Freshwater Biology (2009) **54,** 1876–1887 doi:10.1111/j.1365-2427.2009.02237.x

Behavioural and growth differences between experienced and naïve populations of a native crayfish in the presence of invasive rusty crayfish

NICOLE M. HAYES*, KATRINA J. BUTKAS*, JULIAN D. OLDEN† AND M. JAKE VANDER ZANDEN*

*Center for Limnology, University of Wisconsin-Madison, Madison, WI, U.S.A.

†School of Aquatic & Fishery Sciences, University of Washington, Seattle, WA, U.S.A.

SUMMARY

1. Species invasions are a leading threat to native species and ecosystems. How populations of native species respond to the presence of invasive species will ultimately determine their long-term persistence.

2. In this study, we capitalise on a unique opportunity to compare the behaviour and growth of naïve and experienced virile crayfish (*Orconectes virilis* Hagen) populations in the presence of invasive rusty crayfish (*O. rusticus* Girard). In behavioural trials, experienced *O. virilis* (coexisted with *O. rusticus* for >30 years) showed more aggressive behaviour than their naïve counterparts. Naïve *O. virilis* retreated from aggressive interactions with *O. rusticus* 38% more often than experienced *O. virilis.* Experienced *O. virilis* spent 39% more time occupying shelter than naïve *O. virilis.*

3. There were also differences in *O. rusticus* behaviour: *O. rusticus* spent 24% more time occupying shelter with naïve *O. virilis* relative to experienced *O. virilis*.

4. In field mesocosm experiments with *O. rusticus*, naïve *O. virilis* declined in body mass by 1% while experienced *O. virilis*' body mass increased by 6%, thus highlighting the potential population-level implications of the previously observed behavioural effects.

5. Our work demonstrates significant behaviour and growth differences between naïve and experienced *O. virilis* in the presence of invasive *O. rusticus.* Whether this difference is the result of phenotypic plasticity or evolution by natural selection remains to be determined. Either way, this area of inquiry has implications for managing native populations and species in an increasingly invaded world.

Keywords: behaviour, growth, invasive species, rusty crayfish, virile crayfish

Introduction

Invasive species are a major driver of global ecological change and are a leading cause of native species imperilment in a wide range of ecosystems (Vitousek *et al.*, 1996; Sala *et al.*, 2000; Lodge *et al.*, 2006; Ricciardi, 2007). Although not all introduced species become invasive, those that do have led to the extirpation of native species through predation, competition, hybridisation, and alteration of ecosystems and habitats (Parker *et al.*, 1999; Mack *et al.*, 2000; Sakai *et al.*, 2001). Prevention efforts to date have failed to stem the spread of invasive species (Ricciardi & Rasmussen, 1998). Likewise, eradication has proven challenging, and has been successful only under limited conditions (Myers *et al.*, 2000). In light

Correspondence: M. Jake Vander Zanden, Center for Limnology, University of Wisconsin-Madison, 680 North Park Street, Madison, WI 53706-1492, U.S.A. E-mail: mjvanderzand@wisc.edu
Present address: Nicole M. Hayes, Department of Zoology, Miami University, Oxford, OH 45056, U.S.A.

© 2009 Blackwell Publishing Ltd

of these challenges, the long-term persistence of many native populations and species will depend on whether they are able to co-exist in their current habitats in the inevitable presence of new invasive species (Schlaepfer *et al*., 2005).

Recently, there has been interest in the evolutionary consequences of species invasions (Mooney & Cleland, 2001; Palumbi, 2001; Suarez & Tsutsui, 2008). A growing body of evidence indicates that invasive species can rapidly adapt to novel conditions in their new environment (Lee, 2002; Novak, 2007), thereby facilitating their establishment and spread (Stockwell, Hendry & Kinnison, 2003; Carroll, 2007; Yoshida *et al*., 2007). While a number of studies have found that non-native species can adapt to their introduced environment, an emerging management question concerns the extent to which native species may also adapt to the changing conditions wrought by biological invasions (Schlaepfer *et al*., 2005). Indeed, it has been argued that the potential to respond to rapid environmental change may be essential for the long-term survival of many species (Mooney & Cleland, 2001; Rice & Emery, 2003; Carroll, 2008). There is recent evidence for rapid or 'contemporary' evolution in native populations in response to invasion (Strauss, Lau & Carroll, 2006; Carroll, 2007, 2008). After the arrival of cane toads, native species of Australian snakes have shown a steady reduction in gape size (Phillips & Shine, 2004). In another example, Fisk *et al*. (2007) found that native zooplankton populations have undergone reductions in body size and in the timing of reproduction in response to the introduction of non-native salmonids in alpine lakes of California, U.S.A. Native populations may also exhibit changes lacking a genetic basis stemming from phenotypic or behavioural plasticity (Agrawal, 2003). Plasticity in behaviour or life-history traits may result from environmental or biotic factors such as predation and competition (Relyea, 2004; Gosline & Rodd, 2008). For example, guppies respond to predation by increasing offspring, delaying maturity and increasing size (Gosline & Rodd, 2008).

Both evolutionary and phenotypic responses to invasive species can promote long-term survival of native species (Ghalambor *et al*., 2007). However, there is a need to develop a better understanding of the potential for native species to adapt to ecological changes such as species invasions. The present study capitalises on a unique situation that allows us to compare the behaviour and growth of native crayfish populations with very different histories of coexistence with invasive rusty crayfish, *Orconectes rusticus*. Sparkling Lake, Wisconsin, historically supported a single species of native crayfish, *Orconectes virilis*, but in the 1970s was invaded by two non-native crayfish species from the same genus, *Orconectes propinquus* (Girard) and *O. rusticus*. *O. propinquus* did not persist, though *O. rusticus* became extremely abundant. Correspondingly, the number of *O. virilis* trapped in annual sampling declined until 1999, after which they were no longer observed, and were thought to have been extirpated by invasive *O. rusticus* (Hein, Vander Zanden & Magnuson, 2007). These observed declines in *O. virilis* are consistent with previous studies showing the negative impact of *O. rusticus* on native crayfishes (Olsen *et al*., 1991; Hill & Lodge, 1999).

In 2001, we initiated an intensive whole-lake removal of invasive *O. rusticus* in Sparkling Lake, resulting in dramatic declines in *O. rusticus* abundance (Hein *et al*., 2006, 2007). During the course of removal, the native crayfish *O. virilis* were discovered to have persisted, albeit at very low numbers. With intensive removal of *O. rusticus* over the past 6 years, catch rates of *O. virilis* have increased, and *O. virilis* now appears regularly in crayfish trapping efforts. Individuals from the relic population of *O. virilis* can be viewed as 'experienced' or conditioned because they have managed to persist despite the fact that population size was pushed to low levels as a result of *O. rusticus* invasion. In contrast, several surrounding lakes support a native *O. virilis* population and have never been invaded by invasive crayfish, and thus represent what can be considered 'naïve' populations. By virtue of differential invasion of *O. rusticus* across the landscape and the intensive removal of this species in Sparkling Lake we are provided with a unique opportunity to compare the behaviour and growth of naïve and experienced *O. virilis* populations in response to an invasive congener. We hypothesised that in the presence of *O. rusticus*, experienced *O. virilis* would be more aggressive, and show higher growth rates in mesocsoms relative to their naïve counterparts.

Methods

Study organisms

Orconectes rusticus has expanded its distribution over the last 50 years from its historical range in the Ohio

River drainage to waters throughout the US and Canada. For example, in Wisconsin *O. rusticus* was first reported in 1965, and is now present in hundreds of lakes and rivers throughout the state, whereas the frequency of occurrence of native *O. virilis* has decreased over the same time period (Olden *et al*., 2006). *Orconectes rusticus* can have negative impacts on a wide variety of taxa, including benthic algae, macrophytes, aquatic invertebrates, snails, native crayfish and fishes (e.g. Lodge & Lorman, 1987; Olsen *et al*., 1991; Wilson *et al*., 2004; McCarthy *et al*., 2006).

Our study was conducted during the summer of 2006. We collected *O. rusticus* and experienced *O. virilis* from Sparkling Lake, Vilas County, WI (46°00′N, 89°42′W). 'Naïve' *O. virilis* were collected from Lake Laura, Vilas County, WI (46°04′N, 89°27′W), which does not contain *O. rusticus*. These two lakes are in close proximity to each other and are similar in many respects, particularly in terms of the availability of cobble habitat and fish community composition. Crayfish were hand collected via snorkeling or trapped in baited minnow traps and were temporarily held in 40-L aerated aquaria with lake water and fed daily Tetramin bottom feeder pellets and native snails (*Lymnaea stagnalis*) from nearby lakes. Each aquarium contained only individuals of the same species-lake combination.

Behavioural trials

To test for differences in behaviour between 'naïve' and 'experienced' *O. virilis*, we compared their interactions during behavioural trials with *O. rusticus*. Behaviour is often sex- and size-dependent in crayfish (Bruski & Dunham, 1987; Bergman & Moore, 2003) therefore we conducted observations of same-sex pairs of *O. rusticus* and *O. virilis* in three relative size categories: same size (*O. rusticus* with carapace length ±2 mm *O. virilis* length), *O. virilis* larger (*O. rusticus* with a carapace length 10 ±2 mm shorter than *O. virilis* length) and *O. virilis* smaller (*O. rusticus* with a carapace length 10 ± 2 mm longer then *O. virilis* length). A total of 85 behavioural trials were conducted; 39 were male pairs and 46 were female pairs. Individual crayfish were used in trials from one to three times. There were no significant behavioural differences between male and female pairs for any of the behaviours (*t*-test on transformed data, all *P*-values >0.05), thus sex was not used in the analysis.

Pairs were selected by an individual other than the observer to eliminate potential observer bias, and only after the trials did the observer learn if the *O. virilis* specimen originated from the 'naïve' or 'experienced' population. *Orconectes rusticus* were randomly selected from holding tanks and each individual was used for multiple trials and with both types of *O. virilis*. Behavioural observations were made in 40-L aquaria with gravel and sand substrate, an opaque divider, a clear glass front, and opaque sides to reduce disturbance of crayfish during the trial. We took measurements of wet mass, carapace length and chelae length prior to each trial. The observer positioned the opaque divider in the centre of the tank and placed a crayfish on either side. Crayfish were given 15 min to acclimate to the environment before the divider was removed (following Gherardi & Cioni, 2004) and a 5-inch segment of 1.5-inch diameter PVC (mimicking a shelter) was gently lowered to the centre of the aquarium (following Gherardi & Daniels, 2004). During the 30-min observational period, we recorded selected behaviours following the *Orconectes* ethogram by Bruski & Dunham (1987). These behaviours included the number of minutes each individual occupied a shelter, either in or under the PVC or burrowed into the substrate (shelter), the number of times they approached to within one body length of the other crayfish (approach), and the number of times they moved farther than one body length away from the other crayfish following an approach (retreat).

Comparison of growth rates in mesocosms

To examine potential differences in growth between 'naïve' and 'experienced' *O. virilis* in the presence of *O. rusticus*, we performed a six-week study using large field mesocosms (Hill & Lodge, 1994). Mesocosms contained *O. rusticus* and either 'experienced' or 'naïve' *O. virilis*. The amount of food and shelter available was also varied to examine their effect on crayfish growth. Low-density shelter mesocosms had one brick and one 12 cm segment of 3.8 cm diameter PVC. High density shelter mesocosms had two bricks, two PVC segments, and one cinderblock. High density food mesocosms received approximately 40 g of *Lymnaea stagnalis* (20 snails) on a biweekly basis while the low density food treatment received 20 g (10 snails) at the same frequency.

A total of 16, 1135-liter Rubbermaid stock tanks were filled with water from Sparkling Lake and 18 kg each of cleaned commercial 'play sand' and coarse (1.9 cm diameter) gravel. We employed a factorial experimental design such that there were two mesocosms for each combination of the three factors (food, shelter, virilis type). Eight crayfish were added to each mesocosm, four *O. virilis* and four *O. rusticus*, producing crayfish densities similar to those found in natural lakes in the region (M. J. Vander Zanden, unpubl. data). Crayfish mass (wet weight of blotted individuals) and carapace length measurements were taken on the first and last day of the 6-week experiment. Each crayfish carapace was given a unique marking with nail varnish to track individual mass and length change over the experiment. *Orconectes virilis* and *O. rusticus* were size- and sex-matched within each mesocosm to the extent possible considering the limited number of crayfish available for the experiment. Average carapace length of *O. rusticus* and *O. virilis* within each mesocosm were approximately equal. Twenty-two of the 120 crayfish used in the field experiment died prior to the end of the trial (five experienced *O. virilis*, nine naïve *O. virilis*, six *O. rusticus* paired with experienced *O. virilis*, and two *O. rusticus* paired with naïve *O. virilis*). Though naïve *O. virilis* were the most likely to die, and *O. rusticus* paired with naïve *O. virilis* were the least likely to die, there was a weak association between the occurrence of mortality and these four groups (Pearson's $\chi^2 = 3.14$, 1 d.f., $P = 0.07$). Dead crayfish were removed from the mesocosms and were excluded from further analysis.

Statistical analysis

Statistical analysis for behavioural trials was conducted on non-transformed frequency data for 'number of approaches', 'number of retreats', and 'number of minutes spent in shelter' for *O. rusticus* and *O. virilis* in the 30-min behavioural trials. We used generalised linear model (GENMOD procedure; SAS v. 9.1, Cary, NC, U.S.A.) to examine the effects of *O. virilis* type (naïve versus experienced) on the number of occurrences of the three behaviours (Poisson distribution, log link function). The model also included body size ratio (positive values indicate *O. virilis* larger than *O. rusticus*) as a covariate, and a body size ratio × *O. virilis* type interaction term.

To compare differences in growth rates between naïve and experienced *O. virilis*, we used a mixed model (MIXED procedure, compound symmetry covariance structure, SAS version 9.1). Growth was the dependent variable and was expressed as absolute growth (total mass added during the experiment) and as relative growth (percent change in mass during the experiment). *Orconectes virilis* type (naïve versus experienced), food (high versus low), and structure (high versus low) were modelled as fixed effects, and individual mesocosms were experimental units and modelled as a random effect. Because males and females were mixed among the mesocosms, sex was treated as a within-subject fixed effect.

Results

Behavioural trials

For *O. virilis* behaviours, there was a significant ($P < 0.05$) effect of *O. virilis* type on retreats ($P < 0.0001$) and shelter occupancy ($P < 0.0001$), but not approaches ($P = 0.827$; Table 1; Fig. 1a). Naïve *O. virilis* retreated from *O. rusticus* during encounters 38% more often than experienced *O. virilis* (Fig. 1a). Experienced *O. virilis* spent 39% more time in shelter than naïve *O. virilis* (Fig. 1a). Body size ratio also had a significant effect on number of *O. virilis* approaches ($P < 0.001$), retreats ($P < 0.001$), and time in shelter ($P = 0.030$, Table 1, Fig. 2a–c). There was a significant body size ratio × *O. virilis* type interaction for approaches ($P = 0.040$) and shelter occupancy ($P = 0.0006$), but not for retreats ($P = 0.130$). The

Table 1 Results of generalised linear models examining the effects of *Orconectes virilis* population type (naïve versus experienced), body size ratio and interactions on the behaviours of *O. virilis*. Models tested for differences in the number of approaches, number of retreats, and time spent occupying shelter during 30-min behavioural trials.

	Approaches		Retreats		Shelter occupancy	
Effect	χ^2	P	χ^2	P	χ^2	P
O. virilis behaviours						
O. virilis type	0.05	0.827	20.43	**<0.0001**	15.22	**<0.0001**
Body size ratio	12.22	**0.0005**	10.93	**0.0009**	4.69	**0.030**
Body size ratio × *O. virilis* type	4.20	**0.040**	2.30	0.130	11.69	**0.0006**

Statistically significant differences ($P < 0.05$) are shown in bold.

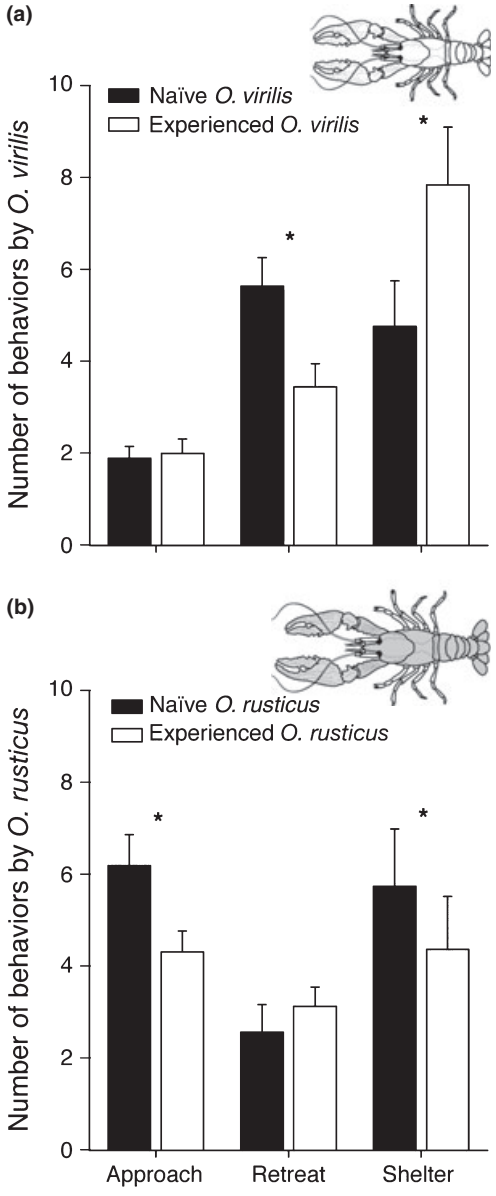

Fig. 1 Comparison of the number of approaches, retreats, and time spent in shelter for naïve and experienced *Orconectes virilis* during 30-min behavioural trials: (a) Behaviours by *O. virilis*, (b) Behaviours by *O. rusticus*. Error bars are ±1SE. Asterisks indicate significant at $P < 0.05$.

significant interaction terms precludes the straightforward comparison of *y*-intercepts of behaviour–size ratio relationships for naïve and experienced crayfish in analysis of covariance (ANCOVA). These significant interaction terms also indicate that the effect of body size ratio on the behaviours differs between the two *O. virilis* types. For comparison, models that used only *O. virilis* type as a predictor variable produced the same general result (approaches, $P = 0.702$; retreats, $P < 0.0001$; shelter occupancy, $P < 0.0001$).

For *O. rusticus* behaviours, there was a significant ($P < 0.05$) effect of *O. virilis* type on approaches ($P < 0.0001$) and shelter occupancy ($P = 0.0007$), but not retreats ($P = 0.077$, Table 2, Fig. 1b). *Orconectes rusticus* approached naïve *O. virilis* 30% more often than they approached experienced *O. virilis* (Fig. 1b). *O. rusticus* spent 24% more time in shelter in the presence of naïve *O. virilis* compared to in the presence of experienced *O. virilis* (Fig. 1b). Body size ratio was significant for *O. rusticus* approaches ($P = 0.003$), retreats ($P = 0.015$), and shelter occupancy ($P = 0.0008$, Fig. 2d–f). For *O. rusticus*, body size ratio × *O. virilis* type interactions were not significant for any of the behaviours (Table 1, Fig. 2d–f), allowing group (naïve versus experienced) intercepts to be compared with ANCOVA. Results were virtually identical as those from the generalised linear models shown in Table 2 (approaches, $P < 0.0001$; retreats, $P = 0.070$; shelter occupancy, $P = 0.001$).

Comparison of growth rates in mesocosms

Over the duration of the mesocosm study, experienced *O. virilis* increased in mass, while naïve *O. virilis* declined in mass, the result being a significant difference in growth rates ($F_{1,11} = 5.09$, $P = 0.045$, Table 3). Expressed in relative terms, experienced *O. virilis* increase in body mass by 6%, while naïve body mass declined by 1% ($F_{1,11} = 6.53$, $P = 0.027$, Table 3, Fig. 3a). Growth rates for males were slightly higher than females, though the effect was not significant for percent growth ($F_{1,11} = 4.25$, $P = 0.073$), or for change in mass ($F_{1,8} = 3.22$, $P = 0.11$). There were no significant effects of food and structure treatments on either measure of *O. virilis* growth rates (Fig. 3a,b).

Discussion

Our study provides experimental evidence for differences between naïve and experienced populations of a native crayfish, *O. virilis*, as evidenced by both behavioural and performance (growth rate) differences in the presence of invasive *O. rusticus*. Such differences derive from *c.* 30 years of coexistence between the native and invasive crayfish in Sparkling Lake, Wisconsin, during which the native population was pushed to such low levels that they were not

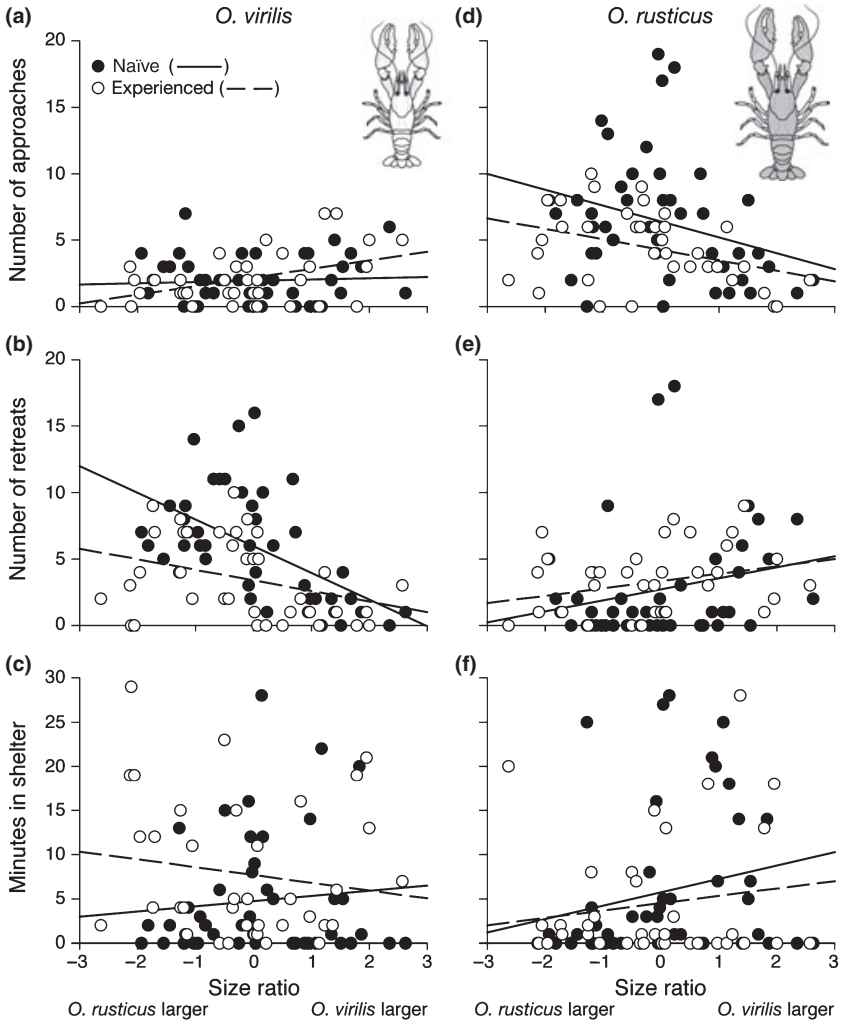

Fig. 2 Comparison of crayfish behaviours for naïve (filled circles) and experienced (open circles) *Orconectes virilis* as a function of relative body size differences (size ratio): (a) Approaches by *O. virilis*, (b) Retreats by *O. virilis*, (c) Time spent in shelter by *O. virilis*. Behaviours exhibited by *O. rusticus*: (d) Approaches by *O. rusticus*, (e) Retreats by *O. rusticus* and (f) Time spent in shelter by *O. rusticus*.

Table 2 Results of generalised linear models examining the effects of *Orconectes virilis* population type (naïve versus experienced), body size ratio, and interactions on the behaviours of *O. rusticus*. Models tested for differences in the number of approaches, number of retreats, and time spent occupying shelter during 30-min behavioural trials

	Approaches		Retreats		Shelter occupancy	
	χ^2	*P*	χ^2	*P*	χ^2	*P*
O. rusticus behaviours						
O. virilis type	15.74	**<0.0001**	3.13	0.077	11.54	**0.0007**
Body size ratio	8.64	**0.003**	5.85	**0.015**	11.27	**0.0008**
Body size ratio × *O. virilis* type	0.02	0.882	1.33	0.248	2.81	0.093

Statistically significant differences ($P < 0.05$) are shown in bold.

Table 3 Results of mixed model ANOVA testing the effects of *Orconectes virilis* population type (naïve versus experienced), sex (male versus female), food level (low versus high) and structure (low versus high) on *O. virilis* growth rates, expressed as absolute change in mass, and percent change in mass during a 6-week mesocosm experiment

	Growth (absolute)			Growth (percent)		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Population type	1,11	5.09	**0.045**	1,11	6.53	**0.027**
Sex	1,8	3.22	0.110	1,8	4.25	0.073
Food	1,11	2.32	0.160	1,11	1.20	0.300
Structure	1,11	0.03	0.860	1,11	0.13	0.720

Statistically significant differences ($P < 0.05$) are shown in bold. d.f., degrees of freedom.

detected in routine long-term sampling. In fact, native *O. virilis* in Sparkling Lake were considered to be locally extirpated until they were detected during intensive sampling that began in 2001 aimed at controlling the invasive *O. rusticus* population (Hein *et al.*, 2006, 2007).

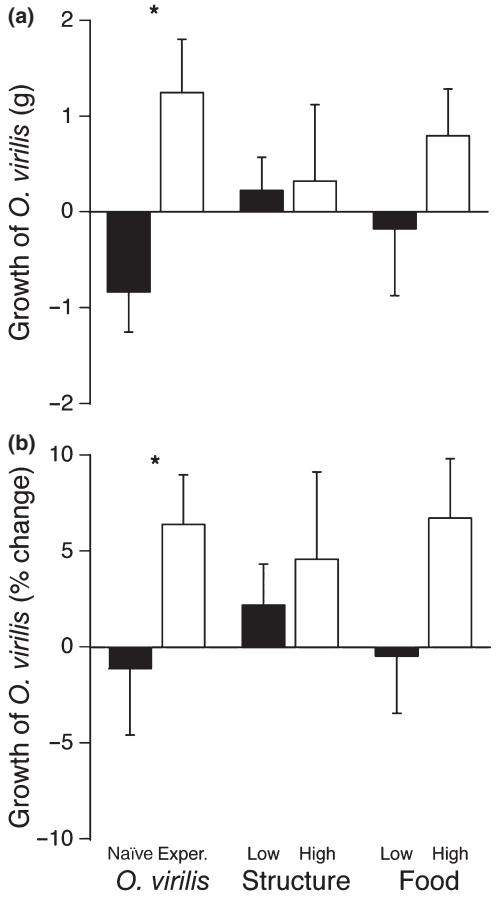

Fig. 3. Effect of *Orconectes virilis* type (naïve versus experienced), structure, and food on *O. virilis* growth rates in the presence of *O. rusticus* in field mesocosms. (a) total growth (g), (b) relative growth (percent change in mass). Bars represent overall means estimated across all mesocosms. Error bars are ±1SE. Asterisks indicate significance of effect in mixed model ANOVA (Table 3) at $P < 0.05$.

Orconectes rusticus are known to displace native crayfishes via several different mechanisms. Through competitive displacement, *O. rusticus* are able to gain access to higher quality shelter and food resources (Bovbjerg, 1970; Hill & Lodge, 1999), and their larger carapace and chelae size also make them less vulnerable to fish predation than native crayfishes (Roth & Kitchell, 2005). In addition, there can be hybridisation and introgression with native populations, thereby swamping native genotypes (Rhymer & Simberloff, 1996). Notably, hybridisation between *O. virilis* and *O. rusticus* was found not to occur in a previous study (Perry, Feder & Lodge, 2001), though hybridisation with *O. rusticus* has led to the decline of other *Orconectes* species (Capelli & Capelli, 1980; Perry *et al.*, 2001). Considering the widely-documented negative impacts of invasive *O. rusticus* on native *O. virilis*, the conditions are suitable for adaptive change in native *O. virilis* populations in response to this aggressive competitor (Hill & Lodge, 1994, 1999).

In our behavioural trials, the naïve *O. virilis* retreated more frequently from aggressive interactions with *O. rusticus* relative to experienced *O. virilis*, and previous studies have shown that competitive exclusion is an important mechanism whereby native crayfish are displaced by *O. rusticus* (Bovbjerg, 1970). The competitively dominant *O. rusticus* are more successful at obtaining food and grow more rapidly as a result. In turn, they are less susceptible to fish predation and have higher fecundity (Hill & Lodge, 1999). The more aggressive behaviours observed in the experienced *O. virilis* population (relative to naïve *O. virilis*) would be expected to increase their ability to compete and coexist with *O. rusticus*, potentially counteracting the tendency of *O. rusticus* to outcompete and replace *O. virilis*.

The competitive differences between *O. rusticus* and *O. virilis* are also apparent in the acquisition and occupancy of limited shelter in behavioural trials. Shelter provides crayfish with a respite from predation, and several studies indicate that use and availability of shelter may limit crayfish populations (Bovbjerg, 1970; Hill & Lodge, 1994; Gherardi & Daniels, 2004). This highlights the potential importance of obtaining shelter for crayfish survival. We found that experienced *O. virilis* gained greater access to shelter (i.e. spent more time occupying shelter) than naïve *O. virilis*, and that *O. rusticus* spent more time in shelter during trials with naïve *O. virilis* than with experienced *O. virilis*. It is generally the aggressively dominant crayfish that inhabit shelter (Vorburger & Ribi, 1999), and these individuals benefit from reduced predation risk from fish (Garvey, Stein & Thomas, 1994; Söderbäck, 1994). Shelter occupancy thus appears to be reflective of individual dominance, and our finding that experienced *O. virilis* spend more time occupying shelter relative to naïve *O. virilis* provides further support for behaviour differences between populations. *Orconectes virilis* type also affected *O. rusticus* shelter occupancy. By reducing *O. rusticus* shelter occupancy, experienced *O. virilis* are presumably in a better competitive position and have an improved ability to coexist with *O. rusticus*.

Body size and chelae length are important in determining the outcome of agonistic interactions

between crayfish (Bergman & Moore, 2003; Vorburger & Ribi, 1999; but see Wilson *et al*., 2007). This was confirmed in behavioural trials with naïve and experienced *O. virilis;* relative body size was an important factor in determining the number of approaches, number of retreats and shelter occupancy. Both species initiated more approaches and retreated less often as relative body size increased, indicating the overall advantage of large body size, regardless of species, during agonistic interactions.

The behavioural differences described above corresponded with differences in crayfish performance: individuals from the experienced *O. virilis* population had higher growth rates in the presence of *O. rusticus*, independent of differences in habitat structure and food availability. The higher growth rates for experienced *O. virilis* (relative to naïve *O. virilis*) indicates better performance, and a greater ability to acquire food resources in the presence of the invader. The growth results are consistent with the hypothesis that experienced *O. virilis* are at a competitive advantage compared to naïve *O. virilis*.

A total of 22 crayfish died during the mesocosm trials. Though the χ^2 test ($P = 0.07$, comparing differences in mortality among the four 'populations' in the trials) is not significant at $P < 0.05$, the mortality results deserve further consideration. Mortality was highest for the naïve *O. virilis* ($n = 9$) and lowest for the *O. rusticus* paired with naïve *O. virilis* ($n = 2$). In the experienced *O. virilis* mesocosms, mortality for *O. virilis* and *O. rusticus* was similar ($n = 5$ and 6 respectively). Total crayfish mortality (both species) for experienced and naïve *O. virilis* mesocosms did not differ (11 each). For naïve, *O. virilis* comprised 82% of total crayfish mortality (nine of 11). For experienced, *O. virilis* comprised only 45% of total crayfish mortality (five of 11). Though comparing mortality was not a goal of this study, these results also suggest the superiority of experienced *O. virilis* relative to naïve *O. virilis*. The fact that total crayfish mortality was the same in both native and experienced treatments also indicates that the observed differences in growth are not due to total crayfish density differences resulting from mortality.

Differences between experienced and naïve *O. virilis* were also manifested in the behaviours of *O. rusticus* in our behavioural experiments. In trials with experienced *O. virilis*, *O. rusticus* display reduced aggression, with fewer approaches and more retreats. Thus, the elevated aggression and potential competitive ability of experienced *O. virilis* appears to also modify *O. rusticus* behaviour when they co-occur. It is intriguing that the effect of experienced versus naïve native crayfish is not only seen in the behaviour of *O. virilis,* but also for *O. rusticus,* and highlights the importance of the behaviour of *both* the native and invasive species in determining the outcome of species interactions during biological invasions.

Though our study design for testing behavioural and growth differences was statistically robust, perhaps the most important limitation of this study was that our comparison involved one naïve and one experienced *O. virilis* population. Though our results are consistent with change having occurred in the experienced population (i.e. the experienced crayfish population is more aggressive and exhibits faster growth in the presence of the invasive), we cannot rule out the possibility that *O. virilis* in Sparkling Lake were more aggressive than *O. virilis* from nearby Lake Laura prior to introduction of *O. rusticus.* Laura and Sparkling have similar fish communities and substrate composition is not notably different, thus we have no specific reason to believe that *O. virilis* behaviour and growth would otherwise vary among these lakes. Still, we cannot rule out the possibility that these differences are independent of *O. rusticus* effects. Comparing behaviour and growth of multiple *O. virilis* populations from each type (i.e. experienced and naïve) would undoubtedly strengthen our findings, though we are not aware of other experienced *O. virilis* populations. In addition, the intensive removal of *O. rusticus* from Sparkling Lake from 2001 onwards allowed *O. virilis* to rebound to the point of being abundant enough to even perform these experiments.

Implications for the long-term management of biological invasions

The long-term persistence of many native species hinges upon their ability to adapt to environments that are increasingly altered by invasive species. A growing literature documents examples of native species adaptation to species invasions (Singer, Thomas & Parmesan, 1993; Carroll & Dingle, 1996; Carroll, 2007, 2008). The observation that a native population 'adapts' to a species invasions can be the result of evolution by natural selection, or alternatively, behavioural or phenotypic plasticity, whereby

multiple phenotypes can derive from a single genotype, depending upon environmental conditions (Miner *et al*., 2005). Determining whether there is a genetic basis for the differences in performance and behaviour between naïve and experienced crayfish populations is beyond the scope of this particular study, but is a critical research question to be addressed in the future. Developing an understanding of the mechanisms underlying these differences will involve further studies, including morphological and genetic comparisons, as well as studies assessing heritability of the traits.

Though we provided experimental evidence for differences between experienced and naïve populations of native crayfish species in the presence of an aggressive invasive crayfish, understanding the mechanism for the observed differences has several important implications for management. Phenotypic plasticity would result in relatively rapid shifts, as environmental cues would be responsible for invoking adaptive responses. Such rapid shifts, the result of within-individual developmental and behavioural changes, would enable individuals to cope with a highly variable, or otherwise altered environment (Carroll, 2008). If the behavioural and performance differences observed in our study are the result of phenotypic plasticity, *O. virilis*, regardless of experience level, could rapidly develop an improved ability to compete with *O. rusticus*. A high degree of phenotypic plasticity will also serve to reduce the magnitude of natural selection (Rice & Emery, 2003). If many individuals within a population are capable of traits that favour coexistence, the population would be expected to be more likely to persist in the presence of *O. rusticus*. The plasticity could act as a buffer to help maintain population levels until the environment shifts, or until longer-term genetic adaptation occurs.

It is also possible that these differences are the result of genetic changes within the population of *O. virilis*, though at present we have no evidence for genetic change. *Orconectes virilis* and *O. rusticus* cohabited in Sparkling Lake for *c.* 30 years, which is on the order of 15 crayfish generations. There are a growing number of studies documenting rapid adaptation of species to environmental change (Kinnison & Hendry, 2001; Meyers & Bull, 2002; Ashley *et al*., 2003; Stockwell *et al*., 2003). Most of these examples occur within 100 years, but in many cases morphological, physiological, and behavioural changes are apparent in <30 years (Able & Belthoff, 1998; Lee, 1999; Quinn, Kinnison & Unwin, 2001).

Sparkling Lake is a seepage lake, and thus lacks surface water connections to other lakes. This reduces the possibility that movement of individuals could swamp local adaptation of the existing *O. virilis* population. On the other hand, this isolation prevents gene flow that would spread presumably adaptive 'experienced' traits to other locations. If genetic change has allowed the coexistence of *O. virilis* and *O. rusticus* in Sparkling Lake, one possible approach would be to facilitate gene flow to other *O. virilis* populations facing adverse impacts of *O. rusticus*. In the beginning phases of *O. rusticus* removal in Sparkling Lake, *O. virilis* populations were at very low levels. Since then, *O. virilis* numbers have increased markedly. These surviving individuals presumably possess traits necessary to co-exist with the aggressive *O. rusticus*, though the current population of *O. virilis* is likely descended from a small number of survivors. Thus, there is a possibility that they have reduced genetic variability resulting from the severe population reduction. Though this population may be well-adapted to coexist with *O. rusticus*, the reduced genetic variability could also have the disadvantage of limiting their ability to adapt to other types of environmental change in this region such as climate change or habitat alteration from shoreline development (Carpenter *et al*., 2007).

Ongoing efforts to manage invasive species should consider creating opportunities for native species to adapt to their changing environment (Ashley *et al*., 2003; Watters, Lema & Nevitt, 2003). Management actions may focus on preserving intraspecific diversity by controlling invasives and maintaining native populations until phenotypic plasticity and natural selection allow natives to develop the ability to cope and coexist with invasive species, without the need for long-term human intervention. Preserving the diversity of native populations is necessary to ensure enough genetic variation to allow an adaptive shift. If management to conserve native populations over the long-term is to succeed, we must more thoroughly consider how we can facilitate the process of native species adapting to a changing environment. In the case of native *O. virilis*, a critical next step will be to estimate the relative importance of evolution versus phenotypic/behavioural plasticity as mechanisms for the apparent adaptive change in response to invasive *O. rusticus*.

Acknowledgments

We thank the University of Wisconsin-Madison Trout Lake Station for use of facilities and technical support. This manuscript was greatly improved by comments from Eric Larson and Denis Réale. Thanks to Ashley Gramza for assistance in collecting the data, Ting-Li Lin for statistical advice, and Pieter Johnson, Erin Vennie-Vollrath, Kira Langree, and Rick Hartson for intellectual contributions and assistance with the mesocosm experiments. Linda Winn facilitated access to state land and the WI Department of Natural Resources Forestry Fire division filled the mesocosms with water. Support for this project was provided by the National Science Foundation, specifically a Research Experience for Undergraduate award to NMH through the North-Temperate Lakes Long-Term Ecological Research (NTL-LTER) program.

References

Able K.P. & Belthoff J.R. (1998) Rapid 'evolution' of migratory behaviour in the introduced house finch of eastern North America. *Proceedings of the Royal Society of London Series B-Biological Sciences (B)*, **265**, 2063–2071.

Agrawal A.A. (2003) Community genetics: new insights into community ecology by integrating population genetics. *Ecology*, **84**, 543–544.

Ashley M.V., Willson M.F., Pergams O.R.W., O'Dowd D.J., Gende S.M. & Brown J.S. (2003) Evolutionarily enlightened management. *Biological Conservation*, **111**, 115–123.

Bergman D.A. & Moore P.A. (2003) Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. *The Biological Bulletin*, **205**, 26–35.

Bovbjerg R.V. (1970) Ecological isolation and competitive exclusion in 2 crayfish (*Orconectes-virilis* and *Orconectes-immunis*). *Ecology*, **51**, 225–236.

Bruski C.A. & Dunham D.W. (1987) The importance of vision in agonisitic communication of the crayfish *Orconectes-rusticus*. I: an analysis of bout dynamics. *Behaviour*, **103**, 83–107.

Capelli G.M. & Capelli J.F. (1980) Hybridization between crayfish of the genus *Orconectes*-morphological evidence (Decapoda, Cambaridae). *Crustaceana*, **39**, 121–132.

Carpenter S.R., Benson B.J., Biggs R. *et al.* (2007) Understanding regional change: comparison of two lake districts. *Bioscience*, **57**, 323–335.

Carroll S.P. (2007) Natives adapting to invasive species: ecology, genes, and the sustainability of conservation. *Ecological Research*, **22**, 892–901.

Carroll S.P. (2008) Facing change: forms and foundations of contemporary adaptation to biotic invasions. *Molecular Ecology*, **17**, 361–372.

Carroll S.P. & Dingle H. (1996) The biology of post-invasion events. *Biological Conservation*, **78**, 207–214.

Fisk D.L., Latta L.C., Knapp R.A. & Pfrender M.E. (2007) Rapid evolution in response to introduced predators I: rates and patterns of morphological and life-history trait divergence. *BMC Evolutionary Biology*, **7**, 22.

Garvey J.E., Stein R.A. & Thomas H.M. (1994) Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology*, **75**, 532–547.

Ghalambor C.K., McKay J.K., Carroll S.P. & Reznick D.M. (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Gherardi F. & Cioni A. (2004) Agonism and interference competition in freshwater decapods. *Behaviour*, **141**, 1297–1324.

Gherardi F. & Daniels W.H. (2004) Agonism and shelter competition between invasive and indigenous crayfish species. *Canadian Journal of Zoology*, **82**, 1923–1932.

Gosline A.K. & Rodd F.H. (2008) Predator-induced plasticity in guppy (*Poecilia reticulata*) life history traits. *Aquatic Ecology*, **42**, 693–699.

Hein C.L., Roth B.M., Ives A.R. & Vander Zanden M.J. (2006) Fish predation and trapping for a rusty crayfish (*Orconectes rusticus*) control: a whole lake-experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 383–393.

Hein C.L., Vander Zanden M.J. & Magnuson J.J. (2007) Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology*, **52**, 1134–1146.

Hill A.M. & Lodge D.M. (1994) Diel changes in resource demand – competition and predation in species replacement among crayfishes. *Ecology*, **75**, 2118–2126.

Hill A.M. & Lodge D.M. (1999) Replacement of resident crayfishes by an exotic crayfish: the roles of competition and predation. *Ecological Applications*, **9**, 678–690.

Kinnison M.T. & Hendry A.P. (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica*, **112**, 145–164.

Lee C.E. (1999) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, **53**, 1423–1434.

Lee C.E. (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386–391.

Lodge D.M. & Lorman J.G. (1987) Reductions in submersed macrophyte biomass and species richness

by the crayfish *Orconectes rusticus*. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 591–597.

Lodge D.M., Williams S., MacIsaac H.J. *et al.* (2006) Biological invasions: recommendations for U.S. policy and management. *Ecological Applications*, **16**, 2035–2054.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M. & Bazzaz F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

McCarthy J.M., Hein C.L., Olden J.D. & Vander Zanden M.J. (2006) Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology*, **51**, 224–235.

Meyers L.A. & Bull J.J. (2002) Fighting change with change: adaptive variation in an uncertain world. *Trends in Ecology and Evolution*, **17**, 551–557.

Miner B.G., Sultan S.E., Morgan S.G., Padilla D.K. & Relyea R.A. (2005) Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution*, **20**, 685–692.

Mooney H.A. & Cleland E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5446–5451.

Myers J.H., Simberloff D., Kuris A.M. & Carey J.R. (2000) Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution*, **15**, 316–320.

Novak S.J. (2007) The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3671–3672.

Olden J.D., McCarthy J.M., Maxted J.T., Fetzer W.W. & Vander Zanden M.J. (2006) The rapid spread of rusty crayfish (*Orconectes rusticus*) with observations on native crayfish declines in Wisconsin (USA) over the past 130 years. *Biological Invasions*, **8**, 1621–1628.

Olsen T.M., Lodge D.M., Capelli G.M. & Houlihan R.J. (1991) Mechanisms of impact of an introduced crayfish (*Orconectes rusticus)* on littoral congeners, snails, and macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 1853–1861.

Palumbi S.R. (2001) Evolution – Humans as the world's greatest evolutionary force. *Science*, **293**, 1786–1790.

Parker I.M., Simberloff D., Lonsdale W.M. *et al.* (1999) Impact: towards a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**, 3–19.

Perry W.L., Feder J.L. & Lodge D.M. (2001) Implications of hybridization between introduced and resident *Orconectes* crayfishes. *Conservation Biology*, **15**, 1656–1666.

Phillips B.L. & Shine R. (2004) Adapting to an invasive species: toxic cane toads induce morphological change in Australian snakes. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 17150–17155.

Quinn T.P., Kinnison M.T. & Unwin M.J. (2001) Evolution of Chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. *Genetica*, **112**, 493–513.

Relyea R. (2004) Fine-tuned phenotypes: tadpole plasticity under 16 different combinations of predators and competitors. *Ecology*, **85**, 172–179.

Rhymer J.M. & Simberloff D. (1996) Extinction by hybridization and introgression. *Annual Review of Ecology, Evolution, and Systematics*, **27**, 83–109.

Ricciardi A. (2007) Are modern biological invasions an unprecedented form of global change? *Conservation Biology*, **21**, 329–336.

Ricciardi A. & Rasmussen J.B. (1998) Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1759–1765.

Rice K.J. & Emery N.C. (2003) Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment*, **1**, 469–478.

Roth B.M. & Kitchell J.F. (2005) The role of size-selective predation in the displacement of *Orconectes* crayfishes following rusty crayfish invasion. *Crustaceana*, **78**, 297–310.

Sakai A.K., Allendorf F.W., Holt J.S. *et al.* (2001) The population biology of an invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Sala O.E. *et al.* (2000) Biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Schlaepfer M.A., Sherman P.W., Blossey B. & Runge M.C. (2005) Introduced species as evolutionary traps. *Ecology Letters*, **8**, 241–246.

Singer M.C., Thomas C.D. & Parmesan C. (1993) Rapid human-induced evolution of insect host associations. *Nature*, **366**, 681–683.

Söderbäck B. (1994) Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia*, **100**, 229–235.

Stockwell C.A., Hendry A.P. & Kinnison M.T. (2003) Contemporary evolution meets conservation biology. *Trends in Ecology and Evolution*, **18**, 94–101.

Strauss S.Y., Lau J.A. & Carroll S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 354–371.

Suarez A.V. & Tsutsui N.D. (2008) The evolutionary consequences of biological invasions. *Molecular Ecology*, **17**, 351–360.

Vitousek P.M., D'Antonio C.M., Loope L.L. & Westbrooks R. (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468–478.

Vorburger C. & Ribi G. (1999) Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology*, **42**, 111–113.

Watters J.V., Lema S.C. & Nevitt G.A. (2003) Phenotype management: a new approach to habitat restoration. *Biological Conservation*, **112**, 435–445.

Wilson K.A., Magnuson J.J., Lodge D.M., Hill A.M., Kratz T.K., Perry W.L. & Willis T.V. (2004) A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 2255–2266.

Wilson R.S., Angilletta M.J., James R.S., Navas C. & Seebacher F. (2007) Dishonest signals of strength in male slender crayfish (*Cherax dispar*) during agonistic encounters. *American Naturalist*, **170**, 284–291.

Yoshida T., Goka K., Ishihama F., Ishihara M. & Kudo S. (2007) Biological invasions as a natural experiment of the evolutionary processes: introduction of the special feature. *Ecological Research*, **22**, 849–854.

(Manuscript accepted 23 March 2009)