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# Possible influences of plasticity and genetic/maternal effects on species coexistence: native *Gammarus fasciatus* facing exotic amphipods

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## Summary

**1.** Native species might be able to persist in the face of a detrimental exotic invader by occupying refuge habitats where the exotic is not successful. These refuges might then provide demographic subsidies that enhance persistence of the native species in areas of coexistence with the exotic species. Phenotypic plasticity of the native species could play a critical role in this process by allowing individuals dispersing from refuge to invaded habitats to adaptively modify their phenotypes, thereby enhancing the subsidy. By contrast, locally adapted genetic or maternal differences between native populations could result in fitness disadvantages for dispersers at invaded habitats, thereby reducing the subsidy.

**2.** We studied phenotypic variation in a native amphipod (*Gammarus fasciatus*) across ion gradients in Lac St. Louis, Québec, Canada. Ion gradients determine refuge vs. invaded habitats because native amphipods are found in both ion-poor and ion-rich habitats, whereas exotic (*Echinogammarus ischnus*) amphipods are found only in the latter.

**3.** We tested for plastic vs. genetic/maternal contributions to spatial variation (ion-rich vs. ion-poor habitats) in native amphipod fitness components: postmoult calcification, body size, larval survival, time to first reproduction and fecundity. We did so by comparing the following: (i) $F_0$ (collected from the wild) individuals between the two habitats – some of which were reared for a time under varying conditions in the laboratory; and (ii) $F_1$ (reared in the laboratory for their entire lives) individuals from the two habitats under a variety of water conditions.

**4.** We found strong plastic effects on postmoult calcification – it was more rapid in ion-rich water. We found genetic/maternal differences in time to reproduction (almost twice as long in ion-poor amphipods), fecundity and larval survival (higher in ion-poor amphipods) in early summer. These plastic effects and genetic/maternal differences are likely to be adaptive.

**5.** Plasticity and genetic/maternal effects both could influence the efficacy of ion-poor populations in providing demographic subsidies that aid native persistence at ion-rich habitats where exotic amphipods are present. These effects could thus have important consequences for native species persistence in the presence of non-native species.

**Key-words:** Crustacea, exotic invasion, local adaptation, maternal effects, phenotypic plasticity, spatial demographic subsidies, uninvaded refuges

## Introduction

Phenotypic variation among populations has the potential to influence ecological interactions at the population, community and ecosystem levels (reviews: Thompson 1997; Agrawal 2001; Fussmann *et al.* 2007; Pelletier, Garant & Hendry 2009; Post & Palkovacs 2009; Matthews *et al.* 2011). This phenotypic variation could be the result of plasticity, maternal effects or genetic differences, with these mechanisms likely producing different evolutionary (Ghalambor *et al.* 2007) and ecological (Urban *et al.* 2008) effects. Phenotypic plasticity is the ability of a genotype to

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change its phenotype in response to different biotic or abiotic environments. It can be adaptive (increases an individuals' fitness by enhancing their chances of leaving genes to succeeding generations) or nonadaptive (neutral with regard to an individual's fitness), active (regulated by the organism) or passive (not regulated by the organism), reversible or permanent and continuous or discontinuous (Agrawal 2001; West-Eberhard 2003).

Phenotypic plasticity is expected to predominate when (i) the environment experienced by a population is variable through time (Stomp *et al.* 2008; Svanbäck, Pineda-Krch & Doebeli 2009; Lande 2009; Schoeppner & Relyea 2009) or (ii) dispersal is frequent across spatially heterogeneous environments (Sultan & Spencer 2002; Richter-Boix, Llorente & Montori 2006; Lind & Johansson 2007; Hollander 2008; Lind *et al.* 2011; Thibert-Plante & Hendry 2011). By contrast, genetic differences are expected to predominate in spatial heterogeneous but temporally stable environments among which dispersal is somewhat limited (Endler 1986; Schluter 2000; Kawecki & Ebert 2004; Ghalambor *et al.* 2007). Maternal effects occur when, in addition to hereditary effects from the mother, the maternal phenotype affects the phenotype of her offspring. Maternal effects, which can be transgenerational and often adaptive (Galloway & Etterson 2007), are expected to be important in heterogeneous environments when environmental cues are predictable (Räsänen & Kruuk 2007). Of course, phenotypic differences among populations can reflect a combination of plastic, genetic and maternal influences, including genetic differences in plasticity (Agrawal 2001; Crispo 2008; Crispo *et al.* 2010). An arena where these various contributions to phenotypic variation are likely to be very important is in mediating interactions between species, such as native and exotic species (Strauss, Lau & Carroll 2006; Carroll 2008).

Our goal is to consider the plastic vs. genetic/maternal (we will not be able to distinguish these latter two) basis for phenotypic variation along a habitat gradient for a native species facing an exotic invader. Plasticity and local adaptation have been shown to be important for the spread of exotic species along environmental gradients (Parker, Rodriguez & Loik 2003; Dybdahl & Kane 2005; Pichancourt & van Klinken 2012). As a reaction to exotic invasion, native species can have adaptive phenotypic responses – whether plastic, genetic or maternal – that then mitigate the otherwise detrimental effects, such as in native prey facing an invasive predator (McIntosh & Townsend 1994; Freeman & Byers 2006; Fisk *et al.* 2007; Latta *et al.* 2007; Edgell *et al.* 2008). As another possibility, adaptive phenotypic variation in a native species along an environmental gradient (e.g. temperature or dissolved oxygen) might influence its persistence throughout the gradient if the exotic is successful along only part of that gradient (Strauss, Lau & Carroll 2006; Chapman & McKenzie 2009). This idea rests on the possibility that the uninvaded (refuge) habitats can provide demographic subsidies that facilitate persistence of the native species in invaded habitats outside the refuge. Theoretical and empirical work has shown that spatial subsidization of individuals between source and sink habitats can influence population persistence (Levins 1969; Hanski & Gaggiotti 2004), competitive outcomes (Amarasekare & Nisbet 2001; Fox 2007; Long, Petchey & Holt 2007) and trophic interactions (Melian & Bascompte 2002; Mouquet, Hoopes & Amarasekare 2005; Orrock *et al.* 2008).

The importance of refuge habitats in providing spatial native demographic subsidies at invaded habitats could depend on the plastic, genetic and maternal contributions to adaptive trait variation in the native regardless of species interactions. A plastic basis for adaptive variation might mean that a native individual that disperses from a refuge to an invaded habitat could adjust its phenotype to improve the probability of establishment success at the invaded habitat – thus enhancing the subsidy. However, a genetic basis for adaptive variation might mean that such an individual would have low fitness at the invaded habitat – thus decreasing the subsidy. Maternal effects might have intermediate effects as they would require at least one generation before the adaptive change could be made. Only a handful of studies have considered the effects of plasticity vs. genetic/maternal effects on demographic consequences in natural populations (Parker, Rodriguez & Loik 2003; Dybdahl & Kane 2005; Pichancourt & van Klinken 2012). Our study is the first to consider these effects in the context of native species persistence in face of exotic invasion.

Amphipods (Crustacea: Amphipoda) are important invertebrates in freshwater ecosystems because they occupy diverse trophic positions, are an important food source for fish and are involved in energy and nutrient transfer (Covich & Thorp 2001). North American *Gammarus fasciatus* (Say, 1818) has been displaced by invasive Ponto-Caspian *Echinogammarus ischnus* (Stebbing, 1899) at many sites in the lower Great Lakes (Dermott *et al.* 1998; Van Overdijk *et al.* 2003; González & Burkart 2004; Haynes *et al.* 2005) since its introduction to North American waters in 1994 (Detroit River; Witt, Hebert & Morton 1997). However, the amphipod species assemblage in the upper St. Lawrence River at Lac St. Louis (Montréal, QC Canada; 45°N, 73°W) continues to be dominated by the native amphipod (Palmer & Ricciardi 2004; Kestrup & Ricciardi 2010). This spatial variation in exotic–native interactions appears to be mediated by the ability of the native amphipod to persist beyond the exotic species along a particular habitat gradient. Whereas native amphipods inhabit both ion-rich and ion-poor habitats, the exotic amphipod is restricted to ion-rich habitats (Palmer & Ricciardi 2004; Kestrup & Ricciardi 2010). In fact, dissolved calcium and ion gradients determine the relative abundance of the two species (Palmer & Ricciardi 2004; Kestrup & Ricciardi 2010), as well as interactions between them (Kestrup & Ricciardi 2009; Kestrup, Dick & Ricciardi 2011).

The biology of the native amphipod in Lac St. Louis suggests that at least some plasticity might be favoured along the habitat gradient. First, Lac St. Louis is characterized by high spatial environmental variation because the lake receives ion-rich water from the St. Lawrence River along its south shore (conductivity 305 μS cm$^{-1}$, calcium 36·3 mg L$^{-1}$, salinity 0·15‰) and ion-poor water from the Ottawa River along its north shore (conductivity 80 μS cm$^{-1}$, calcium 8·3 mg L$^{-1}$, salinity 0·04‰) (Fig. 1; Centre St-Laurent 2003). Second, the native amphipod is characterized by high gene flow. For instance, Hogg, de Lafontaine & Eadie (2000) found low levels of allozyme differentiation (Wright's $F_{ST} < 0{\cdot}03$) even across very large spatial scales in the Great Lakes St. Lawrence River system (>1500 km from Lake Superior to Quebec City). More directly, no genetic differences were observed between the north and south shores of Lac St. Louis (Hogg, de Lafontaine & Eadie 2000). Native adults and larvae attach to floating and uprooted macrophytes (Palmer & Ricciardi 2004) and can be transported among habitats within Lac St. Louis on this vegetation by wind and water currents. Third, temporal variation in ion concentration is high because the relative contribution of water from the St. Lawrence or Ottawa rivers varies seasonally and with precipitation – relative contributions from the latter are higher during spring floods (Centre St-Laurent 2003; Kestrup & Ricciardi 2010). All of the conditions expected to favour the evolution of trait plasticity were present for the native amphipod in Lac St. Louis.

Our objective was to test for plastic vs. genetic/maternal contributions to fitness components (larval survival, time to first reproduction, body size, fecundity and postmoult calcification) in the native amphipod from ion-rich and ion-poor habitats in Lac St. Louis. We tested for such effects by comparing the following: (i) field-collected $F_0$ amphipods between the two habitats and (ii) laboratory-reared $F_1$ individuals from the two habitats under a variety of different water conditions. The relative influence of plasticity vs. genetic/maternal effects in different traits could contribute to overall average fitness in the native amphipod and its system-wide coexistence with the detrimental exotic amphipod.

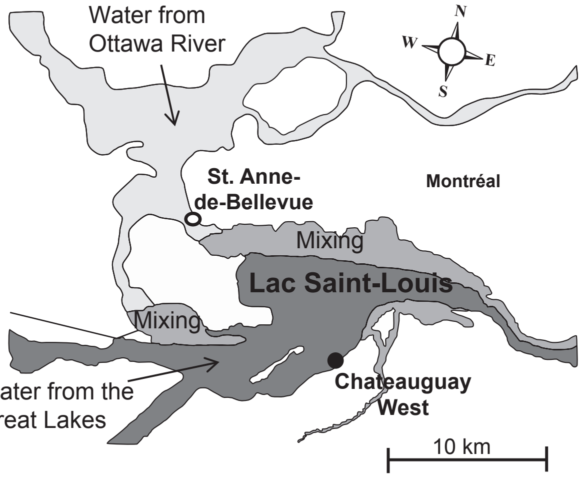

**Fig. 1.** Map of Lac St. Louis, Québec, Canada, with shades of grey indicating heterogeneous water masses with different ion concentrations (light grey: ion-poor water from the Ottawa River, dark grey: ion-rich water from the Great Lakes, intermediate shade of grey: zones of mixing and influence from tributaries entering the lake). Sites of amphipod and water collection for the experiment are indicated by the solid symbol (ion-rich habitat at Chateauguay West;45°21·80N, 73°47·20W) and the open symbol (ion-poor habitat at St. Anne-de-Bellevue; 45°24·141N, 73°56·786W). Water flows from west to east in Lac St. Louis, an embayment of the St. Lawrence River.

## Materials and methods

### FIELD

Live native amphipods (*G. fasciatus*) were collected from two habitats during the summer of 2009 in Lac St. Louis (Fig. 1). One collection habitat (Chateauguay West: 45°21·80N, 73°47·20W) was on the south-western shore and receives ion-rich water from the St. Lawrence River. The other collection habitat (St. Anne de Bellevue: 45°24·141N, 73°56·786W) was on the north-western shore and receives ion-poor water from the Ottawa River. The ion-rich habitat was sampled throughout the summer, and the ion-poor habitat was sampled at the beginning of the summer and in late summer because of logistical constraints (Table S1). $F_0$ females expected to produce $F_1$ offspring for the laboratory experiment were collected in early summer at both habitats (June; Table S1). Native amphipods were collected using a kick net and by lifting rocks and cobble. They were then stored in plastic zip-lock bags in coolers with ice until brought to the laboratory in the Department of Biology at McGill University, Montréal, QC, Canada. Temperature and conductivity of the water above the littoral river bottom were measured with a YSI 85 meter (YSI Incorporated, Yellow Springs, OH, USA). Water samples were collected in clean bottles and stored at 4 °C until analysed within 2 days of collection for dissolved calcium (LaMotte Model PHT-CM-DR-LT; Hoskin Scientific, Saint-Laurent, QC, Canada). Conductivity and dissolved calcium measurements from throughout the summer at both study habitats are summarized in Appendix S1. Since we used water from Lac St. Louis for the experiment, other factors aside from dissolved ions likely varied among study habitats (e.g. dissolved organic carbon). However, (i) ion concentration (in particular, dissolved calcium) varied dramatically between the north and south shores of the lake (Appendix S1) and was most relevant for our study, (ii) 100 μm filtration removed some biological variation (detritus and large crustaceans) and (iii) even if other factors contributed to our results, the use of actual river water rather than artificial media made the experimental conditions more realistic.

### COMPARISON OF $F_0$ AMPHIPODS

We compared potentially adaptive phenotypic traits of $F_0$ native females collected directly from the field between the two habitats (see Fig. 2a for experimental design). We measured body size (head length, mm: length from the tip of the rostrum to the rear edge of the head and dry weight biomass, mg), fecundity (clutch size per female) and postmoult calcification (% dry weight body Ca$^{2+}$). Head length was measured directly on field-caught female amphipods ($F_{0\text{-field}}$) from both habitats in early summer and was measured throughout the summer at the ion-rich habitat. Head length is often used to estimate body size in amphipods (Wilhelm & Lasenby 1998; Kestrup & Ricciardi 2010), and we measured it here with a Leica MZ 12.5 dissecting microscope (Leica Microsystems Inc., Concord, ON, Canada) equipped with digital software

**Table 1.** Physico-chemical characteristics of study habitats in Lac St. Louis during summer 2009. Sampling dates for amphipod generations in the experiments are indicated

| Habitat | Substrate | Sampling dates | Temperature (°C) | Conductivity (µS cm$^{-1}$) | Dissolved $Ca^{2+}$ (mgL$^{-1}$) | Amphipod generation |
|---|---|---|---|---|---|---|
| Ion-rich | Cobble | June 01 | 15·0 | 268·0 | 30·4 | $F_0$ & $F_1$ |
| | | July 29 | 23·5 | 262·0 | 33·6 | $F_0$ |
| | | September 05 | 22·3 | 269·6 | 32·8 | $F_0$ |
| Ion-poor | *Cladophora* sp. and submersed leaves | June 15 | 19·1 | 83·0 | 11·2 | $F_0$ & $F_1$ |
| | | September 20 | 16·0 | 77·3 | 11·2 | $F_0$ |

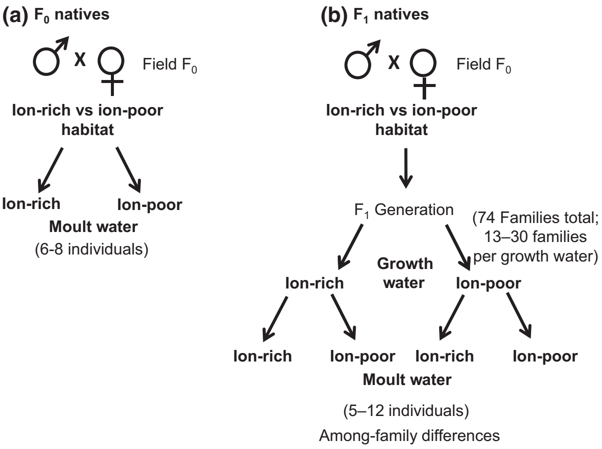

**Fig. 2.** Experimental design to test for plastic and genetic/maternal contributions to phenotypic variation in *Gammarus fasciatus* amphipods: (a) field-collected $F_0$ amphipods from the two habitats exposed to ion-rich vs. ion-poor moult water; (b) laboratory-reared $F_1$ individuals from the two habitats exposed under a variety of different growth and moult water conditions (ion poor vs. ion rich).

(QCapturePro). All other $F_0$ traits (dry weight biomass, fecundity and postmoult calcification) were measured on field-caught individuals that were held in the laboratory under experimental conditions ($F_{\text{0-field/lab}}$).

$F_{\text{0-field/lab}}$ female amphipods were caught in early and late summer at both habitats. Gravid individuals were placed into 200 mL containers with 1 gravid female per container and were incubated at 20 °C and on a 16 : 8 h light/dark photoperiod. We experimentally manipulated the water conditions that $F_{\text{0-field/lab}}$ female amphipods experienced by maintaining them in two types of 100-µm-filtered *moult water: ion rich vs. ion poor*. They were fed *ad libitum* catfish pellets (a mixture of plant and animal matter) and were monitored once every 24 h for larval production and the appearance of the shed exuviae, which indicated the onset of moulting. Fecundity was enumerated for $F_{\text{0-field/lab}}$ female amphipods collected from both habitats in early and late summer. Dry weight biomass at both habitats was measured only for late summer $F_{\text{0-field/lab}}$ female amphipods and was measured within 24 h post-larval release. Postmoult calcification was measured only for late summer $F_{\text{0-field/lab}}$ female amphipods. The appearance of exuviae following larval release is an indication of the onset of a postmoult stage, when there is uptake of dissolved $Ca^{2+}$ from surrounding water to rebuild the exoskeleton (Wright 1980; Greenaway 1985). We therefore performed lethal sampling within 24 h of this moult. Using acid-washed tweezers and wearing gloves, moulted females were rinsed in double-deionized water and dried at 65–70 °C for 24 h. They were cooled in a desiccator, and dry weight biomass (body size) was recorded to the nearest 0·1 µg with a Sartorius microbalance S4 (Sartorius, Cleveland, OH, USA).

Calcification (% dry weight $Ca^{2+}$) of postgravid females was quantified within 24 h of entering the postmoult stage. The samples were digested in 0·5 mL of concentrated nitric acid (Fisher Trace Metal Grade) at 95 °C for 2 h and then placed in a graphite digestion block equipped with a feedback temperature loop (DigiPREP; SCP SCIENCE, Baie D'Urfé, QC, Canada). To ensure quality of the sample preparation method, sample blanks and spiked samples (0·01–0·02 mg L$^{-1}$ $Ca^{2+}$) were digested simultaneously. Spike recovery was 100%. Amphipod digestions, blank controls and spike recovery samples were transferred to preweighed test tubes after cooling to room temperature. Deionized water (3 × 1 mL rinse) was used to rinse any remaining digestion, and this was added to corresponding test tubes. One hundred microlitres of 50% hydrogen peroxide ($H_2O_2$) was added to each test tube, 20 µL at a time. Test tubes containing digestion, rinses and hydrogen peroxide were weighed to 0·0001 g, and the mass of samples for $Ca^{2+}$ analyses was calculated by mass difference. Samples were analysed using a Trace Scan ICP-OES (Thermo) (inductively coupled plasma-optical emission spectroscopy) equipped with a mini-cross flow nebulizer. The calibration standards (0·5–5 ppm) were made from NIST traceable ICP-OES standards. A standard bracketing technique using three quality control standards was used to validate of the sample results.

### COMPARISON OF $F_1$ AMPHIPODS

To test for plastic vs. genetic/maternal differences between the two habitats, we compared potentially adaptive phenotypic traits of $F_1$ female amphipods raised under similar conditions in the laboratory (see Fig. 2b for experimental design). $F_1$ amphipod traits that were analysed included larval survival per brood at week 12, time to first reproduction in females, female body size at sexual maturity (dry weight biomass; mg), fecundity (clutch size per female) and postmoult calcification (% dry weight body $Ca^{2+}$). Replication was at the between-family level, achieved by averaging values across individuals within families. Low within-family sample sizes did not permit us to split clutches among water treatments.

$F_0$ female amphipods were collected in early summer, brought to the laboratory, were allowed to produce larvae and then those larvae (called $F_1$ amphipods) were then raised at 20 °C and on a 16 : 8 h light/dark photoperiod. During this period, we experimentally manipulated the water conditions that $F_1$ amphipod larvae experienced during birth, growth and reproduction (*growth water: ion rich vs. ion poor*). To produce this treatment, we used 100-µm-filtered river water from the ion-rich habitat and the ion-poor habitat. Care was taken to distribute $F_1$ amphipods among treatments of different growth water. $F_1$ amphipod larvae were separated from their mother as soon as they were detected. They were placed into 120 mL containers with three similarly sized pebbles per container, the same growth water treatment that they hatched into. Larvae from each family were grown together, and they remained in these conditions until sexual maturity. Every 5 days, water and food (*ad libitum* catfish pellets) were refreshed and the number of surviving larvae per family was enumerated. When sexual maturity was reached, gravid $F_1$ female amphipods were removed from their families and growth water and divided among individual 118 mL containers with either ion-rich or ion-poor 100-μm-filtered river water. This represented a second $F_1$ experimental water treatment (*moult water: ion rich vs. ion poor*) that was similar as described for $F_0$ amphipods $F_1$ gravid female amphipods were monitored once every 24 h for larval production (fecundity) and the appearance of the shed exuviae, which indicated the onset of moulting. As for $F_0$ amphipods, we performed lethal sampling within 24 h of this moult. Dry weight biomass and postmoult calcification were measured as described for $F_0$ amphipods.

### DATA ANALYSES

Statistical analyses were performed using STATISTICA 9.0 (Statsoft Inc., Tulsa, OK, USA) for almost all cases where data distributions met statistical assumptions following $\ln(x + 1)$ transformation (Kolmogorov–Smirnov test for normality, $P > 0{\cdot}05$; Levene's test for homogeneity of variances, $P > 0{\cdot}05$). Tukey HSD *post hoc* tests were subsequently used to test pairwise differences on observed means following ANOVA where significant interactions were detected. For the single case where the assumption of homogeneity in variance was violated [$\ln(x + 1)$-transformed $F_1$ time to reproduction; Levene's test for homogeneity of variances, $P > 0{\cdot}05$], we conducted a generalized least squares linear model to explicitly allow for heterogeneous variance with the nlme package in R (R Development Core Team 2010). Although we interpret a significant habitat effect in the $F_1$ experiment as indicative of genetic differences, we cannot rule out the possibility of a carry-over of maternal effects. Statistical findings are summarized in Appendices S3–S5.

### *Between-habitat comparisons of $F_0$ amphipods*

For $\ln(x + 1)$-transformed $F_{0\text{-field/lab}}$ fecundity, we used two-way ANCOVA with habitat and moult water (ion rich vs. ion poor) as experimental fixed factors, and $_{F0\text{-field/lab}}$ dry weight biomass as a covariate since it was positively related to $F_{0\text{-field/lab}}$ fecundity (Appendix S2). For $F_{0\text{-field/lab}}$ dry weight biomass and postmoult calcification, we used the same two-way structure but without a covariate (Appendix S3).

### *Seasonal changes in $F_0$ amphipods*

Seasonal changes in $\ln(x + 1)$-transformed $F_{0\text{-field}}$ head length at the ion-rich habitat were tested with one-way ANOVA with month (June, August and September) as a fixed factor (Appendix S4). We conducted a similar one-way ANCOVA to test for seasonal changes in $\ln(x + 1)$-transformed F0-$_{\text{field/lab}}$ fecundity at the ion-rich habitat with $F_{0\text{-field}}$ head length as a covariate because these variables were positively related (adjusted $R^2 = 0{\cdot}16$, $P < 0{\cdot}001$, linear regression). Seasonal differences in $F_{0\text{-field/lab}}$ fecundity between the two habitats were tested with a two-way ANOVA with habitat and time of summer (early vs. late) as fixed factors. We could not include a body size covariate in this latter analysis because our metrics for body size were different between early summer (head length) and late summer (dry weight biomass) for amphipods from the ion-poor habitat.

### *Between-habitat comparisons of $F_1$ amphipods*

For $\ln(x + 1)$-transformed $F_1$ amphipod fecundity, we used three-way ANCOVA with habitat, growth water (ion rich vs. ion poor) and moult water (ion rich vs. ion poor) as experimental fixed factors and $F_1$ dry weight biomass as a covariate since it was positively related to $F_1$ fecundity (Appendix S2). For $F_1$ dry weight biomass and postmoult calcification, we used the same three-way structure but without a covariate (i.e. ANOVA). For ln-transformed larval survival at week 12, we used two-way ANCOVA with habitat and growth water as fixed factors and initial $F_0$ family size [$\ln(F_0 \text{ fecundity} + 1)$] as a covariate to control for the positive dependence of clutch size at week 12 on initial clutch size (adjusted $R^2 = 0{\cdot}55$, $P < 0{\cdot}001$, linear regression). For time to first reproduction in female amphipods, we used two-way ANCOVA with habitat and growth water as fixed factors, and initial $F_0$ family size [$\ln(F_0 \text{ fecundity} + 1)$] as a covariate since time to first reproduction and total clutch size were positively related (adjusted $R^2 = 0{\cdot}24$, $P < 0{\cdot}001$, linear regression) (Appendix S5).

## Results

### BETWEEN-HABITAT EFFECTS IN $F_0$ AMPHIPODS

Differences were evident between $F_{0\text{-field/lab}}$ amphipods collected from the ion-rich and ion-poor habitat for body size (dry weight biomass) and postmoult calcification (Appendix S3, habitat main effect; Fig. 3). $F_{0\text{-field-lab}}$ female amphipods from the ion-poor habitat were smaller than those from the ion-rich habitat (Fig. 3a). Calcification was higher in ion-rich moult water compared with ion-poor moult water after moving $F_0$ amphipods to ion-rich moult water (Appendix S3, moult water main effect; Fig. 3b). Further, calcification at 24 h postmoult was higher in ion-poor origin $F_{0\text{-field/lab}}$ amphipods compared with ion-rich origin $F_{0\text{-field/lab}}$ amphipods (Fig. 3b). Ion-rich $F_{0\text{-field/lab}}$ amphipods were more fecund than ion-poor $F_{0\text{-field/lab}}$ amphipods, but after taking $F_{0\text{-field/lab}}$ body size into account as a covariate, this difference was only detected in ion-poor moult water (Appendix S3, habitat × moult water interaction; $P < 0{\cdot}001$, Tukey test, Fig. 3c).

### BETWEEN-HABITAT EFFECTS IN $F_1$ AMPHIPODS

Differences were evident between $F_1$ amphipods that originated from the ion-rich and ion-poor habitat for larval survival, time to first reproduction and fecundity and in $F_1$ amphipods (Appendix S5, habitat main effect; Fig. 4). $F_1$ amphipod larvae from the ion-poor habitat had higher survival per clutch than $F_1$ amphipod larvae from the ion-rich habitat (Fig. 4a). Further, ion-poor $F_1$ amphipods took longer to start to reproduce (Fig. 4b) and they had higher fecundity than ion-rich $F_1$ (Fig. 4c). No differences were detected in $F_1$ amphipods between habitats for body size or postmoult calcification (Fig. 4d,e). Water conditions during moulting, but not water conditions during growth or reproduction, determined % $Ca^{2+}$ body content because as for $F_0$ amphipods, $F_1$ female amphipods had more $Ca^{2+}$ when they moulted in ion-rich water compared

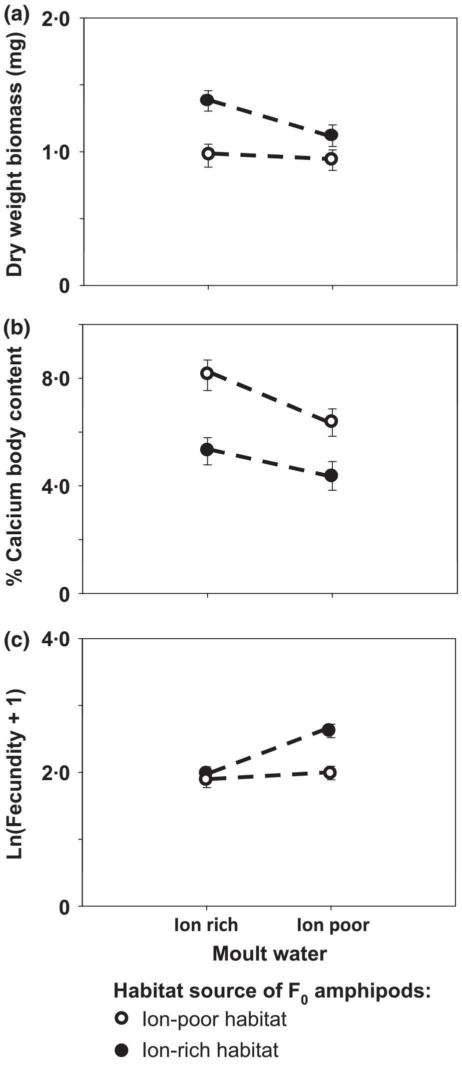

**Fig. 3.** Traits (adjusted least square mean ± standard error) of $F_{0\text{-field/lab}}$ *Gammarus fasciatus* originating from the ion-rich habitat (solid symbols) and the ion-poor habitat (open symbols) incubated in ion-rich or ion-poor moult water (circle symbols and dashed lines): (a) body size (dry weight biomass, mg); (b)% calcium dry weight biomass after 24 h postmoult; (c) fecundity.

with ion-poor water (Appendix S5, moult water main effect; $P < 0{\cdot}001$, Tukey test; Fig. 4e).

## Discussion

Plasticity and maternal/genetic effects may play a special role in enhancing spatial subsidies of dispersing individuals from uninvaded refuges at invaded habitats. We detected plastic and genetic influences on the traits of native amphipods in Lac St. Louis, QC, Canada. In the following sections, we discuss plastic responses to ion concentration, genetic/maternal differences between amphipods from ion-rich and ion-poor habitats and the adaptive significance of these influences. We also discuss the potential implications of plasticity and genetic/maternal effects for native–exotic amphipod coexistence, as well as the potential role of seasonal variability. Overall, we advance a hypothesis for how native trait plasticity and genetic/maternal effects at uninvaded refuges could influence native species persistence in face of exotic invasion along environmental gradients through spatial subsidies. While the most successful invaders tend to be generalists (Romanuk *et al.* 2009) that can displace specialist natives in many cases (Sax *et al.* 2007), our hypothesis suggests that the converse can also be true – generalist natives can persist with more specialized invaders when spatial demographic subsidies are enhanced by plasticity and genetic/maternal effects across environmental gradients.

### PLASTIC RESPONSES TO ION CONCENTRATION

Plastic trait responses in native species are potentially important for coexistence with exotic species because native individuals dispersing from uninvaded refuges might be able to adaptively adjust their phenotype and thus increase their success in invaded habitats (Strauss, Lau & Carroll 2006). We anticipated the possibility of high trait plasticity in *G. fasciatus* from the upper St. Lawrence River because of high gene flow and high spatial and temporal environmental variation (see Introduction). This expectation was confirmed for one fitness component: calcification. In particular, short-term experimental exposure to water with different ion concentrations during post-gravid moulting strongly determined $Ca^{2+}$ content in both the $F_{0\text{-field/lab}}$ and $F_1$ generations (Figs 3b and 4e). This finding is supported by studies of $F_0$ amphipods from other systems (Meyran 1997, 1998). These results provide evidence for plasticity in postmoult calcification, which is likely a passive response to differences in dissolved calcium availability among habitats.

Although plasticity clearly plays a role in postmoult calcification, we also found that field-collected amphipods from the ion-poor refuge recalcified faster than did amphipods from the ion-rich habitat for a given ambient ionic level. This latter finding provides evidence for a pattern of counter-gradient variation, in which ion-poor source amphipods outperformed individuals from the ion-rich habitat across ion water concentrations. Counter-gradient trait variation is often indicative of local adaptation for physiological traits and occurs when genetic and environmental influences on a phenotype have opposing effects (Conover, Duffy & Hice 2009). However, it is unclear if genetic factors influence counter-gradient variation of postmoult calcification because this pattern was not evident for laboratory-reared amphipods in our study. Plasticity in postmoult calcification likely increases the overall fitness of native amphipods along ion gradients within Lac St. Louis (see more details below) and could be important for the maintenance of native populations in ion-poor uninvaded refuges as well as for their continued existence in face of exotic amphipods at invaded habitats.

### GENETIC/MATERNAL DIFFERENCES ALONG ION GRADIENTS

Genetic/maternal differences among populations of native species have potential consequences for coexistence with exotic species because local adaptation should increase relative fitness in home habitats and might thus decrease demographic subsidies between habitats. In particular, individuals dispersing from refuge habitats into invaded habitats might have reduced success because they are poorly adapted for the new environmental conditions (Urban 2006). In the speciation literature, this process whereby dispersers have reduced fitness owing to maladaptation is called ‘selection against migrants’ (Hendry 2004) or immigrant inviability (Nosil, Vines & Funk 2005). Despite these theoretical possibilities, we did not expect to find extensive local adaptation/maternal effects in amphipods from Lac St. Louis because extensive gene flow and spatiotemporal environmental variation would seem more conductive to plasticity (see Introduction).

Interestingly, however, we found between-habitat differences in three of five fitness components that we measured in $F_1$ amphipods. First, $F_1$ larval survival (as measured by week 12) was higher for ion-poor source larvae than for ion-rich source larvae (Fig. 4a). Second, the length of time to first reproduction was about twice as long for $F_1$ female amphipods from the ion-poor habitat as for those from the ion-rich habitat (Fig. 4b). Third, when $F_1$ females from the ion-poor habitat matured, their fecundity was higher than that of $F_1$ female amphipods from the ion-rich habitat (Fig. 4c). The pattern of longer time to maturity, greater larval survival and greater fecundity in amphipods from the ion-poor habitat suggests that different ionic conditions select for different solutions to the trade-off between growth and reproduction (see more details below). The differences that we observed across the ion gradient in Lac St. Louis could reflect some combination of genetic differences or maternal effects, as either can have effects in the first generation of rearing under common conditions (Kawecki & Ebert 2004; Räsänen & Kruuk 2007). More detailed experiments

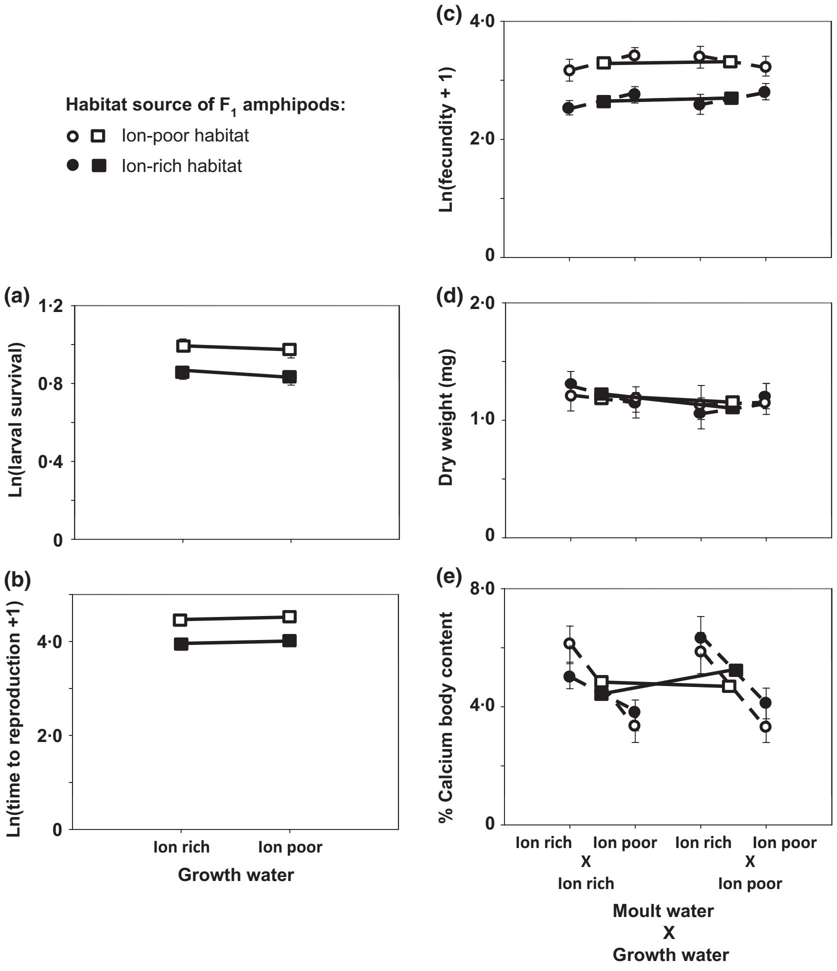

**Fig. 4.** Traits (adjusted least square mean ± standard error) of $F_1$ *Gammarus fasciatus* originating from the ion-rich habitat (solid symbols) and the ion-poor habitat (open symbols) incubated in ion-rich or ion-poor growth water (square symbols and solid lines): (a) larval survival at week 12; (b) time to first reproduction (days); (c) fecundity; (d) body size (dry weight biomass, mg); (e) postmoult calcification (% calcium (dry weight biomass) at 24 h postmoult). For fecundity, body size and postmoult calcification, $F_1$ was also exposed to a moult water treatment (ion rich vs. ion poor) (circle symbols and dashed lines).

involving additional generations under common rearing would be necessary to disentangle these two alternatives.

### ADAPTIVE SIGNIFICANCE

The plastic and genetic/maternal effects that we detected for native amphipods are likely adaptive in the ion gradients of Lac St. Louis. Adaptive interpretations are particularly clear for calcification. Freshwater crustaceans must rebuild their heavily calcified exoskeletons during regular moult cycles that allow for growth and reproduction (Cairns & Yan 2009). Faster postmoult calcification has important fitness benefits because a calcified exoskeleton reduces predation (Bailey *et al.* 2006) and osmotic stress (Zehmer, Mahon & Capelli 2002). We found that field-collected and laboratory-reared amphipods from both habitat sources recalcified their exoskeletons more quickly in ion-rich moult water than in ion-poor water. In addition, field-collected amphipods from the ion-poor refuge recalcified faster than did amphipods from the ion-rich habitat. The elevation of postmoult calcification rate in amphipods at the ion-poor habitat may be maintained to counteract negative fitness consequences of ion-poor environments for traits such as growth and development rates (Conover, Duffy & Hice 2009). Thus, amphipods are able to recalcify more quickly when ambient calcium levels are higher, and amphipods from ion-poor habitats are able to recalcify more quickly for a given ambient ionic level.

Some of the genetic/maternal differences also have reasonable adaptive interpretations. Longer development times (as measured by time to first reproduction) were seen in amphipods from ion-poor habitats, which were associated with slower individual maturity. This trait might be expected to cause a fitness trade-off owing to the lower number of cohorts that can be produced within a given north-temperate growing season. However, this possible trade-off between growth and reproduction could potentially be compensated by increased fecundity and higher larval survival in early summer at the ion-poor habitat. Also, faster postmoult calcification of amphipods at the ion-poor habitat may help to dampen negative effects of low ion concentration on growth and development time (Conover, Duffy & Hice 2009). It might seem surprising that adaptive differences can arise despite high gene flow, but an increasing number of studies are finding small-scale adaptive trait differences despite high gene flow (Blondel *et al.* 1999; Berner, Grandchamp & Hendry 2009; de León *et al.* 2010; Galligan *et al.* 2012).

Given that some plastic and genetic/maternal effects might be positive and others negative for overall average fitness, more work is required to understand the net effect of these multiple influences for native amphipods along ion gradients. Reciprocal transplant experiments might be particularly informative in this regard (Schluter 2000; Kawecki & Ebert 2004; Hereford 2009), such as common-garden environments testing for divergence in average values and reaction norms for ecologically important traits. A meta-analysis of empirical studies that have used this typical approach for studying costs and benefits of plasticity in different environments has found weak or no costs of plasticity on overall fitness (Van Buskirk & Steiner 2009). However, costs of plasticity may be small and difficult to detect because of biases associated with environment-specific responses and correlations between trait values and trait plasticities (Auld, Agrawal & Relyea 2010). Future work could be designed to evaluate how plasticity in metabolic compensation (postmoult calcification) potentially interacts with other traits to reduce possible fitness trade-offs in growth and reproduction across ion gradients, especially when exposed to multiple environmental factors.

### IMPLICATIONS FOR NATIVE–EXOTIC COEXISTENCE

Plastic vs. genetic/maternal sources of phenotypic variation in fitness components could be important for the overall average fitness of native amphipods across ion gradients, which could influence demographic subsidies based on native individuals dispersing from ion-poor refuge habitats to ion-rich invaded habitats. For instance, adaptive plasticity might increase the success of dispersers (Strauss, Lau & Carroll 2006), thereby increasing the abundance of native amphipods. In the present case, plasticity in postmoult calcification could improve the success of refuge individuals from ion-poor habitats following their arrival at ion-rich habitats. This effect should benefit dispersing native amphipods with respect to multiple predators, but it might be particularly relevant to their direct interaction with the exotic amphipods, which are known to be effective predators on postmoulting native amphipods in ion-rich water (Kestrup & Ricciardi 2009; Kestrup, Dick & Ricciardi 2011). We therefore predict that this aspect of plasticity in the native species could help to facilitate coexistence of the native and exotic amphipods at ion-rich habitats in the upper St. Lawrence River.

In contrast to plasticity, genetic/maternal differences would be expected to decrease the fitness of dispersers (Thibert-Plante & Hendry 2011; Weese *et al.* 2011). For instance, locally adapted/maternal variation in time to reproduction could decrease the fitness of ion-poor individuals dispersing to ion-rich habitats because it would allow fewer total cohorts during a north-temperate summer season. However, other genetic/maternal differences might increase the fitness of dispersers. For example, higher larval survival and fecundity in ion-poor amphipods could result in a greater ability of refuge individuals to resist the fitness declines otherwise expected on their arrival at invaded habitats. This result should not be surprising as a number of other instances are known where adaptive divergence between habitats can actually increase the fitness of dispersers relative to residents (Reznick, Rodd & Nunney 2004). Our results therefore imply that both plastic and genetic/maternal differences in different fitness components could influence the overall average fitness and efficacy of ion-poor populations in providing demographic subsidies that influence native amphipod persistence at ion-rich habitats where invasive amphipods are present. Plastic and genetic/maternal effects in amphipods from ion-poor refuges that are positive with respect to fitness at invaded, ion-rich habitats could therefore enhance the effect of demographic subsidies from refuges and increase the probability of native/exotic species coexistence at invaded habitats.

Future research directed towards understanding the relative influences of plasticity and genetic/maternal differences on native demographic subsidies could apply cohort-structured matrix population models (Pichancourt & van Klinken 2012) to estimate overall demographic effects of different plastic, genetic and maternal effects. In doing so, it will be important to determine whether the nonplastic effects, we documented are the result of genetic differences, maternal effects that do not persist beyond several generations (Räsänen & Kruuk 2007) or adaptive transgenerational plasticity (Galloway & Etterson 2007). All of these issues are important because they will influence not only immediate demographic subsidies but also global adaptive divergence in traits that could influence future demographic effects. Few other studies have addressed the global demographic benefit of plasticity and/or genetic changes in natural populations (Parker, Rodriguez & Loik 2003; Dybdahl & Kane 2005; Pichancourt & van Klinken 2012). Our study has thus suggested an initial step to addressing how native trait plasticity and genetic/maternal effects at uninvaded refuges could potentially influence native species persistence in face of exotic invasion along environmental gradients.

### SEASONAL VARIATION

Amphipods are known to exhibit inter- and intra-annual variation in body size and abundance in relation to age class, size-selective predation and food quality/quantity (Johnson & Wiederholm 1992). Thus, although the focus of our study was on spatial phenotypic differences, we also tested for seasonal differences that might influence native–exotic interactions. In particular, we detected seasonal differences in $F_0$ amphipod body size at the ion-rich habitat and in $F_0$ amphipod fecundity at both habitats (Appendix S4). Body size was seasonally variable at the ion-rich invaded habitat because $F_{0\text{field}}$ amphipods decreased in size from early to late summer (Appendix S6a). In early summer, $F_{0\text{-field/lab}}$ amphipods were larger at the ion-rich habitat than at the ion-poor habitat (Fig. 3a), a finding consistent with other studies of amphipods along ion gradients (Meyran 1997). We could not test for seasonal changes in body size of amphipods from the ion-poor habitat because our metrics for body size were different between early summer (head length) and late summer (dry weight biomass) for this habitat. In addition, $F_{0\text{field/lab}}$ fecundity decreased from early to late summer at the ion-poor habitat, but did not vary over the summer at the ion-rich habitat (Appendix S6b). $F_{0\text{-field/lab}}$ amphipods from the ion-poor habitat were more fecund in early summer than those from the ion-rich habitat, whereas the reverse pattern was evident in late summer (Appendix S6b).

Seasonal variation in amphipod traits could arise owing to differential dispersal among habitats (e.g. Garant *et al.* 2005), adaptive genetic variation through the season (Hendry & Day 2005), plastic cues or responses that vary with the seasons (Brakefield, Pijpe & Zwaan 2007) and seasonal differences in population maturity, food quality and quantity and size-selective predation (Johnson & Wiederholm 1992). Seasonal effects in native amphipods could influence their coexistence with exotic amphipods over the summer. For instance, the adaptive advantage of higher fecundity in early summer amphipods for native individuals dispersing from ion-poor refuge habitats to ion-rich invaded habitats (observed for both $F_{0\text{-field/lab}}$ and $F_1$ amphipods) likely ceases to exist by late summer when fecundity of $F_{0\text{-field/lab}}$ amphipods becomes diminished at the ion-poor habitat compared with the ion-rich habitat. We do not currently have a way to distinguish among different factors that can cause seasonal variation in amphipod traits and suggest that a profitable area for future research could examine seasonal effects more explicitly by collecting amphipods from different habitats on finer time scales.

## Conclusions

Our goal was to consider plastic and genetic/maternal contributions to trait variation in native *G. fasciatus* in relation to ion concentration and to interpret how this variation might influence spatial demographic subsidies from a refuge habitat into an invaded habitat where the native amphipod coexists with the exotic amphipod *E. ischnus*. We anticipated high trait plasticity in the native amphipod because of high gene flow and high spatial and temporal environmental variation. Fitting this expectation, we found plasticity in only one fitness component, postmoult calcification. However, we found that three other traits (larval survival, time to first reproduction and fecundity) were influenced by genetic variation or maternal effects that differed between ion-rich and ion-poor habitats. Our findings highlight the conservation value of ion-poor, un-invaded refuges that exotic amphipods cannot tolerate. Adaptive phenotypic variation in native amphipods potentially enhances the effect of ion-poor refuges in providing demographic subsidies of native individuals at invaded habitats where they coexist with exotic amphipods. Given that some plastic and genetic/maternal effects might be positive and others negative for overall fitness, more work is required to understand the net effect of these multiple influences on spatial demographic subsidies of native amphipods from refuge habitats into invaded habitats. If the net influence of plasticity and genetic/maternal effects is positive with respect to overall fitness at invaded habitats for native amphipods dispersing from refuge habitats, then the persistence of generalist natives with more specialized invaders will be enhanced.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Temporal variation in conductivity and dissolved calcium among sampling habitats.

**Appendix S2.** Positive linear relationship between female amphipod body size and fecundity.

**Appendix S3.** Statistical results for $F_0$ *Gammarus fasciatus* from ion-rich and ion-poor habitats in Lac St. Louis.

**Appendix S4.** Statistical results for seasonal patterns in $F_0$ *Gammarus fasciatus* from the ion-rich habitat and the ion-poor habitat in Lac St. Louis.

**Appendix S5.** Statistical results for $F_1$ *Gammarus fasciatus* originating from the ion-rich habitat and the ion-poor habitat in Lac St. Louis.

**Appendix S6.** Seasonal patterns in traits of $F_0$ *Gammarus fasciatus* originating from the ion-rich habitat (solid symbols) and the ion-poor habitat (open symbols) (adjusted least square mean ± standard error): (a) $F_{0\text{-field}}$ body size (head length, mm) at the ion-rich habitat in early (June), mid (late July) and late (September) summer; (b) $F_{0\text{-field/lab}}$ fecundity at the ion-rich and ion-poor habitats in early (June) and late (September) summer.