# The effects of interspecific interactions and environmental heterogeneity on the dominance of an invasive crustacean

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## ABSTRACT

The success and impact of an introduced exotic species may be highly variable across sites, owing to the influence of local abiotic and biotic factors. The upper St. Lawrence River is a physically heterogeneous ecosystem that has been invaded by several Ponto-Caspian species, including an amphipod crustacean (*Echinogammarus ischnus*), a benthic fish (the round goby *Neogobius melanostomus*), and two dreissenid mussels (*Dreissena polymorpha* and *D. bugensis*). *Echinogammarus* is replacing the native amphipod *Gammarus fasciatus* on rocky substrates in the lower Great Lakes, but in the St. Lawrence River the native species remains dominant at many sites ten years after invasion.

While Gammarus is a strictly freshwater species, Echinogammarus is adapted to ion-rich waters. A series of field and laboratory experiments tested the hypothesis that their relative dominance is dependent on local physicochemical conditions. These experiments revealed that both species are mutual predators whose differing physiological tolerances influence the magnitude and direction of predation, such that a dominant predator can reverse to an inferior predator along a conductivity gradient. Additional experiments compared the survival, growth and fecundity of both species across a range of conductivities. At low conductivity, growth of both species is reduced and Echinogammarus also suffers higher mortality. A further study examined predation by adults on the juveniles of opposing species at different conductivities. Contrary to expectations, Gammarus had a higher type II functional response than Echinogammarus in both levels of conductivity. This finding indicates the importance of considering different life stages when assessing the overall effects of intraguild predation. A final series of experiments revealed that amphipod species replacement is not influenced by their respective ability to compete for refugia. Competition for microhabitat on dreissenid mussel-covered substrates does not increase the native amphipod's vulnerability to goby predation. Echinogammarus is more vulnerable to native predators, but the round goby does not have a differential impact on the native amphipod. Thus, the outcome of antagonistic interactions involving these amphipods is mediated more by abiotic factors than by shared evolutionary history with co-occurring exotic species.

## RÉSUMÉ

Le succès et l'impact d'une espèce introduite exotique peuvent être hautement variables à travers des sites à cause des influences des facteurs abiotiques et biotiques locaux. Le Haut-Saint-Laurent est physiquement un écosystème fluviale hétérogène qui a été envahi par plusieurs espèces Ponto-Caspiennes, incluant un crustacé amphipode (*Echinogammarus ischnus*), un poisson benthique (la gobie à tache noire *Neogobius melanostomus*), et deux moules dreissenidae (*Dreissena polymorpha* et *D. bugensis*). *Echinogammarus* est en train de remplacer l'amphipode indigène *Gammarus fasciatus* sur les substrats rocheux des Grands Lacs inférieurs, mais demeure dominant dans le fleuve St-Laurent dans plusieurs sites dix ans après l'invasion.

Alors que Gammarus est une espèce strictement dulcicole, Echinogammarus est adaptée aux eaux riches en ions. Une série d'expériences en laboratoire et sur le terrain ont testé l'hypothèse que leur dominance relative est dépendante des conditions physicochimiques. Ces expériences ont démontré que les deux espèces sont des prédateurs mutuels dont la différence de leurs tolérances physiologiques influence la magnitude et la direction de prédation, de manière à ce qu'un prédateur dominant puisse changer vers un prédateur inférieur au long d'un gradient de conductivité. Des expériences supplémentaires ont comparé la survie, la croissance et la fécondité des deux espèces à travers une gamme de conductivités. En basse conductivité, la croissance des deux espèces est réduite, et *Echinogammarus* subit également une mortalité élevée. Une étude supplémentaire a examiné la prédation des adultes sur les juvéniles de l'espèce opposée à des conductivités différentes. Contrairement aux attentes, Gammarus avait une réponse de type II fonctionnel supérieur à celui de *Echinogammarus* dans les deux niveaux de conductivité. Cette découverte indique l'importance de prendre en compte les différentes étapes de vie lors de l'évaluation des effets totaux de la prédation au sein de la guilde. Une série finale d'expériences a démontré que le remplacement des espèces d'amphipode n'est pas influencé par leur capacité respective de compétitionner pour les refuges; la compétition pour les microhabitats sur les substrats couverts par les moules dreissenidae n'augmente pas la vulnérabilité de l'amphipode indigène envers la prédation du gobie. *Echinogammarus* est plus vulnérable aux prédateurs indigènes, mais le gobie à tache noire n'a pas d'impact différentiel sur l'amphipode indigène. Donc, les résultats des

interactions antagonistes impliquant ces amphipodes sont déterminés plus par les facteurs abiotiques que par l'histoire évolutionnaire partagé avec d'autres espèces exotiques cooccurrentes.

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## CONTRIBUTIONS OF AUTHORS

This thesis consists of four chapters prepared as individual manuscripts for publication in peer-reviewed scientific journals. I designed and executed the entire research presented herein, under the supervision of Dr. Anthony Ricciardi (Department of Biology and Redpath Museum, McGill University). I wrote each of the manuscripts in full. In addition to providing editorial criticisms, Dr. Ricciardi contributed significantly to the development of the ideas presented in this thesis, and the design and execution of the studies. Dr. Ricciardi is therefore listed as a co-author on all four manuscripts. Dr Jaimie T.A. Dick (School of Biological Sciences, Queen's University Belfast), contributed to the ideas discussed in Chapter 3 based on his expertise in amphipod invasions, and is thus a co-author of that manuscript.

Chapter 1 has been published in Biological Invasions, Chapter 2 has been accepted for publication in Fundamental and Applied Limnology, Chapter 3 has been submitted to Biological Invasions, and Chapter 4 has been published in Journal of Great Lakes Research.

## CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

This thesis is the first comprehensive attempt at testing the effect of an environmental gradient and biotic interactions on several aspects of invasion success of a euryhaline amphipod in an aquatic ecosystem. The thesis provides predictive information for forecasting the impact of this invader in freshwater ecosystems. The specific contributions of each chapter are listed below:

**Chapter 1** This study is the first to identify a reversal in the intensity and direction of intraguild predation along an abiotic gradient.

**Chapter 2** This study is the first to simultaneously compare the life history traits of an exotic and a native amphipod along a conductivity gradient.

**Chapter 3** This study is the first to compare the functional responses of a native and an exotic amphipod in different abiotic environments.

**Chapter 4** This is the first study to explicitly test if selective predation by an introduced predator facilitates the invasion by its coevolved prey.

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## **GENERAL INTRODUCTION**

#### **Ecological impacts of invasions**

The rate of biological invasions is increasing as a result of globalization (Cohen & Carlton 1998; Mack & Lonsdale 2001; Levine & D'Antonio 2003; Ricciardi 2007), homogenizing the earth's flora and fauna (Olden & Poff, 2003, 2004; Olden et al. 2004). Biological invasion – the spread of species beyond their native ranges – is the second largest threat to biodiversity after habitat destruction, followed by over-exploitation, disease, pollution, and climate change (Mace et al. 2005). The large-scale humanmediated spread of exotic species stems from intentional and unintentional introductions linked to trade and transport of humans and goods (Mack et al. 2000; Ricciardi 2006). In addition to their environmental effects, invasive species also have impacts on regional economies (Naylor 1996; Pimentel et al. 2001, 2005), and human health (Cash & Narasimhan 1999; Enserink 2007; Wang et al. 2008). Although many exotic organisms appear to cause no detectable changes to the invaded community, a subset of them have strong impacts resulting in population declines or local extinction of resident species through mechanisms such as predation, grazing, competition, habitat alteration, or a combination of factors (Williamson 1996; Mack et al. 2000; Hoddle 2004; Ricciardi & Kipp 2008).

The outcome of an invasion is a result of the interactions between the invader and its new environment, and is thus highly context dependent and difficult to predict (Parker 1999; Ricciardi 2003). The ecological impact of an invader at a given site is a function of its abundance, its ecological traits, and the composition and evolutionary experience of the recipient community (Parker *et al.* 1999; Ricciardi 2003). Depending on the strength and direction of these interactions, the invader can have a strong negative impact on resident native or exotic species resulting in the latter's exclusion, or the resident species may have a strong negative impact on the invader that inhibits the latter's establishment or population growth.

To date, few tools have been developed that enable us to predict the success and impact of aquatic invasions (but see Ricciardi & Rasmussen 1998; Kolar & Lodge 2002; Ricciardi & Atkinson 2004; Ricciardi & Cohen 2007; Romanuk *et al.* 2009). Thus, the best predictor of an invader's impact is its invasion history (Ricciardi 2003; Ward &

Ricciardi 2007). Niche modeling, which is based on the identification of a species' 'fundamental niche' (Hutchinson 1957) and links the functional traits of organisms and their environments, enables us to predict range limits and habitat suitability (Peterson & Robins 2003; Drake & Bossenbroek 2004; Herborg et al. 2007; Kearney & Porter 2009; Roura-Pascual et al. 2009). These models do not include interspecific interactions, which may restrict the "realized niche" of an invader (Herborg et al. 2007). In addition to determining the range limits of the invader, it is now widely recognized that the abiotic environment (e.g. temperature, substrate, salinity) also influences the local abundance and the 'performance' of native and exotic species (e.g. acquisition and allocation of energy and nutrients; Dunson & Travis 1991; Hunt & Yamada 2003; Piscart et al. 2005; Wijnhoven et al. 2003; McGill et al. 2006; Alcaraz & Garcia-Berthou 2007). However, if the per capita effect of an invader in a given abiotic environment is known, it would be possible to predict its ecological impact at sites that have not yet been invaded. This could be done by combining the per capita effect with estimates of the local abundance (Ricciardi 2003; Bollache et al. 2008). The per capita effect of the invader could thereafter be compared with that of functinally similar native and exotic species to identify invaders that might damage recipient communities (Ricciardi 2003; Bollache et al. 2008).

#### The role of species interactions: intraguild predation

Nevertheless, most studies examining the interspecific interactions resulting in invasion success or invasion failure of aquatic organisms have been conducted in an environment without abiotic gradients. If the interacting species respond differently to changes in the abiotic environment, i.e. when environmental conditions optimal to the invader differ from those optimal to the native species, it may affect the outcome of interspecific interactions (Dick & Platvoet 1996; Von Holle 2005; Alcaraz *et al.* 2008). The abiotic environment thus influences both the abundance and the per capita effect of the invader, both of which are linked to its impact. As such, the impact of an invader may vary across environmental gradients, being lower where conditions are suboptimal (Palmer & Ricciardi 2004; Jones & Ricciardi 2005; Alcaraz *et al.* 2008; Jokela & Ricciardi 2008).

The mechanisms driving these impacts are often poorly known. In recent years, the role of behavioural traits has received increasing attention in order to explain the impact of an invader (Holway & Suarez 1999). Direct negative interactions that have been frequently observed and linked to the impact (or, alternatively, the exclusion) of an invader include exploitation (e.g. predator-prey, herbivore-plant, and parasite-host interactions) and mutually antagonistic interactions (e.g. resource competition and interference competition). Predation is easier to measure as it can readily be seen, but the occurrence of competition is often more difficult to demonstrate, as resources may be limiting only under certain conditions. Competition has often been assumed to occur without being properly demonstrated, and in some cases research has later revealed that was never important (Dick 2008). While predation is typically observed across trophic levels, intraguild predation (IGP), defined as "the killing and eating of species that use similar, often limiting, resources and are thus potential competitors" (Polis & Holt 1992; Polis & Strong 1996; Polis et al. 1989), is common in nature, particularly among intermediate trophic levels (Arim & Marquet 2004), but is less frequently observed in species invasions (Dick & Platvoet 1996; Hunt & Yamada 2003; deRivera et al. 2005). IGP has been described as an extreme form of competition, where one species (the IG predator or "top predator") reduces competition for the shared resource while at the same time gaining energy from its competitor (the IG prey or mesopredator, Fig. 1).

In several cases, species that were previously assumed to compete for resources have instead been discovered to be intraguild predators, as competition and IGP may produce similar patterns. IGP includes traits from several community processes: apparent competition, food chains and exploitative competition (Holt & Polis 1997). The predatory interaction between the IG predator and prey can be classified according to its symmetry (Polis *et al.* 1989; Rosenheim *et al.* 1995): unidirectional, mutual symmetric and mutual asymmetric. In unidirectional IGP one species (A) is always the predator and one species (B) is always the prey at any site where the two species co-occur (Fig. 2a). In mutual asymmetric IGP both species prey on each other but A preys more on B than vice versa (Fig. 2b). In symmetric IGP, both species prey on each other equally at a given site (Fig. 2c). Moreover, age structure may be important when IGP is influenced by ontogenetic changes in size or vulnerability (Polis *et al.* 1989). While IGP has received a lot of

attention in terrestrial agricultural systems due to its negative impact on the success of biological control of agricultural pests (Rosenheim et al. 1995; Hoddle 2004), and in terrestrial natural systems in which it poses a challenge to conservation (Zavaleta et al. 2001; Muller & Brodeur 2002; Coomes et al. 2006), its role in invasions in aquatic systems has only recently been studied. However, there is increasing evidence that IGP is common in invaded aquatic communities, and it has been found to occur between exotic and native species as well as amongst exotic species (Dick & Platvoet 1996; Zavaleta et al. 2001). In aquatic systems, IGP has been identified as the mechanism behind species replacement and resistance to invasion within and across taxonomic groups. The introduced European green crab Carcinus maenas and the exotic Asian shore crab Hemigrapsus sanguineus both prey unidirectionally on juveniles of the native American lobster Homarus americanus, with C. maenas in addition being a superior competitor for molluscs (Demeo & Riley 2006; Rossong et al. 2006); C. maenas also preys unidirectionally on native shore crabs Hemigrapsus oregonensis (Grosholz et al. 2000; Jensen et al. 2002). However, on the North American Atlantic coast, the impact of C. maenas is being moderated by the more recently established *H. sanguineus*, which preys on juvenile European green crabs through unidirectional predation (Lohrer & Whitlatch 2002; Griffen *et al.* 2008). In the same area, the native blue crab *Callinectes sapidus* may be a natural control of the predatory Asian rapa whelks Rapana venosa through IGP on juvenile whelks (Harding 2003). In Japan, the introduced rainbow trout Oncorhynchus *mykiss* replaces the native masu salmon O. *masou* through complex size-structured mutual IGP among juveniles (Taniguchi et al. 2002). The exotic eastern mosquitofish Gambusia affinis and the exotic green sunfish Lepomis cyanellus both prey unidirectionally on native aquatic predatory invertebrates, which may result in increased abundances of mosquitoes, their shared prey (Blaustein 1992). The exotic ctenophore *Mnemiopsis leidyi* feeds on zooplankton and also preys on juveniles and eggs of the Black Sea anchovy *Engraulis encrasicolus*, which induced a collapse of the anchovy stock (Purcell et al. 2001).

IGP has repeatedly been identified as a mechanism behind species replacement among amphipods (MacNeil *et al.* 1997). In Europe, the Ponto-Caspian amphipods *Dikerogammarus villosus* and *Echinogammarus ischnus* (*=Chaetogammarus ischnus*) are

replacing the European amphipods *Gammarus roeseli* and *G. fossarum* through asymmetrical IGP (Kinzler & Maier 2003). D. villosus, identified as highly predatory, is also replacing the native *Gammarus duebeni* and the previously established North American amphipod Gammarus tigrinus through uni-directional IGP (Dick & Platvoet 2000; Dick et al. 2002; Bollache et al. 2008). In Ireland, the introduced Gammarus pulex is replacing the native Gammarus duebeni celticus through asymmetrical IGP (Dick et al. 1993, 1999), and replacing the previously established invader G. tigrinus (Dick 1996; Dick & Platvoet 1996). Invasion success of the North American amphipod Crangonyx pseudogracilis is reduced in North Ireland through IGP by the invaders G. pulex, G. tigrinus and the native G. duebeni celticus. In the same area, age-structured IGP allow the exotic amphipod G. tigrinus and the mysid shrimp Mysis relicta to coexist; while G. tigrinus preys unidirectionally on adult M. relicta, both species prey on each other's juveniles (Bailey *et al.* 2006). While there often is a substantial overlap in resource use among different species of amphipods regarding food and habitat, competition has been assumed but rarely demonstrated (MacNeil et al. 1997). Where it has been demonstrated, it involved interference competition for habitat which resulted in altered substrate preference (van Riel et al. 2007, 2009). Predation has thus been identified as an important interaction in species invasions involving ecologically similar species, and has so far been found among fish and crustaceans of the taxonomic groups amphipods and crabs in particular.

Invasion success may also depend on interactions with other species in the invaded community with ecological functions very differerent from that of the invader. Selective predation from a shared predator on either species may result in apparent competition (Holt 1977), which could influence the invader's success and impact (Dudas *et al.* 2005; Bollache *et al.* 2006; Kinzler & Maier 2006). Where the invader and native species compete for refugia, intereference competition can result in increased vulnerability for the weakest competitor to a shared predator (Balshine *et al.* 2005; Jensen *et al.* 2002; van Riel *et al.* 2007; van Riel 2008).

Furthermore, previously established invaders can influence the establishment success and impact of subsequent invaders. Contrary to the biotic resistance model, which predicts that a community will become increasinly more difficult to invade as it

accumulates invaders (Elton 1958), direct positive interactions among invaders are more common in the Great Lakes than purely negative interactions (Ricciardi 2001). Facilitation and synergistic interactions between invaders could make the ecosystem more easily invaded and enhance the impact of invaders, resulting in an "invasional meltdown" (Simberloff & Von Holle 1999).

The invader's vulnerability to predation is in many cases influenced by its evolutionary experience (Cox & Lima 2006; Parker *et al.* 2006; Banks & Dickman 2007). If the predator originates from the invader's native range, their shared evolutionary history may promote interactions that yield a net mutual benefit for both invading species (Simberloff & Von Holle 1999; Ricciardi 2001, 2005) and confer an advantage against native competitors.

#### Objectives

A predictive understanding of the impact of invasions can only be achieved by identifying the key factors and mechanisms that contribute to context-dependent variation in the effects of introduced species (Parker et al. 1999; Ricciardi 2003). Aquatic studies have begun to explore the moderating effect of the abiotic environment on the performance and impact of introduced species across environmental gradients; these species gradient studies include: the Eastern mosquitofish Gambusia holbrooki - salinity (Alcaraz & Garcia-Berthou 2007; Alcaraz et al. 2008), the European green crab Carcinus *maenas* – salinity, temperature and intertidal area:edge ratio (Hunt & Yamada 2003; DeRivera et al. 2005; Jensen et al. 2007), the Pacific oyster Crassostrea gigas - tidal height (Krassoi et al. 2008), Nuttallia obscurata – tidal height and sediment composition (Byers JE 2002), the estuarine mudsnail *Batillaria attramentaria* (Byers 2000), brown trout Salmo trutta and brook trout Salvelinus fontinalis - temperature/elevation (Taniguchi et al. 1998; Peterson et al. 2004) and the amphipods Gammarus tigrinus conductivity (Dick & Platvoet 1996) and depth (MacNeil et al. 2003a), Gammarus pulex and Crangonyx pseudogracilis – oxygen (MacNeil et al. 2000; MacNeil et al. 2004) and Dikerogammarus villosus – conductivity (Dick & Platvoet 2000). Several of these studies involve freshwater species.

My thesis concentrates on the linkages between species interactions and environmental heterogeneity to predict invasion success. My goals are to examine (1) how the post-establishment success (population growth and dominance) of an invading species is affected by interactions with other native and exotic species and how these interactions vary along a physico-chemical gradient, (2) the biotic factors that limit the dominance of the invader where the abiotic environment is optimal, and (3) if species replacement is mediated by positive interactions among invaders that share an evolutionary history, according to the popular invasional meltdown hypothesis, which has hardly been tested in aquatic systems.

#### Study system and organisms

Euryhaline organisms, especially species originating from the Ponto-Caspian region, have successfully invaded fresh and brackish water environments in North America and Europe, as they survive the harsh conditions in the ballast tanks of transoceanic vessels (bij de Vaate *et al.* 2002; Leppäkoski *et al.* 2002; Reid & Orlova 2002; Devin *et al.* 2005; Ricciardi 2006). The species that have invaded the Great Lakes are dominated by crustaceans and molluscs (Ricciardi 2006). The upper salinity tolerances of species have in some cases been examined to assess the likelihood of spread to more saline environments or survival in ballast tanks (Bailey *et al.* 2004; Berezina & Panov 2004; Ellis & MacIsaac 2009), but data on lower salinity tolerances are often scarce. However, this is of importance to predict the range expansion and impact of the invader once it has established.

Amphipods occur in a wide range of habitats in fresh, brackish and salt waters. They have been successfully introduced within and between continents as a result of intentional introductions to enhance fish production (Costello 1993; Berezina 2007), and unintentionally through man-made canals and in the ballast tanks of transoceanic vessels (Costello 1993; bij de Vaate *et al.* 2002). Species that are highly predatory have had adverse effects of the invaded communities (Dick & Platvoet 2000; Dick *et al.* 2002; Kelly *et al.* 2006). Their small size and tolerance to a broad range of environmental conditions makes them valuable model organisms to study the link between interspecific interactions and abiotic variables.

My work was conducted in the upper St. Lawrence River, a physically heterogeneous system that comprises Lake St Louis, a fluvial lake west of the Island of Montreal. Owing to the influx of ion-poor water from the Ottawa River and ion-rich water from the St. Lawrence River, the lake contains a strong gradient in conductivity that provided a natural laboratory for my work. During the 1990s, the system was invaded by a suite of euryhaline Ponto-Caspian species (Ricciardi 2001), which offers a valuable opportunity to study the interactions of a group of invasive species with a shared evolutionary history: a crustacean (the amphipod *Echinogammarus ischnus*), a benthic fish (the round goby Neogobius melanostomus), and dreissenid mussels (zebra mussel Dreissena polymorpha and the quagga mussel D. bugensis). I am focusing on *Echinogammarus ischnus* and its interaction with the North American amphipod Gammarus fasciatus, which it has replaced throughout the lower Great Lakes (Dermott et al. 1998). In contrast, E. ischnus rarely dominates the amphipod community in the upper St. Lawrence River (Palmer & Ricciardi 2005). The mechanisms by which E. ischnus is replacing G. fasciatus are not yet understood, although several hypotheses have been suggested, such as competition for food, with E. ischnus being identified as the most predatory of the two species (Gonzalez & Burkart 2004; Limén et al. 2005), and predation from fish (Palmer & Ricciardi 2005). Another question is why E. ischnus has not had a similarly large impact on G. fasciatus in the St. Lawrence River, more than ten years since its invasion of the river circa 1998 (Palmer & Ricciardi 2004, 2005). Because G. fasciatus is a strictly freshwater species (Bousfield 1958; Holsinger 1972) and E. ischnus is a euryhaline species adapted to ion-rich waters (Cristescu et al. 2004), the spatially heterogeneous concentration of dissolved ions in the river may mediate their interactions (Wijnhoven et al. 2003).

Therefore, my thesis examines the influence of physico-chemical variables, particularly conductivity, on (a) the variation in the outcome of interspecific interactions between the two competitors (Chapter 1), (b) life history traits that may determine the outcome of these interactions (Chapter 2) and (c) the predation rate that may determine the impact of the invader on other species in the invaded community (Chapter 3). In addition, my thesis is (d) testing the invasional meltdown hypothesis by examining differences among amphipod species in their vulnerability to predation from higher

trophic levels by a predator that shares an evolutionary history with the invader (Chapter 4).



Figure 1. Food web with intraguild predation (adapted from Polis et al. 1989).



**Figure 2.** The three different kinds of symmetry in intraguild predation: Unidirectional (a) Mutual asymmetric (b) and mutual symmetric (c). The thickness of the arrow indicates the intensity of the interaction between the Intraguild Predator (A) and the Intraguild Prey (B). Adapted from Polis *et al.* (1989).

## CHAPTER 1:

## ENVIRONMENTAL HETEROGENEITY LIMITS THE LOCAL DOMINANCE OF AN INVASIVE FRESHWATER CRUSTACEAN

Åsa M. Kestrup and Anthony Ricciardi. Environmental heterogeneity limits the local dominance of an invasive freshwater crustacean. Published in Biological Invasions (2009) 11: 2095-2105.

## Abstract

The impacts of an exotic species may vary along environmental gradients. We tested the hypothesis that site-specific heterogeneity in the physico-chemical environment alters the dominance of two competing amphipods: Gammarus fasciatus, the dominant native species in the upper St. Lawrence River, and Echinogammarus ischnus, a euryhaline Ponto-Caspian species that invaded the river in the late 1990s. E. ischnus has replaced G. *fasciatus* as the dominant amphipod at some sites, while remaining inferior at other sites - even several years after invasion. We tested the effect of water chemistry (conductivity) on the outcome of interactions between these two species in the laboratory and in the river in 2006 and 2007. Field experiments involved transplanting both species to different sites along a gradient of conductivity within the river, whereas the laboratory experiments examined interspecific predation in water collected from these sites. The laboratory experiments revealed that these species are mutual predators and their rate of predation on each other varies with conductivity; E. ischnus is the dominant predator at higher conductivity, while G. fasciatus dominates at lower conductivity. The field experiments showed interannual variation, but supported the lab results at high conductivity in 2006 and at low conductivity in 2007. E. ischnus was more sensitive than G. fasciatus to variation in conductivity, which apparently mediates this invader's ability to replace or dominate the native species in a heterogeneous environment.

## Introduction

There can be substantial spatial variation in the establishment success and population growth of an introduced species (Hunt &Yamada 2003; deRivera *et al.* 2005; Jones & Ricciardi 2005) and in its impact on native species populations (MacNeil *et al.* 2001; Ricciardi 2003; Melbourne *et al.* 2007). This site-specific dependence is driven largely by the physiological tolerance of the invader to local conditions, which can affect the outcome of its interactions with other species in the invader that is a superior competitor at one site may be an inferior competitor at other sites, depending on whether the physico-chemical conditions optimal to the invader differ from those optimal to its competitors (Dunson & Travis 1991; Hunt & Yamada 2003; deRivera *et al.* 2005). For example, water quality parameters such as conductivity (specific conductance) have been recently shown to mediate the outcome of invasions by freshwater crustaceans in Europe (Dick & Platvoet 1996; MacNeil *et al.* 2003a, 2004).

The aim of our study is to examine the influence of conductivity on the interactions between an invasive amphipod crustacean of Ponto-Caspian origin, *Echinogammarus ischnus*, and a North American amphipod, *Gammarus fasciatus*. *E. ischnus* is a successful invader of fresh and brackish waters in Europe (bij de Vaate *et al.* 2002; Kinzler & Maier 2003) and more recently in the North American Great Lakes, where it became established in the early 1990s (Witt *et al.* 1997; Dermott *et al.* 1998; Van Overdijk *et al.* 2003). By 1998, it had colonized the upper St. Lawrence River between Lake Ontario and the Island of Montreal (Palmer & Ricciardi 2004). In the Great Lakes, *E. ischnus* has replaced the previously dominant amphipod, *G. fasciatus*, on rocky substrate colonized by other Ponto-Caspian species: dreissenid mussels *Dreissena polymorpha* and *D. bugensis* (Dermott *et al.* 1998; Van Overdijk *et al.* 2003; Gonzalez & Burkart 2004; Haynes *et al.* 2005). By contrast, a decade after its discovery in the St. Lawrence River, *E. ischnus* rarely dominates amphipod communities even on mussel covered substrate (Å.K. pers. obs.; Palmer & Ricciardi 2004, 2005).

The hypothesized mechanisms of this species replacement include resource competition, interspecific (intraguild) predation and interspecific aggression (Gonzalez & Burkart 2004; Limén *et al.* 2005). Many species of amphipods engage in cannibalism and

interspecific predation (Conlan 1994; MacNeil *et al.* 1997, 1999; MacNeil & Platvoet 2005). An individual is particularly vulnerable to predation during moulting (Dick *et al.* 1999); this vulnerability can be influenced by abiotic conditions that deviate from the conditions to which the species is optimally adapted (Dick & Platvoet 1996). However, there is contrasting evidence regarding the mechanisms of dominance and species replacement involving *E. ischnus* and *G. fasciatus* (Dermott *et al.* 1998; Van Overdijk *et al.* 2003; Limén *et al.* 2005), and thusfar no studies have examined the influence of conductivity on *E. ischnus* dominance. Although both amphipods have a freshwater ancestry (Barnard & Barnard 1983), *E. ischnus* is a euryhaline species adapted to fresh and brackish water environments with high conductivity and calcium concentration (Cristescu *et al.* 2004) and its activity is reduced in ion-poor water (Wijnhoven *et al.* 2003), whereas *G. fasciatus* is a strictly freshwater species (Clemens 1950; Holsinger 1972).

We evaluated the hypothesis that water chemistry mediates the impact of *E*. *ischnus* on *G. fasciatus*, and tested whether (1) interspecific predation is a mechanism by which *E. ischnus* dominates amphipod communities in the St. Lawrence River, (2) the magnitude and direction of interspecific predation between *E. ischnus* and *G. fasciatus* is influenced by conductivity, and (3) the mean body size and survivorship of both species varies across a conductivity gradient in the river.

#### Methods

A series of experiments was performed in the laboratory and at field sites in Lake St. Louis, a fluvial lake situated at the Island of Montreal where the Ottawa River joins the St. Lawrence River. The lake is chemically heterogenous because of the mixing of these two water masses (Ottawa River: conductivity 80  $\mu$ S cm<sup>-1</sup>; St. Lawrence River: conductivity 300  $\mu$ S cm<sup>-1</sup>) (Centre St-Laurent 2003). We selected three sites (Fig. 1) that had bottom substrate suitable for amphipods (cobble, sand, and rocks with *Dreissena* colonies) and which also differed in conductivity (Table 1) and in the relative abundance of the two amphipod species (Palmer & Ricciardi 2004); henceforth, these sites are referred to as the low (LC), intermediate (IC) and high conductivity (HC) sites. The LC site receives water mainly from the Ottawa River, the HC site receives water from the St.



**Figure 1.** Map of Lake St. Louis showing the collection site (CS) at PDM and the experimental sites with low (LC), intermediate (IC), and high conductivity (HC). The dashed arrows indicate low conductivity water flow from the Ottawa River and the full arrows indicate high conductivity water flow from the St. Lawrence River.

**Table 1.** Average (A) and range (R) of the values of the physico-chemical properties measured at the sites with low conductivity (LC), intermediate conductivity (IC) and high conductivity (HC) and the collection site (PDM). The number in brackets represents the number of sampling occasions. In 2007, pH was not measured at any site, and conductivity was not measured at PDM.

Site	Year	Conductivity (µS cm⁻¹)	$Ca^{2+}$ (mg l <sup>-1</sup> )	Temp (°C)	O <sub>2</sub> (%)	$O_2 (mg l^{-1})$	рН
PDM	2006	A: 273 (9)	A: 37.5 (5)	A: 25.3 (9)	A: 139 (7)	A: 9.1 (7)	A: 9.1 (7)
		R: 253-295	R: 32-42	R: 23.9-26.2	R: 115-155	R: 7.4-12.6	R: 8.8-9.4
	2007	-	-	-	-	-	-
LC	2006	A: 98 (10)	A: 13.9 (7)	A: 26.0 (10)	A: 119 (9)	A: 9.5 (9)	A: 8.8 (8)
		R: 87-107	R: 13-15	R: 22.7-27.5	R: 101-128	R: 7.6-12.9	R: 8.4-9.2
	2007	A: 110 (4)	A: 15.8 (2)	A: 24.3 (4)	A: 110 (4)	A: 9.2 (4)	-
		R: 83-113	R: 13-18	R: 19.9-27.0	R: 88-132	R: 8.2-10.3	-
IC	2006	A: 149 (8)	A: 23.1 (7)	A: 25.5 (8)	A: 109 (7)	A: 9.1 (7)	A: 8.4 (6)
		R: 121-175	R: 20-27	R: 21.9-27.3	R: 89-126	R: 8.2-11.2	R: 7.9-8.7
	2007	A: 203 (4)	A: 26.9 (2)	A: 24.4 (4)	A: 134 (4)	A: 11.2 (4)	-
_		R: 177-215	R: 21-33	R: 19.6-27.5	R: 82-153	R: 7.5-12.6	-
HC	2006	A: 286 (8)	A: 34.5 (7)	A: 24.0 (8)	A: 116 (8)	A: 9.8 (8)	A: 8.8 (6)
		R: 276-294	R: 32-35	R: 19.8-27.5	R: 94-135	R: 8.5-11.4	R: 8.5-8.9
	2007	A: 281 (4)	A: 34.2 (2)	A: 24.3 (4)	A: 127 (4)	A: 10.7 (4)	-
		R: 280-284	R: 33-35	R: 21.3-26.1	R: 109-150	R: 9.0-12.5	-

Lawrence River, whereas the water at the IC site is a mixture of water from both rivers. Water chemistry was sampled repeatedly at all sites to account for fluctuations in conductivity. The water was collected just above the river bottom in clean plastic bottles, and stored at 4°C in a refrigerator until analysed <3 days later (LaMotte Model PHT-CM-DR-LT). Water chemistry variables (pH, O<sub>2</sub>, conductivity, temperature) were measured *in situ* with an electronic meter (YSI# 85-25-FT). All amphipods used in the experiments were collected from Pointe-du-Moulin (PDM) at the southern end of Ile Perrot, an island immediately west of Lac St. Louis, where both species were found in high abundance (Fig. 1); thus, we avoided using individuals that may have adapted to different microenvironments.

Individuals were identified to species and sex, using the criteria of Witt *et al.* (1997), Holsinger (1972) and Clemens (1950). We measured the total straight length (i.e. from the base of the antennae to the tip of the telson) of a separate group of individuals from each site, to avoid exposing the experimental animals to additional stress. Our study was performed at sites not yet invaded by the round goby (*Neogobius melanostomus*), a Ponto-Caspian fish with a potentially strong predatory impact on amphipod abundance (Barton *et al.* 2005).

#### Survey of the relative abundance of the two amphipod species

The abundance of amphipods on rocky substrate at each site was estimated from samples of 10 *Dreissena*-covered rocks collected by snorkeling. This was done to identify natural patterns of the relative abundance of the two amphipods in relation to conductivity. Rocks were collected randomly from 1m depth on 26-30 July 2007, placed individually in double plastic bags while under water, stored in coolers and transported to the lab within 3 hrs. In the lab, all organisms were removed from the rocks. Amphipods were collected using a sieve with a mesh size of 0.5 mm, preserved in 70% ethanol, and later identified to species. Small juveniles that passed through the sieve were therefore not included, resulting in an underestimate of amphipod abundance. However, newly hatched juveniles are very difficult to distinguish as species, and including them would increase the risk of misidentification. The surface area of the rock was estimated from its three

orthogonal dimensions (length, width, breadth) by an equation that determines the area of an ellipsoidal shape (Dall 1979):

Surface area =  $\pi/3$  (length × width + length × breadth + width × breadth)

Given that amphipod abundance is correlated with *Dreissena* and macrophyte biomass (Palmer & Ricciardi 2004), both parameters were measured. Using an electronic balance, the wet weight of *Dreissena* was measured to the nearest 0.1 g, and the dry weight of *Cladophora* was determined after drying samples in an oven for 12 hr at 60°C. One-way ANOVAs with Tukey multiple comparisons post-hoc tests were performed to identify differences in abundance between sites. The data were log<sub>10</sub>-transformed prior to analysis to meet with assumptions of normality.

#### Laboratory experiment: Interspecific predation on females

Rates of inter- and intraspecific predation on females were examined in experimental arenas using water collected from the three sites. Preliminary experiments showed that the rate of intra- and interspecific predation among males was very low, while inter- and intraspecific predation by males on females was frequent; therefore only predation rates on females were examined in subsequent experiments. Experiments took place in August 2006 using only healthy, sexually mature males and non-gravid females found in precopula pairs collected at PDM (Fig. 1). Prior to the experiment, animals were stored in well-aerated tanks with source water and gravel from the collection site for >24 h provided with food in excess (Vallisneria americana and catfish pellets). Experiments were conducted in an environmental chamber (Lab-Line Environette) at a constant temperature (24°C) and with a cycle of 12h light: 12h darkness, appropriate for the time of the year. Experimental arenas consisted of glass vials (6 cm  $\emptyset$ ), with 2 ceramic filter tubes (1cm long, 0.6 cm  $\emptyset$ ) per vial serving as shelters, filled with 50 ml of source water (LC, IC or HC). Water used in the experiment was allowed to stand in the lab for 1 h prior to the onset of the experiment to ensure acclimation to room temperature. Food was not added to the experimental arenas. The observation time was limited to 12 h during the light cycle. The mortality of single females was used as controls (25 replicates per

species) to account for mortality due to stressors other than predation. Treatments (25 replicates per treatment) consisted of one female in combination with one male (native or exotic) or two males (1 native + 1 exotic). Any acts of ongoing cannibalism or predation and unobserved deaths were recorded at 30 min, 1 h, 2 h, 4 h and 12 h after the initial introduction of amphipods. However, as both species were predators and scavengers, the different causes of mortality are treated as total mortality. Statistical comparisons of raw data were done with the Fisher Exact test (one-tailed).

#### Field experiment: interspecific predation along a conductivity gradient

To examine if the results from the lab experiment could be extrapolated to the field, field transplants were performed in 2006 and 2007. These involved single-species treatments with either 12 E. ischnus or 12 G. fasciatus, and mixed-species treatments with 6 individuals of each species (6 replicates per treatment). Single-species treatments were used to assess differences in the tolerance to abiotic conditions, mortality and the occurrence of cannibalism, while mixed-species treatments were used to examine the rate of interspecific predation. Bioassay tubes (18 in total per site) containing individuals collected at PDM were deployed at 1 m depth at each of the three field sites. In 2006, the experiments were started on July 25-27 and ran for 7 days, whereas in 2007 they were started on July 19-20 and ran for 4 days. Individuals were collected 1 day prior to the onset of the experiment and stored overnight in well-aerated aquaria with source water and food in excess. Only healthy, sexually mature males and non-gravid females were used. The proportion of females and males was random in 2006, whereas equal sex ratios were used in 2007. Bioassay tubes were PVC pipes (length 20 cm,  $\emptyset$  5 cm) with a single terminal opening covered with two layers of nylon mesh of 1 mm pore size; the opposite opening was sealed with a PVC screw cap. Pre-trials indicated that two layers of mesh of this size were required to prevent amphipods from escaping, but still allowed the free movement of water and prevented clogging by silt and debris. Each tube contained two catfish food pellets and two 20 cm strands of eelgrass (V. americana). Triplets of tubes (one with each treatment) secured to two bricks were placed randomly within 1 m of each other throughout each site. Survival of native and exotic species in single-species tubes at the different sites was compared in one-way ANOVAs and Tukey multiple comparisons

post-hoc tests. Survival in mixed-species tubes was compared in paired t-tests with equal variances assumed, as was survival of natives and exotics in single-species tubes within a site.

#### Size measurement of males

Ion-poor water is more stressful to some species than others (Wijnhoven *et al.* 2003). To determine whether differences in mean body size influences which species is more vulnerable to predation in suboptimal conductivities, length measurements were made of individuals of *E. ischnus* and *G. fasciatus* from all three sites in late July 2007. Only males were measured, because they are responsible for predation (Å.K. pers. obs.; MacNeil *et al.* 1997). The body length, i.e. the base of the antennae to the tip of the telson when straightening the back of the animal, was measured to the nearest 0.5 mm using a ruler under a dissection microscope. Males were identified according to Clemens (1950) and Witt *et al.* (1997). The samples from the IC site contained too few male *E. ischnus* to obtain significant results in July, therefore amphipods of both species were collected again from the three sites on October 22, 2007. The size of *G. fasciatus* at the different sites was compared in one-way ANOVAs with a Tukey multiple comparisons post-hoc test, whereas the size of *E. ischnus* was compared across different sites and with co-occurring *G. fasciatus* in independent-samples t-tests with equal variances assumed, unless otherwise indicated.

All analyses were done using SPSS 12.0 for Windows statistical software (release 12.0.0).

#### Results

#### Relative abundance of the amphipod species in relation to conductivity

The native amphipod *G. fasciatus* was more abundant at the IC and HC sites than at the LC site ( $F_{2,27}$ =20.7, p<0.001; Fig. 2). By contrast, *E. ischnus* was abundant only at the HC site, and insignificant or absent at the other sites ( $F_{2,27}$ =16.3, p<0.001). The low abundance of *E. ischnus* at the IC site was not driven by either low *Dreissena* biomass or overgrowth of *Cladophora*, as *Dreissena* was most abundant at this site ( $F_{2,27}$ =6.7,

p=0.004; Fig. 3) and *Cladophora* was absent ( $F_{2,27}$ =58.2, p<0.001; Fig. 4). Furthermore, *E. ischnus* was found at high abundances at the HC site, despite lower *Dreissena* biomass and overgrowth of *Cladophora*.

#### Laboratory experiment: Interspecific predation on females

In August 2006, the total lengths of G. fasciatus males and females that were collected at PDM and used in the experiments were 7.2–9.0 mm and 5.7–7.8 mm, respectively. E. *ischnus* males and females were 8.0–9.1 mm and 5.5–7.5 mm in length, respectively. In the controls, the mortality of single E. ischnus females was higher than the mortality of G. fasciatus females only in HC water (p<0.001; Fig. 5). Both species acted as cannibals, interspecific predators and scavengers – as indicated by our observations of amphipods attacking carcasses of individuals that had died from causes other than predation. Missing bodies could therefore not be interpreted as being the result of predation only. Instead, death rate due to predation was measured indirectly as an increase in death rate compared to single-species treatments. In IC water, there was an increase in the mortality of exotic females in the presence of native males (p=0.037), but not of native females (Fig. 5, Table 2). In HC water, the pattern was reversed and the mortality of native females was increased in the presence of E. ischnus males (p=0.0048). At low conductivity, there was no evidence of increased mortality of females of either species in the presence of males. Neither of the observed increases were significant at the Bonferroni-corrected alpha level of 0.003.

#### Field experiment: interspecific predation along a conductivity gradient

In 2006, we retrieved all but one replicate of the single-species treatment of each species at the HC site and all but one replicate of the mixed-species treatment at the IC site. In single-species treatments, survival of *E. ischnus* was lower at the LC site than at the IC and HC sites, whereas there was no difference between IC and HC sites ( $F_{2,14}=8.7$ , p=0.004; Fig. 6a). The native amphipod *G. fasciatus* survived equally well at all sites ( $F_{2,14}=0.8$ , p=0.5). The survival of both species was similar at the IC and HC sites, and differed only at the LC site (t=4.5, p=0.001). In mixed-species treatments, survival of *G. fasciatus* was lower at the IC and LC sites,



**Figure 2.** Abundance of *G. fasciatus* (open diamonds) and *E. ischnus* (solid diamonds) at the three experimental sites in late July. Error bars denote 1 standard error. Different letters above the bars indicate statistical differences (p<0.001).



**Figure 3.** *Dreissena* biomass (g) per m2. Error bars denote 1 standard error. Different letters above the bars indicate statistical differences (p<0.02).


**Figure 4.** *Cladophora* biomass (g) per  $m^2$ . Error bars denote 1 standard error. Different letters above the bars indicate statistical differences (p<0.001).

Table 2. Statistical comparisons of mortality of females in controls and treatments at the low conductivity
(LC), intermediate conductivity (IC) and high conductivity (HC) sites. p, probability value from Fisher
Exact test. ns, not significant.

Site	<i>E. ischnus</i> female +	р	<i>G. fasciatus</i> female +	р
LC	E male	Ns	G male	ns
	E + G male	Ns	E + G male	ns
	G male	Ns	E male	ns
IC	E male	Ns	G male	ns
	E + G male	Ns	E + G male	ns
	G male	0.037	E male	ns
нс	E male	Ns	G male	ns
	E + G male	Ns	E + G male	ns
	G male	Ns	E male	0.0048



**Figure 5.** Mortality of females in combination with 0, 1 or 2 males in experimental treatments. *E. ischnus* mortality is represented by grey bars and *G. fasciatus* mortality by white bars.

there was no indication of increased mortality due to interspecific predation (t-tests, p>0.2).

In the second series of experiments, performed in July 2007, all replicates were recovered. *E. ischnus* survival varied across sites in single-species treatments ( $F_{2,15}$ =6.2, p=0.011; Fig. 7a); however, survival differed only between the HC and the LC sites. Again, there was no difference in *G. fasciatus* survival across sites ( $F_{2.15}$ =0.587, p=0.568), but the survival of *G. fasciatus* was lower than that of *E. ischnus* at the HC site (t=0.8, p=0.042). In mixed-species treatments, survival of *E. ischnus* was lower than that of *G. fasciatus* at the LC site (t=-4.5, p=0.007; Fig. 7b). There was no difference in survival between species at the IC and HC sites (t-tests, p>0.4).

#### **Relative size of male amphipods**

In July, *G. fasciatus* males were larger at the IC site than at the LC site, but there was no difference in size between the HC and the other sites ( $F_{2,77}=3.0$ , p=0.056; Fig. 8a). There were too few male *E. ischnus* to perform a similar comparison.

In late October, the size of both *G. fasciatus* and *E. ischnus* differed among sites  $(F_{2,54}=8.9, p<0.001, and t=-5.9, p<0.001, Fig. 8b)$ . *G. fasciatus* achieved its largest size at the IC site. *E. ischnus* was largest at the HC site (t=4.5, p<0.001). *E. ischnus* was smaller than *G. fasciatus* at both sites in which they co-occurred (IC: t=14.7, p<0.001, HC: t=2.4, p=0.025).

## Discussion

In our lab experiments, the rate and direction of interspecific predation varied with conductivity. Consistent with previous studies, males of the dominant species preyed almost exclusively on females of the inferior species and mortality was reduced in females guarded by a conspecific male (Dick *et al.* 1999; MacNeil *et al.* 1999). However, the contrasts are not very strong, and we found an increase in mortality only when comparing the survival of single females and females together with males. Male *E. ischnus* were more predaceous at high conductivity, whereas male *G. fasciatus* were more predaceous at high conductivity found that the vulnerability of an individual to predation is influenced by suboptimal abiotic conditions (Dick &



**Figure 6.** Number of survivors in field transplants in 2006 for (a) single species and (b) mixed species treatments. *E. ischnus* survival is represented by grey bars and *G. fasciatus* survival by white bars. In single-species treatments, survival of *E. ischnus* was lower at the LC than at the IC (p=0.007) and HC (p=0.009) sites, while survival of the native species did not differ among sites. In mixed-species treatments, survival of the native species did not differ among sites. Error bars denote 1 standard error.



**Figure 7.** Number of survivors in field transplants in 2007 for (a) single species and (b) mixed species treatments. *E. ischnus* survival is represented by grey bars and *G. fasciatus* survival by white bars. In single-species treatments, survival of *E. ischnus* was higher at the HC than at the LC site (p=0.008). In mixed-species treatments, survival of *E. ischnus* was lower than that of *G. fasciatus* at the LC site. Error bars denote 1 standard error.

Platvoet 1996; MacNeil *et al.* 2003a). However, to our knowledge, this is the first observation of a native crustacean replacing an invader as the superior predator along an environmental gradient. In previous studies of predatory interactions between native and exotic crustaceans, the impact of the superior species on the inferior species was reduced in unfavourable abiotic conditions, but the predator-prey relationship was not reversed (Dick & Platvoet 1996; Hunt & Yamada 2003; MacNeil *et al.* 2003a; deRivera *et al.* 2005).

In both years of our field experiment, survival of *E. ischnus* increased with conductivity in the absence of interspecific interactions, while survival of the native species did not change. This finding is consistent with the predominant occurrence of *E. ischnus* in ion-rich waters within its native range (Wijnhoven *et al.* 2003; Cristescu *et al.* 2004). Although both species tolerate fresh water, the conductivities at our IC and LC sites could be at the lower tolerance limit of the invader. In our field and laboratory experiments, the presence of *E. ischnus* increased the mortality of *G. fasciatus* at high conductivity. In contrast to the laboratory results, *G. fasciatus* had no negative influence on *E. ischnus* survival at the IC site. However, the native species had an antagonistic effect that resulted in a reduction in the survival of *E. ischnus* at low conductivity. There was interannual variation in the outcome of interspecific interactions; we found a negative effect of the exotic on the native amphipod only in 2006 and a negative effect of the native on the exotic amphipod only in 2007, suggesting that other external variables (e.g. temperature, oxygen) might contribute to the relative competitiveness of a species and its vulnerability to predation (cf. Wijnhoven *et al.* 2003; MacNeil *et al.* 2004).

The native amphipod occurred at all three field sites, but was found in lower abundances at the site with low conductivity. At this site, *E. ischnus* was absent; its abundance increased with conductivity at the other two sites. *E. ischnus* abundance is strongly influenced by both *Dreissena* and *Cladophora* biomass (Palmer & Ricciardi 2004). Although both species of amphipods can use *Cladophora* as food, *E. ischnus* is more susceptible to predation from fish on this substrate because of its conspicuous coloration (Gonzalez & Burkart 2004). Selective predation therefore excludes *E. ischnus* from becoming abundant on plants. However, despite more favourable conditions at the IC site regarding *Cladophora* and *Dreissena* biomass, *E. ischnus* was found in low



**Figure 8.** Average size of male *G. fasciatus* (open diamonds) and *E. ischnus* (solid diamonds) in (a) late July and (b) late October. Error bars denote 1 standard error. Different letters above the bars indicate statistical differences of p < 0.05.

abundance at this site, suggesting that these two variables are not driving the patterns found in our field survey.

At sites where the two species co-occurred, the native males were generally larger than the exotic males, but exotic males became larger with increasing conductivity. The reduction in size of *E. ischnus* in ion-poor water may be due to increased physiological stress that limits their growth (cf. Wijnhoven *et al.* 2003), possibly also affecting their predatory activities.

The impact of *E. ischnus* in North America is similar to that in its introduced range in western Europe, where it is a superior predator on other gammarid amphipods (Kinzler & Maier 2003). However, the negative impact of *E. ischnus* on *G. fasicatus* in the Great Lakes-St. Lawrence River system apparently occurs only in water of high conductivity. Low conductivity limits the range of environments that *E. ischnus* could potentially invade, but even where it becomes established its dominance may be further limited by interactions with *G. fasciatus* in areas where the physico-chemical environment is suboptimal to the invader.

Our results reveal the importance of considering physiological tolerances and variation in water quality when examining antagonistic interactions between native and exotic species. They support the hypothesis that increasing environmental heterogeneity facilitates species coexistence (Chesson & Huntly 1997; Chesson 2000) – a concept applicable to species invasions. A spatially heterogenous environment offers closely-spaced habitats suitable for either native or exotic species, or both, and is therefore more invasible than homogenous environments (Davies *et al.* 2005). Furthermore, heterogenous environments provide refugia for competitively inferior species in the presence of a dominant native or exotic species (Hunt & Yamada 2003; deRivera *et al.* 2005). Indeed, there is an increasing understanding of the role of habitat heterogeneity and biotic interactions on invasion success and impact (Davies *et al.* 2005; Mitchell *et al.* 2006; Melbourne *et al.* 2007), with examples that include bivalves and their various enemies (Reusch 1998; Reusch & Williams 1999; Byers 2002; Hunt & Yamada 2003; deRivera *et al.* 2005), fishes (Alcaraz *et al.* 2007), ants (Holway *et al.* 2002), and terrestrial plants (Von Holle 2005).

To our knowledge, this is the first experimental study in North America to test the role of water chemistry in regulating the impact of an aquatic invader. Our findings suggest that conductivity mediates the rate and direction of interspecific predation between the invasive amphipod *E. ischnus* and the native amphipod *G. fasciatus* in the St. Lawrence River. We conclude that interspecific predation is at least one mechanism by which *E. ischnus* is replacing *G. fasciatus* in the Great Lakes basin, but via this mechanism either amphipod may dominate, depending on local water chemistry.

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## **Connecting statement**

In the preceding chapter, I discovered that the intensity and direction of intraguild predation on adult females varies with conductivity, with the euryhaline Ponto-Caspian amphipod *E. ischnus* being the dominant predator in water with high concentrations of dissolved ions while being the inferior predator in water with low concentrations of dissolved ions. In the following chapter, I explore the influence of the abiotic environment on other aspects of the biology of the species, namely life history traits, in order to reveal additional mechanisms that contribute to creating the observed distribution pattern of *E. ischnus*.

## CHAPTER 2:

## INFLUENCE OF CONDUCTIVITY ON LIFE HISTORY TRAITS OF EXOTIC AND NATIVE AMPHIPODS IN THE ST. LAWRENCE RIVER

Åsa M. Kestrup and Anthony Ricciardi. Influence of conductivity on life history traits of exotic and native amphipods in the St. Lawrence River. Accepted for publication in Fundamental and Applied Limnology.

## Abstract

We compared the survival, growth and fecundity of the Ponto-Caspian amphipod Echinogammarus ischnus and the North American amphipod Gammarus fasciatus across a range of conductivities in a fluvial lake at the confluence of the St. Lawrence and Ottawa rivers (Quebec, Canada). Previous work has found that water chemistry (conductivity) regulates the direction and intensity of intraguild predation between these two species. Because *E. ischnus* evolved in ion-rich waters, we hypothesized that low conductivity has a negative effect on its life history traits, whereas G. fasciatus would be more tolerant. Consistent with this hypothesis, E. ischnus is abundant along the south shore of the lake in water of high conductivity (300  $\mu$ S/cm), but rare along the north shore where conductivity is subject to strong fluctuations (85-230 µS/cm). In laboratory experiments using water with low (108  $\mu$ S/cm), high (300  $\mu$ S/cm) and intermediate (50:50 mix) conductivities, both species showed reduced growth at low conductivity, but E. ischnus also suffered higher mortality. E. ischnus collected from both shores did not differ in size-specific fecundity, size at reproduction, or size of adults. On the north shore, E. ischnus was present in low abundance at one site and absent from another site where it had been recorded in previous years. We conclude that low conductivity negatively affects the growth rate and mortality of *E. ischnus*, which is apparently eliminated from the north shore during periods of strong influx of ion-poor water from the Ottawa River, but re-establishes populations temporarily when higher conductivity is restored.

## Introduction

Amphipods have successfully invaded fresh- and brackish water systems in North America and Europe (Jazdzewski 1980; Dermott et al. 1998; bij de Vaate et al. 2002), sometimes causing the decline or exclusion of native species (Dick & Platvoet 2000; Kinzler & Maier 2003). The invasion success and impacts of aquatic invaders vary through space and time, and studies have recently begun to relate this variation to local physico-chemical conditions (Costanzo et al. 2005; Thomsen & D'Antonio 2007; Alcaraz et al. 2008). Among amphipods, variation in the concentration of dissolved ions (conductivity) has been shown to govern whether species replacement or exclusion will occur, by regulating the intensity and direction of intraguild predation between native and exotic species (Dick & Platvoet 1996; Kinzler & Maier 2003; MacNeil et al. 2003a; Kestrup & Ricciardi 2009). However, to understand why a particular abiotic factor limits the success of an invader, we must examine the effect of the factor at different stages of its life history (Berezina & Panov 2004; Alcaraz & Garcia-Berthou 2007) and how its response may differ from that of native competitors (Kestrup & Ricciardi 2009). Very little is known about the influence of the abiotic environment on the life history traits of aquatic invaders, although dissolved ions (salinity or conductivity) are among the most commonly studied factors (Haynes & Cashner 1995; Work & Gophen 1999; Berezina & Panov 2004; Alcaraz & Garcia-Berthou 2007). Furthermore, to date, no study has simultaneously examined the influence of variation in water chemistry on the life history traits of an aquatic invader and the native species with which it is interacting.

A concentration of dissolved ions beyond the tolerance range of an aquatic organism results in osmotic stress (Sutcliffe 1984). Among crustaceans in particular, calcium is a key element for maintaining an impermeable membrane necessary for osmoregulation, especially at the time of moult (Zehmer *et al.* 2002), and is essential for growth of the exoskeleton and for regulating intracellular processes (Wright 1979). When exposed to levels of conductivity or calcium at the lower limit of their tolerance range, crustaceans may suffer reduced growth, lower reproduction and increased mortality (Rukke 2002; Zehmer *et al.* 2002; Jeziorski & Yan 2006; Ashforth & Yan 2008). Identification of such tolerance limits is therefore necessary for predicting (i) which

environments are susceptible to invasion by a crustacean and (ii) the conditions in which its performance and fitness (and thus impact) are maximal.

In this study, we examined the life history traits of native and exotic amphipods exposed to different concentrations of dissolved ions, to determine how two competing species differ in their response to local variation in water chemistry. The Ponto-Caspian amphipod *Echinogammarus ischnus* has replaced the North American amphipod *Gammarus fasciatus* throughout much of its invaded range in the North American Great Lakes (Dermott *et al.* 1998), but more than a decade after the invasion the two species coexist in Lake St. Louis, a fluvial lake near Montreal, Quebec, with strong gradients in the concentration of dissolved ions (Palmer & Ricciardi 2004). Although *E. ischnus* is a euryhaline species with a reported upper salinity tolerance of 23‰ and able to survive short-term exposure to highly saline water (30‰), its lower ionic tolerance is not known (Jazdzewski 1980; Ellis & MacIsaac 2009). This is of interest as *E. ischnus* has invaded lakes and rivers with salinities of <0.1‰ in Europe and North America (Jazdzewski 1980; Dermott *et al.* 1998; Palmer & Ricciardi 2004).

Here, we examine the role of low conductivity as a factor limiting the invasion success of *E. ischnus* and contrast this species' performance to that of *G. fasciatus*. Given that *E. ischnus* has evolved in ion-rich waters (Cristescu & Hebert 2005), while *G. fasciatus* is a freshwater species, we hypothesized that exposure to low conductivity would have a negative influence on the life history traits of the invader, but little or no influence on the life history traits of the native amphipod. Specifically, we predict that at low conductivity the invader would suffer from reduced growth and lower survival, and be less fecund (i.e. a lower proportion of adult females would be ovigerous, and ovigerous females would carry fewer eggs). However, if the individuals on both sides of the river have adapted to local water conditions, those in low-conductivity habitats may be more tolerant than individuals from high-conductivity habitats (Bijlsma & Loeschcke 2005).

## Methods

These hypotheses were tested in a combination of laboratory experiments and field observations using Lake St Louis as a study and collection site. A natural gradient of

conductivity exists in the lake because it receives ion-rich water from the St. Lawrence River along its south shore (conductivity 305 µS/cm, calcium 36.3 mg/L, salinity 0.15‰) and ion-poor water from the Ottawa River along its north shore (conductivity 80 µS/cm, calcium 8.3 mg/L, salinity 0.04‰) (Centre St-Laurent 2003). E. ischnus is abundant along the south shore and scarce along the north shore, where conductivity fluctuates greatly depending on the relative discharge of the two rivers (Å Kestrup, unpubl. data; Palmer & Ricciardi 2004). We used two study sites on the south shore (Chateauguay West, 45°21.80 N, 73°47.20 W and Chateauguay East, 45°22.52 N, 73°46.55 W, Fig. 1) and two sites on the north shore (Valois Bay, 45°26.37 N, 73°46.48 W and Lachine, 45°26.01 N, 73°41.17 W), where both species have previously been recorded. The natural fluctuations of conductivity and calcium concentration in the study system were observed by measuring these two parameters biweekly at all four sites from mid-May to mid-September 2008 (10 occasions). Water chemistry variables (O<sub>2</sub>, conductivity, temperature) were measured with an electronic meter (YSI 85 DO/Cond/Salinity Meter), while water for calcium measurements was collected just above the river bottom in clean plastic bottles, and stored at 4°C in a refrigerator until analysed <3 days later (LaMotte Model PHT-CM-DR-LT). The relationship between conductance and calcium concentration at the different sites was tested in least squares regressions.

#### Laboratory experiments

The survival and growth of juveniles of both species in different conductivity and calcium levels were tested in a laboratory experiment. Water with high conductivity and calcium concentration (HC) (conductance: 300  $\mu$ S/cm, calcium: 37.8 mg/L) was collected from Chateauguay East on the south shore, and water from the Ottawa River (LC) with low conductivity and calcium concentration (conductance: 107.7  $\mu$ S/cm, calcium: 16.8 mg/L) was collected from a site on the north shore at the western end of the Island of Montreal (Place St Louis, 45°25.03 N, 73°52.68 W). During late July and early August 2008, ovigerous females and precopula pairs of both species were collected from Chateaguay West. In Lachine on the north shore, only native females were collected, as very few ovigerous *E. ischnus* females were encountered. In the laboratory, the precopula pairs were allowed to mate and the ovigerous females were placed in individual trays



Figure 1. Sites for collection of amphipods and sampling of water chemistry.

(400 mL) of filtered water: initially HC water for those from the south shore and LC water for those from the north shore. All trays were kept in a chamber at constant temperature (20°C) and a light regime of 15:9 hrs light:dark. The amphipods were exposed to the following treatments: 1) E. ischnus in HC, IC or LC water; 2) G. fasciatus from the south shore in HC, IC or LC water; and 3) G. fasciatus from the north shore in LC water. The last treatment served to test if survival of G. fasciatus individuals from the south and north shore differ in their tolerance to LC water. To acclimatize the females to low conductivity-conditions, two thirds of the females from the south shore were placed in a 50:50 mix of low- and high- conductivity water (IC). The following day, half of these females were placed in pure LC water. The trays were checked daily over a period of 11 days, and hatched juveniles were transferred to individual trays (118 mL) with filtered water of the same type as in the parental tray. The E. ischnus juveniles came from 26 females, while the G. fasciatus juveniles came from 17 females from the south shore and 5 females from the south shore. Animals were fed 'catfish food pellets' (a mixture of plant and animal matter) ad libitum. Five days after hatching, 30 juveniles of visibly good condition were chosen for each treatment. The head length (i.e. from the tip of the rostrum to the rear edge of the head) of each individual was measured using a Wild Heerbrugg stereo microscope with a micrometer evepiece. Head length was used as a proxy for the total body length (Gonzalez & Burkart 2004); we confirmed this relationship by measuring the head length and the total straight body length (tip of the rostrum to the tip of the telson) of 80 individuals of both species and found a strong positive correlation (linear regression:  $R^2=0.97$  for *E. ischnus* and  $R^2=0.94$  for *G. fasciatus*, both p<0.001). To prevent the amphipods from moving while being measured, they were placed on a piece of mesh (mesh size 63  $\mu$ m or 1 mm, depending on the size of the individual) in a Petri dish. The juveniles were grown for 6 weeks. Mortality was measured weekly and head length was measured every 2 weeks. Following measurement, the amphipod was transferred to a container with clean water and fresh food. After 6 weeks, the animals were preserved in 70 % alcohol, and both head length and total body length were measured immediately after preservation. Differences in growth between treatments (using head length) were analyzed in repeated-measures ANOVAs, one per species, with water type/origin as the fixed factor and head size as the repeated measure,

followed by Tukey's post-hoc test. Differences in the total length at the end of the experiment were tested in one-way ANOVAs. The specific growth rate (% length increase/day) per two-week period was determined and tested in a repeated-measures ANOVA with species, water and origin as fixed factors, and time as repeated measures. Individuals were used as replicates in the analysis. We did not control for juveniles coming from the same female. Differences in survival among treatments during the 5 days after hatching and during the experiment were tested using Chi-square analyses followed by the Marascuilo (1966) procedure to allow comparison of all possible pairs of proportions.

#### **Field sampling**

To examine growth rate and fecundity in situ, amphipods were collected from the two sites on the south shore and the two sites on the north shore on July 17-18 and August 7-8, 2008. Amphipods were collected using a variety of methods: Plastic trays (21 cm width, 34 cm length, 12 cm depth) filled to the brim with cobble and nylon mesh (1x1 m)attached to bricks were deployed at each site at 0.5-1 m depths and left undisturbed for 3 weeks to be colonized by amphipods. Rocks were also collected from each site on the sampling date in order to collect a minimum of 300 individuals per species per site. As all size classes of both species were collected using these methods, it is unlikely that differences in sampling techniques between sites may have affected the results. In the lab, all amphipods were stored in 70% alcohol and later identified to species. The total straight length of each individual (from the tip of the rostrum to the base of the telson) of all E. ischnus and subsamples of ca. 300 G. fasciatus per site was measured using a stereo microscope with an eye-piece micrometer. Individuals of lengths below 2.4 mm were not included in the study because species identification characters were not fully developed (although E. ischnus uropods are generally quite distinct, the inner rami on newly hatched G. fasciatus are difficult to discern). Females were identified by the presence of brood plates, and males by the presence of genital papillae. G. fasciatus individuals larger than 5.3 mm and *E. ischnus* individuals larger than 4.5 mm were classified as adults, based on the size of the smallest ovigerous female found in the samples. Females carrying eggs or hatched juveniles in their brood pouches were recorded as gravid. The number of eggs

per female was counted only if all eggs in the brood were intact (i.e. none had hatched). Our data on fecundity was compared with data collected in 1996 at sites in Lake Erie and western Lake Ontario (Dermott *et al.* 1998), where conductivity and calcium concentrations are relatively high (conductivity: 234-290µS/cm, and calcium 30-38 mg/L; R. Dermott, Great Lakes Laboratory for Fisheries, Burlington, Ontario, pers. comm.).

Storage in alcohol may cause reductions in the weight and size of aquatic invertebrates (Lasenby *et al.* 1994; Black & Dodson 2003; Wetzel *et al.* 2005), but this is unlikely to have biased our length measurements because all of the specimens we collected were measured consistently after a time interval of approximately 4 weeks since preservation.

The size-specific fecundity of females was analyzed in ANCOVAs with body length as the covariate, site as a fixed factor and the number of eggs per female as the dependent variable. Egg numbers were square-root transformed to meet the assumption of homogeneity of variances. Each species was analyzed separately. Where slopes were heterogenous, the Wilcox procedure was used to identify ranges of the covariate for which the group means were significantly different (significance level p<0.05). The partial fecundity index, defined as the mean brood size/female size, was also estimated for each species at the different sites and compared in one-way ANOVAs followed by Tukey's post-hoc test (Grabowski *et al.* 2007). Differences in the partial fecundity index of the two species at the sites where they co-occur were tested in independent samples ttests with Bonferroni correction. The size of males and females at the different sites were also compared in independent samples t-tests with Bonferroni correction. Deviation of the sex ratio from 1:1 was tested in a binomial test. Differences in the proportion of ovigerous individuals among adult females at the different sites were tested using Chisquare analyses followed by the Marascuilo procedure.

To estimate growth rate in the field, cohorts were followed between sampling dates. Cohorts were identified by Bhattacharya's (1967) method, using the software FiSAT II. Differences in growth rate between sites were tested in a one-way ANOVA (*G. fasciatus*) or an independent samples t-test (*E. ischnus*).

## Results

Conductivity remained high and fluctuated less throughout the season at the south shore sites (283-297.5  $\mu$ S/cm at Chateauguay West and 281.0-298.0  $\mu$ S/cm at Chateauguay East) than at the north shore (85-230.5  $\mu$ S/cm at Valois Bay and 90.9-202.7  $\mu$ S/cm at Lachine; Fig. 2). The calcium concentration was also higher at the south shore, but its fluctuations were of similar magnitude on both shores (28.4-38.8 mg/L and 27.2-40.8 at Chateauguay West and East, respectively, and 11.8-24.4 mg/L and 10.4-25.4 mg/L at Valois Bay and Lachine, respectively). Temporal variation in calcium concentration and conductance were positively correlated at the north shore sites (Valois Bay: p=0.010, R<sup>2</sup>=0.59, F<sub>1,9</sub>=11.43, Lachine: p=0.017, R<sup>2</sup>=0.53, F<sub>1,9</sub>=8.99), but negatively correlated at Chateauguay West (p=0.016, R<sup>2</sup>=0.54, F<sub>1,9</sub>=9.30), whereas there was no correlation at Chateauguay East (NS; Fig. 3).

#### Laboratory experiment

The eggs of both species hatched successfully to produce viable offspring in all three types of water. *G. fasciatus* juveniles hatched after 8-10 days and *E. ischnus* juveniles hatched after 9-11 days, following fertilization. Juvenile *E. ischnus* suffered higher mortality in LC water during the acclimatization period (the first 5 days after hatching), with a survival of only 61%, compared to 83% and 89% in the HC and IC treatments, respectively ( $\chi^2$ =11.8, df=2, p=0.002). By contrast, *G. fasciatus* survival was similar across treatments, 81-92% (NS). Throughout the experiment, the number of dead individuals increased gradually. After two weeks, mortality of *E. ischnus* in LC water was higher (>50%) than in the other treatments (HC-10% and IC-20%), while mortality of *G. fasciatus* was relatively low (17-30%) in all treatments. After 6 weeks, survival differed among treatments for *E. ischnus* ( $\chi^2$ =20.40, df=2, p<0.0001; Fig. 4a), whereas *G. fasciatus* survival did not differ among treatments ( $\chi^2$ =2.83, df=3, NS; Fig. 4b). The survival of *E. ischnus* was significantly lower (p<0.05) at LC (20 %) than at IC (60%) and HC (77%), while there was no significant difference in mortality at IC and HC.

Similarly, the growth (change in head length as a function of time) of *E. ischnus* (Fig. 5a) differed between treatments (rmANOVA,  $F_{2.44}$ =13.08, p<0.001), being lower at LC than at IC and HC (p<0.001), although there was no difference between IC and HC



**Figure 2.** Variation in conductivity (A) and calcium concentration (B) in Chateauguay West (filled circles) and East (open circles) on the south shore, and Valois Bay (filled triangles) and Lachine (open triangles) on the north shore of Lake St. Louis.



**Figure 3.** Relationship between calcium concentration and conductivity at the sample sites on the north shore (Valois Bay – filled triangles and Lachine – open triangles) and the south shore (Chateauguay East – open circles and Chateauguay West – closed circles).

(Tukey's post-hoc, p=0.75). The growth of *G. fasciatus* also varied across treatments (p<0.001,  $F_{3.65}$ =20.77, Fig. 5b); its growth in both LC-treatments was lower than in HC and IC treatments (p≤0.001), but there was no significant difference between the two LC treatments or between IC and HC treatments. Using the total length after 6 weeks in a one-way ANOVA gave identical results. The specific growth rate of both species decreased with increasing age ( $F_{2,116}$ =18, p<0.001) (as has been observed for other amphipods; Piscart *et al.* 2003), increased with conductivity ( $F_{2,116}$ =14.33, p<0.001), and was higher for *G. fasciatus* than *E. ischnus* in all treatments ( $F_{1,116}$ =341.31, p<0.001; Table 1). There was no effect of origin ( $F_{1,116}$ =0.58, NS).

#### **Field sampling**

#### Fecundity

The size-specific fecundity of *E. ischnus* did not differ among the HC sites and the lower Great Lakes (Fig. 6a, Table 2). Too few gravid females were found in Valois Bay to allow a statistical comparison of both sides of the river; however, the size-specific fecundity of the few females found in Valois Bay was within the same range as the other samples (Fig. 6a). By contrast, the size-specific fecundity of *G. fasciatus* differed among sites (i.e. there was an interaction between site and female length; Table 2). Large *G. fasciatus* females were less fecund in Valois Bay than at the other four sites (Fig. 6b); these differences were significant for individuals of length >7.3 mm at Lachine, >6.5 mm at Chateauguay East, >6.7 mm at Chateauguay West, and >5.8 mm in the lower Great Lakes. In addition, smaller females in the lower Great Lakes were more fecund than those in Lachine (<7.0 mm) and Chateauguay West (<7.3 mm). The highest number of eggs recorded in gravid *G. fasciatus* females at the different sites were 17 (Valois Bay), 30 (Lachine), 24 (Chateauguay West) and 30 (Chateauguay East). For *E. ischnus* females, the highest number of eggs ranged from 12 at Valois Bay to 19 at Chateauguay East.

The partial fecundity index of *E. ischnus* was not significantly different between sites ( $F_{3,162}$ =0.2, N.S., Table 3). The partial fecundity of *G. fasciatus* differed between sites ( $F_{4,243}$ =11.1, p<0.001), and was higher in the lower Great Lakes than at all sites in Lake St Louis (p≤0.001), except for Chateauguay East (NS), and was higher in Chateauguay East than in Valois Bay (p=0.001). The partial fecundity of *G. fasciatus* was



**Figure 4.** Survival of *E. ischnus* (a) and *G. fasciatus* (b) from the north shore (NS) and the south shore (SS) in water of low (LC), intermediate (IC) and high conductivity (HC).



**Figure 5.** Growth rate of *E. ischnus* (a) and *G. fasciatus* (b) from the north shore (NS) and the south shore (SS) in water of low (LC), intermediate (IC) and high conductivity (HC).

higher than that of *E. ischnus* at only two sites: the lower Great Lakes and Chateauguay East ( $t_{95}=5.1$ , p<0.001 and  $t_{104}=3.8$ , p<0.001 respectively). At the other sites, the partial fecundity of the two species did not differ significantly.

*E. ischnus* generally began reproducing at a smaller size than *G. fasciatus* (Table 4). In both species, males were generally larger than females (t-tests, p<0.01, unequal variances assumed), with the exception of *E. ischnus* at Valois Bay. The larger size classes were comprised almost entirely of males.

#### Sex ratio and proportion of ovigerous females

Populations of both species were dominated by males at one site (Chateauguay East), but the sex ratio was not significantly different from unity at the other sites (Table 5). The proportion of ovigerous individuals among adult *G. fasciatus* females varied across sites  $(\chi^2 = 27.232, df=3, p<0.0001)$ , differing between Chateauguay West (79%) and the two sites on the north shore (Valois Bay 58%, and Lachine 67%), and between Chateauguay East (73%) and Valois Bay. There was no significant difference between sites on the same shore. By contrast, the proportion of ovigerous adult females of *E. ischnus* did not differ among sites ( $\chi^2=0.678$ , df=2, NS), and was 60% at Chateauguay West, 56% at Chateauguay East, and 50 % at Valois Bay.

#### Growth rate in the field

There was no difference in growth rate between sites (*G. fasciatus*:  $F_{2,8}=2.28$ , NS; *E. ischnus*: t=-0.27, NS). The growth rate of *E. ischnus* appeared lower at Valois Bay (1.1%) than at the two HC sites (1.5-2.1%), but the small number of individuals found at the site makes the estimate uncertain. For both species, it was possible to identify and follow multiple cohorts from the first to the second sampling dates (Fig. 7 and 8, Table 6). During this time period, conductivities at Valois Bay and Lachine varied from 103-153  $\mu$ S/cm and 112-130  $\mu$ S/cm (and [Ca<sup>2+</sup>] was 15-21 mg/L and 15-17 mg/L), respectively. For *G. fasciatus*, 2 and 4 cohorts were followed in Chateauguay West and East respectively, 3 cohorts in Lachine, and only one cohort in Valois Bay. For *E. ischnus*, two cohorts were followed at each of the HC sites, and one cohort in Valois Bay.

		Specific growth rate					
Species	Treatment	0 – 2 wks	2 – 4 wks	4 – 6 wks			
E. ischnus	HC	2.1	1.1	0.7			
	IC	1.2	1.1	1.4			
	LC	0.8	0.8	0.7			
G. fasciatus	HC	3.1	2.5	1.9			
	IC	2.7	2.0	2.3			
	LC	2.0	2.1	2.0			
	LC-LC	2.3	1.4	1.7			

**Table 1.** Specific growth rate (% length increase/day) of native and exotic amphipods exposed to water with high, intermediate and low conductivity in the laboratory.

 Table 2. Analysis of size-specific fecundity of E. ischnus and G. fasciatus.

Size-specific fecundity								
E. ischnus								
Linear regression								
Site	Ν	F (df)	Р	$R^2$	Equation			
Chateauguay W	46	25.495 (1,45)	<0.001	0.37	y= -1.399 + 0.664 * female length			
Chateauguay E	56	37.916 (1.55)	<0.001	0.41	y= -1.203 + 0.637 * female length			
Valois Bay	6		>0.05					
Lower Great Lakes	55	91.069 (1,54)	<0.001	0.63	y= -1.952 + 0.748 * female length			
ANCOVA								
Independent variables	df	F	Р					
Site	2	0.273	0.761					
female length	1	139.825	<0.001					
Total	157							
		(	G. fasciatus					
Linear regression								
Site	Ν	F (df)	Р	$R^2$	Equation			
Chateauguay W	50	65.617 (1,49)	<0.001	0.58	y= -1.816 + 0.720 * female length			
Chateauguay E	50	78.080 (1,49)	<0.001	0.62	y= -2.403 + 0.824 * female length			
Valois Bay	53	8.665 (1,52)	0.005	0.15	y= -0.30 + 0.393 * female length			
Lachine	49	22.375 (1,48)	<0.001	0.32	y= -2.976 + 0.880 * female length			
Lower Great Lakes	42	134.178 (1,41)	<0.001	0.77	y= -0.871 + 0.641 * female length			
ANCOVA								
Independent variables	df	F	Р					
Site	4	2.336	0.056					
female length	1	180.653	<0.001					
Interaction site*fem lgth	4	2.528	0.041					
Total	244							



Figure 6. Size-specific fecundity of E. ischnus (a) and G. fasciatus (b) at the different sampling sites.

Partial fecundity	(	G. fasciatus	E	E. ischnus		
index	Ν	mean ± SE	Ν	mean ± SE		
Lachine	49	1.41 ± 0.10		N. A.		
Valois	53	$1.10 \pm 0.07$	6	1.20 ± 0.23		
Chateauguay	50	$1.60 \pm 0.09$	56	1.16 ± 0.07		
Peninsula	50	$1.33 \pm 0.06$	46	1.13 ± 0.08		
Lower Great Lakes	42	1.93 ± 0.13	55	1.21 ± 0.08		

Table 3. Partial fecundity index (mean brood size/female size).

Table 4. Size at the onset of reproduction and of the largest females and males found.

Species	Site	Smallest size at female sexual maturity		Largest male		Largest female	
		Ν	mm	Ν	mm	Ν	mm
E. ischnus	Chateauguay W	46	4.6	129	9.6	120	7.8
	Chateauguay E	56	4.7	159	10.7	130	8.1
	Valois Bay	6	5.8	14	8.3	12	7.9
G. fasciatus	Chateauguay W	50	5.4	258	11.9	286	8.9
	Chateauguay E	50	5.5	217	17.0	168	9.2
	Valois Bay	53	5.4	233	10.9	247	10.7
	Lachine	49	5.5	239	10.7	272	8.9

			Sex ratio	Binomial tests p <sub>(sex ratio)</sub>			
Site N		gravid females nongravid males females				males	(f/m)
		G.	fasciatus				
Chateauguay W 544		0.414	0.112	0.474	1.109	ns	
Chateauguay E	385	0.317	0.119	0.564	0.774	0.0072	
Valois Bay	480	0.300	0.215	0.485	1.060	ns	
Lachine 511		0.358 0.174		0.468	1.138	ns	
		E.	. ischnus				
Chateauguay W	249	0.289	0.193	0.518	0.930	ns	
Chateauguay E	289	0.253	0.197	0.550	0.818	0.0497	
Valois Bay	26 0.231		0.231	0.538	0.857	ns	

**Table 5.** Sex ratio in the adult population tested against a ratio of 1:1.

Table 6. Mean cohort length and growth between sampling dates for *E. ischnus* and *G. fasciatus*.

South shore		G. fasciatus E. ischnus								
Site	TL (	(mm)	Siz	e increase		TL	(mm)	Size increase		
	July 18	Aug 8	Absolute (mm)	Relative (%)	Daily (%)	July 18	Aug 8	Absolute (mm)	Relative (%)	Daily (%)
Chateauguay W	2.80	3.85	1.05	38	1.53		3.0			
		6.63				3.12	4.84	1.72	55	2.11
	6.38	9.02	2.64	41	1.66	4.91	6.67	1.76	36	1.47
	8.66					6.16				
Chateauguay E	3.70	4.62	0.92	25	1.06		2.90			
	4.86	6.70	1.84	38	1.54	3.40	5.00	1.60	47	1.85
	6.35	8.99	2.64	42	1.67	4.85	7.21	2.36	49	1.91
	8.19	10.62	2.43	30	1.24	6.55				
	11.44									
North shore			G. fasciatus				E. ischnus			
	TL (	(mm)	Size increase			TL (mm)		Size increase		
	July 17	Aug 7	Absolute (mm)	Relative (%)	Daily (%)	July 17	Aug 7	Absolute (mm)	Relative (%)	Daily (%)
Valois Bay		2.96								
	3.40									
		6.07								
	6.58	8.18	1.60	24	1.04	3.80	4.80	1.00	26	1.12
	8.35						6.12			
	10.22									
Lachine		3.37								
	3.33	4.98	1.65	50	1.93					
	4.66	6.73	2.07	44	1.77					
	6.27	8.65	2.38	38	1.54					
	7.34									
	8.78									

## Discussion

#### Growth experiment in the laboratory

Low conductivity appears to be stressful to native and exotic amphipods, as it resulted in reduced growth rate for both species in our laboratory experiments. However, it has a stronger negative effect on the invader, which suffered higher mortality. The pattern of mortality is similar to what was observed for *Gammarus pseudolimnaeus* along a calcium gradient (Zehmer *et al.* 2002). In that study, although general osmoregulatory failure did not prevent intermoult *G. pseudolimnaeus* from living in water with low Ca-levels, animals died quickly as they moulted, resulting in a gradual increase in mortality as moulting was not synchronized within the population (Zehmer *et al.* 2002). The loss of the exoskeleton during moulting exacerbates osmotic stress as the permeability to water increases, and the influx of water is higher for animals in a medium with lower concentrations of dissolved ions (Lockwood & Inman 1973). Surprisingly, *G. fasciatus* individuals from the north shore were not more tolerant to low conductivity than those from the south shore, suggesting no local adaptation of *G. fasciatus* to low conductivity.

In the laboratory experiment, the specific growth rate of *E. ischnus* was lower than that of *G. fasciatus* in all treatments. The specific growth rate of *G. fasciatus* was higher than what has been recorded for other species of freshwater amphipods at similar temperatures and comparable to that recorded for *Dikerogammarus villosus* (1.6-2.6 for males, 1.7-2.3 for females) – another Ponto-Caspian amphipod that has successfully invaded European inland waters (Piscart *et al.* 2003). In the lab, the growth rate of *E. ischnus* was as high as that of *G. fasciatus* only among newly hatched individuals in favourable conditions (i.e. HC). In the field, we found no clear differences in the growth rate of *E. ischnus* at both shores of the lake as it was found in very low abundances at one site on the north shore, and absent from another site where it had been recorded previously (Palmer & Ricciardi 2004). However, our laboratory results indicate that the growth rate of the native amphipod is higher than that of the exotic amphipod, contrary to the popular assumption that invasive species exhibit more rapid growth than closely-related native species (Piscart *et al.* 2003).

The sex ratios of both species were in accordance with previous records from



Figure 7. Size distribution of G. fasciatus at the four sampling sites in July and August 2008.



Figure 8. Size distribution of *E. ischnus* at the three sampling sites in 2008.

other regions. Previous studies found the sex ratio to be dominated by males (e.g. *G. fasciatus* in Lake Erie; Clemens 1950) or to fluctuate in dominance between genders (e.g. *E. ischnus* in Lichenskie Lake, Poland; Konopacka & Jesionowska 1995). *E. ischnus* females started reproducing at a smaller size than *G. fasciatus*, regardless of the water chemistry. The size-specific fecundity of *G. fasciatus* was more variable across sites than of *E. ischnus*, and was lower at Valois Bay than in the lower Great Lakes. *E. ischnus* size-specific fecundity did not differ between the sites with high conductivity and the lower Great Lakes. The smallest maximal brood size of both species was recorded at Valois Bay. The variation in fecundity across sites appears not to depend on conductivity, as the sites on the north shore followed different patterns.

Few studies have examined the relationship between water chemistry and brood size. Arnér and Koivisto (1993) observed that the size-specific fecundity of the cladoceran *Daphnia magna* peaked at 4 ‰, whereas Glazier *et al.* (1992) found no relationship in a study of *Gammarus minus* across a wide range of calcium and conductivity (5-72 mg/L and 36-424  $\mu$ S/cm). Alcaraz and Garcia-Berthou (2007) measured an increase in reproductive investment of the mosquitofish (*Gambusia holbrooki*) in more stressful environments. In our study, the partial fecundity of *E. ischnus* did not differ between sites and, unexpectedly, was much lower in our study system (1.13-1.21) than in central Europe (2.21; Grabowski *et al.* 2007). Our results thus contrast the observed European pattern of exotic amphipods having a higher partial fecundity than native amphipods (Grabowski *et al.* 2007; Pöckl 2007).

The majority of the females of both species were gravid, the exception being *E*. *ischnus* in Valois Bay, where only 50% of the females carried eggs. The proportion of *G*. *fasciatus* females carrying eggs was also lowest in Valois Bay. Thus, Valois Bay may be more stressful for *G*. *fasciatus* than the other sites for reasons unrelated to conductivity. The lack of relationship between water chemistry and the percentage of ovigerous females of gammarid amphipods is consistent with other studies (Glazier *et al.* 1992).

#### Influence of conductivity on life history of amphipods

There is very limited published information on the conductivity and calcium requirements of *G. fasciatus* and *E. ischnus*, even though the tolerance of *G. fasciatus* to

other abiotic factors such as temperature and oxygen has been examined (e.g. Sprague 1963; Thibault & Couture 1982). Creaser (1935) recorded the presence of *G. fasciatus* in a West Virginia stream at a calcium concentration of 10.8 mg/L and its absence downstream at a calcium concentration of 4 mg/L. The recorded levels of calcium along the north shore in our survey (minimum  $[Ca^{2+}]=10.4$  mg/L) are therefore within the known tolerance range of *G. fasciatus*. Moreover, this species was found at all sites on both shores in this study as well as in a previous survey (Palmer & Ricciardi 2004). While our lab experiment suggests that a conductivity and calcium concentration of 108  $\mu$ S/cm and 17 mg/L, respectively, result in high mortality of juvenile *E. ischnus*, the species was present at low abundance at Valois Bay when conductivity was 102.7  $\mu$ S/cm. This species may tolerate short exposure to low conductivity, but is weakened in this environment. Previously, we found that *E. ischnus* survived well with conspecifics in enclosures *in situ* at conductivities of 169-177  $\mu$ S/cm and calcium levels of 21-27 mg/L, but was vulnerable to intraguild predation from *G. fasciatus* (Kestrup & Ricciardi 2009).

The populations of *E. ischnus* along the north shore of Lake St. Louis are not persistent (this study; Palmer & Ricciardi 2004); individuals are found there only occasionally and at low abundances. The presence of juveniles and ovigerous females in our study and in archived samples (Palmer & Ricciardi 2004) suggests that either E. ischnus establishes reproducing populations infrequently along the north shore, or these are sink populations of individuals dispersed from sites upstream where E. ischnus are abundant (e.g. Pointe-du Moulin on Ile Perrot). Its intolerance to low conductivity is the most probable reason why E. ischnus has not established permanent self-sustaining populations along the north shore. Conductivity regularly fluctuates to levels near 100  $\mu$ S/cm, which results in increased mortality among juveniles, whereas adult individuals generally are more tolerant to unfavourable conditions (Berezina & Panov 2004; Bravo et al. 2007; Roche et al. 2009). E. ischnus may be able to survive exposure to low conductivity water during intermoult periods, but suffers higher mortality when moulting (Zehmer *et al.* 2002). In addition to mortality caused by osmotic stress, crustaceans are also more vulnerable to predation during this period (Bailey *et al.* 2006). Our study covered only a 5-month period during the summer, and it is possible that it omitted periods in which the lowest values of conductivity and calcium occurred. While there are

no long-term data series of these parameters in Lake St Louis, daily measurements throughout 2007 of the conductivity in the Ottawa River at Carillon show fluctuations between 69-152  $\mu$ S/cm, with the periods with the lowest values occurring in February-March (69 $\mu$ S/cm) and June-July 65 ( $\mu$ S/cm). If these periods coincide with periods of high influx into Lake St Louis, it is possible that the conductivity along the north shore can be even lower than 100  $\mu$ S/cm (Myriam Rondeau, Environment Canada, unpubl. data).

The physiological tolerance of an invader is a major determinant of the potential range it can invade, as shown for amphipods (Wijnhoven *et al.* 2003; Berezina & Panov 2004), cladocerans (Work & Gophen 1999) and mosquitofish (Haynes & Cashner 1995; Alcaraz & Garcia-Berthou 2007). However, in order to properly assess the tolerance of a species, it is important to consider multiple life history stages, which may respond in a different way to different levels of the same factor. An example is a study of the Baikalian amphipod *Gmeniloides fasciatus* in the Gulf of Finland, which revealed strong differences in salinity tolerance among adults, juveniles and embryos that limit the amphipod's occurrence in brackish waters (Berezina & Panov 2004). Thus, despite a broader tolerance of many invaders to abiotic factors, many euryhaline species are less tolerant than native freshwater species to low conductivity and salinity (Wijnhoven *et al.* 2003; Jokela & Ricciardi 2008). Ion-poor environments therefore constitute natural barriers to the spread and impact of many euryhaline invaders.

#### Conclusion

Conductivity influences the growth and survival of amphipods, but has no strong influence on their size-specific fecundity and size at reproduction. Although its tolerance to a wide range of salinities has allowed *E. ischnus* to successfully invade new environments in Europe and North America, its survival is diminished in areas of low conductivity, which provides an advantage or refuge for native competitors such as *G. fasciatus* in Lake St. Louis.

Human activities (e.g. organic pollution) have facilitated some invasions (Jazdzewski *et al.* 2004; MacNeil *et al.* 2004; Piscart *et al.* 2005), but declining calcium levels in northern lakes resulting from acidification (Jeziorski *et al.* 2008) may ultimately

exclude invaders with high calcium requirements, such as Ponto-Caspian crustaceans. High biotic potential (i.e. a strategy for rapid colonization, involving early maturation, high number of generations per year and high fecundity) is a trait often shared by invaders (Devin & Beisel 2007; Grabowski *et al.* 2007). However, as pointed out by other authors (Devin & Beisel 2007; Dick 2008), high biotic potential alone does not guarantee successful establishment or dominance following establishment. Indeed, our results show that the success and dominance of *E. ischnus* in the lower Great Lakes (Dermott *et al.* 1998) cannot be attributed to greater reproductive ability. Devin & Beisel (2007) claim that invasive species exhibit a particular ecological profile (traits related to tolerance to abiotic conditions) rather than a biological one (e.g. life history traits such as body size, life span and reproduction). However, we believe that a better predictor of the invasion success and relative dominance of amphipods is a broad tolerance to the abiotic environment combined with being a stronger intraguild predator.

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## **Connecting statement**

In the preceding chapters I explored the influence of conductivity on the rate of intraguild predation on adult females and on the life-history traits of the two species of amphipods. Both studies indicated that water with low concentrations of dissolved ions is more stressful to the euryhaline invader *E. ischnus* than to the native amphipod *G. fasciatus*. In the next chapter, I build upon this knowledge when measuring the rate of intraguild predation on juveniles in two types of water using the functional response, in an attempt to compare their potential impacts in different environments.

## CHAPTER 3:

# INTERACTIONS BETWEEN INVASIVE AND NATIVE CRUSTACEANS: DIFFERENTIAL FUNCTIONAL RESPONSES OF INTRAGUILD PREDATORS TOWARDS JUVENILE HETERO-SPECIFICS

Åsa M. Kestrup, Jaimie T. A. Dick, and Anthony Ricciardi. Interactions between invasive and native crustaceans: differential functional responses of intraguild predators towards juvenile hetero-specifics. The manuscript has been submitted to Biological Invasions.

## Abstract

Intraguild predation (IGP) between invasive and native species can lead to species exclusions or co-existence, dependent on the direction and strength of the interaction. Recently, derivation of 'functional responses' has been identified as a means of comparing the community impacts of invasive and native species. Here, we employ a novel use of this methodology to evaluate any IGP asymmetries between the invasive Ponto-Caspian amphipod *Echinogammarus ischnus* and the North American native Gammarus fasciatus. The direction and magnitude of intraguild predation of adult males on hetero-specific adult females has previously been shown to reverse across a water conductivity gradient, with the invader dominating in high-conductivity waters and the native dominating in low-conductivity waters. This partially explains field patterns, but does not predict the co-existence of the two species observed in many habitats and locations. Here, we compared IGP by both species on each other's juveniles in high- and low- conductivity water. We found that G. fasciatus has a higher type II functional response towards E. ischnus juveniles compared to the reciprocal interaction. Conductivity did not influence the predation rate on juveniles of either species. Thus, the male/female IGP advantage to G. fasciatus in low conductivity waters is compounded by a juvenile IGP asymmetry, which also counteracts the male/female advantage to E. ischnus in high conductivity waters, helping to explain field patterns of exclusion and coexistence.
## Introduction

Intraguild predation (IGP; Polis *et al.* 1989) is increasingly recognized as a powerful force in structuring biological communities and determining the outcomes of species invasions (Arim and Marquet 2004; Dick 2008; Hatcher *et al.* 2008). Where IGP is mutual between two species, any biotic or abiotic mediation of the interaction, or inherent asymmetries in ability to engage in the interaction, can profoundly influence patterns of species exclusion and co-existence. For example, many amphipod species replacements are caused by asymmetrical engagement in IGP (Dick 2008), while parasitism of such IGP participants can determine species co-existence (MacNeil *et al.* 2003b; Hatcher *et al.* 2008). Recently, it has also been demonstrated that environmental factors, such as water chemistry, can modulate IGP (Kestrup & Ricciardi 2009; Piscart *et al.* 2009). Thus, resolving the strength and direction of IGP in various environmental contexts should help us understand and predict the structuring of communities and the outcomes of invasions.

Bollache *et al.* (2008) showed that the functional response (*sensu* Holling 1959) of an introduced species can be used to quantify and compare its effect on prey populations with that of native species. This involves interspecific comparisons of the relationship between prey density and prey consumption. Such an approach can help forecast the community impacts of invaders (Bollache *et al.* 2008). Here, we took the novel approach of using functional responses in an IGP context, deriving and comparing the functional responses of co-occurring native and invader amphipods towards each others' juveniles, under differing environmental regimes, in order to understand spatial variation in the population and community level outcomes of the invasion.

Amphipod crustaceans feature as aquatic invaders throughout much of the world, frequently resulting in the displacement of native species and previously established invaders (e.g., Dick & Platvoet 2000; Kelly *et al.* 2003; Dick 2008). Many invasive amphipods are highly predatory and feed on a broad range of small invertebrates including other amphipods; some of these species can thereby reduce the diversity and biomass of benthic communities (Dick & Platvoet 1996; Kelly *et al.* 2002a; Kelly *et al.* 2003, 2006; Krisp & Mayer 2005; Kestrup & Ricciardi 2009).

The Ponto-Caspian invader *Echinogammarus ischnus* has displaced the native *G*. *fasciatus* on hard substrate throughout much of the lower Great Lakes (Dermott *et al.* 

1998). The invader may be more carnivorous than *G. fasciatus* (Limén *et al.* 2005) and appears to be replacing the latter through mutual but asymmetric IGP by adult males on females (Kestrup & Ricciardi 2009). However, these two species continue to co-exist in a variety of habitats (e.g. in the St. Lawrence River; Kestrup & Ricciardi 2009), even 10 years after invasion, in contrast to other asymmetrical amphipod IGP systems where complete replacements have been rapid (Dick *et al.* 1993; Dick and Platvoet 2000; Dick 2008). One factor contributing to the complex pattern of co-existence is the lower tolerance of *E. ischnus* to water of low ionic content, such that it is the dominant intraguild predator only at sites with high conductivity (i.e.  $\geq$ 300 µS/cm). In contrast, the roles are reversed in ion-poor waters (175-200 µS/cm), in which *G. fasciatus* males are the dominant intraguild predators (Kestrup & Ricciardi 2009). However, this pattern implies that the species should not co-exist, since even small asymmetries in IGP should lead to species exclusions (Dick *et al.* 1993; Dick 2008; Hatcher *et al.* 2008).

The vulnerability of a species to IGP may vary with life history stage (Polis *et al.* 1989). Generally, larger amphipod individuals prey on smaller hetero-specifics, particularly males on females, and the asymmetric loss of females from the population of the inferior IGP participant reduces population size and recruitment (e.g. Dick *et al.* 1993). However, juvenile amphipods are also subject to IGP by adults and any asymmetries in this interaction will clearly have implications for population recruitment and the dynamics of co-existence. Thus, in the present invasion scenario, it is necessary to examine the intraguild predatory pressure of the two species on each other's juveniles under different water conductivities, to gain a more complete understanding of this interaction. We therefore compare the functional responses of *E. ischnus* and *G. fasciatus* towards hetero-specific juvenile prey in ion-rich and ion-poor waters in order to assess the magnitude and any asymmetry of such IGP.

## Methods

In August and September 2009, *E. ischnus* (invader) and *G. fasciatus* (native) were collected at Chateauguay West ( $45^{\circ}21.80$  N,  $73^{\circ}47.20$  W), a site south of the Island of Montreal that receives ion-rich water from the St. Lawrence River. High-conductivity water ( $290 \mu$ S/cm, 26 mg Ca<sup>2+</sup>/L) was collected from this site and low-conductivity water

(98  $\mu$ S/cm, 13 mg Ca<sup>2+</sup>/L) from Place St-Louis (45°25.03 N, 73°52.68 W), a site on the north shore of Lake St Louis that receives water from the Ottawa River. Water for the two experimental treatments was high-conductivity (HC) water and intermediate-conductivity (IC) water (175  $\mu$ S/cm, 15 mg Ca<sup>2+</sup>/L), the latter a mixture of the two source waters. Conductivity was measured using an electronic meter (YSI# 85-25-FT), while the calcium concentration was analysed using a LaMotte hardness kit (model PHT-CM-DR-LT).

In the laboratory (20°C and 10:14hr L:D), animals were treated in a bath of 15 ppm Malachite green in filtered source water (11 µm filter paper) for 15 minutes to reduce the risk of fungal infection (Melendre et al. 2006). The species were kept separately in aquaria with filtered source water and cobble and fed *ad libitum* with dried winter squash (Cucurbita maxima), which was readily consumed. Emerging juveniles were collected from gravid females held individually in plastic cups with 150 ml filtered source water and stored for 3 days in trays with food. Juvenile length (tip of rostrum to tip of telson) was then measured on a sample of juveniles (n=20 per species) using a stereo microscope and micrometer eyepiece, and the mean sizes of juveniles of the two species were compared by t-test. We selected similar-sized adult males of the two species (measured after the experiment; mean body length of the two species in both experimental water conductivities examined by ANOVA) and starved them for 24 hours in either HC or IC water before use in the experiment. Individual males of the two species were then presented with hetero-specific juveniles in both HC and IC water at nine prey densities (4, 6, 8, 10, 16, 20, 30, 60, 90; n=4 per density) or until an asymptote was reached; G. fasciatus males were therefore also offered a density of 120 juveniles. Four replicates of each prey density in both HC and IC water without adult amphipods acted as controls. Replicates were examined after 24 and 40 hours.

The mean number of juvenile prey eaten was examined in a five-Factor ANOVA with respect to predator status (i.e. adults present/absent), predator species, prey density, time (repeated measure) and water conductivity type. Functional responses were modelled in SigmaPlot v. 10.0 using a Monod function y=ax/(1+bx), which provided estimates of *a* (the scale parameter), *b* (the saturation parameter), the maximum feeding rate (given by the asymptote a/(bh), where *h* is experimental time), and the R<sup>2</sup> values for

the fitted curves. Mean maximum feeding rate estimates were examined with respect to predator species by a t-test. All analyses were conducted with SPSS 17.0 for Windows (release 12.0.0).

## Results

*E. ischnus* juveniles were marginally, if consistently, smaller in length than *G. fasciatus* juveniles (1.5 mm  $\pm$  0.02 SE vs. 1.7 mm  $\pm$  0.02 SE, t<sub>38</sub>=-11.9, p<0.001). Experimental adult males of the two species did not differ significantly in length (*E. ischnus* IC water=7.6 mm  $\pm$  0.10 SE and HC water=7.7  $\pm$  0.07 SE; *G. fasciatus* IC water=7.8 mm  $\pm$  0.06 SE and HC water=7.9  $\pm$  0.07 SE; F<sub>3,151</sub>=2.6, N.S). Survival of control juveniles at 24 and 40 hours was, respectively, 98.4 % and 96.8 % for *E. ischnus* in HC water, 98.2 % and 95.2 % for *G. fasciatus* in HC water, 98.3 % and 97.3 % for *E. ischnus* in IC water and 98.2 % and 95.9 % for *G. fasciatus* in IC water. Predation was thus concluded to be the principal cause of juvenile mortality in the predator treatments and this activity was indeed frequently observed.

The presence of adults significantly increased juvenile mortality ( $F_{1,288}$ =898.8, p<0.001), with significantly more hetero-specific juvenile prey eaten by the native *G. fasciatus* than the invader *E. ischnus* ( $F_{1,288}$ =140.1, P<0.001; Fig. 1). Significantly more prey were consumed as prey density increased ( $F_{8,288}$ =647.2, P<0.001) and over time ( $F_{1,288}$ =149.2, P<0.001; Fig. 1). However, there was no significant difference in predation between the two water conductivities ( $F_{1,288}$ =0.06, NS; Fig. 1). All two- and three-way interactions were significant (P<0.001), except for those including water conductivity type.

Since the Monod models achieved high goodness of fit ( $\mathbb{R}^2$  values, Table 1), the functional responses of *E. ischnus* and *G. fasciatus* (Fig. 1) can be described as type II (see also Bollache *et al.* 2008). In addition, maximum predation rate (see Table 1) was significantly higher for *G. fasciatus* than for *E. ischnus* ( $t_6$ =3.82, P<0.01).

## Discussion

In its invasion of North American freshwaters, the Ponto-Caspian amphipod E. ischnus is disadvantaged at low and intermediate water conductivities, both through its lower tolerance to ion-poor waters in terms of survival ability and to the asymmetry in IGP on adult females that favours the native G. fasciatus under such conditions (Kestrup & Ricciardi 2009). The present study shows clearly that E. ischnus suffers another differential predation pressure, that of a higher predatory impact on its juveniles from G. fasciatus compared to the reciprocal interaction, as revealed through our derivation of functional responses. This is fully consistent with field patterns, where the invader is excluded or exists in very low abundance alongside G. fasciatus in low and intermediate conductivity waters (see Kestrup & Ricciardi 2009). Low levels of co-occurrence of the two species may be explained by immigration of *E. ischnus* into *G. fasciatus* areas, a situation found in another amphipod invasion scenario by Dick (1996). In contrast, E. *ischnus* is the stronger intraguild predator with respect to adult male/female predation in high conductivity waters, and indeed is successful in invading areas of high G. fasciatus abundance (Dermott et al. 1998; Palmer and Ricciardi 2004; Kestrup & Ricciardi 2009). However, the present experiment helps explain why the invader does not always entirely replace the native in high conductivity waters, since the native retains its IGP advantage over E. ischnus juveniles under such conditions, counteracting the IGP asymmetry on females favouring *E. ischnus*. Thus, the field patterns of exclusion and co-existence are much better understood when the asymmetries in IGP, in relation to water conductivity, are resolved for a number of life-history stages.

In the experiment, *E. ischnus* juveniles were slightly smaller than those of *G. fasciatus*. However, the difference in prey size was insignificant compared to the difference in predation rate and it therefore seems unlikely that a small size discrepancy alone explains the large difference in functional responses between these species. Further, the adult males of the two species were matched for size in the experiment. However, since *G. fasciatus* generally grows larger than *E. ischnus* (Dermott *et al.* 1998; Kestrup & Ricciardi unpubl. data), the species differences in functional responses shown here are most likely conservative. Additionally, at the population level, the effect of the higher functional response of *G. fasciatus* towards juvenile *E. ischnus* as compared to the



**Figure 1.** Relationship between prey density and the number of prey consumed (the functional response) for *E. ischnus* and *G. fasciatus* in water of different conductivities, where prey were hetero-specific juveniles, after (a) 24 hours and (b) 40 hours. Squares = *G. fasciatus*, circles = *E. ischnus*, filled symbols = high conductivity (HC), and open symbols = intermediate conductivity (IC).

Adult predators	Juvenile prey	Water (µS/cm)	time	a	В	$R^2$	a/(bh)	S.E.
G. fasciatus	E. ischnus	290	24	1.402	0.015	0.803	3.82	9.89
			40	1.384	0.010	0.857	3.33	10.31
G. fasciatus	E. ischnus	175	24	1.329	0.012	0.867	4.77	8.86
			40	1.195	0.006	0.924	5.34	8.24
E. ischnus	G. fasciatus	290	24	0.452	0.008	0.864	2.38	2.81
			40	0.617	0.008	0.881	1.93	3.67
E. ischnus	G. fasciatus	175	24	0.483	0.009	0.817	2.34	3.63
			40	0.556	0.005	0.878	2.95	4.10

**Table 1.** Functional response parameters with goodness of fit ( $R^2$ ) for each species/water type/time combination and estimated mean maximum intake rate, a/(bh), with standard errors (S.E.).

reciprocal interaction may be enhanced by the lower fecundity of the latter. Even though the size-specific fecundity of the two species is similar, the reproductive output of *G*. *fasciatus* females is higher due to their larger size (Dermott *et al.* 1998; Kestrup & Ricciardi unpubl. data). Indeed, Dick and Platvoet (1996) showed that a reversal in species dominance can occur with changes in the balance of IGP and reproductive output.

There is a growing body of evidence that local environmental factors are key to understanding variation in IGP interactions and hence patterns of species exclusions and co-existence. Dick and Platvoet (1996) and MacNeil et al. (2004) showed that patterns of dominance by either G. pulex, G. tigrinus or G. duebeni celticus are partially driven by the changing balance of IGP under different water qualities, while an unknown feature of water chemistry reduced IGP asymmetries and hence slowed the replacement of G. duebeni celticus by G. pulex in one Irish river (Piscart et al. 2009). With respect to the current invasion by E. ischnus, we can predict its expansion into high conductivity and polluted waters, such as parts of the Mississippi and the Missouri Rivers (USGS Water Quality Watch 2009), persistence of the native species in low conductivity and more pristine waters, such as rivers draining the Canadian Shield (e.g. the Ottawa River and north shore tributaries to the St. Lawrence River; Rondeau 1993), and co-existence in intermediate conductivity waters such as Lake Champlain and the Richelieu River (Jokela and Ricciardi 2008; Vermont Department of Environmental Conservation 2009a,b). Further, the range of parasites that these amphipod species harbour can modulate IGP asymmetries (MacNeil et al. 2003b; Hatcher et al. 2008), such that species exclusions, reversals of such exclusions, and complex patterns of co-existence are now being unravelled (Dick 2008). This requires further study in the current system.

It should be noted that we did not examine the influence of any anti-predator behaviour of juveniles, such as phototaxis (Hunte & Myers 1984), which may have an influence on the relative vulnerability of juveniles to predation. In addition, substrate heterogeneity, oxygen levels, alternative prey and other factors may alter functional responses (see Bollache *et al.* 2008) and require further investigation in the present context.

With regards to the wider aquatic community, predation is the primary mechanism associated with invasive amphipod impacts (Kelly *et al.* 2003; Kelly & Dick

2005; van Riel et al. 2006). The invasion of Northern Ireland by G. pulex resulted not only in a replacement of the similar-sized native G. duebeni celticus, but also in a reduction in the abundance and diversity of other benthic invertebrates, owing to the invader's voracious feeding habits (Kelly et al. 2002b; Kelly et al. 2003; Kelly & Dick 2005). Similarly, the invasive and predatory *Dikerogammarus villosus* has had a substantial impact on invaded food webs in Europe (Dick et al. 2002; van Riel et al. 2006). By contrast, while the displacement of G. fasciatus by E. ischnus in the lower Great Lakes is well documented, the impact of E. ischnus on other benthic invertebrates is largely unknown. It would be informative to compare functional responses of these amphipods on different individual or multiple prey species. Nonetheless, the differences in functional reponses revealed by our study tentatively suggest that the replacement of G. fasciatus by E. ischnus, where it occurs, may alter amphipod predation rates in benthic food webs. If we assume that E. ischnus has a lower per-capita effect than G. fasciatus on small invertebrate prey, as suggested by the results of this study, we can make some predictions. E. ischnus rarely reaches high abundances in the upper St. Lawrence River, where the benthic community is often dominated locally by G. fasciatus. Therefore, the total predation pressure per unit area of the invader in the upper St. Lawerence River may be low compared to the native amphipod. However, in areas of the lower Great Lakes where the invader thrives, high local abundances may overcome the low per-capita effect, resulting in a larger total impact (as observed for in G. pulex in Ireland; Kelly et al. 2003; Bollache et al. 2008). This requires pre- and post-invasion monitoring of the composition of the benthic community.

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## **Connecting statement**

In the preceding chapters, I examined the influence of variations in conductivity on different aspects of the performance of the two species of amphipods. I demonstrated that the ion-rich water in the St. Lawrence River is optimal to both species, while the ion-poor water in the Ottawa River is more stressful to the exotic than to the native amphipod. Water chemistry is thus the factor explaining why the invader has not successfully invaded ion-poor waters. However, the invader has not successfully displaced the invader in ion-rich waters, and water chemistry is therefore unlikely to be the single determinant of invasion success. In the next chapter, I explore the influence of other agents in the aquatic community on invasion success by testing the vulnerability of *E. ischnus* to predation from a co-evolved benthivorous fish, to examine if the presence of a co-evolved predator is facilitating its invasion.

# CHAPTER 4: ARE INTERACTIONS AMONG PONTO-CASPIAN INVADERS

## DRIVING AMPHIPOD SPECIES REPLACEMENT IN THE ST.

LAWRENCE RIVER?

Åsa M. Kestrup and Anthony Ricciardi. Are interactions among Ponto-Caspian invaders driving amphipod species replacement in the St. Lawrence River? Published in Journal of Great Lakes Research (2009) 35: 392-398.

## Abstract

In Lake Erie and Lake Ontario, the Ponto-Caspian amphipod Echinogammarus ischnus has replaced the native amphipod Gammarus fasciatus on rocky substrates colonized by dreissenid mussels, which provide interstitial refugia for small invertebrates. Based on the premise that an invader's vulnerability to predation is influenced by its evolutionary experience with the predator and its ability to compete for refugia, we hypothesized that amphipod species replacement is facilitated through selective predation by the round goby Neogobius melanostomus, a Ponto-Caspian fish that invaded the Great Lakes in the early 1990s and is now colonizing the St. Lawrence River. In laboratory experiments, we determined if E. ischnus excludes G. fasciatus from mussel patches, and if the vulnerability of G. fasciatus to predation by gobies is increased in the presence of the invasive amphipod. E. ischnus and G. fasciatus did not differ in their use of mussel patches, either when alone or in each other's presence. Both species were equally vulnerable to predation by the round goby. In field experiments, we determined if the round goby exerts a stronger impact than native predators on the relative abundance of amphipod species. Our results suggest that E. ischnus is more vulnerable to native predators, but the round goby does not have a differential impact on the native amphipod. We conclude that competition with E. ischnus does not increase the vulnerability of G. fasciatus to goby predation, and that the round goby does not promote the replacement of G. fasciatus by E. ischnus in the St. Lawrence River. The outcome of antagonistic interactions between exotic and native amphipods is mediated more by abiotic factors than by shared evolutionary history with other co-occurring exotic species.

## Introduction

The abundance and impact of an exotic species can vary greatly across its invaded range. This variation is largely attributable to heterogeneity in the physico-chemical environment (Berezina & Panov 2004; Palmer & Ricciardi 2004; Alcaraz & Garcia-Berthou 2007) and the composition of the invaded community, particularly the presence of predators (Reusch 1998; Crooks 2002; Hunt & Yamada 2003), competitors (Jensen et al. 2002; van Riel et al. 2007) and facilitators (Ricciardi 2001, 2005). Vulnerability to a shared predator may be mediated by competition between the invader and native species for refugia (Balshine et al. 2005; Jensen et al. 2002; van Riel et al. 2007; van Riel 2008), and selective predation on either species may influence the invader's success and impact (Dudas et al. 2005; Bollache et al. 2006; Kinzler & Maier 2006). The invader's vulnerability to predation is in many cases influenced by its evolutionary experience (Cox & Lima 2006; Parker et al. 2006; Banks & Dickman 2007). If the predator originates from the invader's native range, their shared evolutionary history may promote interactions that yield a net mutual benefit for both invading species (Simberloff & Von Holle 1999; Ricciardi 2001, 2005) and confer an advantage against native competitors. Alternatively, vulnerability of the invader to predation might depend more on the specific traits of resident predators (e.g. physiological tolerances, foraging behaviour) than on the evolutionary experience of the species involved.

In this study, we examine the relative abundance of competing native and exotic crustaceans, the North American amphipod *Gammarus fasciatus* and the Ponto-Caspian amphipod *Echinogammarus ischnus*, in the presence of other Ponto-Caspian species in the St. Lawrence River. The latter include a benthivorous fish (the round goby, *Neogobius melanostomus*) and dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*), whose colonies provide interstitial refugia for amphipods (Ricciardi *et al.* 1997; Gonzalez & Downing 1999) and are commonly inhabited by *E. ischnus* in its native range (Köhn & Waterstraat 1990). *E. ischnus* was discovered in the Great Lakes in the early 1990s (Witt *et al.* 1997), and by 1998 it had colonized the St. Lawrence River as far downstream as Montreal (Palmer & Ricciardi 2004). This species has increased its biomass more than 20-fold in the presence of *Dreissena* in western Lake Erie (Stewart *et al.* 1998a). *E. ischnus* appears to have a higher affinity for *Dreissena* patches than the

native species G. fasciatus, and has replaced G. fasciatus as the dominant amphipod on substrates fouled by dreissenids in Lake Erie and Lake Ontario (Dermott et al. 1998; Van Overdijk et al. 2003; Haynes et al. 2005). E. ischnus is assumed to have excluded G. fasciatus from mussel patches in the lower Great Lakes (Dermott et al. 1998; Van Overdijk *et al.* 2003), but the mechanisms behind this species replacement are not fully understood (Gonzalez & Burkart 2004; Limén et al. 2005). They may involve selective predation by fish (Gonzalez & Burkart 2004; Palmer & Ricciardi 2005) or intraguild predation, with *E. ischnus* being the dominant predator in water with high concentrations of dissolved ions (Kestrup & Ricciardi 2009). However, no studies have determined if competition for dreissenid-covered substrate with E. ischnus increases the vulnerability of G. fasciatus to fish predation, which has been implicated as a driver of species replacement among other crustaceans (DiDonato & Lodge 1993; van Riel 2008). Although intraguild predation is recognized as an important mechanism of species replacement (Dick & Platvoet 1996; MacNeil & Platvoet 2005), its importance relative to other mechanisms such as predation from higher trophic levels has not been tested (Dick 2008).

The round goby forms dense populations locally in its introduced range (Corkum *et al.* 2004) and can have a strong impact on the abundance of benthic invertebrates (Kuhns & Berg 1999; Barton *et al.* 2005; Lederer *et al.* 2006). Among its most important prey items are gammarid amphipods (Simonovic *et al.* 2001; Diggins *et al.* 2002; Barton *et al.* 2005). The round goby was already abundant in Lake Erie and Lake Ontario during the 1990s, when *E. ischnus* became dominant (Barton *et al.* 2005). By contrast, the round goby has only recently become abundant at some sites in the upper St. Lawrence River (Å. Kestrup, pers. obs.).

If the exclusion of *G. fasciatus* from *Dreissena* patches is caused by increased vulnerability of *G. fasciatus* to predation by gobies, it seems plausible that the rapid species replacement previously observed in the Great Lakes has failed to occur in the St. Lawrence River thus far because of the time delay in the goby's invasion. We hypothesize that predation by the round goby facilitates the replacement of *G. fasciatus* by *E. ischnus* on substrates densely colonized by dreissenids, consistent with the "invasional meltdown" model that predicts increased facilitation amongst invaders with a

shared evolutionary history (Simberloff & Von Holle 1999; Ricciardi 2001, 2005). Alternatively, despite its evolutionary history, *E. ischnus* may not be better adapted to using *Dreissena* patches or may be equally or more vulnerable to round goby predation than *G. fasciatus*. We tested whether (1) the presence of *E. ischnus* alters the use of dreissenid patches by *G. fasciatus*; (2) the round goby selects *G. fasciatus* over *E. ischnus* as prey; (3) the vulnerability of *G. fasciatus* to round goby predation is increased in the presence of *E. ischnus*; and (4) the round goby has a stronger negative impact than native benthivorous fish on the abundance of native amphipods.

## Methods

#### Collection and treatment before lab experiments

The abundances of *E. ischnus* and the round goby are highly variable in the upper St. Lawrence River (Å. Kestrup & R. Kipp, unpubl. data; Palmer & Ricciardi 2004). The round goby has rapidly expanded its populations in the river in recent years. Its distribution is still highly scattered such that sites where it is abundant are in close proximity (< 1 km) to sites where it is scarce or absent (Å. Kestrup, pers. obs.). Exotic and native amphipods used in our laboratory experiments were collected continuously throughout the study at Pointe-du-Moulin on Ile Perrot (an island in the St. Lawrence River adjacent to Montreal), which was not yet colonized by the round goby. Amphipods were collected from dreissenid-covered rocks while wading and snorkelling, and were transported within 3 hrs to the lab, where they were placed in aerated aquaria containing river water (conductivity 280-300 µS), cobble, sand, dreissenid shells, macrophytes and catfish pellets. All aquaria were kept in the same temperature-controlled chamber at 19°C in a light regime appropriate for the time of the year (15:9 hrs light:dark). Round gobies were captured using minnow traps in Lake Saint-François, a fluvial lake in the St. Lawrence River. During an acclimatization period of at least one week, fish were stored in aerated 40-L aquaria (L  $50 \times 27 \times 30$  cm) with filtered river water, gravel and rocks and were fed frozen chironomid larvae and both species of amphipods. They were exposed to the same temperature and light regime as the amphipods.

#### Habitat choice experiment

Laboratory experiments were conducted in 54-L aquaria ( $62 \times 31 \times 31$  cm) filled to 18 cm depth with filtered water of high conductivity (280-300  $\mu$ S) from the St. Lawrence River. The bottom of each aquarium was covered with a 1-cm thick layer of washed aquarium sand. Placed on the sand were two dreissenid patches, consisting of a monolayer of empty mussel shells attached to  $7 \times 7$  cm transparent plexiglass sheets with nontoxic silicone glue to achieve an average mussel density of 4600 m<sup>-2</sup> (total area of both patches was 0.0098  $m^2$  or 5% of the bottom surface area of the aquarium) and weighted down by a stainless steel plate. The shells were glued at their base with their valves closed and were placed in contact with each other to mimic a single-layered dreissenid colony. To examine if habitat choice was density dependent, we added to the aquarium either a low or high abundance of adult amphipods of both sexes (20 or 40 individuals, equivalent to 104 and 208 individuals m<sup>-2</sup>, respectively, which is within the range of natural densities in the river; Palmer & Ricciardi 2004). In both high- and lowdensity experiments, single species treatments involved either E. ischnus or G. fasciatus, whereas mixed species treatments consisted of both species in equal ratios. In mixed species treatments, G. fasciatus were introduced first and allowed to settle for 5 minutes before E. ischnus were added to the aquaria. After 24 hours, the mussel patches were retrieved by covering them with a plastic container that was sealed with a plastic sheet before being lifted out of the aquarium. Amphipods were removed from the mussel patches and counted in a sorting tray. The aquaria were emptied of water and sand and the remaining amphipods were located and counted. Individuals that had been consumed or died were recorded as mortality. No individuals were used in more than one experiment. All experiments had 10 replicates – with the exception of the low-density treatment with single G. fasciatus, which had 11 replicates. The low-density experiment was run July 12 – August 19, 2007, and the high-density experiment was run September 13 - 17, 2007. We determined whether the results were influenced by differences in mortality among treatments, by comparing the proportions (arcsine transformed) of surviving amphipods on mussel patches.

#### **Goby predation: Lab experiment**

The experimental design and procedure were similar to the habitat choice experiment. Aquaria were divided into two compartments of equal size by transparent plexiglass sheets, and the walls of the aquaria were covered with opaque paper to minimize disturbance. A goby was placed into one of the compartments 24 hours prior to an experiment and received no food during this time. A total of 40 amphipods (single or mixed species) were added to the other compartment containing two mussel patches. In the single species treatments, the amphipods were allowed to settle for 5 minutes, whereas in the mixed species treatment G. fasciatus individuals were added 5 minutes prior to the addition of E. ischnus. Thereafter, both species were allowed to settle for another 5 minutes before the divider was removed. The goby was then allowed to forage for 24 hours. At the end of the experiment, the mussel patches were retrieved, the fish removed using a hand net, and the remaining amphipods counted. Individual amphipods that had been consumed or died were recorded as mortality. All experiments had 7 replicates and were run August 23 – September 6, 2007. To determine if mussels served as a refuge from predation, the proportions of surviving amphipods in mussel beds with and without predation from round gobies were compared in a two-way ANOVA with amphipod species (single *E. ischnus*, single *G. fasciatus*, mixed species) and goby predation (with, without) as fixed factors. Fish of similar size were used in each experiment (57-75 mm TL, average 66.1 mm  $\pm 1.2$  SE). During the experiment, the round gobies did not disrupt the dreissenid colonies with their feeding activities, as the total number of mussels per colony was the same at the onset and the end of each experiment.

#### **Goby Predation: Field experiment**

We tested the effect of fish predation on the relative abundance of both amphipod species at two sites in the St. Lawrence River west of Montreal. At one site, an island near Coteau Landing (45°15'11 N, 74°12'26W), round gobies were found in high abundance. At another site, Pointe-du-Moulin (54°21'57 N, 73°51'06W), situated 30 km downstream, gobies were found in very low abundance. They began to colonize this site in detectable numbers during the month preceding the experiment and were observed for the first time on September 9, 2007, when 5 individuals of <5 cm length were encountered while

snorkelling (Å. Kestrup & R. Kipp, pers. obs.). Both sites, henceforth referred to as the "High Goby" and "Low Goby" sites, are similar in terms of substrate (rocks, boulders and dreissenid colonies) and exposure (facing the main channel of the river), and receive well-oxygenated (8.7-11.5 mg/L, 91-114 %) and highly mineralized water (conductance  $290-302 \mu$ S) from the Great Lakes. Both sites also have a similar composition of native fishes (A. Ricciardi, pers. obs.). The presence of small (<7 cm) and large (>7 cm) individual gobies at the High Goby site was confirmed on August 9 by two SCUBA divers surveying two 10 m transects, 1 m wide, at 2 m depth. Each transect was surveyed by both divers, and the average of both observations was used as an estimate of the goby abundance along that transect. The abundance of gobies varied between 1.2-4.5 small individuals/m<sup>2</sup> and 0.4-1.8 large individuals/m<sup>2</sup>, with means of 2.8 small and 1.1 large individuals/m<sup>2</sup>. Experimental treatments consisted of predator-exclusion cages and artificial substrates (cement bricks  $19.1 \times 9.1 \times 5.7$  cm) covered with a monolayer of empty dreissenid shells (2.5-3.0 cm shell length). Shells were glued at their base with their valves closed to resemble a natural dreissenid colony, attached to the bricks with nontoxic aquarium silicone. The average number of mussels per brick was  $53.8 \pm 0.7$  SE. Predator-exclusion cages  $(29.5 \times 19.5 \times 18 \text{ cm})$  were constructed of stainless-steel wire (mesh size 0.64 cm) and their sides were connected with plastic cable ties. Preliminary trials revealed that small gobies (< 7 cm) could easily enter cages with a mesh size of 1.27 cm, but were excluded by the smaller mesh size. In early September 2007, 20 bricks were deployed haphazardly at least 1 m apart at a depth of 0.5–0.8 m at each site. Ten of these bricks were placed individually inside predator-exclusion cages, while another ten bricks remained uncaged and thus served as a predator-access treatment. Bricks were retrieved after 38 and 40 days at the Low Goby and High Goby sites, respectively. Cages were cut open under water and bricks gently lifted and enveloped within double plastic bags. In the lab, amphipods were removed from the bricks with forceps, and the water in each bag was filtered through a 500 µm mesh sieve. Amphipods were preserved in 70 % ethanol and identified to species using a Hund Wetzlar SM33 microscope. Amphipods that were unidentifiable due to the loss of uropods were not included in the analyses.

All analyses were done using SPSS 12.0 for Windows (SPSS Inc. 2003). Statistical tests included independent sample t-tests (single species treatments), paired samples t-test (mixed species treatments) and one-way ANOVAs. Variances were equal unless otherwise indicated. Prior to analysis, numbers were  $log_{10}$ -transformed and proportions were transformed using  $sin^{-1} (x^{0.5})$ , where necessary, to meet with the assumption of normality.

## Results

#### Habitat choice experiment

#### Single species treatment

Individuals of either species were rarely seen swimming in the water column of the aquarium unless disturbed, but instead swam close to the bottom or hid amongst mussel shells. Both species strongly preferred to occupy mussel patches, with densities on the patches more than  $5 \times$  higher than on sand (paired t-tests,  $p \le 0.001$ ). At low abundances (Fig. 1a), both species showed a similar preference for mussel patches in single species treatments (t = 1.336, p = 0.197) and their mortality did not differ (*E. ischnus*  $0.5 \pm 0.2$  SE, *G. fasciatus*  $1.6 \pm 0.5$  SE, t = -1.994, p = 0.066, unequal variances). At high abundances (Fig. 1a), *E. ischnus* showed a stronger preference for mussel patches (t = 2.369, p = 0.029) and its mortality was higher than that of *G. fasciatus* (*E. ischnus*  $1.7 \pm 0.4$  SE, *G. fasciatus*  $0.4 \pm 0.2$  SE, t = 2.867, p = 0.015, unequal variances). The final abundance of *E. ischnus* on mussel patches did not differ between high and low abundance treatments (t = 0.44, p = 0.66), whereas the final abundance of *G. fasciatus* on mussel patches was lower in the high abundance treatment (t = 2.15, p = 0.045). Similar results were obtained using proportions instead of absolute numbers.

#### Mixed species treatment

In mixed species treatments, both species preferred to occupy mussel patches over sand at both high and low abundances (paired t-tests,  $p \le 0.018$ ). In the low abundance treatment (Fig. 1b), *E. ischnus* was found in higher densities on mussel patches than was *G. fasciatus* (t = -6.626, p <0.001). There was no difference in the level of mortality for both species (*E. ischnus* 0.4 ± 0.3 SE, *G. fasciatus* 0.7 ± 0.3 SE, t = 1.406, p = 0.193). In the high abundance treatment (Fig. 1b), there was no significant difference in the number of individuals found among mussel patches (t = -1.665, p = 0.130) or in the level of

mortality (*E. ischnus*  $0.4 \pm 0.3$  SE, *G. fasciatus*  $0.7 \pm 0.3$  SE, t = 0.605, p = 0.560). Similar results were obtained using proportions instead of absolute numbers. The average number of amphipods found on mussel patches across all replicates was 13.4 ( $\pm$  0.7 SE), and the total number did not differ significantly between treatments (one-way ANOVA,  $F_{5.55}$ =0.003, p=0.093).

#### **Goby predation: Lab experiment**

Both amphipod species were equally vulnerable to predation from gobies in single species treatments (t=0.289, p=0.778; Fig. 2a) and in mixed species treatments (t = 0.881, p = 0.412; Fig. 2b). Mortality of these species did not vary between single and mixed species treatments (*E. ischnus* t=0.26, p=0.80, *G. fasciatus* t= -0.13, p=0.90). Furthermore, total mortality in the mixed species treatment did not differ from that of the single species treatments (one-way ANOVA,  $F_{2,18}$ =0.057, p=0.95). A higher proportion of surviving amphipods was found on mussel patches after exposure to predation from round gobies than before predation (two-way ANOVA, p<0.001,  $F_{1,51}$ =38.361; Fig. 3). There was no significant effect of species treatment, nor any significant interaction between species and predator treatments.

#### **Goby predation: Field experiment**

All bricks were retrieved from the Low Goby site, whereas 5 bricks were lost at the High Goby site (4 caged bricks and 1 uncaged brick). The total abundance of amphipods in the predator-access treatment was higher than in the predator-exclusion treatment at the Low Goby site ( $224.5 \pm 19.1$  SE and  $154.9 \pm 18.7$  SE respectively, t = 2.608,  $p_{2-tailed} = 0.018$ ; Fig. 4a), but it did not differ between treatments at the High Goby site ( $182.6 \pm 9.2$  SE and  $160.2 \pm 22.3$  SE respectively, t = 1.057,  $p_{2-tailed} = 0.310$ , Fig. 4b). At both sites, *G. fasciatus* outnumbered *E. ischnus* in predator-exclusion and predator-access treatments (paired samples t-tests, p  $\leq 0.001$ ). However, fish predation did not have the same effect on the relative abundance of *E. ischnus* at both sites. At the Low Goby site, *E. ischnus* comprised a higher proportion of amphipods in the predator-exclusion treatment than in the predator-access treatment ( $0.25 \pm 0.03$  versus  $0.09 \pm 0.01$ , respectively, t=-5.871, p<0.001, arcsine transformed data; Fig. 5). Also in absolute numbers, the number of *E.* 



**Figure 1.** Number of amphipods on mussel patches of constant size in single (a) and mixed (b) species treatments at low and high abundances of amphipods. Error bars denote 1 standard error. Different letters above the bars indicate significant differences (p<0.03).



**Figure 2.** Total amphipod mortality in single (a) and mixed (b) species treatments after exposure to round goby predation. Error bars denote 1 standard error.



**Figure 3.** Proportions of surviving amphipods found on mussel patches in single and mixed species treatments after round goby predation compared with treatments without goby predation. Error bars denote 1 standard error.

*ischnus* was higher in the predator-exclusion treatment ( $36.8 \pm 4.2$  versus  $19.6 \pm 2.2$ ; t = -3.60, p = 0.002), while the number of *G. fasciatus* was lower in the predator-exclusion treatment ( $118.1 \pm 16.4$  versus  $204.9 \pm 19.0$ ; t = 3.46, p = 0.003) (Fig. 4a). At the High Goby site, the relative abundance of *E. ischnus* was similar in the two treatments ( $0.09 \pm 0.01$  and  $0.05 \pm 0.02$ , respectively; t =-1.73, p=0.11, arcsine transformed data; Fig. 5). In absolute numbers, the abundance of *E. ischnus* was also similar in the two treatments ( $8.9 \pm 2.6$  and  $13.0 \pm 1.8$ , respectively; t=-1.16, p=0.268), as was the abundance of *G. fasciatus* ( $147.2 \pm 21.5$  and  $173.7 \pm 10.3$ ; t=1.24, p>0.2) (Fig. 4b).

#### Discussion

#### **Habitat Choice Experiment**

Despite having evolutionary experience with Dreissena, E. ischnus does not appear to be better adapted than G. fasciatus to using mussel patches as habitat. Both species occupied mussel patches more frequently than surrounding sand. There was no increased mortality in mixed species treatments, suggesting that interspecific predation among amphipods may not be significant at time scales as short as our experiment. In the low abundance treatment, the low numbers of G. fasciatus on mussel patches suggested that E. ischnus might exclude G. fasciatus from dreissenid colonies, but this pattern was not repeated in the high abundance treatment. Therefore, the hypothesis that E. ischnus alters the habitat choice of G. fasciatus is not supported, even though E. ischnus is a dominant intraguild predator in water of high conductivity (Kestrup & Ricciardi 2009). Although the relative abundance of amphipods on mussel patches in mixed species treatments was variable, the total abundance was constant, suggesting that a limited number of amphipods can inhabit a discrete mussel patch regardless of their species composition. However, few studies have shown evidence for competition for habitat among amphipods (Dick 2008). Where it has been shown, competitive ability appears to be mediated by size, with the larger



**Figure 4.** Amphipod abundance on bricks in predation experiments at field sites with (a) low and (b) high abundances of the round goby. Error bars denote 1 standard error. Different letters above the bars indicate significant differences ( $p \le 0.002$ ).



**Figure 5.** Proportion of *E. ischnus* on bricks in predation experiments at field sites with low and high abundances of round gobies. Different letters above the bars indicate significant differences (p<0.001, based on transformed data).

species excluding the smaller species (van Riel et al. 2007, 2008).

#### **Goby Predation Experiments**

Surprisingly, E. ischnus and G. fasciatus were equally susceptible to goby predation in the presence of mussel patches, despite differences in their evolutionary experience with the round goby and dreissenid mussels. Gobies can reduce total amphipod abundance, but although some previous studies have indicated lower vulnerability of E. ischnus compared to other gammarid species in the presence of fish predators (Palmer & Ricciardi 2005; Kinzler & Maier 2006), we found no evidence that gobies are selective in their consumption of amphipods or that G. fasciatus is more vulnerable to fish predation in the presence of *E. ischnus*. *E. ischnus* is generally more active than *G. fasciatus* (Å. Kestrup, pers. obs.; Pennuto & Keppler 2008), yet both species respond to the scent of round gobies similarly by spending more time immobile and moving shorter distances. In prolonged exposure to round goby scent, E. ischnus tends to increase its avoidance behaviour while G. fasciatus acts oppositely (Pennuto & Keppler 2008), but our study shows that these behavioural differences do not affect their vulnerability to predation. Apparently, predator recognition does not overcome the risk posed by the efficient hunting tactics of round gobies (Banks & Dickman 2007), which, being an ambush predator that spends most of its time in close proximity to bottom sediments, may be better at detecting small prey than actively foraging visual predators. The complex interstitial habitat provided by dreissenid mussels may serve as a refuge for amphipods from fish predation (Dermott et al. 1998; Gonzalez & Downing 1999; Mayer et al. 2001), but whether any particular species is favored depends on the specific behaviour of the predator species. An unpublished study found that a native benthivore, rock bass Ambloplites rupestris, preferentially selected G. fasciatus over E. ischnus (C.M. Mayer, Department of Environmental Sciences, University of Toledo, pers. comm.). Kolar et al. (2002) found similar results when exposing the North American amphipod Gammarus *pseudolimnaeus* to predation from a benthic ambush predator with a well developed sensory system – the Eurasian ruffe (*Gymnocephalus cernuus*), and a visual predator – the North American yellow perch (*Perca flavescens*); amphipods reduced their activity

more in the presence of ruffe, but nevertheless suffered more intense predation from the ruffe.

An appropriate question is whether the use of live mussels instead of intact shells would have produced different results in our experiments by making the mussel patches more attractive to amphipods and gobies. Previous studies showed that both *G. fasciatus* and *E. ischnus* respond primarily to the physical structure of the *Dreissena* patch, rather than to the characteristics of live mussels (Ricciardi *et al.* 1997; Stewart *et al.* 1998a, 1998b). Moreover, round gobies of the size used in our lab experiment strongly prefer amphipods over mussels as a prey item (Diggins *et al.* 2002). Therefore, it seems unlikely that the use of live mussels would have altered the results.

Selective predation by the fish community as a whole may account for the lower relative and absolute abundance of *E. ischnus* in predator-access treatments during the field experiment. Indeed, a previous study found evidence that *E. ischnus* was more vulnerable than *G. fasciatus* to native predators in the St. Lawrence River (Palmer & Ricciardi 2005). Owing to the small mesh size required to exclude small gobies, there may have been a cage effect that reduced the colonization of bricks by amphipods at both sites. However, such an effect would not explain the higher abundance of *E. ischnus* in the predator-exclusion treatment at the Low Goby site; we would expect reduced flow to negatively affect *E. ischnus*, which prefers habitats with at least moderate current (Palmer & Ricciardi 2004). At the High Goby site, the total abundances of amphipods in the predator-access and predator-exclusion treatments were not significantly different, perhaps indicating that predation pressure from gobies in predator-exclusion treatment.

#### Conclusions

The growing frequency of invasions in aquatic systems worldwide has increased the likelihood that species with shared evolutionary histories will be reunited in new regions. Positive interactions are common among such invaders (Simberloff & Von Holle 1999; Ricciardi 2001) and can augment their ecological impact (Ricciardi 2001, 2005). For example, co-adaptation may reduce the intensity of predator-prey interactions and confer a competitive advantage to an exotic prey introduced with its predator over predator-

naïve enemies in the invaded community (Case & Bolger 1991; Adams *et al.* 2003). Coadapted relationships that are re-established through multiple invasions in a new region, as is occurring in the Great Lakes, may thus have synergistic effects on native species (Ricciardi 2005).

However, as we have shown here, the co-occurrence of exotic species with shared evolutionary histories does not guarantee that one or more of them will have a competitive advantage over native species. The exotic and native gammarid amphipods in our study are quite similar morphologically and ecologically, which might account for their similar use of *Dreissena* patches and the lack of selective predation by the round goby. Moreover, in general, species interactions are highly context-dependent and can vary across space and time (Bruno *et al.* 2003), particularly across physically heterogeneous environments (e.g. Alcaraz & Garcia-Berthou 2007). We conclude that the outcome of antagonistic interactions among these amphipods in the St. Lawrence River is mediated more by their relative tolerance to local physico-chemical conditions (particularly calcium concentration and conductivity; Kestrup & Ricciardi 2009) than by the presence of other Ponto-Caspian species.

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### FINAL CONCLUSIONS AND SUMMARY

As demonstrated here, the success and impact of an invasion are highly contextdependent and can vary greatly across sites even within a single heterogeneous system. Although potentially many variables may contribute to this context-dependence, only a few key variables might have overriding importance and their identification is crucial for developing models that predict the impacts of specific invaders. A major governing factor in the occurrence and dominance of *E. ischnus* in the St. Lawrence River appears to be conductivity, which was revealed to mediate the magnitude and direction of its intraguild predation with native amphipods (Chapter 1). Conductivity also influences the growth rate and mortality of the two species; however, the invader was more negatively affected by low conductivity than the native species (Chapter 2). Interactions between the two species were shown to be more complex than previously assumed. Contrary to expectations, the native amphipod had a higher type II functional response in intraguild predation than the exotic amphipod, at both levels of conductivity (Chapter 3). This finding indicates the importance of considering different life stages when assessing the overall effects of intraguild predation. Intriguingly, it also suggests (following the argument by Bollache et al. 2008), that G. fasciatus may cause greater predatory impacts on benthic communities than E. ischnus if introduced outside its native range.

Conductivity was found to be more important than the effect of co-occurring invaders that have a shared evolutionary history (Chapter 4), suggesting that water chemistry could aid predictions of the invaded range and relative impact of *E. ischnus* in different habitats. Low-conductivity waters could function as refugia for native species having a higher tolerance to ion-poor environments. Similar patterns have been found in Europe, where exotic amphipods are dominant in larger rivers, but are absent from smaller streams and rivers with low conductivity (Grabowski *et al.* 2009). A reduction in impact in ion-poor water has also been shown for other Ponto-Caspian invaders in the St. Lawrence River (the quagga and zebra mussel; Jones & Ricciardi 2005; Jokela & Ricciardi 2008), and probably applies also to other recent euryhaline invaders, such as the highly predatory Ponto-Caspian mysid shrimp *Hemimysis anomala* – which has to date only been recorded at sites with high conductivity (Kestrup & Ricciardi 2008; J. Marty, St. Lawrence River Institute, Cornwall, Ontario, pers. comm.), and potential future

invaders such as the highly predatory Ponto-Caspian amphipod *Dikerogammarus villosus* (Ricciardi & Rasmussen 1998). Future research will reveal how the impact of these invaders on the invaded community also varies with site-specific environmental factors.

Other key factors may have a substantial influence the invasion success of *E*. *ischnus*. During the course of this project, a previously undescribed parasitic water mold (the oomocyte *Saprolegnia* sp.) was discovered and linked to spring die-offs of amphipods – particularly of *E. ischnus* – in the upper St. Lawrence River (Kestrup *et al.* in preparation). The origin of the parasite is unknown. It remains to be determined whether recurring outbreaks of this disease facilitate the persisting dominance of the native amphipod at many sites in the river. If so, it would be another factor that contributes to context-dependent variation in the dominance of amphipod communities in the river.

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