *Pseudorasbora parva* (adult *n* = 20–8; 0+ *n* = 6–12) in each treatment

	Treatment	δ ¹³ C (‰)	δ ¹⁵ N (‰)
Pellet fishmeal (<i>n</i> = 3)		-23.88 ± 0.24	5.80 ± 0.10
Natural terrestrial insects (<i>n</i> = 6)		-27.28 ± 0.44	6.12 ± 0.53
Algae	Pellet	-23.92 ± 0.58	3.99 ± 0.65
	Both	-23.14 ± 0.71	4.83 ± 0.67
	Natural	-24.94 ± 0.46	1.63 ± 0.27
	None	-22.65 ± 1.02	3.89 ± 0.58
Zooplankton	Pellet	-25.69 ± 0.26	8.02 ± 1.13
	Both	-24.51 ± 0.56	7.49 ± 0.62
	Natural	-25.33 ± 0.51	6.00 ± 0.53
	None	-23.45 ± 0.62	4.36 ± 0.47
Chironomidae	Pellet	-24.70 ± 0.45	6.07 ± 0.31
	Both	-23.68 ± 0.16	6.45 ± 0.19
	Natural	-24.57 ± 0.29	2.78 ± 0.19
	None	-22.97 ± 0.24	3.53 ± 0.41
Adult <i>P. parva</i>	Pellet	-26.08 ± 0.22	8.18 ± 0.10
	Both	-25.41 ± 0.26	8.29 ± 0.10
	Natural	-28.57 ± 0.33	7.15 ± 0.09
	None	-27.66 ± 0.37	7.08 ± 0.11
0+ <i>P. parva</i>	Pellet	-23.07 ± 0.21	9.55 ± 0.21
	Both	-23.25 ± 0.24	9.46 ± 0.13
	Natural	-24.72 ± 0.23	5.53 ± 0.14
	None	-22.55 ± 0.30	6.15 ± 0.18

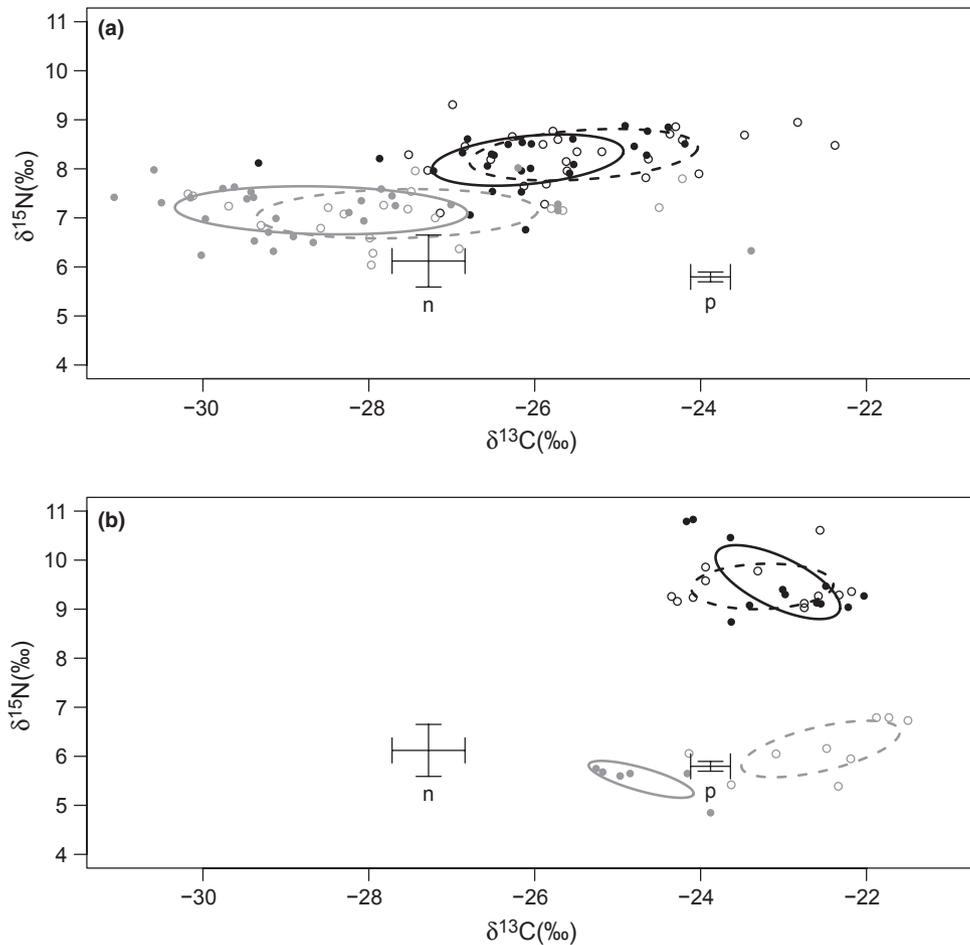


Fig. 2 Carbon and nitrogen stable isotope bi-plot of all (a) adult and (b) 0+ *Pseudorasbora parva* from all mesocosms at the end of the experiment. Data points represent individual fish from the pellet (filled black circles), both (empty black circles), natural (filled grey circles) and none (empty grey circles) treatments. The lines enclose the standard ellipse area (SEA_c) for each population in the pellet (solid black), both (dashed black), natural (solid grey) and none (dashed grey) treatments. Pellet (a) and natural (n) resource subsidies are shown by the labelled black lines (mean \pm standard error).

The 0+ fish from the treatments with and without pellets occupied distinct areas in isotopic space, with enriched $\delta^{15}N$ when the pellets were available (Fig. 2b). Moreover, the 0+ fish had enriched $\delta^{15}N$ values compared with adults in the same treatments (Fig. 2). Finally, there

was no interactive effect of the two subsidy types on the stable isotope values of adult or 0+ fish.

Measures of the niche width of adult fish (measured as SEA_c , CR_b and NR_b), trophic diversity (measured as $SDCD_b$) and trophic evenness (measured as $SDNND_b$)

Table 2 Mean stable isotope-derived metrics of the adult and 0+ fish from each treatment

Population	Treatment	SEA_c (‰ ²)	CR_b (‰)	NR_b (‰)	$SDCD_b$ (‰)	$SDNND_b$ (‰)
Adults	Pellet ($n = 26$)	1.78	1.23 (0.42–2.12)	2.72 (0.82–5.14)	0.54 (0.17–1.12)	0.46 (0.09–1.09)
	Both ($n = 26$)	2.18	1.26 (0.51–2.21)	3.31 (1.42–5.14)	0.61 (0.21–1.08)	0.47 (0.11–1.08)
	Natural ($n = 28$)	2.7	1.18 (0.43–1.78)	4.12 (1.38–7.68)	0.80 (0.22–1.69)	0.64 (0.11–1.75)
	None ($n = 20$)	2.65	1.20 (0.39–1.92)	4.02 (1.29–5.96)	0.78 (0.23–1.33)	0.60 (0.12–1.28)
0+	Pellet ($n = 12$)	1.32	1.57 (0.32–2.09)	1.67 (0.79–2.14)	0.34 (0.14–0.55)	0.39 (0.08–0.70)
	Both ($n = 12$)	1.24	0.97 (0.20–1.58)	1.80 (0.78–2.17)	0.31 (0.11–0.51)	0.31 (0.07–0.65)
	Natural ($n = 6$)	0.49	0.61 (0.05–0.90)	1.18 (0.33–1.38)	0.22 (0.06–0.36)	0.26 (0.03–0.47)
	None ($n = 9$)	1.38	1.18 (0.66–1.40)	2.03 (0.75–2.64)	0.36 (0.14–0.60)	0.35 (0.08–0.70)

Numbers in parentheses show the 2.5–97.5% quantile range.

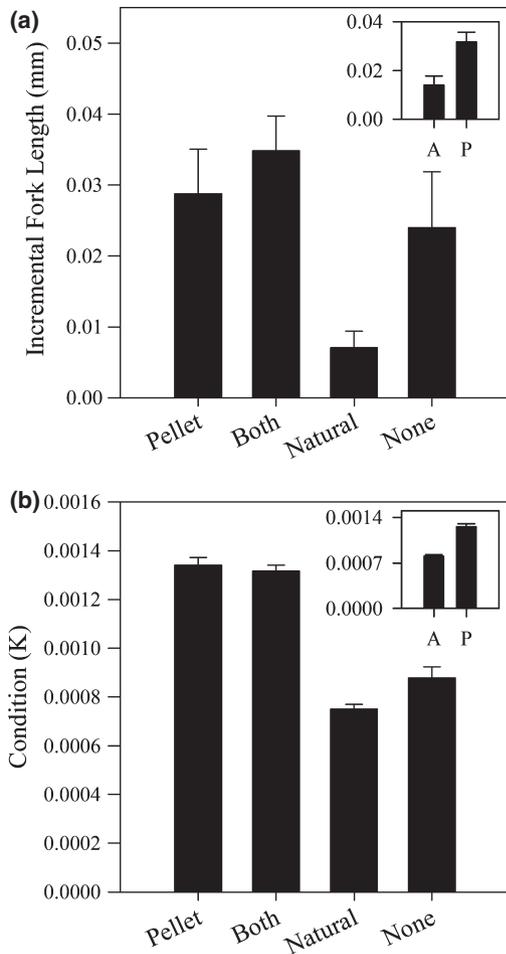


Fig. 3 *Pseudorasbora parva* (a) incremental fork length, IL and (b) condition, K in each treatment at the end of the experiment (mean \pm standard error; $n = 20$ – 29). Graph insets show the significant differences in IL and K between pellet subsidy presence (P) and absence (A).

were higher in treatments without pellet subsidies (except CR_b; Table 2), suggesting a more diverse diet. In contrast, measures of the niche width, trophic diversity and trophic evenness of 0+ fish were generally higher in treatments without natural subsidies (except NR_b; Table 2). Hence, adult and 0+ fish had more restricted niches in the presence of pellets and natural terrestrial prey, respectively.

Fish growth and reproductive output

The growth and condition of adult fish was high when pellets were available but were not affected by the natural subsidy (Fig. 3); that is, there was a significant independent effect of pellets on both metrics (PERMANOVA: $n = 105$, Pseudo- $F_{1,101} = 6.50$, $P = 0.024$; $n = 105$, Pseudo- $F_{1,101} = 27.58$, $P < 0.001$, respectively). The pellet subsidy also had significant consequences on the fitness of the

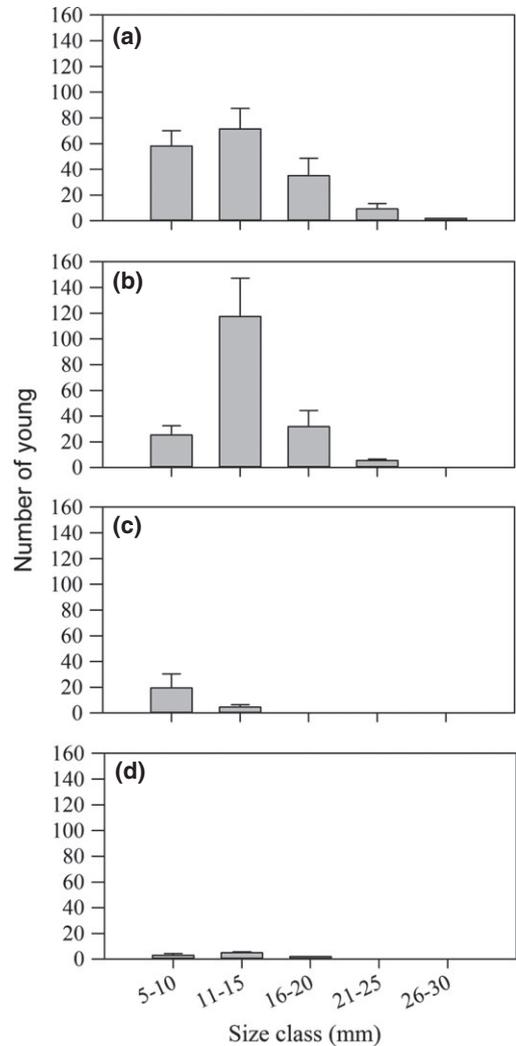


Fig. 4 The total number of 0+ (young) fish (mean \pm standard error, $n = 4$) in treatments (a) Pellet, (b) Both, (c) Natural and (d) None at the end of the experiment, grouped by size class.

adult fish, with significantly higher numbers of offspring produced than in mesocosms without pellets (PERMANOVA; $n = 16$, Pseudo- $F_{1,12} = 17.39$, $P = 0.002$; Fig. 4). Moreover, when pellets were available, the 0+ fish were 5–30 mm in length, whereas without pellets, there were few 0+ fish above 15 mm (Fig. 4). There was no interactive effect of the two subsidy types on any measure of fish growth and reproductive output. Finally, total reproductive output had a significant inverse correlation with adult niche width (measured as SEA_c; $n = 16$, Pearson $r = -0.53$, $P = 0.042$).

Discussion

There were significant increases in the growth, fitness and density of *P. parva* in the mesocosms with pellets

compared with those with natural terrestrial prey. This suggests that where nutrient availability is enhanced in freshwater ecosystems through anthropogenic subsidies of food, then the likelihood of establishment and invasion of non-native fish may be increased. Whilst the growth of the adult fish in the "None" treatment was similar to that in the "Pellet" and "Both" treatments, this was likely to be due to the response of the mature fish to their conditions, which diverted assimilate into somatic growth rather than reproduction. Whilst many factors can influence invasion success, such as propagule pressure (Lockwood *et al.*, 2005; Britton & Gozlan, 2013) and biotic resistance (Britton, 2012), these results indicate that anthropogenic trophic subsidies, where they occur, can play a role in determining the establishment success of non-native fish.

Pellets significantly raised phosphate and chlorophyll-*a* concentrations in the water column, which indirectly enhanced the growth of the adult fish via food-web interactions whilst, in turn, stable isotopes suggested that fish with access to the contrasting subsidies had differing resource bases. Indeed, the $\delta^{13}\text{C}$ value of fish was higher when the pellet subsidy was available, reflecting the higher $\delta^{13}\text{C}$ of the pellets themselves compared with terrestrial insects. Despite the $\delta^{15}\text{N}$ signatures of the two subsidy types being similar, there were raised $\delta^{15}\text{N}$ signatures in the algae, zooplankton and chironomids when the pellet subsidy was available, with this being reflected in the fish. In real lakes, whilst pellets could also be beneficial to native fish as well as invasive, our results nevertheless highlight that the anthropogenic stressors of introduced species and feeding attractants used by anglers can interact and alter aquatic ecosystems (Ormerod *et al.*, 2010).

In contrast to the pellets, terrestrial prey did not alter system productivity and there was no effect on the resource base of the adult fish. This was reflected in their slow somatic growth and low reproductive output, suggesting that resource availability could have been limiting. Nonetheless, the resource base of the 0+ fish with access to natural subsidies did differ from that of those that were reliant solely on the aquatic prey within the mesocosms, suggesting that terrestrial subsidies may be important when others are absent. Ontogenetic dietary shifts are common in fish (Britton *et al.*, 2009; Zimmerman *et al.*, 2009), and our results suggest that the diet of the 0+ fish included more terrestrial prey than that of adults. The ponds that received both terrestrial prey and pellets had the highest growth and reproductive output, again indicating that the terrestrial inputs were being used by the fish in addition to the higher-energy fishmeal.

Adult fish had smaller niche widths and lower measures of trophic diversity and evenness (both indicators of individual spread in isotopic space) in the presence of the pellet subsidy, implying convergence of all individuals on the highly profitable subsidy and its associated consumers (Popa-Lisseanu *et al.*, 2007). This implies that the pellets, both consumed directly and via food-web linkages, provided sufficient energy for the founding fish to survive and reproduce; this being emphasised by the fact that fish production was inversely correlated with adult niche width. In contrast, the niche width, trophic diversity and trophic evenness in the 0+ fish were all smaller in the presence of terrestrial prey, again suggesting that the latter were an important dietary component for these fish.

The potential implications of our results for managing invasions of fish are twofold. Firstly, they suggest that the risks of invasions by non-native fish in fresh waters may change with trophic subsidies. When aquaculture and angling involves the use of feeds of high nutritional value, then the invasion risk may be increased. Although speculative, this may potentially create invasion "hotspots" around hubs of fishery and aquaculture activity, especially those in habitats that are already disturbed such as impounded rivers that are already prone to fish introductions (Havel, Lee & Vander Zanden, 2005). Whilst it is acknowledged that there are management difficulties in regulating allochthonous inputs into fresh waters generally (particularly in fisheries), their role in increasing the invasion risk of non-native fish should be considered (e.g. Britton, 2012), which is not the case at present.

The second implication for risk management relates primarily to *P. parva* and similar small, invasive pest fish. Ecological concerns about *P. parva* relate to its ability to colonise new habitats, to compete with native species (Britton, Davies & Harrod, 2010b) and to transmit diseases to native fish (Andreou *et al.*, 2012). Our results suggest that its ability to colonise new habitats and reach high population density is affected by trophic subsidies from angling and aquaculture, and it should be noted that invasive populations are strongly associated with recreational fisheries (Britton *et al.*, 2011a) and aquaculture (Gozlan *et al.*, 2010). Thus, in freshwater ecosystems not used in these ways, and so with relatively low allochthonous inputs (at least from humans), their establishment and invasion risk may be substantially lower than in more perturbed systems. Moreover, the ongoing control of *P. parva* in U.K. fresh waters, involving the eradication of populations, is driven by ecological concerns about its ability to compete with

native fish (Britton *et al.*, 2010b). However, our data suggest these abundant populations might only develop in systems where fisheries have already caused disturbance. Thus, the benefits of eradicating *P. parva* from these fisheries relate primarily to preventing its wider dispersal into the environment and subsequent disease transmission.

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References

- Alston K.P. & Richardson D.M. (2006) The roles of habitat features, disturbance, and distance from putative source populations in structuring alien plant invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biological Conservation*, **132**, 183–198.
- Anderson M.J., Gorley R.N. & Clarke K.R. (2008) *PERMANOVA+ for PRIMER: A Guide to Software And Statistical Methods*. PRIMER-E, Plymouth, U.K.
- Andreou D., Arkush K., Guegan J.F. & Gozlan R.E. (2012) Introduced pathogens and native freshwater biodiversity, a case study of *Sphaerothecum destruens*. *PLoS ONE*, **7**, e36998.
- Arlinghaus R. & Niesar M. (2005) Nutrient digestibility of angling baits for carp, *Cyprinus carpio*, with implications for groundbait formulation and eutrophication control. *Fisheries Management and Ecology*, **12**, 91–97.
- Baxter C.V., Fausch K.D., Murakami M. & Chapman P.L. (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology*, **85**, 2656–2663.
- Baxter C.V., Fausch K.D. & Saunders W.C. (2005) Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology*, **50**, 201–220.
- Besaw L.M., Thelen G.C., Sutherland S., Metlen K. & Callaway R.M. (2011) Disturbance, resource pulses and invasion: short-term shifts in competitive effects, not growth responses, favour exotic annuals. *Journal of Applied Ecology*, **48**, 998–1006.
- Bosley K.L., Witting D.A., Chambers R.C. & Wainright S.C. (2002) Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuro-*
- nectes americanus* with stable isotopes. *Marine Ecology Progress Series*, **236**, 233–240.
- Britton J.R. (2012) Testing strength of biotic resistance against an introduced fish: inter-specific competition or predation through facultative piscivory? *PLoS ONE*, **7**, e31707.
- Britton J.R., Copp G.H., Brazier M. & Davies G.D. (2011a) A modular assessment tool for managing introduced fishes according to risks of species and their populations, and impacts of management actions. *Biological Invasions*, **13**, 2847–2860.
- Britton J.R., Cucherousset J., Grey J. & Gozlan R.E. (2011b) Determining the strength of exploitative competition from an introduced fish: roles of density, biomass and body size. *Ecology of Freshwater Fish*, **20**, 74–79.
- Britton J.R., Davies G.D. & Brazier M. (2010a) Towards the successful control of *Pseudorasbora parva* in the U.K. *Biological Invasions*, **12**, 25–31.
- Britton J.R., Davies G.D. & Harrod C. (2010b) Trophic interactions and consequent impacts of the invasive fish *Pseudorasbora parva* in a native aquatic foodweb: a field investigation in the U.K. *Biological Invasions*, **12**, 1533–1542.
- Britton J.R. & Gozlan R.E. (2013) How many founders for a biological invasion? Predicting introduction outcomes from propagule pressure. *Ecology*, DOI: 10.1890/13-0527.1.
- Britton J.R., Jackson M.C., Muchiri M., Tarras-Wahlberg H., Harper D.M. & Grey J. (2009) Status, ecology and conservation of an endemic fish, *Oreochromis niloticus baringoensis*, in Lake Baringo, Kenya. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **19**, 487–496.
- Catford J.A., Vesik P.A., Richardson D.M. & Pysek P. (2012) Quantifying levels of biological invasion: towards the objective classification of invaded and invulnerable ecosystems. *Global Change Biology*, **18**, 44–62.
- Davies K.F., Chesson P., Harrison S., Inouye B.D., Melbourne B.A. & Rice K.J. (2005) Spatial heterogeneity explains the scale dependence of the native-exotic diversity relationship. *Ecology*, **86**, 1602–1610.
- Davis M.A., Grime J.P. & Thompson K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, **88**, 528–534.
- Doane T.A. & Horwath W.R. (2003) Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, **36**, 2713–2722.
- Elton C.S. (1958) *The Ecology of Invasions by Plants and Animals*. Methuen, London.
- Fernandez-Jover D., Martinez-Rubio L., Sanchez-Jerez P., Bayle-Sempere J.T., Lopez Jimenez J.A., Martinez Lopez F.J. *et al.* (2011) Waste feed from coastal fish farms: a trophic subsidy with compositional side-effects for wild gadoids. *Estuarine Coastal and Shelf Science*, **91**, 559–568.
- Fridley J.D., Stachowicz J.J., Naeem S., Sax D.F., Seabloom E.W., Smith M.D. *et al.* (2007) The invasion paradox: reconciling pattern and process in species invasions. *Ecology*, **88**, 3–17.

- Gozlan R.E., Andreou D., Asaeda T., Beyer K., Bouhadad R., Burnard D. *et al.* (2010) Pan-continental invasion of *Pseudorasbora parva*: towards a better understanding of freshwater fish invasions. *Fish and Fisheries*, **11**, 315–340.
- Grey J. (2006) The use of stable isotope analyses in freshwater ecology: current awareness. *Polish Journal of Ecology*, **54**, 563–584.
- Griffen B.D., Altman I., Bess B.M., Hurley J. & Penfield A. (2012) The role of foraging in the success of invasive Asian shore crabs in New England. *Biological Invasions*, **14**, 2545–2558.
- Havel J.E., Lee C.E. & Vander Zanden M.J. (2005) Do reservoirs facilitate invasions into landscapes? *BioScience*, **55**, 518–525.
- Jackson A.L., Inger R., Parnell A.C. & Bearhop S. (2011) Comparing isotopic niche widths among and within communities: Bayesian analysis of stable isotope data. *Journal of Animal Ecology*, **80**, 595–602.
- Jackson M.C. & Grey J. (2013) Accelerating rates of freshwater invasions in the catchment of the River Thames. *Biological Invasions*, **15**, 945–951.
- Jackson M.C., Jackson A.L., Britton J.R., Donohue I., Harper D. & Grey J. (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS ONE*, **7**, e31757.
- Jones R.I., Grey J., Sleep D. & Quarmby C. (1998) An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 105–110.
- Kawaguchi Y., Taniguchi Y. & Nakano S. (2003) Terrestrial invertebrate inputs determine the local abundance of stream fishes in a forested stream. *Ecology*, **84**, 701–708.
- Kolar C.S. & Lodge D.M. (2002) Ecological predictions and risk assessments for alien fish in North America. *Science*, **298**, 1233–1236.
- Layman C.A., Arrington D.A., Montaña C.G. & Post D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.
- Li W. & Stevens M.H.H. (2012) Fluctuating resource availability increases invasibility in microbial microcosms. *Oikos*, **121**, 435–441.
- Lockwood J.L., Cassey P. & Blackburn T. (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution*, **20**, 223–228.
- Lonsdale W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**, 1522–1536.
- Marczak L.B., Thompson R.M. & Richardson J.S. (2007) Meta-analysis: trophic level, habitat, and productivity shape the food web effects of resource subsidies. *Ecology*, **88**, 140–148.
- McArdle B.H. & Anderson M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82**, 290–297.
- McCutchan J.H. Jr, Lewis W.M. Jr, Kendall C. & McGrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.
- McIntyre P.B. & Flecker A.S. (2006) Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia*, **148**, 12–21.
- Miranda K.M., Espey M.G. & Wink D.A. (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide-Biology and Chemistry*, **5**, 62–71.
- Murphy J. & Riley J.P. (1986) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.
- NEMI (2013) *Natural Environmental Methods Index*. <https://www.nemi.gov/apex/f?p=237:1:5108928664713822>. (accessed on 3 June 2013).
- Ormerod S.J., Dobson M., Hildrew A.G. & Townsend C.R. (2010) Multiple stressors in freshwater ecosystems. *Freshwater Biology*, **55**, 1–4.
- Popa-Lisseanu A.G., Delgado-Huertas A., Forero M.G., Rodriguez A., Arlettaz R. & Ibanez C. (2007) Bats' conquest of a formidable foraging niche: the myriads of nocturnally migrating songbirds. *PLoS ONE*, **2**, e205.
- Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Pysek P., Jarosik V., Hulme P.E., Kuehn I., Wild J., Arianoutsou M. *et al.* (2010) Disentangling the role of environmental and human pressures on biological invasions across Europe. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12157–12162.
- R Development Core Team (2011) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A. *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **30**, 5–332.
- Tacon A.G.J. & Metian M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, **285**, 146–158.
- Wellburn A.R. (1994) The spectral determination of chlorophyll-a and chlorophyll-b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, **144**, 307–313.
- Zimmerman M.S., Schmidt S.N., Krueger C.C., Vander Zanden M.J. & Eshenroder R.L. (2009) Ontogenetic niche shifts and resource partitioning of lake trout morphotypes. *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 1007–1018.

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