

# Feeding behaviour, predatory functional responses and trophic interactions of the invasive Chinese mitten crab (*Eriocheir sinensis*) and signal crayfish (*Pacifastacus leniusculus*)

PAULA J ROSEWARNE\*, ROBERT J.G. MORTIMER<sup>†,‡</sup>, ROBERT J. NEWTON<sup>†</sup>, CHRISTOPHER GROCOCK\*, CHRISTOPHER D. WING\* AND ALISON M. DUNN\*

\*School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, U.K.

<sup>†</sup>School of Earth and Environment, University of Leeds, Leeds, U.K.

<sup>‡</sup>School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, Nottinghamshire, U.K.

## SUMMARY

1. Freshwaters are subject to particularly high rates of species introductions; hence, invaders increasingly co-occur and may interact to enhance impacts on ecosystem structure and function. As trophic interactions are a key mechanism by which invaders influence communities, we used a combination of approaches to investigate the feeding preferences and community impacts of two globally invasive large benthic decapods that co-occur in freshwaters: the signal crayfish (*Pacifastacus leniusculus*) and Chinese mitten crab (*Eriocheir sinensis*).
2. In laboratory preference tests, both consumed similar food items, including chironomids, isopods and the eggs of two coarse fish species. In a comparison of predatory functional responses with a native crayfish (*Austropotamobius pallipes*), juvenile *E. sinensis* had a greater predatory intensity than the native *A. pallipes* on the keystone shredder *Gammarus pulex*, and also displayed a greater preference than *P. leniusculus* for this prey item.
3. In outdoor mesocosms ( $n = 16$ ) used to investigate community impacts, the abundance of amphipods, isopods, chironomids and gastropods declined in the presence of decapods, and a decapod >gastropod >periphyton trophic cascade was detected when both species were present. *Eriocheir sinensis* affected a wider range of animal taxa than *P. leniusculus*.
4. Stable-isotope and gut-content analysis of wild-caught adult specimens of both invaders revealed a wide and overlapping range of diet items including macrophytes, algae, terrestrial detritus, macroinvertebrates and fish. Both decapods were similarly enriched in  $^{15}\text{N}$  and occupied the same trophic level as Ephemeroptera, Odonata and Notonecta. *Eriocheir sinensis*  $\delta^{13}\text{C}$  values were closely aligned with macrophytes indicating a reliance on energy from this basal resource, supported by evidence of direct consumption from gut contents. *Pacifastacus leniusculus*  $\delta^{13}\text{C}$  values were intermediate between those of terrestrial leaf litter and macrophytes, suggesting reliance on both allochthonous and autochthonous energy pathways.
5. Our results suggest that *E. sinensis* is likely to exert a greater per capita impact on the macroinvertebrate communities in invaded systems than *P. leniusculus*, with potential indirect effects on productivity and energy flow through the community.

*Keywords:* invasive species, mesocosm, prey choice, stable isotopes, Type II functional response

## Introduction

Freshwaters are particularly vulnerable to human-mediated introduction of invasive species due to their physical connectivity and high levels of human disturbance (Dudgeon *et al.*, 2006), hence biological invasions are currently one of the most widespread and deleterious pressures on freshwater ecosystems (Leprieur *et al.*, 2009; Ricciardi & Macisaac, 2011). Understanding and quantifying the impacts invasive species have on the communities and ecosystems they invade is crucial for effectively targeting the limited resources available for management and control (Parker *et al.*, 1999; Keller *et al.*, 2011). As the mechanisms by which invaders influence ecosystems are frequently complex and vary greatly between species, assessing invader impacts is often difficult (Ruiz *et al.*, 1999; Simberloff *et al.*, 2013). Further, the accelerating rate of biological invasions means many ecosystems already support several sympatric invasive species (Simberloff & Von Holle, 1999; Jackson & Grey, 2013). The potentially complex interactions between invaders can mediate ecological outcomes, for example, by facilitating subsequent establishment of introduced species in an 'invasion meltdown' (Simberloff & Von Holle, 1999; Ricciardi, 2001), or modifying structural and functional impacts on the invaded community through additive or counteractive effects (Strayer, 2010; Preston, Henderson & Johnson, 2012; Jackson *et al.*, 2014).

Trophic interactions are a key mechanism by which invaders influence communities. Invaders can reduce the abundances of native species by direct predation and, through a variety of mechanisms, may exert a stronger predation pressure than functionally equivalent native predators (Noonburg & Byers, 2005; Salo *et al.*, 2007). Invaders may compete with native species for food resources and are often more successful competitors due to r-selected traits such as boldness, faster growth rate and higher fecundity (Williamson & Fitter, 1996; Karatayev *et al.*, 2009). As well as frequently reaching higher densities than native predators (Parker *et al.*, 2013), invaders may exert a stronger per capita effect on prey, if naïve native prey exhibit less effective predator-avoidance strategies for the exotic predator (Diamond, Ashmole & Purves, 1989) or if invasive predators capture or handle prey more effectively (Bollache *et al.*, 2008; Haddaway *et al.*, 2012; Dick *et al.*, 2013). Due to the often complex nature of freshwater food webs, with high connectance (Polis & Strong, 1996; Woodward *et al.*, 2005), predation by invaders at one trophic level can cause cascading effects. Further, where invaders that

exploit similar food resources occur in sympatry, they may switch prey or broaden their resource base (Jackson & Britton, 2014; Rothhaupt, Hanselmann & Yohannes, 2014), resulting in increased predatory pressure on these alternative prey species. To disentangle the effects of multiple invaders in an ecosystem, it is necessary to quantify impacts (e.g. predation) for each species separately, but also in combination to detect potential niche shifts and behavioural plasticity when invasive predators occur in sympatry.

The invasive North American signal crayfish (*Pacifastacus leniusculus*: Astacidae) and Chinese mitten crab (*Eriocheir sinensis*: Varunidae) are both listed within the top 100 worst invaders (Lowe *et al.*, 2000). *Pacifastacus leniusculus* has spread rapidly through Europe since its introduction for aquaculture, largely extirpating and replacing native crayfish populations through its role as a vector of *Aphanomyces astaci*, the cause of crayfish plague (Alderman, Holdich & Reeve, 1990). *Eriocheir sinensis* is native to eastern Asia, but has spread, mainly via ships' ballast, to Europe and more recently to North America (Cohen & Carlton, 1997; Dittel & Epifanio, 2009). Unlike *P. leniusculus*, which completes its lifecycle entirely within freshwater, *E. sinensis* is catadromous, whereby reproduction and larval development occurs in estuarine waters, with the main growth phase (~3 years in Europe) in freshwater (Panning, 1939; Gilbey, Attrill & Coleman, 2008). Although their life histories are quite different, *P. leniusculus* and *E. sinensis* increasingly overlap in freshwaters as they undergo range expansion. In the UK, advancement of *E. sinensis* inland has created overlap zones with *P. leniusculus*, which presently occurs in 83% of sub-catchments in England and Wales (Rogers & Watson, 2011). Further, the extent of overlap is likely to be far greater than recorded due to underreporting of *E. sinensis* occurrence (Mitten Crab Recording Project, 2013). Despite their expanding populations and increasing sympatry, no study has compared the feeding ecology of the two species, and for *E. sinensis*, we currently lack any quantitative data on feeding rates, both of which hinder reliable impact assessment (Ojaveer *et al.*, 2007). Interspecies comparison is a valuable tool for forecasting potential impacts of established invaders for which very little ecological information is available (e.g. *E. sinensis*), or indeed, those recently introduced or at high risk of future introduction, when there exist similar invaders (with likely functional equivalence) for which there are better documented impacts, in this case *P. leniusculus* (Dick *et al.*, 2013, 2014). Given the likely complexity of their trophic interactions in freshwaters, we

employed a variety of approaches to elucidate potential impacts of *P. leniusculus* and *E. sinensis* (both in allopatry and sympatry), including quantitative comparison of predatory functional response (the relationship between prey density and prey consumption by a predator) with a functionally equivalent native crayfish species.

Our study had three main aims. First, to compare the dietary preferences and feeding habits of *P. leniusculus* and *E. sinensis* using a combination of laboratory prey choice experiments, along with gut analyses and stable-isotope analysis of wild-caught specimens. Second, to quantify the predatory impact of *P. leniusculus* and *E. sinensis* on a key prey species and keystone shredder in the community using predatory functional responses (Holling, 1959; Bollache *et al.*, 2008; Dick *et al.*, 2014), determined through laboratory experiments, and compared with that of the native crayfish species *Austropotamobius pallipes* (Astacidae). Third, to examine the effects of *P. leniusculus* and *E. sinensis* on freshwater communities using a field mesocosm experiment. Mesocosms provide a more realistic representation of the natural environment than laboratory experiments, but still with a level of control and replication difficult to obtain in the field. This scaling of approaches from laboratory and mesocosm manipulation to field observations was used with a view to reduce potential bias created by the inherent limitations of each, and thereby strengthen interpretation.

## Methods

### Study species

As an omnivorous keystone consumer and ecosystem engineer, *P. leniusculus* has the potential to modify communities through trophic interactions (Nyström, Bronmark & Graneli, 1996; Crawford, Yeomans & Adams, 2006) and physical changes, for example as bioturbators modifying sediment transport and increasing turbidity (Harvey *et al.*, 2011; Johnson, Rice & Reid, 2011). There is concern that it preys on the eggs and tadpoles of amphibians (Axelsson *et al.*, 1997), and on the emerging fry of commercially important fish (Edmonds, Riley & Maxwell, 2011). In invaded systems, *P. leniusculus* causes a reduction in the biomass and species richness of macrophyte and macroinvertebrate communities (Stenroth & Nyström, 2003; Crawford *et al.*, 2006), with an accompanying shift towards predation resistant (e.g. sediment-dwelling) taxa (Nyström, 1999), although recent work in boreal lakes, suggests community

impacts may be habitat specific in some contexts (Ruokonen *et al.*, 2012).

Global concerns about *E. sinensis* derive primarily from its burrowing activities which undermine river banks and flood defences causing huge economic cost (ca. € 80 million since 1912 in Germany alone) (Gollasch, 2006), and also from impediment of commercial fishing operations due to bait interference and clogging of fishing gear (Van Der Velde *et al.*, 2000; Veldhuizen & Stansih, 1999, unpubl. data). Very little attention has been given to the potential ecological impacts of *E. sinensis* either through its role as an ecosystem engineer, or through trophic interactions. Evidence, mainly from estuarine habitats, suggests *E. sinensis* is omnivorous, exploiting a range of food sources including macrophytes, algae, detritus, aquatic invertebrates and small fish (Rudnick & Resh, 2005; Czerniejewski, Rybczyk & Wawrzyniak, 2010). Similar patterns of resource use in freshwater environments would render it likely to affect a range of trophic levels directly through consumption, and also indirectly through cascading effects.

### Collection and maintenance of animals for experiments

Given the catadromy of *E. sinensis*, its residence and therefore impacts in freshwater mostly occur during the sexually immature juvenile stage; hence, juvenile decapods were used for all laboratory and mesocosm experiments. Decapods were collected from multiple locations in the UK and combined to form a laboratory stock. *Eriocheir sinensis* were collected from the estuarine River Thames at Chiswick Eyot (51°29'13.97"N, 0°14'44.81"W) using hand search, and from the tidal limit of the river Blackwater at Beeleigh (51°44'34.31"N, 0°39'41.85"E) as by-catch within an elver monitoring trap. *Pacifastacus leniusculus* were collected from freshwater reaches of the River Pant (51°55'28.14"N, 0°31'16.59"E), and the nearby River Glem (52° 5'33.44"N, 0°41'36.69"E) using hand search. Juvenile native *A. pallipes* were collected under license (Natural England #20122661) from Adel Beck (53°51' 20.80", -1° 34' 29.91") using hand search and were returned to the collection site after study completion. *Eriocheir sinensis* ranged from 20.4 to 30.5 mm carapace width, 3.6 to 10.8 g wet mass, corresponding to sexually immature juveniles of less than 2 years age (Dittel & Epifanio, 2009). *Pacifastacus leniusculus* ranged from 19.2 to 32.7 mm carapace length, 3.4 to 10.6 g (wet mass), corresponding to sexually immature crayfish of less than 2 years (Guan & Wiles, 1999). *Austropotamobius pallipes* ranged from 25.2 to 28.3 mm carapace length, 5.1 to 6.8 g

(wet mass), corresponding to 2–3 year old juveniles (Pratten, 1980).

Decapods were maintained in aquaria (38 L) filled with dechlorinated tap water (17°C, 16 h light: 8 h dark) and fed a diet of crab pellets (Hinari) and algal wafers (King) for a minimum of 3 weeks prior to the start of experiments. Species were maintained separately, with up to eight animals per tank. Shelters (plastic pipe sections) were provided to reduce aggressive interactions and risk of injury. To ensure animals had experience of encountering all the freshwater prey types to be offered in experiments, a kick sample of macroinvertebrates collected from Meanwood Beck, UK (53°49' 51.60", -1°34' 37.19") was added to each tank weekly. Terrestrial leaf litter (20 g) as leaves of beech (~80%) and alder (~20%) soaked for over 2 months, and fresh algae (*Cladophora* sp.) (5 g) were also added each week.

Macroinvertebrates were collected from streams and ponds located within 100 km of Leeds, U.K., using a combination of kick sampling, hand searching and sweep netting, with the exception of chironomid larvae which were purchased live from a pet retailer. After collection, invertebrates were transported to the laboratory, sorted into taxa and maintained separately in aerated aquaria (8 L) until required.

The eggs of two common UK freshwater coarse fish species, roach (*Rutilus rutilus*) (Cyprinidae) and common bream (*Abramis brama*) (Cyprinidae), were collected on the day following fertilisation from a restocking facility. Eggs were retained on the spawning medium (Matala filter mat, CA, U.S.A.) in aerated water (17 °C) until use (<3 days).

#### Prey preference experiments

The prey preferences of *E. sinensis* and *P. leniusculus* were compared using four mobile prey items widespread in UK freshwaters: the amphipod *Gammarus pulex* (Gammaridae), the isopod *Asellus aquaticus* (Asellidae), the gastropod *Radix peregra* (Lymnaeidae) and chironomid larvae (Chironomidae). Size-matched juvenile *E. sinensis* and *P. leniusculus* ( $5 \pm 0.4$  g, wet mass) were isolated in individual aquaria (8 L) filled with 2 L water (50 mm depth), aerated via an air stone and maintained at 17 °C, 16 h light: 8 h dark. The sides of the aquaria were covered in black plastic to reduce stress and promote foraging. Decapods were starved for 24 h prior to the start of the experiment at which point 20 individuals of each prey type were added to each aquarium, with one prey type per corner of the tank. To reduce the possibility of total prey depletion, the experiment lasted

four hours in light conditions (Guan & Wiles, 1998; Jin *et al.*, 2001). At the end of the experiment, the remaining prey items were counted. A total of 10 replicates were carried out per treatment group (*E. sinensis* and *P. leniusculus*), along with five controls with no decapod present. Each decapod was used only once.

In a second experiment, predation by *E. sinensis* and *P. leniusculus* on the eggs of two common species of coarse fish was investigated by means of a simple pairwise choice. *Eriocheir sinensis* and *P. leniusculus* ( $9.5 \pm 1.5$  g, wet mass) were isolated and starved as before, then 50 eggs of *Rutilus rutilus* and 100 eggs of *Abramis brama* were introduced to the aquaria. Twice as many *A. brama* eggs were used because they were approximately half the size of the *R. rutilus* eggs. Due to the fragile and sticky nature of the eggs, it was not feasible to remove them from the spawning medium. Instead, this was cut into small squares (~6 cm<sup>2</sup>), ensuring the appropriate number of eggs were present on each. The experiment ran for 23 h, after which the remaining eggs were retrieved and counted. There were seven replicates per treatment group (*E. sinensis* and *P. leniusculus*), along with seven controls with no decapod present. Each decapod was used only once.

#### Predatory functional response experiments

The invasive decapods *E. sinensis* and *P. leniusculus* and the native *A. pallipes* were tested for differences in their predatory functional response towards *Gammarus pulex*, a prey item widely distributed in both lotic and lentic water bodies and a keystone shredder. Size-matched decapods ( $6 \pm 1$  g) were isolated in individual aquaria (8 L) and starved for 24 h, as previously described, before prey was added at 17:00 hours. A section of plastic pipe (50 mm diameter, 120 mm length) provided refuge. *Gammarus pulex* were size matched ( $12 \pm 1$  mm, TL) to both standardise biomass between trials and reduce cannibalism (Dick, 1995). Prey were introduced to each treatment group (*E. sinensis* and *P. leniusculus* and *A. pallipes*) at ten different densities (5, 10, 16, 20, 30, 40, 60, 80, 120 and 160). These prey densities in the experimental arena corresponded to 120, 240, 385, 480, 720, 962, 1442, 1923, 2885 and 3840 individuals m<sup>-2</sup>. There were four replicates of each density per treatment group, yielding a total of 120 trials. Each trial lasted 24 h, after which the decapod was removed and the number of intact remaining prey items counted. Controls were five replicates of each prey density in the absence of decapods to assess natural mortality and cannibalism among the prey. In between trials, the decapods were

returned to the communal aquaria (38 L) after being marked on the carapace with non-toxic correction fluid to enable identification of individuals. Trials were conducted in a randomised order with each decapod used between one and five times for different prey densities with a recovery period of at least 2 days between successive uses; it was necessary to replace animals as they grew beyond the permitted mass range. To check that reuse of animals did not affect their behaviour, a generalised mixed-effects model (GLMM) was run to identify significant predictors of the proportion of prey eaten in each trial as a function of (i) initial prey density, (ii) species and (iii) number of previous trials in which animal was used, with individual as a fixed factor and weighted to take account of the total number of trials in which an individual was used. The number of previous trials was not a significant predictor of proportion of prey eaten during the trial ( $P = 0.42$ ); the only significant predictors were species and initial prey density. The mean mass of decapods used was  $5.62 \pm 0.7$ ,  $6.14 \pm 0.7$  and  $6.10 \pm 0.7$  g ( $\pm$  SD) for *E. sinensis*, *P. leniusculus* and *A. pallipes*, respectively, and did not vary between groups (linear mixed-effect model;  $\chi^2 = 0.67$ ,  $P = 0.41$ ). Data from individuals that moulted within the 3 days following the experiment were excluded because crabs and crayfish reduce or desist from feeding prior to ecdysis (Zhou, Shirley & Kruse, 1998; Reynolds & O'Keefe, 2005).

#### Mesocosm experiment

To compare the community impacts of *E. sinensis* and *P. leniusculus*, both independently and in combination, an outdoor mesocosm experiment of 4 weeks duration was conducted in summer 2012 using circular plastic pools ( $0.78 \text{ m}^2$ ,  $0.65 \text{ m}$  depth) ( $n = 16$ ) sunken into a meadow at the University of Leeds Field Research Unit, UK. Biosecurity measures such as new fencing around the ponds were implemented following consultation with Cefas. Pools were tightly lined with polyester netting ( $0.9 \text{ mm}$  mesh), to aid recovery of invertebrates at the end of the experiment. The bases were covered with a mix of pure sand and dried loam soil (60:40) with a sporadic covering of stones ( $\sim 20$ – $30 \text{ mm}$ , long axis) and the pools filled with groundwater to a depth of  $0.5 \text{ m}$  ( $0.39 \text{ m}^3$ ). An aliquot ( $1 \text{ L}$ ) of lake water was added to each pool to seed the zooplankton community.

Macrophytes (*Ceratophyllum demersum*,  $55 \text{ g}$  wet mass; *Callitriche stagnalis*,  $15 \text{ g}$ ; *Potamogeton perfoliatus*,  $25 \text{ g}$ ) were planted in the pools 9 days before the experiment. Filamentous algae (*Cladophora* sp.,  $8 \text{ g}$ ), terrestrial leaf lit-

ter ( $100 \text{ g}$ , 80:20 beech and alder, soaked for over 2 months) and periphytic algae colonised in a large outdoor pond for 6 weeks on two ceramic tiles ( $16 \text{ cm}^2$  surface area each) in identical conditions were also added to each pool to test the effects of *P. leniusculus* and *E. sinensis* on basal resources. All macrophytes, algae, detritus and ceramic tiles were hand cleaned of invertebrates before they were added to the pools.

Macroinvertebrates representing a range of functional feeding groups were added to each pool in abundance ratios approximating those observed during collection. The community added to each pool comprised: 27 Mollusca (8 *Radix peregra*: 7 of size  $\sim 11 \text{ mm}$ , longest axis, and 1 of size  $\sim 16 \text{ mm}$ , longest axis; 2 *Lymnaea stagnalis* (Lymnaeidae):  $\sim 23 \text{ mm}$ , longest axis; 17 *Physa fontinalis* (Physidae): 12 of size  $\sim 6 \text{ mm}$ , longest axis, and 5 of size  $\sim 3.5 \text{ mm}$  longest axis); 5 Trichoptera (Limnephilidae); 56 Isopoda (*Asellus aquaticus*); 135 Amphipoda (*Gammarus pulex*), and 500 chironomid larvae. Macroinvertebrates were added 4 days prior to the start of the experiment to allow acclimation in the absence of decapod predators.

Decapods were added to the pools within three treatments: *E. sinensis*, *P. leniusculus*, and both *E. sinensis* and *P. leniusculus*, in addition to a no decapod control. Pools were assigned using a randomised block design with four replicates in each treatment group and control. Four juvenile decapods were assigned to each treatment pool, with two individuals of each species in the mixed treatment. *Eriocheir sinensis* ranged from 13 to 22 mm in carapace width ( $19.14 \pm 1.84$ ; mean  $\pm$  SD) and *P. leniusculus* ranged from 19 to 26 mm in carapace length ( $23.11 \pm 1.20$ ; mean  $\pm$  SD). Sex ratios were 50 : 50 in all pools. Total decapod biomass ranged from 19.5 to 22.9 g across all treatment pools and did not vary between treatments ( $F_{2,9} = 0.75$ ,  $P = 0.50$ ). Eight sections of PVC pipe ( $2 \times 50 \text{ mm}$  diameter,  $120 \text{ mm}$  length;  $6 \times 25 \text{ mm}$  diameter,  $80 \text{ mm}$  length) were added as refugia. After addition of the decapods, the lining nets were closed using cable ties and pools were covered with Enviromesh<sup>R</sup> (Agralan, Swindon, U.K.) secured with shock cord to prevent animals escaping and disturbance by birds.

Pools were checked after 2 weeks for decapod mortalities and evidence of moult; moults were removed if found. Midday water temperature ranged from  $17.2$  to  $18.9 \text{ }^\circ\text{C}$  and did not vary between treatments (ANOVA:  $F_{3,12} = 0.73$ ,  $P = 0.55$ ). Sub-surface water samples collected at the end of the experiment for chemical analysis showed no difference between treatments for the main parameters (ANOVA: Nitrate  $F_{3,12} = 1.47$ ,  $P = 0.27$ ;

Phosphate  $F_{3,12} = 0.56$ ,  $P = 0.65$ ; Sulphate  $F_{3,12} = 1.49$ ,  $P = 0.27$  and Calcium  $F_{3,12} = 1.33$ ,  $P = 0.31$ ).

At the end of the experiment, the decapods were collected, the ceramic tiles were frozen and the net linings transported to the laboratory in plastic bags for processing. Macroinvertebrates, algae, terrestrial leaf and macrophyte fragments were carefully recovered using a net (1 mm mesh size) and sorted. Macroinvertebrates were counted and macrophytes, algae and leaf fragments (exceeding ~4 mm) were hand cleaned of macroinvertebrates, blotted dry and weighed. Total chlorophyll was used as a proxy measure for the remaining biomass of periphytic algae on the ceramic tiles. Each tile was soaked overnight in 90% ethanol, then, extractants were centrifuged at  $4 \times 10^4$  rpm for 20 min and analysed using a spectrophotometer (Biochrom WPA Biowave II) to measure absorbance at 750 nm, 664 nm, 647 nm and 630 nm wavelengths (1 cm path length). Total chlorophyll ( $\mu\text{g}$ ) per tile was calculated as the sum of chlorophyll-a and b (Huang & Cong, 2007).

#### Stable-isotope and gut-content analysis of wild-caught specimens

Wild *E. sinensis* and *P. leniusculus* were collected from two sites on the River Stour, Suffolk, U.K., during October and November 2012. The two species have been sympatric in this lowland watercourse for at least 10 years, and have been observed at locations within 22 km (Adam Piper, Environment Agency, pers. comm.). *Eriocheir sinensis* were collected immediately upstream of the tidal limit (51°57'17.59"N, 1°1'32.31"E) and *P. leniusculus* were collected 62 km further upstream (52°3'31.55" N, 0°29'32.58"E). Sites exhibited similar channel morphology (9 m to 15 m width) with macrophyte communities dominated by *Sparganium* spp., *Phalaris arundinacea* and *Nuphar lutea*, with overhanging *Salix* and *Alnus* spp.

Baited fladen traps were deployed at both sites and checked daily. The bait (detrital leaves, chironomid larvae and sardine in oil) was encased within a nylon mesh (1 mm) and metal mesh box (5 cm<sup>2</sup>) to ensure that animals could not consume it. Captured decapods were immediately frozen. Collections of potential diet items were made at both sites during the same period using a combination of kick sampling, dredge trawling and hand collection. All macroinvertebrates (*Lymnaea* sp., *Theodoxus fluviatilis*, chironomid larvae, Ephemeroptera, *Gammarus pulex*, *Asellus aquaticus*, Limnephilidae, Notonectidae and Odonata) were maintained live in dis-

tilled water for 24 h to clear their gut contents before being frozen. Plant material (*Elodea canadensis*, *Nuphar lutea*, *Cladophora* sp., *Phalaris arundinacea*, decaying *Sparganium erectum*, *Rorippa nasturtium-aquaticum*, *Myosotis scorpioides* and assorted terrestrial detritus) was carefully rinsed in distilled water prior to freezing. Three small fish (*Perca fluviatilis*, *Rutilus rutilus* and *Gasterosteus aculeatus*) found dead in the trap netting and a juvenile *Gobio gobio* accidentally killed during a dredge trawl were filleted to isolate the muscle tissue before freezing.

Only adult decapods were captured during the sampling. Claw muscle tissue from *E. sinensis* ( $n = 5$ ) (54 to 87 mm carapace width) and *P. leniusculus* ( $n = 4$ ) (37–46 mm carapace length) was extracted, freeze dried, weighed and analysed for stable isotope ratios (<sup>13</sup>C:<sup>12</sup>C and <sup>15</sup>N:<sup>14</sup>N) expressed as  $\delta$  values (‰). In addition to the decapods, samples of 14 and 17 potential diet items were analysed from the *E. sinensis* and *P. leniusculus* collection sites respectively. All macroinvertebrates were separated into genera, freeze dried and combusted whole, with the exception of gastropods for which only the muscle tissue of the foot was used. Fish muscle was freeze dried and weighed. All plant material was freeze dried, then immersed in liquid nitrogen and ground to a fine homogenous powder using a pestle and mortar before weighing. Due to restrictions on the number of samples that could be analysed and to ensure sufficient mass of material, composite samples were used for the following animal groups: chironomids (10–23 individuals), *Asellus aquaticus* (2 individuals), Notonectidae (2–3 individuals), *Theodoxus fluviatilis* (4 individuals) and Odonata (2 individuals). All plant samples comprised a minimum of three leaves/stems.

Samples were analysed at the University of Leeds using an Isoprime continuous flow mass spectrometer coupled to an Elementar Pyrocube elemental analyser. Standards of ammonium sulphate USGS-25 (−30.1‰) and USGS-26 (+53.7‰) for Nitrogen; and ANU-sucrose (−10.47‰) and IAEA-CH-7 (polyethylene film, −31.83‰) for carbon, were interspersed every 8–12 samples to calibrate the system and compensate for drift. Stable isotope ratios are expressed in conventional notation as parts per thousand (‰) using delta notation ( $\delta$ ), relative to international standards (Pee Dee Belemnite for carbon and atmospheric nitrogen). Analytical precision on both isotope measurements was 0.2‰ or better.

Foregut contents of *E. sinensis* ( $n = 5$ ) (37–46 mm CW) and *P. leniusculus* ( $n = 10$ ) (37–52 mm CL) were examined under a dissecting microscope using a gridded Petri dish with 24 squares (25 mm<sup>2</sup>) sub-divided into smaller squares (1 mm<sup>2</sup>). First, the number of small

squares (1 mm<sup>2</sup>) with material present was recorded as a percentage of each larger square (25 mm<sup>2</sup>). Second, the material in each small square was assigned to one of seven categories: inorganic; algae; macrophyte; moss; leaves (terrestrial); unidentifiable plant matter and macroinvertebrates. Where possible, the macroinvertebrate fragments were identified to order, and occasionally genera.

#### Data analysis

All statistical analyses were run in R (version 3.0.0, R Core Team 2013) and all mean values are quoted  $\pm$  standard error (SE). The numbers of prey items remaining in the treatment groups in both the prey preference and egg predation experiments were corrected for the mean reduction recorded during control trials, then the mean total number of prey items (all prey types combined) consumed compared between treatments using a *t*-test. Selection indices  $w_i$  were subsequently quantified for each prey type (Manly, McDonald & Thomas, 1993):

$$w_i = \frac{c_i}{a_i}$$

where  $c_i$  is the proportion of prey  $i$  consumed (corrected for reduction during controls) and  $a_i$  is proportion of prey  $i$  available (corrected for reduction during controls). Indices were standardised by dividing each index by the sum of the four indices then arcsine square root transformed (Rehage, Barnett & Sih, 2005). *T*-tests were used to compare the mean indices for each prey type between treatments for both experiments, and compare indices between prey types in the egg predation experiment. Mann–Whitney *U*-tests were used where data could not be normalised. Kruskal–Wallis with *post hoc* Nemenyi–Damico–Wolfe–Dunn tests were used to compare prey type indices within each treatment for the prey preference experiment.

Differences in decapod mass across species groups in the functional response experiment were tested using linear mixed-effects models (LMEs) with identification number as a random factor because individuals were used multiple times across densities. A chi-square test was used to test for a significant difference in log likelihoods between models with and without species as an explanatory variable. Logistic regression of the proportion of prey consumed against initial prey density indicated that all three decapods exhibited a Type II functional response whereby consumption rate decelerates with increasing prey density (Murdoch, 1973). Therefore, functional response data for each species

were modelled using Rogers random predator equation (Rogers, 1972), modified with the Lambert *W* function, to obtain coefficients of  $a$  (attack rate) and  $h$  (handling time):

$$N = N_0 - \left( \frac{W(ahN_0e)^{-a(-hN_0)}}{ah} \right)$$

where  $N$  is the number of prey eaten,  $N_0$  is the number of prey supplied,  $a$  is attack rate,  $h$  is handling time and  $W$  is the Lambert *W* function (Bolker, 2008). This model accounts for decreasing prey density during the trial as prey were not replaced. Data were bootstrapped ( $n = 2000$ ) and 95% confidence intervals for  $a$  and  $h$  calculated within the 'frair' package (Pritchard, 2014).

Mesocosm data on the remaining biomass of macrophytes, chlorophyll concentration (averaged from 2 tiles) and absolute abundances of macroinvertebrate taxa were tested for normality using Shapiro–Wilk test and were log 10 + 1 transformed where necessary. Levene's test was used to determine compliance with the assumption of homogeneity of variance between groups. One-way ANOVA with treatment as a factor and Tukey's HSD *post hoc* test was used to detect and identify differences between treatment groups. Kruskal–Wallis with *post hoc* Nemenyi–Damico–Wolfe–Dunn tests were used where data could not be normalised. A between groups test was not conducted for *Lymnaea* snails as there were too few individuals. Shannon diversity and evenness indices were calculated for each pool and compared among treatments using one-way ANOVA. Percentage change in the biomass/abundance of each taxon was calculated using the final value minus the initial value, as a percentage of the initial value.

Four *E. sinensis* individuals moulted during the course of the study. Two *P. leniusculus* individuals in separate pools in the *P. leniusculus* treatment were missing at the end of the experiment. There was no evidence that the animals had climbed out of the tanks or broken through the netting, so it was assumed that they had died (perhaps during moult) and been consumed by the other decapods and detritivores. It was decided not to exclude these pools from the dataset because checks on day 14 revealed that all decapods were still present, hence pools had their intended decapod biomass for at least half the duration of the experiment. Further, preliminary analyses of the data revealed that macroinvertebrate abundance (all species) and macrophyte biomass (all species) of the two pools in question did not differ significantly from other pools within the same treatment.

Volumetric proportions of food types from gut-contents analysis were arcsine root transformed and compared between decapod species using independent samples t-tests. Delta values for stable isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$  measured in field samples were also compared between decapod species and between functional groups across the two collection sites using independent samples t-tests. Bayesian stable-isotope mixing models (SIAR; Parnell *et al.*, 2008) were used to estimate the relative contributions of the potential food sources sampled to the diet of *E. sinensis* and *P. leniusculus*. Assumed fractionation factors of  $2.4 \pm 0.18$  ‰ for  $\delta^{15}\text{N}$  and  $0.5 \pm 0.17$  ‰ for  $\delta^{13}\text{C}$ , based on a meta-analysis of studies using non-acidified samples (McCutchan *et al.*, 2003), were used to adjust the isotopic values of food sources. Elemental concentrations of C and N within each of the food sources were also incorporated in the model to account for concentration-dependent variation in fractionation (Phillips & Koch, 2002).

## Results

### Prey preference and egg predation

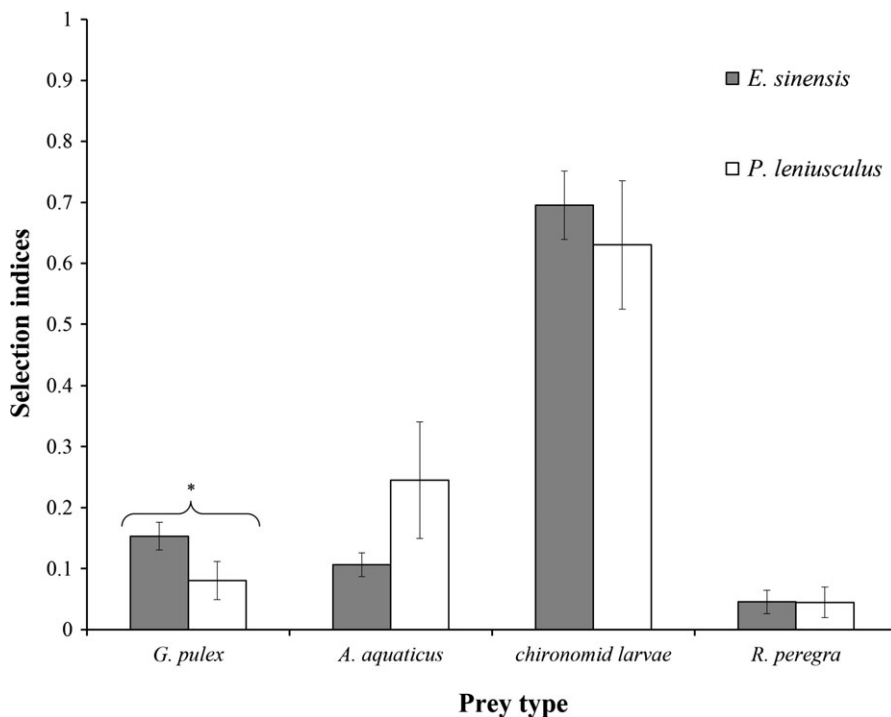
Overall, *E. sinensis* consumed more prey items per trial than *P. leniusculus* (mean  $26.04 \pm 2.86$  and  $16.48 \pm 2.61$  respectively) ( $t_{18} = 2.5$ ,  $P = 0.02$ ) and all four prey types were consumed by the decapods to some extent.

Consumption in the control (by *G. pulex* and/or *A. aquaticus*) was less than half the consumption in the presence of the decapods ( $t_{23} = 4.4$ ,  $P < 0.01$ ), although there was a considerable reduction in chironomid larvae in the control (mean 14 prey items).

Comparison of selection indices between the decapod predators indicated a greater preference for *G. pulex* among *E. sinensis* compared to *P. leniusculus* ( $t_{18} = 3.22$ ,  $P < 0.01$ ) (Fig. 1). There was no difference between predators for the other three prey types (Mann–Whitney *U*-tests,  $U = 0.33$ ,  $P = 0.35$ ;  $U = 0.53$ ,  $P = 0.63$ ;  $U = 0.69$ ,  $P = 0.53$  for *A. aquaticus*, chironomid larvae and *R. peregra* respectively). Chironomid larvae were the most preferred prey type of both *E. sinensis* ( $H_{3,40} = 28.4$ ,  $P < 0.01$ ) and *P. leniusculus* ( $H_{3,40} = 15.2$ ,  $P < 0.01$ ). Both invasive decapods preyed heavily on the fish eggs relative to the control (Mann–Whitney *U*-test,  $U = 3.33$ ,  $P < 0.01$ ), eating the majority offered (60 to 100% across all trials). There was no difference in overall consumption between decapod species ( $t_{12} = 0.30$ ,  $P = 0.77$ ), but there was a preference for *R. rutilus* eggs among both *E. sinensis* and *P. leniusculus* ( $t_{12} = 20.63$ ,  $P < 0.01$  and  $t_{12} = 4.17$ ,  $P < 0.01$  respectively).

### Predatory functional response

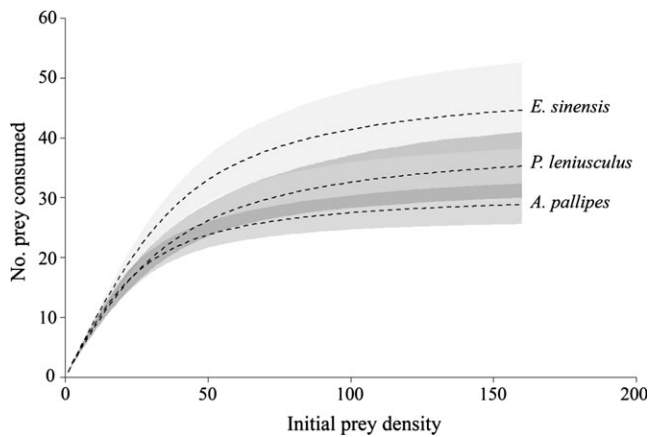
The maximum predatory functional response of *E. sinensis* (44 prey items) was 57% higher than that of the



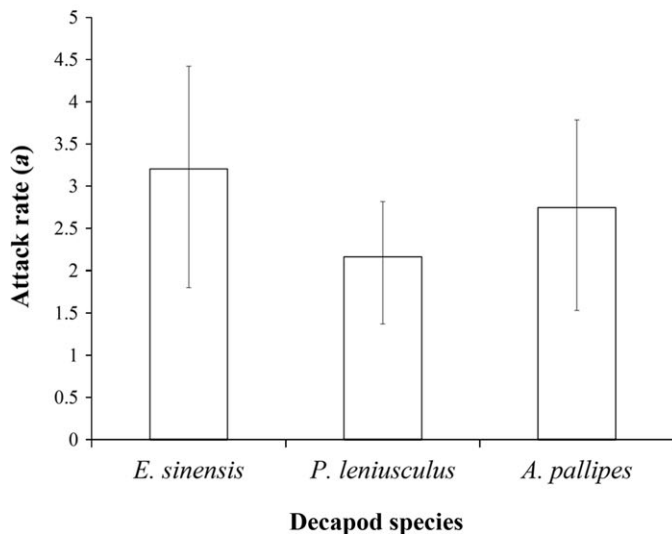
**Fig. 1** Prey selection indices (mean  $\pm$  SE) during trials ( $n = 10$ ) in which predators and *Eriocheir sinensis* and *Pacifastacus leniusculus* were offered four prey items simultaneously: *Gammarus pulex* (amphipod), *Asellus aquaticus* (isopod), chironomid larvae and *Radix peregra* (gastropod). Data corrected for mean prey reduction during control trials ( $n = 5$ ) when no decapod was present. \*denotes significant difference at 0.05 level.



native *A. pallipes* (28 prey items). *Pacifastacus leniusculus* had an intermediate maximum between *E. sinensis* and *A. pallipes* of 35 prey items. The functional response curve of *E. sinensis* was significantly higher than *A. pallipes*, whereas the upper 95% confidence interval of the *P. leniusculus* curve overlapped with the lower 95% confidence interval of the *E. sinensis* curve indicating no significant difference between the two species (Fig. 2). The lower 95% confidence interval also overlapped with the upper *A. pallipes* confidence interval, similarly indicating no difference in consumption between the species



**Fig. 2** Fitted functional response curves (dashed lines) for three decapod predators *Eriocheir sinensis*, *Pacifastacus leniusculus* and *Austropotamobius pallipes* preying upon *Gammarus pulex*. Shaded polygons indicate empirical 95% confidence intervals generated by bootstrapping ( $n = 2000$ ).

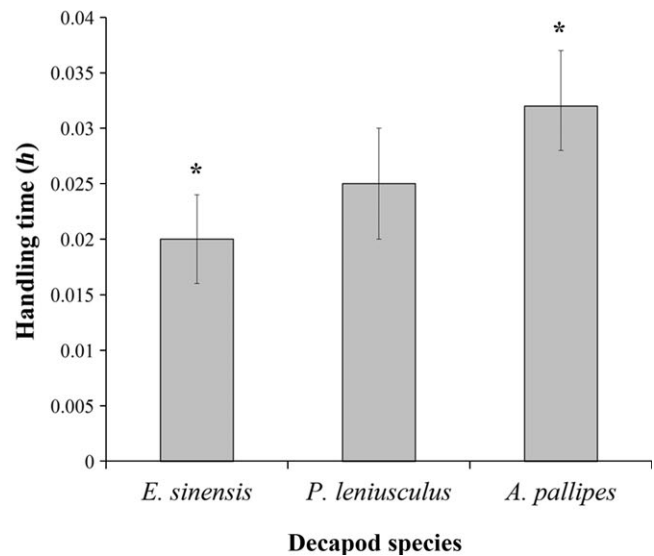


(Fig. 2). Attack rate did not vary between decapod species, but handling time did ( $P < 0.05$ ). *Eriocheir sinensis* exhibited a faster handling time than *A. pallipes*, but handling time by *P. leniusculus* did not differ from the other two decapod species (Fig. 3).

*Outdoor mesocosm experiment*

**Basal resources.** The mass of terrestrial leaf litter was reduced by on average 43% ( $\pm 3.9$ ) in the decapod treatments and 35% ( $\pm 2.6$ ) in the control; there was no significant difference in detrital mass among treatments at the end of the experiment (Table 1). Change in biomass of filamentous algae (*Cladophora* sp.) varied greatly between individual pools, with no significant difference between treatments (Table 1). Periphyton chlorophyll at the end of the experiment was on average 48% higher in the combined *E. sinensis* and *P. leniusculus* treatment relative to control, but did not differ between other treatments (Fig. 4, Table 1). There was a general reduction in the biomass of all three macrophyte species during the experiment. *Callitriche stagnalis* was absent from all except four pools (which were within a range of treatments), and so was omitted from further analyses. Remaining macrophyte biomass did not vary between treatments (Table 1).

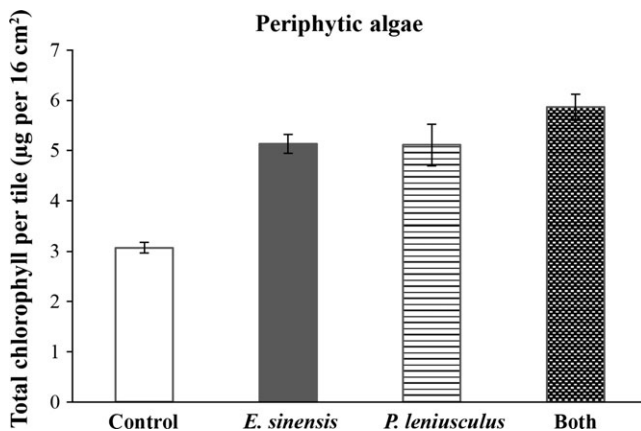
**Shredders.** The abundance of *G. pulex* was significantly reduced in both treatments containing *E. sinensis*, relative to the control, but not in the *P. leniusculus* only



**Fig. 3** Functional response attack rates ( $a$ ) and handling times ( $h$ ) for three decapod predators *Eriocheir sinensis*, *Pacifastacus leniusculus* and *Austropotamobius pallipes* preying upon *Gammarus pulex*. Error bars indicate empirical 95% confidence intervals generated by bootstrapping ( $n = 2000$ ). \*denotes difference at 0.05 significance level.

**Table 1** Biomass of macrophytes, mass of detritus and abundances of invertebrate taxa remaining in pools ( $n = 16$ ) after four weeks compared across treatments: *Eriocheir sinensis*, *Pacifastacus leniusculus*, both *Eriocheir sinensis* and *Pacifastacus leniusculus*, and no decapod control using one-way ANOVA and Tukey's HSD *post-hoc* tests (or Kruskal–Wallis and Nemenyi–Damico–Wolfe–Dunn *post hoc* tests where data could not be normalised). Bold values indicate difference at 0.05 significance level.

	Treatment effect test statistic		Pairwise comparisons					
			<i>E. sinensis</i> versus control	<i>P. leniusculus</i> versus control	Both versus control	<i>E. sinensis</i> versus <i>P. leniusculus</i>	<i>E. sinensis</i> versus both	<i>P. leniusculus</i> versus both
	F/H <sub>3,12</sub>	P	P	P	P	P	P	P
<b>Basal resources</b>								
<i>Potamogeton perfoliatus</i>	0.845	0.495	–	–	–	–	–	–
<i>Ceratophyllum demersum</i>	2.424	0.116	–	–	–	–	–	–
<i>Cladophora sp.</i>	1.675	0.225	–	–	–	–	–	–
Terrestrial leaf litter	0.857	0.49	–	–	–	–	–	–
Periphyton	4.251	<b>0.017</b>	0.075	0.080	<b>0.013</b>	0.999	0.768	0.751
<b>Grazers</b>								
All	8.571	<b>0.003</b>	<b>0.002</b>	<b>0.042</b>	<b>0.017</b>	0.317	0.603	0.946
<i>Radix peregra</i>	12.91(H)	<b>0.005</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.014</b>	1.000	0.973	0.973
<i>Physa fontinalis</i>	4.370	<b>0.027</b>	<b>0.022</b>	0.259	0.081	0.471	0.873	0.880
<i>Lymnaea stagnalis</i>	n/a							
<b>Shredders</b>								
<i>Gammarus pulex</i>	13.560	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.075	<b>0.006</b>	<b>0.021</b>	0.220	0.518
<i>Asellus aquaticus</i>	28.09	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.733	0.583	0.993
Trichoptera <i>sp.</i>	11.76(H)	<b>0.008</b>	<b>0.009</b>	0.454	<b>0.004</b>	0.571	0.997	0.401
<b>Filterers/collectors</b>								
Chironomid larvae	8.99(H)	<b>0.029</b>	<b>0.043</b>	<b>0.015</b>	<b>0.046</b>	0.992	0.979	0.909



**Fig. 4** Periphyton biomass (mean  $\pm$  SE) on tiles after four weeks in mesocosm pools ( $n = 16$ ) within the treatments: *Eriocheir sinensis*, *Pacifastacus leniusculus*, both *Eriocheir sinensis* and *Pacifastacus leniusculus*, and no decapod control.

treatment relative to control (Table 1). Conversely, the other shredders, *Asellus aquaticus* and larvae of the order Trichoptera (*Limnephilid sp.*) were significantly reduced in all the decapod treatments relative to the control (Fig. 5, Table 1).

**Grazers and collectors.** The abundance of Gastropoda increased in the controls during the experiment, but was

significantly reduced in all the decapod treatments with no differences among them (Table 1, Fig. 5). The abundance of chironomid larvae was similarly significantly reduced in all decapod treatments relative to the control, but did not vary among the three decapod treatments (Table 1).

The Shannon diversity index in each pool at the end of the experiment ranged from 0.44 to 1.44 and did not vary between treatments ( $F_{3,12} = 1.43$ ,  $P = 0.283$ ). Shannon evenness ranged from 0.28 to 0.86 and also did not vary between treatments ( $F_{3,12} = 0.28$ ,  $P = 0.839$ ).

#### Stable-isotope analysis

*Eriocheir sinensis* had a significantly lower  $\delta^{13}\text{C}$  value than *P. leniusculus* with a mean of  $-29.90 \pm 0.21$  ‰ compared to  $-28.9 \pm 0.17$  ‰ ( $t_{6,3} = 3.85$ ,  $P < 0.01$ ). Mean  $\delta^{15}\text{N}$  values were  $17.04 \pm 0.41$  ‰ and  $17.40 \pm 0.25$  ‰ for *E. sinensis* and *P. leniusculus*, respectively, and did not vary between species ( $t_{6,3} = 0.76$ ,  $P = 0.48$ ). With regard to potential food sources, some species sampled varied between the two collection sites but isotope signatures of functional groups were similar, with the exception of Gastropoda for which  $\delta^{13}\text{C}$  of the single composite sample collected at Flatford was shifted ( $-38.9$  ‰) compared to the three samples collected at Wixoe ( $-30.0$  to  $31.8$  ‰) (Fig. 6).

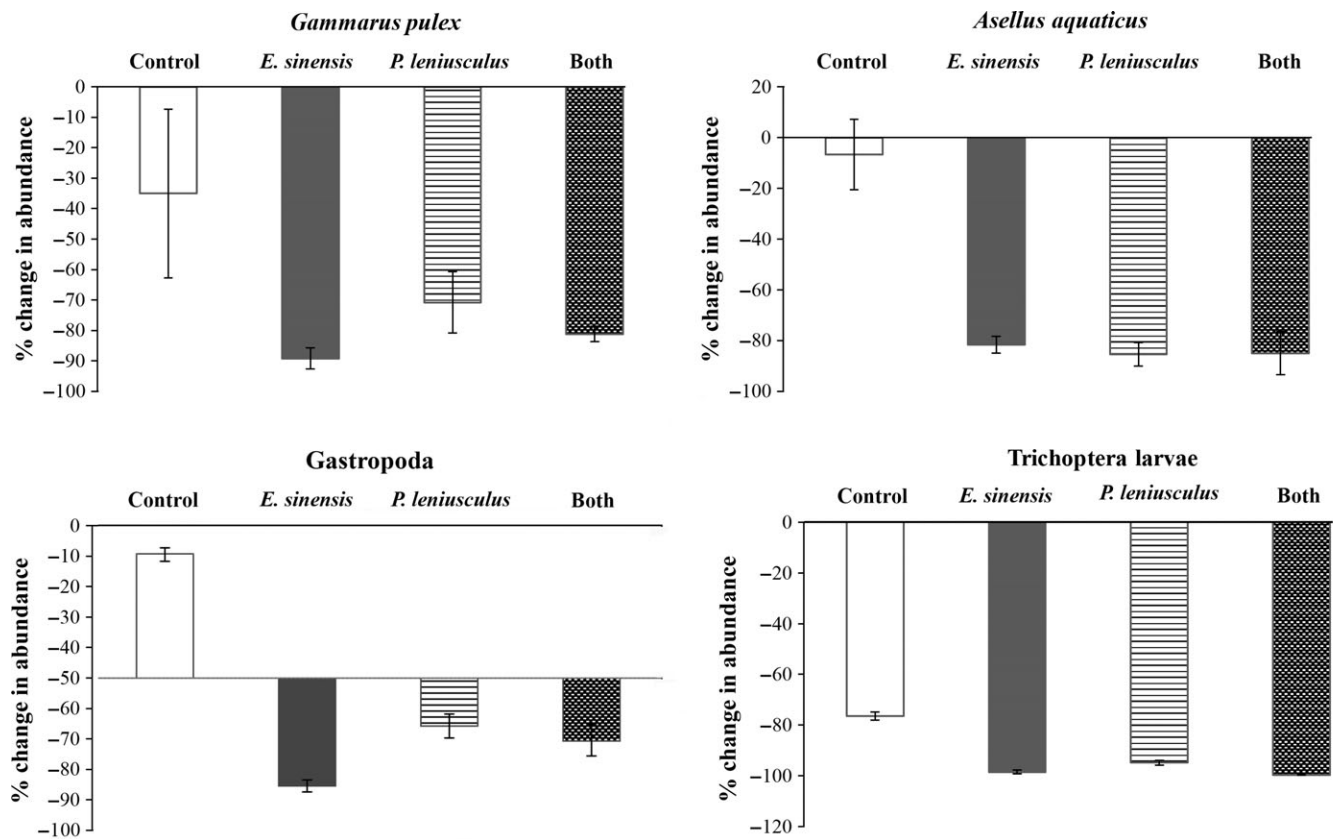


Fig. 5 Percentage change (mean  $\pm$  SE) in abundance of *Gammarus pulex*, *Asellus aquaticus*, Gastropoda and Trichoptera larvae after four weeks in mesocosm pools ( $n = 16$ ) within the treatments: *Eriocheir sinensis*, *Pacifastacus leniusculus*, both *Eriocheir sinensis* and *Pacifastacus leniusculus*, and no decapod control.

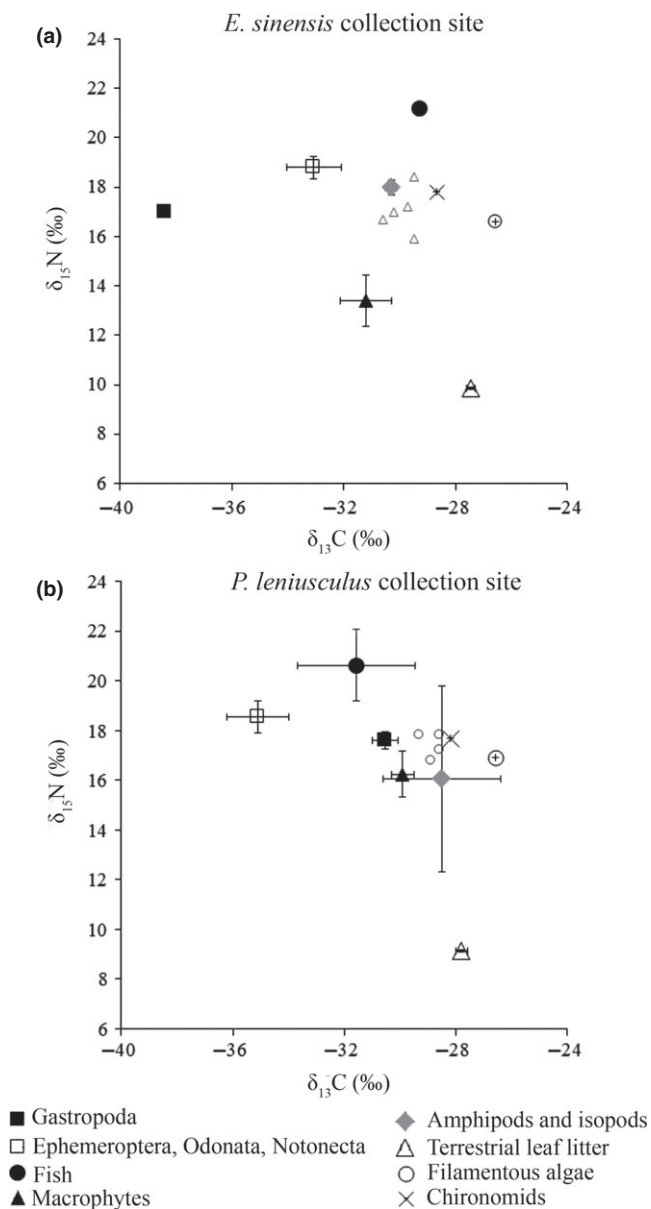
*Eriocheir sinensis* were more  $^{15}\text{N}$ -enriched than chironomids, amphipods and isopods ( $t_{4.4} = 2.85$ ,  $P = 0.04$ ) and Gastropoda, and  $^{15}\text{N}$ -depleted relative to fish, thereby occupying the same trophic level as the Ephemeroptera, Odonata and Notonecta, which were similarly  $^{15}\text{N}$ -enriched ( $t_{4.9} = 1.05$ ,  $P = 0.34$ ) (Fig. 6). Isotopic signatures indicated a similar trophic position for *P. leniusculus* at the second collection site (Fig. 6). Equivalent basal resources did not differ between the two collection sites in their  $\delta^{13}\text{C}$  values (macrophytes:  $t = 1.31$ ,  $P = 0.24$ , 5.4 d.f.; terrestrial leaf litter:  $t = 1.4$ , d.f. = 1.86,  $P = 0.31$ ). For filamentous algae, the one composite sample differed marginally between sites, though the difference (0.02‰ for  $\delta^{13}\text{C}$ ) was less than analytical precision. In both sites,  $\delta^{13}\text{C}$  values of the decapods were intermediate between those of macrophytes and terrestrial leaf litter; however, *E. sinensis* was shifted towards macrophytes and *P. leniusculus* towards terrestrial detritus and filamentous algae, indicating differences in the basal energy sources used by these invaders.

Concentration-dependent mixing models estimated that basal resources comprised the majority of the

diet of both decapods. *Eriocheir sinensis* relied most on macrophytes followed by terrestrial leaf litter, whereas *P. leniusculus* was most dependent on terrestrial leaf litter and filamentous algae (Fig. 7). Estimated contributions of the remaining potential diet items sampled were broadly similar between the two decapod species, though chironomids appeared slightly more important for *P. leniusculus* than *E. sinensis* (Fig. 7).

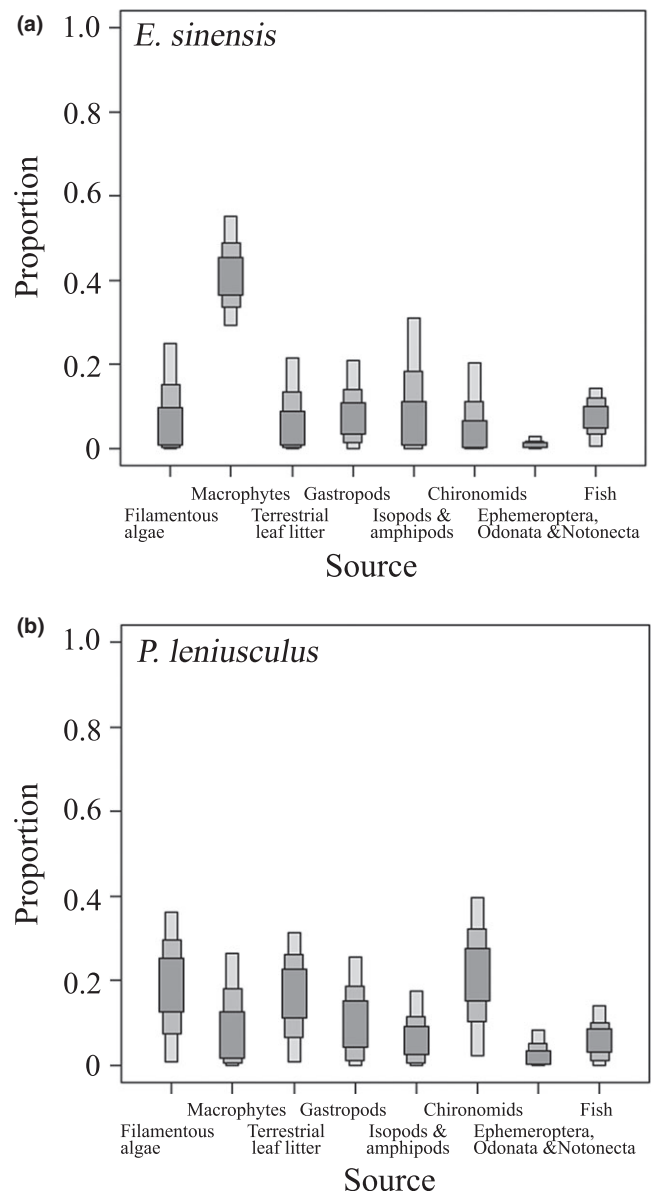
#### Gut-content analysis

Invertebrate material comprised the largest proportion of *E. sinensis* gut contents ( $n = 5$ ) 21.1% ( $\pm 1.53$ ), followed by macrophytes (16.7  $\pm$  2.7%) and algae (10.7  $\pm$  1.7%). Terrestrial detritus was the least detected category, comprising on average 6.5  $\pm$  1.04%. The invertebrate taxa detected in *E. sinensis* gut contents included Trichoptera, Coleoptera, Ephemeroptera and Gastropoda, with Trichoptera the most commonly encountered. In contrast to *E. sinensis*, the gut contents of *P. leniusculus* ( $n = 10$ ) was significantly more



**Fig. 6** Stable-isotope biplots for (a) *Eriocheir sinensis* (triangles) ( $n = 5$ ) and (b) *Pacifastacus leniusculus* (open circles) ( $n = 4$ ), and potential food sources ( $n = 1$  to 5) in the lower River Stour, U.K. Where more than one source or composite sample was analysed, signatures denote mean values ( $\pm 1$  SE) adjusted for trophic enrichment factors.

dominated by terrestrial leaf litter ( $38.2\% \pm 2.7$ ) ( $t_{13} = 9.04$ ,  $P < 0.01$ ). Algae was second most common ( $14.3\% \pm 1.3$ ) and did not differ between decapods ( $t_{13} = 1.85$ ,  $P = 0.087$ ). Invertebrates were found in lower proportion in *P. leniusculus* ( $12.9\% \pm 1.4$ ) than *E. sinensis* ( $t_{13} = 3.39$ ,  $P < 0.01$ ), and included the taxa Gastropoda, Isopoda and Trichoptera. Fish scales were also detected in three *P. leniusculus* individuals.



**Fig. 7** Results of SIAR (95, 75 and 50% credibility intervals) showing estimated contribution of each potential food source to the diet of a) *Eriocheir sinensis* and b) *Pacifastacus leniusculus*.

### Discussion

Using multiple approaches at a range of scales, our study identified differences in the trophic interactions and potential impacts of two omnivorous decapods which are rapidly expanding their invasive freshwater ranges. Quantitative comparison of predatory functional response showed a higher per capita impact of *E. sinensis* than native *A. pallipes* on a keystone shredder. Preference of *E. sinensis* for this prey item was supported by prey choice trials. Community impacts of the decapods

investigated through a mesocosm experiment were broadly similar with marked declines in all animal taxa; however, there was again greater impact on the shredder *G. pulex* by *E. sinensis* compared to the invasive crayfish. Presence of the decapods in sympatry caused a trophic cascade resulting in elevated periphyton levels.

*Eriocheir sinensis* and *P. leniusculus* both appeared to operate as 'generalist omnivores' consuming a wide variety of food items, which accords with previous studies (Guan & Wiles, 1998; Rudnick & Resh, 2005; Stenroth *et al.*, 2006; Czerniejewski *et al.*, 2010), although clear preferences for certain prey types were detected. The selectivity hierarchy of both decapods generally reflected a decline in preference that may reflect ease of capture and handling, with chironomids most preferred and the gastropod least preferred. Prey used in laboratory experiments and field mesocosms were from sites where crayfish and *E. sinensis* had not yet been detected and were therefore presumed naïve to these predators. Prey may exhibit diverse and often complex predator-avoidance behaviours (Covich *et al.*, 1994; Cotton, Rundle & Smith, 2004; Sih *et al.*, 2010), which may be evolved over time and passed to successive generations genetically, or reflect localised behavioural plasticity (Alvarez & Nicleza, 2003); therefore, use of naïve prey may have led to overestimation of predation rates in this study.

Both *E. sinensis* and *P. leniusculus* preyed heavily upon the eggs of coarse fish, with apparent preference for *R. rutilus*, perhaps reflecting greater ease of handling of these larger eggs. In several trials, decapods consumed all the eggs that were accessible to them, i.e. not deeply embedded in the spawning medium, suggesting that they would have consumed more than they had been provided. Only fish eggs were available in this trial so we cannot assess the preference for fish eggs relative to other items, however our results do support previous suggestions that eggs are likely to be present in the diet of both *E. sinensis* (Culver, 2005, unpubl. data; Morrith *et al.*, 2013) and *P. leniusculus* (Edmonds *et al.*, 2011). These data suggest that invasion by *P. leniusculus* and *E. sinensis* may impact recruitment of these common fish species which spawn on vegetation in the mid to lower reaches of rivers, where both *E. sinensis* and *P. leniusculus* reach their highest densities in freshwaters (Rudnick *et al.*, 2003; Weinlaender & Fuereder, 2009).

Invasive *E. sinensis* displayed a 57% higher per capita consumption rate on a keystone freshwater shredder, *G. pulex*, compared to the native crayfish *A. pallipes*. There was also a non-significant trend suggesting that *E. sinensis* may also be a stronger predator than *P. leniusculus* for this prey type, supported by prey choice

trials in which *E. sinensis* consumed more prey items overall and displayed a greater preference for *G. pulex* than did *P. leniusculus*. Further, in the mesocosm experiment, the abundance of this prey was reduced more in the presence of *E. sinensis* than *P. leniusculus*. A stronger predatory functional response among invaders compared to natives has been demonstrated previously for crayfish (Haddaway *et al.*, 2012); amphipods (Bollache *et al.*, 2008), and gambusias (Rehage *et al.*, 2005). Haddaway *et al.* (2012) showed that *P. leniusculus* preyed at a 10% higher rate than *A. pallipes*, and although this trend was also observed in this study with the same species, no clear species difference was apparent due to high intraspecific variability. From the higher predation rate of *E. sinensis* relative to the native decapod, mediated through a faster ability to handle this prey item, we infer that *E. sinensis* is likely to negatively impact native prey species as it invades.

Our ability to control for predator density afforded by the mesocosm and laboratory approaches enabled measurement of per capita effects on native prey. This is one of the three key elements considered important for prediction of invader impact, along with area invaded and abundance (Parker *et al.*, 1999; Dick *et al.*, 2014). There is evidence that *P. leniusculus* reaches higher densities (10–15 individuals  $m^{-2}$  in a U.K. lowland river, Guan & Wiles, 1996; 26–39 individuals  $m^{-2}$  in a U.K. stream, Peay *et al.*, 2014) than the native *A. pallipes* (2–4 individuals  $m^{-2}$  in a stream in France, Grandjean *et al.*, 2000; 5.3  $m^{-2}$  in a U.K. river, Pearson, unpubl. data), although direct comparison of densities in similar habitat are understandably lacking due to the spread of crayfish plague and generally rapid replacement of *A. pallipes* by *P. leniusculus* where they co-occur. There is generally a dearth of knowledge on population densities and the long-term dynamics of *E. sinensis* in invaded areas, particularly in its freshwater range. In one comprehensive study over 6 years, mean crab abundance increased to an estimated 30 individuals  $m^{-2}$  in 1999 in the southerly freshwater tributaries of San Francisco Bay, before a decline to 21.1 individuals  $m^{-2}$  in 2000 (Rudnick *et al.*, 2003). In the upper tidal zone of the River Thames, abundances ranged from 0.6 to 2.25 juvenile crabs  $m^{-2}$  of boulder habitat (Gilbey *et al.*, 2008). The higher per capita consumption by *E. sinensis* observed in our functional response experiment, combined with higher densities of both invasive species in the wild, suggest that impacts on native prey abundances are likely to be higher in invaded areas than in the presence of the native decapod alone. Further data concerning the population densities of both decapods in

their invasive range is necessary to build on our quantitative per capita consumption rates to inform predictions of invader impacts in the wild.

In the simplified mesocosm communities, the effects of decapod presence were generally similar for *E. sinensis* and *P. leniusculus*, with a strong decline in the abundances of all animal taxa. The stronger impact of *E. sinensis* than *P. leniusculus* on *G. pulex* is consistent with its demonstrated preference and tendency towards a higher functional response for this prey item, providing strong evidence that invasion by this decapod is likely to reduce shredder abundance in freshwater systems to a greater extent than would be the case where only crayfish are present. This could have cascading effects whereby reduced shredder abundance results in a dramatic decline in detrital processing. Woodward *et al.* (2008) found the predatory impacts of bullhead on *G. pulex* in a chalkstream caused a dramatic decline in detrital processing. Conversely, macroconsumers of detritus such as crayfish may decouple such a cascade, functionally replacing the more specialised shredders and thereby still creating availability of nutrients to pass to higher levels (Usio & Townsend, 2000; Vanni, 2002; Moore *et al.*, 2012), although crayfish effects are likely to be species- (Dunoyer *et al.*, 2014) and size-dependent (Mancinelli, Sangiorgio & Scalzo, 2013). Our combined evidence from the mesocosms, gut contents and stable isotopes indicated that *E. sinensis* is also a significant consumer of terrestrial leaf litter, so, depending on comparative processing rates and the degree to which shredder populations are reduced, this invader could similarly decouple such a cascade.

There was evidence of additive community impacts in the combined presence of both decapods. Periphyton biomass increased significantly in the joint presence of both invasive decapods, but did not differ in single species and control treatments. The observed increase in periphyton is likely to be a consequence of a top-down cascade created by the decapods feeding on gastropods, and thereby reducing grazing pressure on algae. A similar cascade has been reported caused by reduction of gastropods in the presence of single species invasion by the crayfish *Orconectes rusticus* (Weber & Lodge, 1990; Charlebois & Lamberti, 1996). One explanation for this cascade in the sympatric treatment may be a synergistic effect of the invasive decapod species. *Eriocheir sinensis* had a stronger impact than *P. leniusculus* on the abundance of the gastropod *P. fontinalis* in mesocosms; however, impacts on the other grazer *R. peregra* were similar, so it is unclear why *E. sinensis* in isolation did not also cause a trophic cascade. An alternative explanation may

be that, along with grazing pressure by the gastropods, periphyton was directly consumed by *E. sinensis* to a greater extent than by *P. leniusculus*. The importance of periphyton in the diet of mitten crabs has been reported previously (e.g. Czerniejewski *et al.*, 2010 for Chinese mitten crab, Kobayashi, 2009 for Japanese mitten crab), and although crayfish may graze directly on periphyton, they are inefficient consumers compared to gastropod grazers (Nyström, 1999). Gastropods in the sympatric treatment were reduced by an intermediate amount relative to the allopatric treatments; hence, the observed increase in periphyton may reflect reduced grazing pressure. However, compared to the *E. sinensis* allopatric treatment with four crabs per pool, there was likely to be half as much direct consumption by the decapods (two crabs per pool), potentially causing the outcome of higher periphyton than the allopatric treatments and control.

Stable-isotope analysis of wild specimens supports the indication from laboratory and mesocosm results that the invasive decapods share a varied diet, with potential for overlap and therefore competition for resource use. Both decapods were similarly enriched in <sup>15</sup>N and therefore occupy the same trophic level. The alignment of *P. leniusculus* towards algal basal resources, compared to the closer alignment of *E. sinensis* with macrophytes was supported by the gut-content analysis and may reflect dietary preference or variation in their availability between the two study reaches. Mixing models suggest that algal and plant materials constitute the majority of the diet for both species. Due to limitations in sample collection and analysis, only small samples of the wild decapods were captured and analysed, and only adult specimens, so the stable-isotope and gut-content analyses presented here provide only an initial indication of dietary patterns and interpretation must be cautious. For example, there may be undetected temporal variation or ontogenetic differences in diet, though there is little evidence for ontogenetic diet shift among *P. leniusculus* (Bondar & Richardson, 2009; Stenroth *et al.*, 2006; Usio *et al.*, 2009; but see Bondar & Richardson, 2013) or *E. sinensis* (Rudnick, Halat & Resh, 2000, unpubl. data).

Comparisons of invader impacts are crucial for managers to assess where best to target limited resources for invasive species control. Our study provides the first quantitative comparison of potential impacts of *P. leniusculus* and *E. sinensis* on the communities they invade. The most widely reported impacts of *E. sinensis* are for estuarine environments where it causes substantial bank erosion (Dittel & Epifanio, 2009). However, our results

suggest that the spread of *E. sinensis* into freshwaters is also cause for concern due to structural ecosystem effects including reduction in the abundances of native prey and altered community composition; particularly as it is likely that this invader will have an equal, if not higher, per capita impact on prey species than *P. leniusculus*. The community impacts of an invader in the wild will depend on an array of interlinked factors including population density, the availability of prey, habitat complexity and other biotic interactions (Parker *et al.*, 1999). Our use of several approaches, ranging from fully controlled laboratory experiments to analysis of wild-caught specimens, highlights the benefits of supplementing quantitative per capita measurements with community experiments to better understand the mechanisms of potential community impacts and facilitate prediction of invader impacts.

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