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Effects of osmotic and thermal shock on the invasive aquatic mudsnail *Potamopyrgus antipodarum*: mortality and physiology under stressful conditions

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Abstract

Invasive freshwater species, such as the exotic mollusc *Potamopyrgus antipodarum* (New Zealand mudsnail), can frequently survive under harsh conditions, including brackish and hypoxic environments. We experimentally assessed the effects of osmotic (0, 10, 20, 25 and 30 psu) and thermal (20 °C) shock on mortality, activity and physiology of *P. antipodarum* collected at Capitol Lake, Olympia, Washington, USA, during winter and spring seasons when environmental temperature was 5 and 10 °C respectively. We measured standard metabolic rate and enzymatic activities (malate dehydrogenase, lactate dehydrogenase, alanopine dehydrogenase) in snails after a 10-day acclimation period at high salinity. Significantly higher mortalities were observed at higher salinities; the strongest effects occurred on snails collected at the end of winter, and exposed to 30 psu and 20 °C (100% mortality in 3 days). When snails were collected during the spring, 100% mortality was observed after 40 days at 30 psu and 20 °C. Standard metabolic rates were significantly lower when snails were exposed to salinities of 25 and 30 psu, even after 10 days of acclimation. Enzymatic activities showed small but significant declines after 10 days at 30 psu reflecting the declines observed in overall metabolism. The physiological tolerances to temperature and salinity displayed by this population of *P. antipodarum* make its eradication from Capital Lake difficult to achieve.

Keywords

Ecophysiology, enzymatic activity, invasive species, mortality, New Zealand mudsnail, salinity

Introduction

After their initial introduction and establishment, exotic species often become invasive, spread to new territories, and cause major ecosystem changes with negative socioeconomic impacts (Pimentel et al. 2000; Sala et al. 2000). Due to their fast spread and wide range of impacts, exotic freshwater molluscs are a major concern in ecosystems around the planet (Sousa et al. 2014; Boltovskoy 2015). Development of control methods is an important step to stop the spread and mitigate the impacts produced by invasive molluscs.

The effects of environmental conditions on physiological performance are relevant to understanding changes in the behaviors and distributions of species (Pörtner and Farrell 2008; Somero 2012). Moreover, the study of ecologically relevant physiological variables has direct application in models predicting the spread and control of invasive species (e.g., Fly and Hilbish 2013; Xiao et al. 2014). Particularly, variables that can be used to indicate stress under extreme conditions, such as metabolic rate or enzymatic activity, can be of major importance to understand distribution limits and the efficiency of control methods. For example, the effect of extreme salinity conditions on mortality and physiological variables of several invasive molluscs were studied in order to control and predict their distribution (Duncan and Klekowski 1967; Alexander Jr and McMahon 2004; Sylvester et al. 2013; Boltovskoy 2015; Yang et al. 2018; Underwood et al. 2019).

The New Zealand mudsnail (*Potamopyrgus antipodarum*; Tateidae, Mollusca) is an aquatic freshwater species native to New Zealand that has been frequently introduced, becoming invasive, in Oceania, Asia, Europe, and North America (Ponder 1988; Kerans et al. 2005). It has recently been found in Chile, establishing a foothold in South America, too (Collado 2014; Collado et al. 2019). This species can be spread passively by other organisms (on the feet, pelts, or plumage of birds and mammals) and by human vectors such as in the mud adhered to boats or motor vehicles (Alonso and Castro-Díez 2008). *Potamopyrgus antipodarum* has biological and physiological characteristics that allow it to survive in dry environments, freezing temperatures, and a wide range of salinity conditions (Hylleberg and Siegismund 1987; Siegismund and Hylleberg 1987; Costil et al. 2001; Alonso and Castro-Díez 2008, 2012; Leclair and Cheng 2011). In a review of the salinity tolerance of *P. antipodarum*, Leclair and Cheng (2011) described this species surviving under salinities up to 20–27 psu (practical salinity units). Hoy et al. (2012) found populations living and reproducing at higher salinities in the Columbia River estuary, USA.

This species was first detected in our study site, Capitol Lake, Olympia, Washington (WA), USA, in 2009 (Leclair and Cheng 2011). This artificial lake is isolated from the southern Puget Sound estuary by a dam that when opened can allow seawater into the freshwater, changing the salinity to values close to 30 psu (Leclair and Cheng 2011). Flushing with seawater was practiced as control method in March 2010 when authorities attempted to eliminate this species (Leclair and Cheng 2011). Although more snails died than predicted by Leclair and Cheng's model, survival rate was usually over 70–80%. Consequently, this species still remains in the lake, reproducing and spreading successfully. In the current study, we examined the effects of salinity and temperature on mortality and activity of *P. antipodarum*. Additionally, we measured the effects of temperature and salinity on Standard Metabolic Rate (SMR) and enzymatic activities of *P. antipodarum* in order to study its metabolic response to stressful environments. Our working hypothesis is that mortality will increase at higher salinity concentrations regardless of temperature and season. However, based on previous field studies, we predict that season (winter or spring) or/and temperature will affect results by increasing mortality at higher temperatures for each season. It is expected that SMR will increase with higher temperatures. However, oxygen consumption rate, and consequently SMR will decrease at higher salinities, due to the operculum closing behavior of this species affecting survival capacity under extreme conditions. Additionally, we measured the effects of decreasing oxygen concentration on respiration rates.

Methods

Specimen collection

Snails were collected from the nearshore benthos in the vicinity of Marathon Park, Capitol Lake, Olympia WA, USA (47°02'14"N, 122°54'39"W) in two seasons: winter (March 1, water temperature 5 °C) and spring (April 5, water temperature 10 °C). The undersides of submerged stones and rocks were examined for snails. Specimens were manually removed and placed in Ziploc bags. These bags were transported in coolers to the laboratory and placed into small holding aquaria (40 L) kept in a temperature-controlled room set at 5 and 10 °C, for the first and second rounds of experiments, respectively. Sampling was carried out under a permit of the Washington State Department of Fish and Wildlife, and followed Level 2 decontamination procedures of their Invasive Species Management Protocols (Tweit et al. 2012). Additionally, all materials used to collect and handle snails were maintained at −30 °C for at least one week after use to ensure total disinfection. Final identification was carried out in the laboratory under a stereoscopic microscope using field guides and descriptions of *P. antipodarum* and native snails (Frest and Johannes 1999; Crosier and Molloy 2017). We note that more than 99% of the snails we collected in Capitol Lake were *P. antipodarum*.

Tolerance to osmotic and thermal shock

In order to test the effects of osmotic and thermal shock on the mortality of *P. antipodarum*, two experiments were carried out under controlled conditions. The two exposure experiments, one per collection date, were conducted at five salinity concentrations (0, 10, 20, 25, and 30 psu) and three temperatures (5, 10, and 20 °C). Each experiment was performed in a complete factorial design, including all combinations of salinity concentrations with 5 and 20 °C, or 10 and 20 °C for the first and second experiment, respectively. After 4 days of maintenance in the lab at the collection temperature, snails were randomly transferred to glass chambers (300 ml) filled with 250 ml of water at one of the salinity concentrations. Experimental chambers were capped with plastic mesh, and placed in 40-L aquaria with freshwater filled to same level as the water in the experimental chamber; the whole aquarium was covered using a plastic film to further guard against escape. The free space in the experimental chambers between the water surface and the top mesh allowed snails to remain outside of the water, simulating real conditions at the shore of the lake. Five chambers at each salinity/temperature were used totaling 50 experimental chambers per experiment. The number of snails per experimental chamber was increased from five to ten in order to improve the results in spring experiments when more snails were available.

The five different salinities (0, 10, 20, 25, and 30 psu) were obtained using Instant Ocean sea salt (0, 10, 20, 25, and 30 g/L) and water from the City of Olympia's Artesian Well (water quality data available at http://olympiawa.gov/~/media/Files/PublicWorks/Utility-Inserts/WQR/Artesian-Test-Results.pdf). Target salinity levels were verified using a Red Sea seawater refractometer (Model R12018) after initial preparation and during experiments. Water at the different salinity concentrations and temperatures were stored in large (40 L) carboys in the same experimental room and used to exchange the water in the experimental chambers each week. Although minimal, when evaporation occurred in the experimental chambers, water level was raised to the original level by adding distilled water.

For each experiment, two water temperatures were used, one at the same acclimation value (the same temperature of the lake when snails were collected, either 5 or 10 °C) and one at 20 °C without any acclimation time (thermal shock). While, the acclimation temperature was set for the room in general, two of the aquaria were kept at 20 °C using aquarium heaters, and the temperature in each aquarium was monitored using four data loggers, one per 40-L aquaria (thermal bottom sensors, iButton).

Each experimental chamber and the aquaria themselves were gently aerated, and snails were fed with food grade *Spirulina* three times per week. Mortality was checked every day during the first week and every few days afterwards. Snails were regarded as dead when no reaction was detected under a stereomicroscope after stimulation with a dissection needle in the operculum area. Dead snails were kept for 1–2 h in water at 0 psu to verify that there was no recovery after they were removed from the experimental chambers. Dead snails were kept at −30 °C for at least a week before being discarded. In addition to mortality, activity (active/inactive snails) as the number of open or closed snails (Pascual and Drake 2008) and reproductive activity (presence/absence of neonates released into the chambers and observed under a stereomicroscope) were recorded for each experimental chamber. Experiments were finished when most of the treatments reached more than 90% mortality, resulting in different time durations for each experiment, 7 and 105 days for winter and spring, respectively.

Oxygen consumption

Standard metabolic rates of *P. antipodarum* were measured at 10 and 20 °C on 231 specimens collected in the spring and kept in experimental chambers as described for mortality experiments. These additional experimental chambers were kept in the same 40 L tanks used for the spring mortality experiments. Due to the change in the activity level observed during the mortality experiments at the two temperatures, respiration rates were measured at several salinities, acclimation times and oxygen levels to test metabolic changes associated with these variables. First, a respiration consumption baseline was determined measuring oxygen consumption at 0 psu for 10 and 20 °C. Then, for the remaining salinities (10, 20, 25, and 30 psu), specimens were acclimated for 0, 2, 4, 6, 8, and 10 days before oxygen consumption measurements. Consequently, at least 8 additional chambers at four salinities and two temperatures were used for respiration experiments. All these snails were fed with *Spirulina* at 48 h, just after three snails were removed and used for respiration measurements.

Rates of oxygen consumption were measured on individual specimens using PreSens type B2-NTH fiber optic oxygen optodes connected to a PreSens Microx TX3 temperature-compensated oxygen meter (Precision Sensing, Regensburg, Germany). Sensors were calibrated at two points using an aqueous 5% sodium sulfite solution for oxygen-free water and gently stirred filtered water (at 10 and 20 °C) for oxygen-saturated water. Data were recorded on a personal computer through a serial connector. Three specimens were chosen at random from one experimental chamber, transferred into three different glass syringes with oxygen saturated (100%) filtered water (0.22 μm) containing antibiotics (100 mg l^{-1} each of erythromycin and ampicillin) at each salinity, and incubated until oxygen saturation reached 0%. Antibiotics were added to decrease bacterial effects (Rutherford and Thuesen 2005). The volume of the syringe was set at 0.1 or 0.2 ml for measurements carried out at 10 and 20 °C respectively, which lasted around 1–2 h. Syringes were sealed with Luer-type fittings with Teflon septa for insertion of the oxygen sensor similar to methods described by Rutherford and Thuesen (2005) and Paolucci et al. (2010).

Total lengths of snails were measured before respiration experiments using an electronic caliper, and weight was calculated according to the length-ash free dry weight (AFDW) relationships of Eklöf et al. (2017). All snails were fixed in liquid N_2 after respiration and kept for further enzymatic analyses. Control experiments were performed in an identical fashion to respiration experiments for ~4 h using empty snail shells. Controls showed no significant bacterial respiration over the time in which oxygen consumption experiments were conducted. Linear regressions of oxygen concentrations against time were performed using Graphical Analysis Vernier Software (Sarasota, FL, USA). The rate of oxygen consumption was estimated from the 50–75% oxygen concentration interval and used in statistical comparisons. In order to examine the effect of oxygen concentration on metabolism, additional oxygen consumption rates were estimated at 75, 45, 25, and 5% of saturation for snails measured at 0 psu.

Enzymatic activity

The following enzymes were screened to select appropriate indicators of aerobic and anaerobic metabolic potential: malate dehydrogenase (MDH, E.C. 1.1.1.37), lactate dehydrogenase (LDH, E.C. 1.1.1.27), octopine dehydrogenase (E.C. 1.5.1.11), alanopine dehydrogenase (ADH, E.C. 1.5.1.17), tauropine dehydrogenase (E.C. 1.4.99.2) and strombine dehydrogenase (E.C. 1.5.1.22). Malate dehydrogenase, an important metabolic enzyme that provides oxalacetate to citrate synthase for the first step of the citric acid cycle, was selected as an indicator of aerobic metabolic potential. Lactate dehydrogenase, the terminal enzyme in glycolysis that contributes to both aerobic and anaerobic metabolic pathways, was selected as an indicator of glycolytic potential. Molluscs can use several different –opine dehydrogenases for anaerobic respiration, and in our survey of enzymatic activities, alanopine dehydrogenase displayed activities an order of magnitude higher than the others, and ADH was chosen for analyses. Enzymatic activities of MDH, ADH, and LDH were measured on freshly collected snails in spring (0 psu-0 acclimation time), and after 10 days in the lab at two different temperatures (10 and 20 °C) at 30 psu, since these are the most extreme conditions in which we expected to see differences.

Whole animals were weighed on a Mettler analytical balance while still frozen and homogenized using Duall hand held glass homogenizers kept on ice. Specimens were diluted at 1:99 parts weight/volume with 0.01 M tris homogenization buffer, pH 7.5 at 10 °C. Aliquots of homogenate were transferred to microfuge tubes and centrifuged at 6600 g for 10 minutes at 5 °C. All assays were performed within 1 h of homogenization using a Hewlitt-Packard diode array spectrophotometer equipped with a water-jacketed cuvette holder. Measurements of enzyme activity were made in 2-ml quartz cuvettes at 20 °C under non-limiting conditions in order to estimate maximum metabolic potential and followed procedures essentially as those described previously (Childress and Somero 1979; Seibel et al. 2000). Enzyme activities are expressed as units (µmoles of substrate converted to product per minute) per gram ash-free dry weight of animal (AFDW).

MDH activity measurements were carried out in a cocktail solution containing 50 mM Imidizole/HCl buffer (pH 7.0 at 20 °C), 20 mM $MgCl_2$, 0.4 mM oxaloacetate, and 150 µM NADH. LDH activity measurements were performed in a cocktail solution containing 80 mM tris/HCl buffer (pH 7.2 at 20 °C), 2 mM sodium pyruvate, 150µM NADH, and 100 mM KCl. LDH assay reactions were started by addition of the sample supernatant, and the decrease in absorbance at 340 nm due to NADH oxidation was recorded. For ADH, LDH activity after the addition of homogenate supernatant was recorded as background activity, and this background rate was then subtracted from the overall rate after the assay reaction was initiated by addition of alanine (2 mM) to arrive at the ADH activity of the sample.

Statistics

The effects of salinity and temperature (independent variables) treatments on mortality (dependent variable) were analyzed in two different two-way ANOVA, one for the winter and another for the spring experiments. The effects of the same two independent variables on activity (dependent variable) were assessed again using two-way ANOVA (one for winter and another for spring). The average number of active snails in each of the five chambers per salinity treatment across the full experimental time was used as a variable, rather that the accumulative mortality. Relationships between metabolism (SMR, response variable) and two categorical independent variables were performed using two General Linear Models with Analysis of Covariance (GLM-ANCOVA) and Tukey HSD post hoc test, controlling for the effects of AFDW as covariate. While one GLM-ANCOVA used salinity and temperature as categorical independent variables, the second GLM-ANCOVA used acclimation time and temperature. Differences between oxygen consumption rates of *P. antipodarum*, at different oxygen levels (75, 45, 25, and 5%) at 10 and 20 °C were assessed using a two-way ANOVA and Tukey post hoc comparisons. All analyses were performed in Statistica 7.0. Data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Cochran's test and Levene's test).

Results

Tolerance to osmotic and thermal shock

Snail mortality was significantly higher at higher salinities, but it was also affected by water temperature, showing significant interaction between these two variables in both seasons (Table 1). In winter, experiments only lasted 7 days, because thermal shock at higher salinity resulted in 100% mortality after several days (Fig. 1). In spring, experiments were carried out for 105 days as snails were much more resistant to higher temperature and salinities (Fig. 1). In general, salinity had a strong and significant effect on mortality in both seasons and temperature conditions (two-way ANOVA, $F = 33.17$, $p < 0.001$ and $F = 63.69$ and $p < 0.001$ for the winter and spring, respectively; Fig. 2, Table 1). The strongest effect was found in snails collected during the winter and exposed to 20 psu or more, reaching between 60 and 100% of mortality in only 3 days (Tukey HSD test, $p < 0.001$; Fig. 2 upper panel; Suppl. material 1: Table S1). Conversely, at higher salinities during spring, significantly higher mortalities were observed only at 25 and 30 psu as compared to lower salinities (Tukey HSD test, $p < 0.01$; Fig. 2 lower panel; Suppl. material 1: Table S1). Mortality reached 100% after 40 days in the highest salinity concentration. In general, there was not a strong effect on mortality when snails were kept at low salinities, 0 or 10 psu.

Thermal shock had a lower impact on mortality (~55% at 0 psu after 7 days at 20 °C in winter) compared to the effect of higher salinity (100% at 30 psu after 5 days at 5 °C in winter). A similar trend was seen in the spring (Fig. 2). In general, snail mortality due to higher salinity was slightly higher when a thermal shock treatment (20 °C) was applied (Fig. 2), but this effect was only significantly higher than acclimation temperature on two occasions (0 and 20 psu for winter and spring, respectively; Tukey HSD test, $p < 0.01$; Suppl. material 1: Table S1). No other significant differences in mortality between temperature treatments at a determined salinity concentration were observed (Table 1).

Table 1. Results of two-way ANOVA test assessing effects of salinity (0, 10, 20, 25, and 30 psu) and temperature (5, 10, and 20 °C) on mortality and mean activity of *Potamopyrgus antipodarum* during winter and spring. DF = degrees of freedom.

Mortality				
Winter	**DF**	**MS**	***F***	***P***
Salinity	4	15100.0	38.92	<0.001
Temperature	1	3200.0	8.25	<0.007
Interaction	4	1420.0	3.66	<0.05
Residuals	39	388.0	–	–
Spring	**DF**	**MS**	***F***	***P***
Salinity	4	12890.6	75.31	<0.001
Temperature	1	12409.9	72.51	<0.001
Interaction	4	3048.1	17.81	<0.001
Residuals	39	171.2	–	–
Activity				
Winter	**DF**	**MS**	***F***	***P***
Salinity	4	4187.8	16.92	<0.001
Temperature	1	70.7	0.29	0.595925
Interaction	4	438.1	1.77	0.153958
Residuals	40	247.5	–	–
Spring	**DF**	**MS**	***F***	***P***
Salinity	4	2227.4	83.63	<0.001
Temperature	1	628.4	23.59	<0.001
Interaction	4	12.5	0.47	0.758753
Residuals	40	26.6	–	–

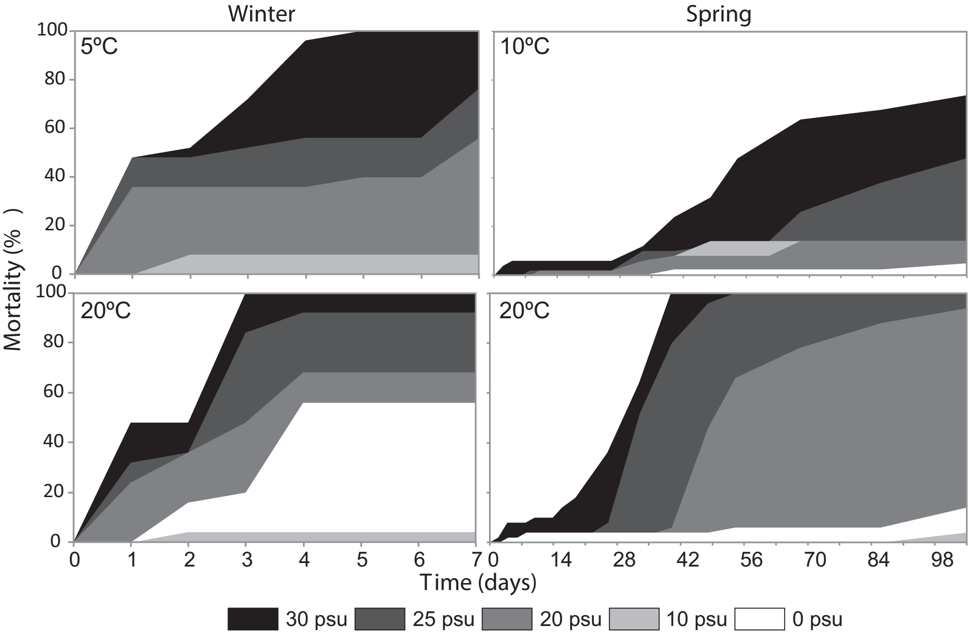

Figure 1. Mortality of the New Zealand mudsnail, *Potamopyrgus antipodarum*, during two exposure experiments (winter and spring season) at different salinities (0, 10, 20, 25, and 30 psu) and temperatures (lake temperature and 20 °C). $n = 5$ for winter experiments and $n = 10$ for spring experiments.

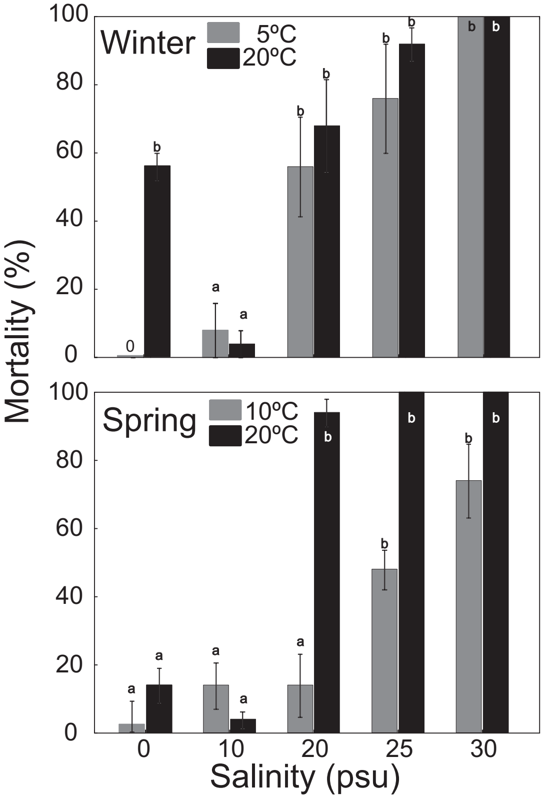

Figure 2. Mortality of the New Zealand mudsnail, *Potamopyrgus antipodarum*, collected during the winter and spring at five salinities. Mortality values (mean percentage ± SE) are given at the end of 5 and 50 days for the winter and spring experiments, respectively. Grey and black bars show water temperature (5 and 10 °C) of Capitol Lake at the time of capture and thermal shock treatments (20 °C), respectively. Different letters indicate significant difference in mortality between salinity treatments within each experimental temperature ($p < 0.05$ ANOVA, Tukey post hoc comparisons).

Snail activity

The mean activity significantly decreased at higher salinities in both seasons, but thermal shock significantly reduced activity only during spring (Table 1). In both experiments, snails remained mostly open and active at salinities of 0 and 10 psu in all temperature treatments, and the percentage of active snails only occasionally fell below 80% at low salinities (Fig. 3). At 20 psu, snails remained active at the start of all the experiments and treatments, however the percentage of active snails decreased to values around 50 or even 0% after 4 days of exposure in winter, or after 21 days in spring at 20 °C (Fig. 3). Snails that survived longer than this remained active to the end of the experiment. In the 10 °C experiments at 25 and 30 psu, most snails died, but the remaining snails also remained active.

The New Zealand mudsnail is parthenogenetic and ovoviviparous, and snails reproduced during some experiments. Neonate snails were mostly observed in 0 and 10 psu experiments, but occasionally at 20 psu. Neonates were only present during the spring experiment after day 40 and 54 in the experimental chambers at 20 and 10 °C, respectively. Neonates were not quantified, and no neonates were observed in the much shorter experiments in winter.

Oxygen consumption

Oxygen consumption rates of 231 snails were measured individually in respiration chambers in the spring (Figure 4; Suppl. material 1: Table S2). The mean length (± SD) of specimens used for respiration measurements was 3.93 ± 0.36 mm, and the calculated mean ash free dry weight (± SD) was 0.61 ± 0.11 mg. At least 3 specimens were measured at each combination of temperature (10 and 20 °C), salinity (0, 10, 20, 25, and 30 psu), and acclimation time (0, 2, 4, 6, 8, and 10 days). Standard metabolic rate of *P. antipodarum* was significantly affected by temperature, salinity, and acclimation time (Figs 4, 5). For comparisons, oxygen consumption rates measured between 50–75% air saturation were use. The oxygen consumption rates of *P. antipodarum* at 0 psu were significantly higher at higher temperatures with 35.9 ± 9.4 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$ at 10 °C and 67.7 ± 15.2 μmol $O_2 g_{AFDW}^{-1} h^{-1}$at 20 °C, respectively (Tukey post hoc, $p < 0.001$; Fig. 4, Table 2; Suppl. material 1: Table S3). The resulting effect of temperature is equivalent to a Q_{10} of 1.9.

Osmotic shock from 0 to 10 psu (no acclimation period) produced a decrease in the average SMR to 23.9 ± 2.1 and 55.2 ± 11.9 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$ for 10 and 20 °C, respectively ($Q_{10} = 2.3$), showing significant differences between these temperature conditions (Tukey post hoc, $p < 0.01$; Fig. 4, Table 2; Suppl. material 1: Table S3), but not between salinities. SMR decreased significantly when snails were exposed to higher salinities of 20 psu or more at the same temperature (Fig. 4, GLM-ANCOVA, Tukey-HSD post hoc test $p < 0.01$), reaching the lowest respiration rates at 30 psu and 10 °C (1.5 ± 1.8 μmol $O_2\, g_{AFDW}^{-1}\, h^{-1}$).

After 2 days of acclimation at 20 °C, the overall average SMR of snails showed a significant increase (Fig. 5). Conversely, at 10 °C SMR remained low without significant differences across acclimation days. At 30 psu, oxygen consumption was always lower than oxygen consumption at lower salinities regardless of acclimation time at 20 °C (Fig. 5; lower panel). This effect of high salinity on respiration rate was not as clear at 10 °C (Fig. 5).

Standard metabolic rate at low oxygen concentration

At 10 °C, SMR was not significantly affected by oxygen concentration above 25% saturation (Fig. 6; Suppl. material 1: Table S5). At 20 °C, SMR was significantly higher at 75% saturation compared with intermediate oxygen levels (Fig. 6; Suppl. material 1: Table S5). A significant drop in SMR was observed when oxygen levels reached ~5% of saturation (two-way ANOVA, Tukey post hoc test, $p < 0.05$; Fig. 6).

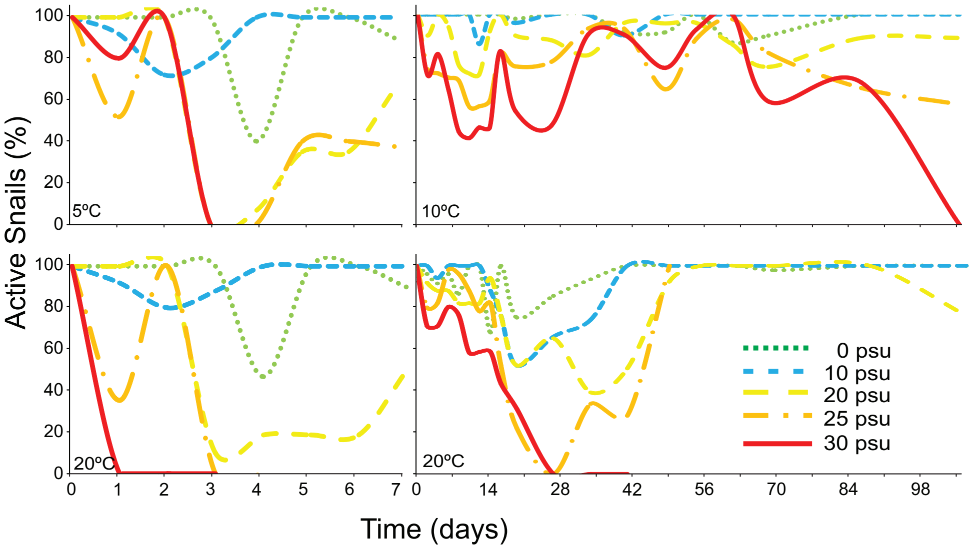

Figure 3. Activity of the New Zealand mudsnail, *Potamopyrgus antipodarum*, under different salinity conditions. Active snails were snails with an open operculum. Experiments were carried out during the winter (left panels) and spring (right panels). Values are based on the number of snails remaining after mortality.

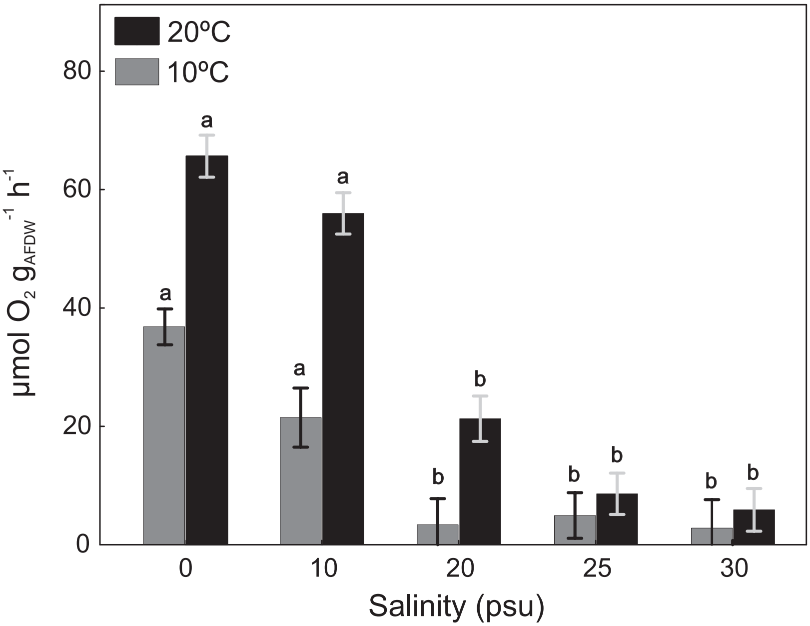

Figure 4. Oxygen consumption rates of *Potamopyrgus antipodarum* at two temperatures and five salinities. Different letters indicate significant difference ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) between standard metabolic rate (mean ± SE) between salinity treatments within the same temperature conditions. Rates were measured between 50–75% air saturation.

Table 2. Results of GLM-ANCOVA analysis assessing effects of salinity (0, 10, 20, 25, and 30 psu), temperature (5, 10, and 20 °C), and acclimation time (0, 2, 4, 6, 8, and 10 days) on the standard metabolic rate of *Potamopyrgus antipodarum*. DF = degrees of freedom. AFDW = ash free dry weight.

	DF	MS	*F*	*P*
AFDW	1	637.8	8.76	0.0051
Temperature	1	3477.8	47.74	<0.001
Salinity	4	4774.8	65.54	<0.001
Temp*Salinity	4	470.9	6.46	0.0004
Residuals	41	72.8		
	DF	**MS**	***F***	***P***
AFDW	1	15167.3	33.01	<0.001
Temperature	1	75814.3	165.02	<0.001
Acclimation time	5	3831.8	8.34	<0.001
Temp*Acclimation	5	2175.1	4.73	0.0004
Residuals	220	459.4		

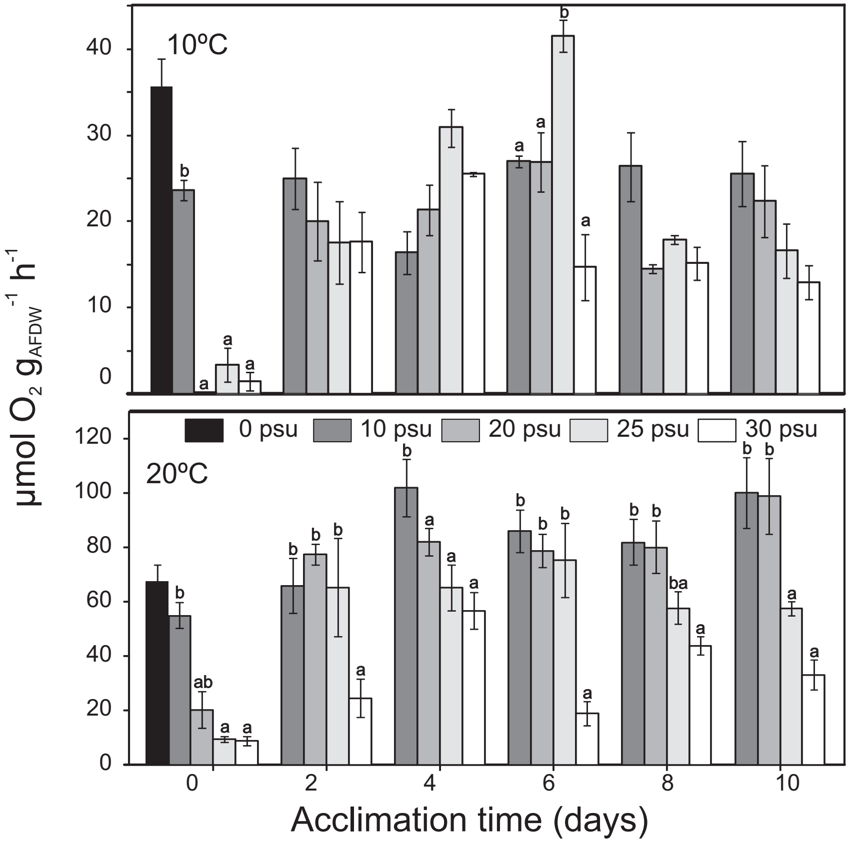

Figure 5. Average oxygen consumption rates for *Potamopyrgus antipodarum* at different acclimation times (0–10 days) when the snail was exposed at different water temperatures (10 and 20 °C) and at different salinities. Different letters indicate significantly different oxygen consumption rate ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) between salinity treatments within each acclimation time. Snails were collected during spring. Rates were measured between 50–75% air saturation.

Table 3. Enzymatic activities of the New Zealand mudsnail, *Potamopyrgus antipodarum*, under three experimental conditions: Temperature, 10 °C, salinity 0 and 30 psu, and acclimation time 0 and 10 days. AFDW: ash free dry weight. n.d.: no data.

	Enzymatic Activity (units g_{AFDW}^{-1}, mean ± SE, *n*)		
	Treatment Conditions (T, S, Incubation period)		
Enzyme	10 °C, 0 psu, 0 days	10 °C, 30psu, 10 days	20 °C, 30 psu, 10 days
Malate dehydrogenase	19.143 ± 1.675, 9	14.298 ± 0.711, 3	10.023 ± 1.303, 6
Lactate dehydrogenase	1.140 ± 0.496, 9	0.410 ± 0.043, 3	0.312 ± 0.032, 6
Alanopine dehydrogenase	3.653 ± 0.241, 9	2.808 ± 0.387, 3	2.572 ± 0.244, 6
Octopine dehydrogenase	0.320 ± 0.128, 5	n.d.	n.d.
Tauropine dehydrogenase	0.710 ± 0.206, 6	n.d.	n.d.
Strombine dehydrogenase	0.953 ±0.169, 7	n.d.	n.d.

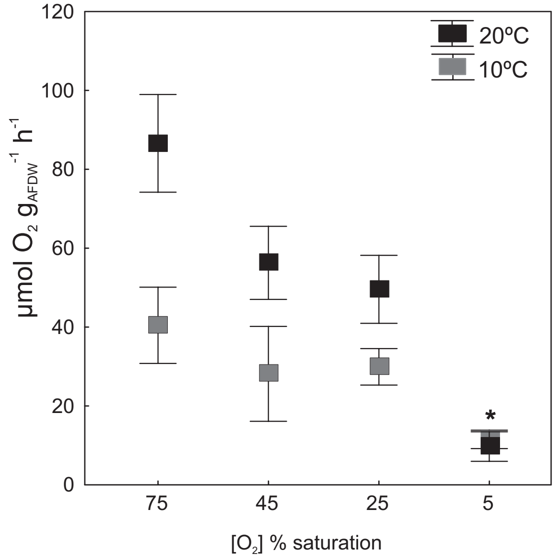

Figure 6. Average oxygen consumption rates of the New Zealand mudsnail, *Potamopyrgus antipodarum*, at different oxygen levels when exposed to 0 psu salinity conditions at 10 and 20 °C. Values are mean ± SE. Rates at 5% saturation are significantly lower than the other rates at the same temperature (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons).

Enzymatic activities

Activities of MDH, LDH, ADH and other –opine dehydrogenases are given in Table 3. Alanopine dehydrogenase was found to be the main anaerobic enzyme in *P. antipodarum*, and ADH activities are ~10× higher than those of LDH. Activities of all enzymes were significantly lower after 10 days at higher salinities (Fig. 7, Table 3). Lactate dehydrogenase showed the largest average difference (73% lower); MDH activity was on average 39% lower, and ADH activity had the smallest average difference (–26%).

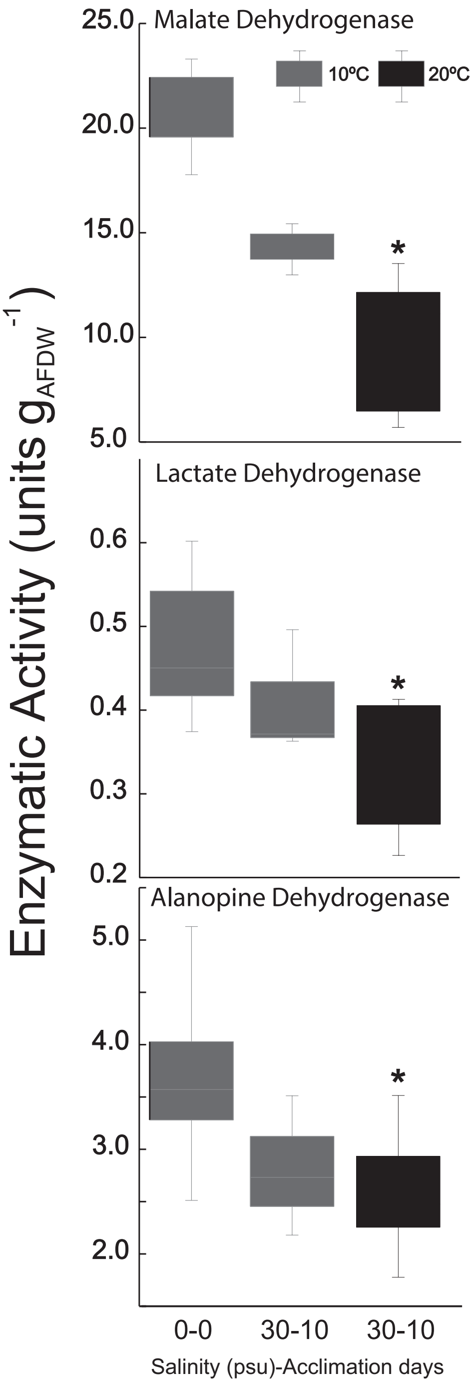

Figure 7. Enzymatic activities of the New Zealand mudsnail, *Potamopyrgus antipodarum*, in freshly collected specimens and under two experimental conditions. Boxes represent 50% of enzyme activities, and whiskers are the total range of data. Enzymatic activities in specimens incubated for 10 days at 20 °C and 30 psu are significantly different from those at 0 psu for all three enzymes (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons). All activities were measured at 20 °C.

Discussion

Mortality

The increase in the mortality rate of *P. antipodarum* with an increase in salinity observed during this study agrees with results obtained in previous studies (Duncan and Klekowski 1967; Costil et al. 2001; Leclair and Cheng 2011; Hoy et al. 2012). The time of exposure and the high salinity needed to reach mortality rates close to 100% render high temperature and high salinity control methods difficult to apply successfully at an environmental scale such as Capitol Lake, Washington. The mortality results demonstrate that controlling this species by osmotic shock will probably fail due to its high tolerance, behavior and plasticity to become acclimated to high salinity concentrations in agreement with previous studies (Duncan and Klekowski 1967; Hoy et al. 2012). In the Columbia River estuary, populations of *P. antipodarum* displayed high salinity tolerance with 80% survival after three weeks at 34 psu (Hoy et al. 2012).

The previous attempt to control *P. antipodarum* in Capitol Lake through backflushing the lake with seawater (Leclair and Cheng 2011) probably failed due to the short period of time (4 days), low salinity (7.5–24.9 psu) and moderate water temperature ~9 °C. Our results demonstrated that a salinity of 30 psu needs to be applied for at least 7–40 days, depending mostly on season and temperature, to reach mortalities close to 100%. A higher tolerance to salinity in our population sample, as compared to that predicted by Leclair and Cheng (2011), may be partially explained by a possible selection process of the surviving snails after backflushing the lake with seawater in 2010. Increased salinity tolerances were also reported for this species in its invasive range as compared to native range populations (Drown et al. 2011). Our mortality results (Figs 1, 2) support the hypothesis of Drown et al. (2011) that the 'performance optimum' of invasive lineages of *P. antipodarum* is shifted to higher salinities.

Seasonal acclimation temperature affected both the salinity concentration at which the highest mortality was observed as well as the speed of mortality. These experimental results were consistent with those reported in previous studies (Hylleberg and Siegismund 1987; Cheng and LeClair 2011; Leclair and Cheng 2011; Moffitt and James 2012). Our results demonstrate that mortality at different salinities can be greatly affected by the season. At temperatures approaching freezing, populations of *P. antipodarum* may be highly stressed (Moffitt and James 2012), and winter may be the best season to carry out control measures. Although logistically intriguing, backflushing the lake with high temperature (20 °C) seawater (>30 psu) during three days in winter could potentially be used as an eradication strategy. Clearly, these snails prefer slightly brackish water over freshwater, and using low salinity seawater in eradication methods will not be effective.

Activity

Snails were significantly most active at lower salinities, and the effect of temperature reducing activity was clear only during the spring. The capacity to avoid stressful conditions by retracting into shells and closing the operculum as observed during our experiment can provide temporary tolerance to high salinity, but this is only a short-term solution to osmotic stress. This species can survive more-or-less normally at 20 psu or less. Although Duncan and Klekowski (1967) found that *P. antipodarum* (as *P. jenkinsi*) could not reproduce at over 12–18 psu, we observed the release of living neonates even at 20 psu. Although we did not quantify number of neonates, these were first observed at 20 °C after 40 days at 0 and 10 psu. Two weeks later the first snails at 10 °C were also observed at these same temperatures. In our study, neonates displayed active locomotor activity even at 20 psu. The presence of neonates during experiments has also been reported by other investigators as a response to stressful conditions (Bruce et al. 2009; Moffitt and James 2012; Romero-Blanco and Alonso 2019). Similar to survival rate, differences in reproduction probability have been observed between populations of this species (Drown et al. 2011), with higher reproduction in invasive or clonal populations compared to native sexually reproducing populations.

Standard metabolic rate

The oxygen consumption rates we measured for *P. antipodarum* are similar to those measured by Duncan and Klekowski (1967) and Hudson (1975). Our results showed that *P. antipodarum* can acclimate to higher salinities and that this acclimation is affected by temperature. Following an initial shock response to higher salinities where aerobic metabolism was suppressed, SMR become depressed at 25 and 30 psu after 8–10 days. This decline in metabolism is more noticeable at 20 °C than at 10 °C. Although, we did not measure SMR at different acclimation times at 0 psu, it is reasonable to suppose that these values will remain stable across time as was observed at 10 and 20 psu for both temperatures. Additionally, our results demonstrated a significant decrease in oxygen consumption rates of *P. antipodarum* at lower oxygen concentrations. Below 70–50% oxygen saturation, *P. antipodarum* becomes an oxy-conformer. This is similar to the oxyconformation response observed for *P. antipodarum* in its native range (Hudson 1975) and for other snail species (McMahon and Russell-Hunter 1978).

Enzymatic activities

It is well known that many molluscs can survive under prolonged periods of hypoxia or even anoxia by exploiting various anaerobic pathways (Grieshaber et al. 1994). Malate dehydrogenase, lactate dehydrogenase, and alanopine dehydrogenase activities in *P. antipodarum* showed significant declines after 10 days at 30 psu. These declines in both anaerobic and aerobic metabolic potentials track the overall decline in whole animal metabolism as measured by oxygen consumption.

Similar to some other gastropods (Baldwin and England 1982; Grieshaber et al. 1994), alanopine dehydrogenase was found to be the main anaerobic enzyme in

P. antipodarum. The –opine dehydrogenases have generally low substrate specificity and can utilize a variety of substrates (Gäde and Grieshaber 1986). The lower activities of octopine dehydrogenase, strombine dehydrogenase and tauropine dehydrogenase could all be due to ADH using those different substrates. Genetic identifications of those other enzymes are needed to confirm their presence in the New Zealand mudsnail. Regardless, MDH, LDH, and ADH were all successfully used herein as indicators of environmental stress (cf. Dahlhoff 2004) for *P. antipodarum*.

Impacts

The future of Capitol Lake remains uncertain. If the dam is permanently breached and the lake restored to estuarine conditions, it is unlikely that wintertime temperatures and salinities would often exceed the thermal and osmotic tolerances of this population of *P. antipodarum*. However, it seems possible that summertime temperatures at low tide and summertime salinities at high tide could pass the upper thermal and osmotic tolerances of this population. This could result in the eradication of the New Zealand mudsnail from this ecosystem. However, if this species makes its way upstream into areas of permanent freshwater, it will likely continue to successfully seed the estuary for many years to come.

Additionally, it seems possible that tolerance to high salinity conditions, probably based on phenotypic plasticity (Drown et al. 2011), will allow this species, not only to survive if Capitol Lake is restored to an estuarine ecosystem, but also to spread to other coastal environments of mid-range salinities. Potentially, its high tolerance to environmental stress due to its physiological plasticity and anaerobic potential may give this species a seemingly greater competitive capacity over other aquatic species as has been previously suggested (Sardiña et al. 2015; Rakauskas et al. 2018). However, this advantage may change under future contexts of increasing temperatures (Sardiña et al. 2015).

Data resources

The data underpinning the analysis reported in this paper are deposited in the Zenodo Data Repository (https://doi.org/10.5281/zenodo.3567136).

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Supplementary material 1

Tables S1–S5

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Data type: measurements.

Explanation note: **Table S1.** Complementary statistic result (Tukey post hoc comparisons) for figure 2, which is showing results of two-way ANOVA test assessing effects of salinity (0, 10, 20, 25, and 30 psu) and temperature (5, 10, and 20°C) on mortality of *Potamopyrgus antipodarum* during winter and spring. DF = degrees of freedom. **Table S2.** Standard Metabolic Rate of the New Zealand mudsnail, *Potamopyrgus antipodarum*, in freshly collected snails and 2–10 days of acclimation at five salinity levels (0, 10, 20, 25, and 30 psu) and two temperature conditions (10 and 20°C). **Table S3.** Statistic results (GLM-ANCOVA, Tukey post hoc comparisons) for the oxygen consumption rates of *Potamopyrgus antipodarum* at two temperatures and five salinities (Figure 4). Rates were measured between 50–75% air saturation. **Table S4.** Lower panels. Statistical results for the average oxygen consumption rates for *Potamopyrgus antipodarum* at different acclimation times (0–10 days) when the snail was exposed to higher salinity conditions at different salinities (Fig. 5 lower panels). Contrast show significantly different oxygen consumption rate ($p < 0.05$ GLM-ANCOVA, Tukey post hoc comparisons) across the same temperature (upper panel) or between salinity treatments within each acclimation time (lower panels). Snails were collected during spring. Rates were measured between 50–75% air saturation. **Table S5.** Statistical results (ANOVA, Tukey post hoc comparisons) for the Figure 6. Average oxygen consumption rates of the New Zealand mudsnail, *Potamopyrgus antipodarum*, at different oxygen levels when exposed to 0 psu salinity conditions at 10 and 20 °C. Values are mean ± SE. Rates at 5% saturation are significantly lower than the other rates at the same temperature (*, $p < 0.05$, ANOVA, Tukey post hoc comparisons).

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RESEARCH ARTICLE

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The functional composition of the neophytic flora changes in response to environmental conditions along a rural-urban gradient

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Abstract

Compared to rural environments, cities are known to be extraordinarily rich in plant species. In particular, the proportion of alien plant species is higher in urban areas. This is attributed to specific urban conditions, which provide a large variety of habitats due to high geological heterogeneity. It can also be attributed to the role of cities as centres for plant introductions and the consequential increased propagule pressure. Neophytes, alien plant species introduced after the discovery of the Americas, appear to contribute especially strongly to the increased proportion of alien plants in cities. To investigate whether the plant traits of neophytes can be explained by environmental variables, we modelled the composition of their pollination types and growth forms as well as their diaspore weight and the onset of flowering in response to a selection of climatic, geological, land cover and traffic network variables with data from Germany. To test for a specific urban effect, we included their interactions with the area of urban land use.

In general, we found that climatic variables were the most important predictors for all traits. However, when considering interactions with urbanisation, non-climatic variables, which often were not significant as the main effect, remained in the final models. This points to an existing 'urban effect'. However, it is much smaller compared to the purely climatic effects. We conclude that interferences and alterations mainly related to urbanisation and human activity in general are responsible for the different ecological processes found in cities compared to rural areas. In addition, we argue that considering functional traits is an appropriate way to identify the ecological mechanisms related to urbanisation.

Keywords
Alien plants, cities, growth form, phenology, pollination, seed mass, traits, vector generalised linear model

Introduction

Next to climate and land-use change, biological invasions are regarded as one of the main drivers for the recent loss of biodiversity (Elton 1958, IPBES 2019). However, the arrival of new species in foreign regions does not necessarily lead to their successful establishment. In fact, some of these alien species rather benefit from changes in ecosystems (they are also called passengers of change) instead of causing them (also called drivers of change, Didham et al. 2005). In particular, those species successfully invading degraded ecosystems tend to be rather passengers of change (MacDougall and Turkington 2005). In turn, heavily disturbed habitats, such as urbanised areas, are the ones that are most strongly invaded (Kowarik 1990).

The increase in urban population (United Nations 2014) leads to an expansion of the urban built area at the cost of unsealed land (Douglas and James 2015). Therefore, urbanisation can be regarded as one of the main drivers of land-use change, often at the expense of natural ecosystems (Vitousek et al. 1997), and it will probably facilitate invasions in the future.

Due to their particular characteristics, cities differ from their surrounding non-urban areas by decreased mean annual air humidity and consequently drier soils (Bridgman et al. 1995), increased average annual temperature (Oke 1982, Bowler et al. 2010), and reduced wind speeds (Nowak et al. 2010). Further, the highly heterogeneous structure of urban areas offers a wider range of different habitats (Kühn et al. 2004). In addition, cities represent hotspots of human mobility and transportation which are considered important factors determining the number of invasive species (von der Lippe and Kowarik 2008, Jehlík et al. 2019, Seebens 2019).

In terms of cities also being hotspots for alien plant species, it is important to understand which plant traits benefit from, and which are disfavoured by urbanisation. Several studies have focussed on functional traits and whether they are either promoted or suppressed in cities. Their results seem to imply that plants that thrive in nutrient rich, alkaline soils as well as in warm and bright conditions are more successful in urban areas. Plants that prefer moist conditions are suppressed in cities (Williams et al. 2015). For example, in Germany, when compared to non-urban grid cells, urbanised grid cells had higher proportions of wind-pollinated plants, plants with scleromorphic leaves, and plants that are dispersed by animals. At the same time, these urban grid cells had lower proportions of insect-pollinated plants, plants with hygromorphic leaves, and plants dispersed by wind (Knapp et al. 2008b).

The concept of the rural-urban gradient (Forman 2014) is widely used to highlight the variability of several environmental factors when transitioning from rural areas to the city centre. Kühn et al. (2017) tested for a potential urban effect on neophytes (i.e. species introduced after the discovery of the Americas, Pyšek et al. 2004), or more precisely, on their richness and proportion. Based on the assumption that the alien flora in cities differs from the alien non-urban flora, they expected changes in the responses of neophyte richness to environmental variables along the rural-urban gradient. If this holds true, they would speak of an existing urban effect. Their results, however, indicate that conditions that explain neophyte richness in cities are mostly the same as in rural environments but are more effective towards the urban end of the gradient (Kühn et al. 2017).

In this study, we aim to extend this approach and to test whether selected traits of neophytes are affected differently by environmental conditions along the rural-urban gradient. Firstly, working with traits allows to get beyond a purely taxonomic characterisation to a more functional approach (Kühn et al. 2006, McGill et al. 2006), secondly, we are not aware of many studies, that take a trait-based approach to urban flora (but see e.g. Knapp et al. 2008b, 2010, Fischer et al. 2013). In doing so, we hypothesise that the following traits will change in a rural-urban gradient: (1) pollination type, (2) growth form, (3) diaspore mass and (4) flowering phenology.

Pollination type describes the vector a plant employed for pollen transfer. We included this trait in the analysis because the main vectors, wind and insects, are known to differ between urban and rural landscapes (Connor et al. 2002, Nowak et al. 2010). We aim to test whether these differences also reflect variances in the proportion of insect-, wind- and self-pollinated neophytes in their response to environmental conditions. According to some studies, urbanisation favours wind pollination over biotic pollination (Lososová et al. 2006, Sodhi et al. 2008, Knapp et al. 2008a, 2010).

Growth form in this study is a combination of life form (Raunkiær 1934) and lifespan, a specification of lifetime and the number of possible generative reproductions. Therefore, growth form provides information on the duration of a plant's life cycle and whether it is woody or not. We analysed this trait because the different frequencies and intensities of disturbance in cities compared to rural areas might favour or disadvantage certain growth forms. Previous studies revealed that urbanisation tends to favour woody plants, i.e. trees and shrubs (Knapp et al. 2008a, Williams et al. 2015, but Chocholoušková and Pyšek 2003), therophytes/annuals (Sukopp and Werner 1983) as well as biennials (Chocholoušková and Pyšek 2003, Lososová et al. 2006), and disfavours geophytes (Williams et al. 2015, but Knapp et al. 2008a).

Diaspore mass is important, because large diaspores contain more resources and therefore have competitive advantages in the establishment of seedlings compared to small seeds (Westoby et al. 1996). Small diaspores, however, are more likely to be dispersed further and therefore are more likely to reach new appropriate habitats (Meyer and Carlson 2001). Hence, diaspore mass is linked to both processes of dispersal and establishment. It has been assumed that for every environment there is an optimal seed mass (Smith and Fretwell 1974). Urban environments should therefore also represent habitats ideally suited for a certain (rather lower) seed mass, because nutrients are usually sufficient but newly disturbed patches need to be colonised.

Flowering phenology refers to information about timing and range of flowering events and is of great importance for pollination and reproduction. Despite genetic determination, the beginning, end and the duration of flowering can be modified, mainly by climatic conditions (Trefflich in Klotz et al. 2002). Here, we test the hypothesis that flowering begins early in urban areas and in areas with higher temperatures and moderate precipitation.

In this study, we test whether there is an urban effect on the proportions of traits (pollination, growth form) or the average state (diaspore mass, phenology) of neophytes in the rural-urban gradient. To this end, we explain the functional composition of grid cells of the mapping scheme of the flora of Germany by different environmental variables. In addition, we added the interactions of each variable with the area of urban land-use to detect possible urban effects in comparison with the main effects of specific environmental variables.

Data and methods

Floristic data

The data for plant species occurrence was extracted from the latest version (2013) of the floristic mapping of Germany (NetPhyD and BfN 2013), which contains more than 14 million records of plant occurrences in Germany. Data is available at a grid resolution corresponding to 10' longitude and 6' latitude (c. 12 km × 11 km). Since fragments of grid cells in border regions or along coastlines have considerably fewer species, only grid cells equal to, or greater than, the size of the smallest full cell of Germany (c. 117 km^2) were kept for analysis. As the survey efforts in different regions and the botanical knowledge of volunteers are heterogeneous (Manceur and Kühn 2014), we followed Kühn et al. (2006) in only including grid cells with at least 45 out of 50 control species (those widely spread which should be present in each well-mapped grid cell), resulting in n = 2599 grid cells.

Only naturalised occurrences of neophytes from 1950 onwards were considered. Data on floristic status was retrieved from BiolFlor (Klotz et al. 2002). In total, 497 different neophyte species were included in the analysis, varying widely in frequency of occurrence (Suppl. material 1, Table S1).

Trait data

Data on the four traits (pollination, growth form, diaspore mass, flowering phenology) was also retrieved from BiolFlor (Klotz et al. 2002) and merged with the plant occurrence data based on species names or synonyms. The categorial traits *pollination type* and *growth form* comprise several trait states (Table 1). For some species, multiple

Table 1. Description, abbreviation and source of the traits and response variables used in the analyses.

Trait	Description	Response var.	Abbreviation	Source
Pollination type	Type of pollen transfer to the stigma. Either abiotic or biotic. Only the three most common types were considered and only those which were assigned as *always, often* or *the rule* for each plant in BiolFlor.	Insect pollination (entomophily)	i	Durka in Klotz et al. (2002)
		Wind pollination (anemophily)	w	
		Self-pollination (autogamy)	s	
		Multiple	m	
Growth form	Trait combining lifespan and life form.	Annuals	an	Krumbiegel in Klotz et al. (2002)
		Biennials	bn	
		Herbaceous perennial	hp	
		Woody plants	wd	
		Multiple	mu	
Diaspore mass	Mean mass of diaspore (germinal plus any dispersal-assisting tissue).	mean(log(diaspore mass))	-	Otto in Klotz et al. (2002)
Flowering phenology	Mean month at which neophytes of a grid cell begin to flower.	mean(beginning month of flowering)	mean(BFM)	Trefflich in Klotz et al. (2002)

pollination types were recorded or the state of life span can have several states. In these cases, an additional state called ‘multiple’ was introduced.

Environmental data

Data for model prediction is comprised of data on climate, geology, land cover and traffic network. A total of 19 initial environmental predictors (see Table 2) were selected based on physiological principles known to be related to species richness in general, and alien species richness in particular (Kühn et al. 2017).

Statistical analysis

Model specification and simplification

All environmental predictors were centred to zero mean and unit standard deviation. Collinearity between the environmental predictors was assessed, but none of the pairs of predictors showed high collinearity ($|\text{Kendall's } \tau| > 0.7$; Dormann et al. 2013).

One problem with compositional data is that the proportions are not independent of each other: if one proportion increases, at least one of the others must decrease and vice versa. This is called the unit-sum-constraint and can be obviated by using the logarithms of ratios (log-ratios) instead of the observed proportions (Aitchison 1986, Billheimer et al. 2001, Kühn et al. 2006). While, mathematically, the choice of denominator and numerator for the log-ratios is arbitrary and does not impair the results (Aitchison 1986), for the purpose of interpretation, the choice depends on the respective ecological interest. Therefore, to model the proportions of the states of pollination type and growth form in response to the environmental variables, we used a vector generalised linear model (VGLM; Yee 2015) with a multinomial distribution family based on log-ratios (Lososová et al. 2012, Menzel et al. 2016). This approach

Table 2. Environmental data and sources used for analysis of the response variables. Variables are known to be related to species richness in general and alien species richness in particular. We provide units and variable range for linear but not for quadratic predictors. In such cases, the units (and min/max values) do not make sense and the transformation was performed to account for non-linear relationships.

Variable	Description of variable	Units	Variable range (min - max)	Source
TmpJul	Average July temperature	°C	12.8–19.8	Fronzek et al. (2012), observation period 1961-2000
TmpJul^2	Squared average July temperature			
TmpAnnualRange	Annual temperature range, i.e. average difference between January and July temperature	°C	21.5–29.1	
PrecipitationSummer	Average summer precipitation (June, July, August)	mm	168–494	
PrecipitationSummer^2	Squared average summer precipitation			
WindSpeed	Average wind speed	m/s	1.9–5.6	
#GeoPatch	Number of geological patches		1-51	Bundesanstalt für Geowissenschaften und Rohstoffe (1993)
#GeoType	Number of geological types		1-24	
LoessArea	Area covered by loess	km^2	0–114.1	
SandArea	Area covered by sand	km^2	0–135.2	
LimestoneArea	Area covered by limestone	km^2	0–135.4	
#LcPatch	Number of land cover patches		26–353	LBM_DE2012 – Bundesamt für Kartographie und Geodäsie (2012)
#LcType	Number of land cover types		5-22	
ForestArea	Forest area	km^2	0–131.5	
AgriculturalArea	Agricultural area	km^2	0.5–124.7	
UrbanArea	Urbanised area	km^2	0–79.7	
RiverArea	River area	km^2	0–8.5	
RoadLength	Total length of roads	km	0.2–29.2	Open Street Map Project and MapCruzin (undated)
RailwayLength	Total length of railways	km	0–6.1	

accounts for the above-described problem of the unit-sum-constraint. For both categorical traits, ‘multiple’ was used as the denominator. For simplicity, the ratios of the unambiguously assignable trait states over ‘multiple’ hereafter are simply called ‘ratios’. Diaspore mass and flowering phenology are metric. Therefore, generalised linear models (GLM) were fitted.

In order to detect a potential ‘urban effect’, a different model was fitted to each of these four traits (either VGLM or GLM) with all the environmental variables (except wind speed, see below) as initial predictors plus the interactions of each variable with the area of urban land cover (*UrbanArea*; hereafter called ‘urbanised area’). In addition, the initial models for pollination type, diaspore mass and flowering phenology also included the variable of average wind speed and its interaction with the urbanised area. The model simplification process for the generalised linear models (GLM) and vector generalised linear models (VGLM) followed the recommendations of Crawley (2012) by implementing a backward selection based on error probabilities such that a predictor was significant for at least one log-ratio.

Spatial autocorrelation

To test for spatial independency of the residuals, Moran's Index (the autocorrelation equivalent of Pearson’s correlation coefficient) was calculated using the R package ncf by Bjørnstad (2013). All initially fitted models (VGLM and GLM) showed highly spatially autocorrelated residuals (Moran's $I > 0.04$ [$p < 0.05$]) up to a distance of 10 grid cells and therefore were not suitable for drawing robust conclusions.

To account for spatial autocorrelation, a method called the 'residuals autocovariate approach' was applied (Crase et al. 2012). As this approach calculates an autocovariate from the residuals (hereafter called 'residual autocovariate', *RAC*) instead of deriving it from the response variables themselves, it does not suffer from biased parameter estimates that were reported by Dormann (2007). For VGLMs, the number of *RACs* equals the number of log-ratios that are included in the model. In generalised linear models with only one response variable, only one residual autocovariate was calculated.

During each step of model simplification (see above), new *RACs* for the updated model were calculated and the process was repeated until the final model was selected. This procedure ensured that the most accurate autocorrelation structure was utilised at every step. The residual autocorrelation could be reduced to a satisfying level for all final models.

Model evaluation

To evaluate the model fits, their explained deviance D^2 was calculated (Guisan and Zimmermann 2000). To assess whether the D^2 of the final models (D^2_{tot}) is mainly due to the environmental variables or the residual autocovariates, it was partitioned into the deviance explained by the environmental-only (D^2_{env}) and by the residual autocovariate-only model (D^2_{rac}; Borcard et al. 1992). We never observed an overlap between the two groups.

Software

All statistical analysis was performed with the software R, version 3.4.1 (R Core Team 2017). The following packages were used, mainly for data processing purposes: raster (Hijmans et al. 2016), sp (Pebesma et al. 2017), corrgram (Wright 2017), and VGAM (Yee 2017).

To calculate area and length per grid cell, it was necessary to indicate the intersections of the land-cover data and traffic network systems with the lattice used for the floristic mapping. This was performed with the geographical information systems (GIS) ArcGIS 10.5 (ESRI 2016) and QGIS 2.18 (QGIS Development Team 2015).

Results

While trait information on pollination, growth form and flowering phenology was available for the majority of species, information on seed mass was scarcer (for details see Table 3). The most common pollination type was insect pollination and most species were either annuals or herbaceous perennials (Figure 1). In the following, we will focus on the most pertinent results of the statistical analyses (Tables 4–6).

Table 3. Number of neophytes per trait and trait state used for analysis. For minimum, maximum, median and/or mean values see Suppl. material 1, Table S2.

Trait	Trait state	Number of species	
		per trait state	total
Pollination type	Insect pollination	262	438
	Wind pollination	69	
	Self-pollination	82	
	Multiple	25	
Growth form	Annuals	157	492
	Biennials	32	
	Herbaceous perennial	193	
	Woody plants	74	
	Multiple	36	
Diaspore mass			188
Flowering phenology			482

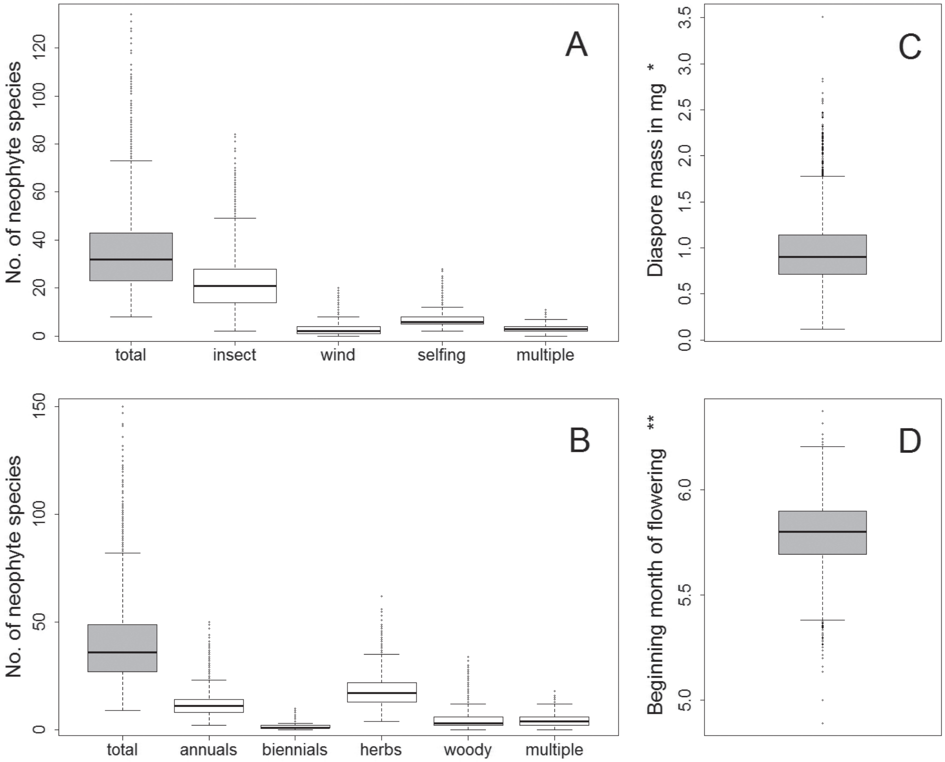

Figure 1. Overview of trait states of neophytes across the 2599 grid cells of Germany **A** pollination type **B** growth form **C** diaspore mass (*back-transformed mean of log transformation) and **D** time (in months) at which neophytes begin to flower (**mean/grid cell). Bold black lines represent medians, boxes 25–75% interquartiles, whiskers samples with less than 1.5 times of the interquartile range and dots are outliers. For detailed values see Suppl. material 1, Table S2.

Pollination type

The explanatory power of the model was quite good, though only half of the variation explained refers to environmental information (Table 4). Wind speed had the strongest negative effect on the relative ratio of insect-pollinated neophytes (Table 4A). Geological characteristics and land cover features were of additional, but much less, importance. We found a significant interaction: in more urbanised areas insect pollination decreases with low July temperatures and increases again with temperatures rising higher than the mean for July.

The log-ratio of wind pollination (log[w/m]) had by far the highest number of significant predictors of all log-ratios in the model (Table 4B). In turn, climatic variables had the absolute largest parameter estimates by far. For July temperatures, there seems to be an optimum pollination ratio around average temperatures. Very low temperatures lead to a strong decrease, and high temperatures to a moderate decrease of wind-pollinated species. Further results indicate that highly urbanised

Table 4. Estimates of modelling the log ratios of (A) insect pollination, (B) wind pollination, and (C) selfing over multiple pollination types. For modelling, a vector generalised linear model (VGLM) was used that included a multinomial distribution family and residual autocovariates (RAC) to account for spatial autocorrelation (SAC). D^2_{tot} – deviance of final model; D^2_{env} – deviance of environmental-only model; D^2_{rac} – deviance of residual autocovariate-only model. Bold numbers indicate significant values. Asterisks represent error probabilities: ◦ 0.1 > $p \leq .05$; * .05 > $p \leq .01$; ** .01 > $p \leq .001$; *** $p < .001$. For abbreviations of predictors see Table 2.

Predictor	(A) log(i/m)	(B) log(w/m)	(C) log(s/m)
Intercept	**+1.791** ***	**-0.482** ***	**+0.746** ***
UrbanArea	+0.027 °	**+0.097** ***	+0.010
TmpAnnualRange	+0.038 °	**+0.277** ***	+0.003
TmpJul	-0.245	**+1.189** *	-0.653
TmpJul^2	+0.072	**-1.172** *	+0.679
PrecipitationSummer	-0.021	**-1.114** ***	**+0.626** ***
PrecipitationSummer^2	+0.057	**+1.116** ***	**-0.491** ***
WindSpeed	**-0.150** ***	**-0.128** ***	-0.021
#GeoPatch	**-0.027** *	-0.008	-0.022
LoessArea	**+0.035** **	**+0.035** *	+0.013
SandArea	**-0.048** ***	+0.038 °	-0.022
#LcType	-0.004	**+0.050** *	+0.002
#LcPatch	+0.007	**-0.021** **	**+0.055** ***
AgriculturalArea	**+0.062** ***	+0.020	-0.001
RiverArea	+0.013	**+0.085** ***	**+0.029** *
UrbanArea:TmpJul	**+0.044** **	**+0.057** **	**+0.051** **
UrbanArea:WindSpeed	+0.020	**+0.067** ***	-0.003
UrbanArea:SandArea	-0.008	**-0.051** **	-0.013
RAC1	**+9.565** ***	+0.373	-0.079
RAC2	+0.013	**+8.942** ***	+0.024
RAC3	-0.254	+0.026	**+9.626** ***
D^2_{tot}		**0.61**	
D^2_{env}		**0.29**	
D^2_{rac}		**0.30**	

areas seemingly harbour more wind-pollinated neophytes. It is also noteworthy that the ratio of wind pollination was the only log-ratio in the model with more than one significant interaction. In more urbanised areas, the ratio of wind pollinated neophytes increases even more with intermediate to high July temperatures and decreases with low July temperatures. Further, the ratio of anemophily decreased less in more urbanised areas with higher wind speeds than in non-urban areas. While the effect of sandy areas was not significant in non-urban areas, it had a negative influence in more urbanised areas.

The ratio of self-pollinators was mostly related to climatic variables, and most strongly to the linear and cubic terms of summer precipitation with an optimum around the average amount of precipitation (Table 4C). July temperature appeared to be the only variable that significantly interacted with the urbanised area. With temperatures higher than the mean, selfing is slightly more frequent in more urbanised areas. With lower temperatures, the frequency of selfing slightly decreases in more urbanised areas.

Growth form

The explained deviance of growth form was comparable to that of pollination (Table 5). The ratio of annual neophytes decreased with a wider annual temperature range and to a lesser degree with a larger number of land cover patches per grid cell (Table 5A). It increased with higher July temperatures and a larger river area. Agricultural area had a significant and negatively related interaction with urbanised area, however, it was not significant as a main effect.

The most important positively related variable explaining the ratio of biennials was the main effect of urbanised area (Table 5B). Most important in general was precipitation (linear and cubic forms). Similar to annuals, the quadratic effect of summer precipitation revealed a minimum ratio of biennials around average amounts of precipitation. Again, land cover and geological predictors had minor effects.

Except for the quadratic effect of summer precipitation, the ratio of herbaceous perennials was explained only by negatively related variables: annual temperature range, July temperature, urbanised area and river area (Table 5C). Most of the land cover and geological predictors were insignificant.

The ratio of woody plants increased with a wider annual temperature range and decreased with increasing average July temperatures (Table 5D). Again, as summer precipitation was negatively related and squared summer precipitation was positively related, the ratio of woody species first decreased with low to intermediate amounts of precipitation and increased with intermediate to high precipitation in summer. Again, non-climatic predictors were less important and even the many interactions they entailed were weak, even if significant.

Table 5. Estimates of modelling the log ratios of (A) annuals, (B) biennials, (C) perennial herbs, and (D) woody plants over multiple types. A vector generalised linear model (VGLM) was used that included a multinomial distribution family and residual autocovariates (RAC) to account for spatial autocorrelation (SAC). D^2_{tot} – deviance of final model; D^2_{env} – deviance of environmental-only model; D^2_{rac} – deviance of residual autocovariate-only model. Bold numbers indicate significant values. Asterisks represent error probabilities: ◦ 0.1 > $p \leq .05$; * .05 > $p \leq .01$; ** .01 > $p \leq .001$; *** $p < .001$. For abbreviations of predictors see Table 2.

Predictor	(A) log(an/mu)	(B) log(bn/mu)	(C) log(hp/mu)	(D) log(wd/mu)
Intercept	**0.979** ***	**-1.055** ***	**+1.443** ***	**-0.040** **
UrbanArea	-0.034	**+0.083** *	**-0.053** *	+0.038
TmpAnnualRange	**-0.106** ***	**+0.058** *	**-0.071** ***	**+0.129** ***
TmpJul	**0.070** ***	-0.051 °	**-0.062** ***	**-0.068** ***
PrecipitationSummer	**-0.651** ***	**-1.009** ***	**-0.253** *	**-1.442** ***
PrecipitationSummer²	**0.546** ***	**+0.911** ***	**+0.232** *	**+0.992** ***
#GeoPatch	0.018	-0.009	+0.010	**+0.037** **
LoessArea	-0.015	+0.009	-0.001	**+0.035** **
SandArea	0.012	**-0.056** **	-0.014	+0.016
LimestoneArea	-0.011	**-0.037** °	+0.004	**-0.050** **
#LcType	**-0.046** **	**+0.063** **	+0.013	+0.025
AgiculturalArea	0.002	**-0.095** ***	+0.010	-0.009
RiverArea	**0.020** *	**-0.046** **	**-0.020** *	-0.009
RoadLength	-0.035	**-0.154** ***	-0.027	**-0.095** ***
UrbanArea:PrecipitationSummer	-0.014	-0.003	-0.016	**+0.042** **
UrbanArea:#GeoPatch	-0.011	-0.001	-0.007	**-0.025** *
UrbanArea:LimestoneArea	0.023	-0.002	+0.003	**+0.061** **
UrbanArea:AgiculturalArea	**-0.019** *	+0.002	**-0.019** *	-0.019 °
RAC1	**9.646** ***	-0.060	-0.032	-0.248
RAC2	-0.165	**+8.870** ***	-0.100	+0.010
RAC3	-0.120	+0.073	**+9.439** *	-0.440
RAC4	0.127	+0.036	+0.150	**+9.750** ***
D^2_{tot}	**0.63**			
D^2_{env}	**0.30**			
D^2_{rac}	**0.30**			

Diaspore mass

The explained deviance of diaspore mass was quite high, but almost twice as much deviance was explained by the autocorrelation structure than was by environmental factors (Table 6A). The most important predictor, again, was summer precipitation. It was quadratically related with diaspore mass and furthermore was significant as quadratic interaction with urbanised area. Hence, minimum masses can be detected just above mean amounts of precipitation; lower and higher amounts of precipitation predict heavier diaspores. The interaction indicates that this main effect of precipitation on diaspore mass is strongly amplified in more urbanised areas. Once again, the non-climatic variables were less important.

Table 6. Estimates of modelling (A) the mean log-transformed diaspore mass per grid cell and (B) untransformed beginning-month of flowering. Generalised linear models (GLM) were fitted including residual autocovariates (RAC) to account for spatial autocorrelation (SAC). D^2_{tot} – deviance of final model; D^2_{env} – deviance of environmental-only model; D^2_{rac} – deviance of residual autocovariate-only model. Bold numbers indicate significant values. Asterisks represent error probabilities: ◦ 0.1 > p ≤ .05; * .05 > p ≤ .01; ** .01 > p ≤ .001; *** p < .001. For abbreviations of predictors, see Table 2.

Predictor	(A) Diaspore mass	(B) Onset of flowering
Intercept	**+0.115** ***	**+5.793** ***
UrbanArea	**-0.046** ***	-0.007
TmpAnnualRange	-0.002	**-0.013** ***
TmpJul	+0.270 °	**+0.021** ***
TmpJul^2	**-0.013** **	
PrecipitationSummer	**-0.398** ***	**+0.123** ***
PrecipitationSummer^2	**+0.308** ***	**-0.132** ***
WindSpeed	**-0.214** ***	**-0.027** ***
#GeoPatch	**-0.026** ***	+0.003
SandArea	**-0.018** ***	+0.003
LoessArea	**+0.031** ***	
LimestoneArea	**-0.018** ***	
#LcType		**0.014** ***
#LcPatch	**+0.028** ***	
ForestArea		**-0.028** ***
AgiculturalArea	**+0.090** ***	**-0.032** ***
RiverArea		**+0.017** ***
RoadLength	**+0.020** *	**+0.021** ***
UrbanArea:TmpAnnualRange	**-0.039** ***	
UrbanArea:TmpJul	**+0.026** ***	**+0.010** ***
UrbanArea:PrecipitationSummer	**-0.217** ***	
UrbanArea:PrecipitationSummer^2	**+0.225** ***	
UrbanArea:#GeoPatch		**-0.009** **
UrbanArea:SandArea		**-0.009** ***
UrbanArea:LoessArea	**-0.014** **	
UrbanArea:LimestoneArea	**-0.029** ***	
UrbanArea:ForestArea		**-0.018** ***
UrbanArea:AgiculturalArea		**-0.016** ***
UrbanArea:RoadLength		**-0.018** ***
RAC	**+9.842** ***	**+9.947** ***
D^2_{tot}	**0.66**	**0.51**
D^2_{env}	**0.21**	**0.16**
D^2_{rac}	**0.39**	**0.33**

Flowering phenology

The explained deviance of flowering phenology was lower than for the other traits with even less explanatory power of the environmental variables (Table 6B). As in the previous traits, summer precipitation was quadratically related to flowering phenology with an optimal amount of precipitation around the average. While urbanised area was not a significant variable itself, six significant interactions with urbanised area remained in the final model. The significant interactions were usually much weaker than their main effects.

Discussion

We analysed trait compositions of alien plant species assemblages with respect to their pollination type, growth form, diaspore mass and flowering phenology at an intermediate spatial scale (i.e. extent is the area of Germany and c. 130 km^2 resolution). We tested whether their composition (for categorical traits) or mean traits values (for metric traits), respectively, are linked to the geographical variation of different environmental conditions, specifically in the rural-urban gradient. Despite the coarse resolution of our input data and rather small shifts in the composition of pollination types, growth forms, mass of diaspores and flowering phenology, the results revealed distinct responses to environmental factors (Tables 4–6). Other studies, e. g. Williams et al. (2015), also analysed a suite of other plant characteristics. However, they chose many niche related features that are not functional traits in the strict sense of the term, but are instead environmental associations (following the concept of Garnier et al. 2017) or compound and derived features such as Grime's CSR strategy, both of which we avoided in order to focus our study. We did not aim, however, to cover the full spectrum of traits available.

Pollination type

In most other studies, pollination did not differ between urban and non-urban environments (see studies reviewed in Williams et al. 2015). In these studies, pollination was shown to be mainly affected by climatic conditions. While wind pollination benefits from low to moderate wind speed, low humidity and infrequent precipitation, insect pollination is promoted by low wind speed, higher humidity rates and infrequent to common rainfall (Kühn et al. 2006). Obviously, insect pollination is limited by insect abundance. However, there is no sufficient data for study available at an appropriate resolution and extent. Still, wind speed across Germany is around 100 times faster than necessary to dispatch pollen from anthers (Kühn et al. 2006, based on data from Whitehead 1968). Therefore, wind is not a limiting factor and hence we conclude that insect activity declining with increasing wind speed is responsible for the observed pattern (c.f. Digby 1958, van Swaay et al. 2002). The higher ratio of anemophily is also hypothesised to be related to phylogeny and correlates, for example, with a high proportion of Poaceae and Chenopodiaceae (which are mainly wind-pollinated) in the flora of settlements (Lososová et al. 2006, Knapp et al. 2008a). Lososová et al. (2006) further discuss that anemophily might benefit from a competitive advantage over entomophily due to a lack of suitable habitats for insects in urban areas.

Although the positive relationship between agricultural land use and insect pollination matches the results of Kühn et al. (2006), an explanation remains elusive as arable fields are usually species poor in general and weeds occurring there are mainly wind- or self-pollinated (Baker 1974). Interestingly, neither the main effects of urbanised area nor July temperatures were significantly related to insect pollination. However, the interaction of both was significantly positively related. This points toward a positive urban effect that mediates the general relationship between July temperatures and the ratio of insect-pollinated neophytes, even reversing it under cooler conditions. Hence, higher or lower temperatures than the mean lead to a slight (non-linear) increase in the ratio of insect-pollinated neophytes. The mediating effect of urbanisation might be explained by a lower habitat availability of insect-pollinated species while the positive effect in cooler regions might especially be due to the urban heat island effect.

Self-pollination is associated with poor climatic conditions and unpredictable environments (Baker 1955), and is often explained as a strategy of reproduction in the absence of pollinators or mates (Kalisz et al. 2004), for example, occurring towards the edge of the geographic range of a species (Crawley 1997). Besides, alien species are more frequently self-pollinated than natives (Klotz et al. 2002). Hence, one would expect a higher frequency of selfing in urban areas and in large river valleys, as both landscapes are known to harbour a relatively high proportion of alien plants (Deutschewitz et al. 2003, Kühn et al. 2004, 2006). Our model, however, only revealed a significant (positive) response of selfing to rivers. Urbanised area remained insignificant as a predictor. Hence, it seems that, in urban regions, this reassurance option is not needed. On the contrary, there is increasing evidence that anthropogenic disturbance can negatively affect outcrossing and hence promote selfing (Eckert et al. 2009). Although the effect of temperature on selfing differed significantly between less and more urbanised areas, the difference was very small and difficult to explain.

Growth form

As expected, climatic variables were most important for explaining the ratio of all of the four growth forms in the model. Furthermore, we detected an urban effect on agriculture for annual and perennial herbs and urban effects of three predictors for woody plants. The annual temperature range is often used as a rough proxy for two important climate types in Europe. While a small temperature range characterises oceanic climate, a wide range indicates a continental climate (Driscoll and Yee Fong 1992). According to our results, annual temperature range was a significant variable that explained ratio changes in all four growth forms. The ratio of annuals decreased with the increasing influence of continental climate.

Annuals are often associated with arable fields, as fields are disturbed relatively regularly (Lososová et al. 2006) and offer a large number of microsites, which are fundamental for the establishment of annuals. However, our results do not support this assumption, as agriculture was not significant as a main effect. This might be because very few of the neophytes are species that are associated with agriculture (Klotz et al. 2002). Highly urbanised areas harbour a high ratio of annuals in absence of agricultural land and a small ratio of annuals in presence of agricultural land. On the other hand, in areas with a high proportion of agricultural land, an additional high proportion of urban area leads to a decrease in the ratio of annuals. Hence, it seems that neophytes cannot cope with two strong types of disturbance simultaneously. The occurrence of two different types of disturbance at the same time might be too strong in magnitude and/or frequency, hinting towards the "intermediate disturbance hypothesis" (Huston 1979).

As in previous studies (Chocholoušková and Pyšek 2003, Lososová et al. 2006, Knapp et al. 2008a), we detected an increase in the ratio of biennials with an increase in urbanised area. Lososová et al. (2006) ascribe this pattern to the existence of so-called mosaics of early to mid-successional stages in cities that offer habitats to all lifespan categories. We did not find any significant interactions that explained the ratio of biennials. One might argue that urban areas simply offer more favourable (or less unfavourable) conditions for biennial neophytes (see the positive relationship with *UrbanArea*). It might also simply be that there is no interaction due to the low number of biennials per grid cell, i.e. a low overall variability.

Our results revealed a large ratio of perennial herbs in more urbanised areas if the proportion of agricultural area was small. In contrast, increasing agricultural area led to a much smaller share of perennial herbs in highly urbanised areas. A possible reason for this could be that the large majority of the neophytes in Germany are intentionally introduced ornamental plants (Lambdon et al. 2008) rather than neophytes that colonize agricultural fields.

Although we did not find that urbanised area had a significant main effect on woody neophytes, our results revealed differences in the rural-urban gradient for three variables. A potential explanation for the benefit that trees and shrubs experience from increased summer precipitation could be deduced from the trade-off between shade- and drought-tolerance. As adaptions of shade-tolerant species preclude a tolerance to drought conditions, they would benefit from moister soils caused by increased summer rainfall (Smith and Huston 1989). This could also perhaps explain the outcome that the positive effect of increased summer precipitation on woody plants is even greater in urban areas. This is because urban areas tend to be even warmer and drier, hence the dependency of shade-tolerant plants on water is even increased. However, we can only speculate about this, as woody plants are not shade-tolerant in general and we did not include the functional types associated with tolerance to shade or drought, neither for trees nor shrubs, in our analysis.

In areas with the lowest rates of urbanisation, an increasing number of geological patches (i.e. coherent areas of the same geological type) had a slightly positive effect. This is probably related to habitat heterogeneity and is in line with the findings of Kühn et al. (2004). Towards the urban end of the gradient, however, this relation is firstly flattened and then even changes direction. Hence, in less heterogeneous regions, urbanisation promotes shrubs and trees again. Most of them, though, are ornamental species that are planted in parks and gardens (Hanspach et al. 2008). Hence, these species are easily promoted by urbanisation. Admittedly, at the moment no reasonable explanation can be given for the changing effect of the area of limestone in conjunction with increasing urbanised area.

Diaspore mass

While four of six studies that were examined by Williams et al. (2015) reported an increase in species with heavier seeds in urban areas, the results of Kalusová et al. (2017) suggest the opposite.

Our results revealed a great number of variables that explain the mass of diaspores. As already encountered by Salisbury (1942), species with larger seeds are more frequent in habitats with higher temperatures. More recently these findings were corroborated by Murray et al. (2004). This relationship between larger seeds and higher temperatures is often explained by the better competitive performance of larger seeds over smaller seeds under poor or stressful conditions such as nutrient deficiency (Westoby et al. 1996), shade (e.g. Leishman and Westoby 1994, Grubb and Metcalfe 1996), and drought (Hendrix et al. 1991).

It is known that seed mass is positively correlated with growth form or adult longevity (Salisbury 1942, Baker 1972, Thompson and Rabinowitz 1989). Thus, annual herbs have smaller seeds than perennial herbs, which again have smaller seeds than shrubs and trees (e.g. Westoby et al. 1996). Several mechanisms generate this pattern. Firstly, due to mechanical constraints, small and short-lived species are not able to produce large seeds. Secondly, when released from the same height, lighter seeds travel further than heavier seeds of similar morphology. As heavy seeds would lead to difficulties of dispersal, small plants tend to produce lighter seeds (Rees 1997).

We showed that urbanised area itself is negatively related to the diaspore mass of neophytes. This matches the results of Kalusová et al. (2017) but contradicts the papers which were reviewed by Williams et al. (2015). Williams et al. argue that heavier seeds are better adapted to hazardous conditions (e.g. drought, competition, deep shade or burial) which are often present in cities. Baker (1972) reported that larger seeds are favoured in arid areas since they provide sufficient resources for the seedling. Our results partly support this suggestion; however, they also point out that large seeds benefit from wet but urban conditions. One might suggest that in rather dry conditions additional precipitation can provide sufficient water to allow photosynthesis for carbon allocation into large seeds.

Most interesting is that the interaction of urban land use amplifies the effect of climatic variables but dampens that of geological conditions. This is a hint towards homogenisation of geological subsoils (Groffman et al. 2014), for example, due to translocation of soil material and bedrock. Extremes in climatic conditions are thus amplified, for example, by changing the physical conditions of the environment (Heisler and Brazel 2010) or by promoting an urban effect on trait compositions.

Flowering phenology

Phenology – the timing of biological events – is known to be linked to climate change. Change in climate leads to change in phenology. Despite climate, changes of further environmental conditions (e.g. land use change) can also lead to shifts in timing (Cara-

Donna et al. 2014). Our models revealed multiple climatic, geological and land-cover variables that explain shifts in the timing of the first flowering. CaraDonna et al. (2014) detected advanced first flowering with increased temperatures over time in the same plot and so we expected higher temperatures that would lead to advanced first flowering over geographical space.

However, our results denote a slight delay with higher July temperatures and even more delay in more urbanised areas with high July temperatures. It is well known that many urban neophytes tend to flower late in the year (Celesti-Grapow et al. 2003, Knapp and Kühn 2012). Hence, it is likely that human preference selected for late-flowering species (i.e. to have colourful gardens when native flowers are already in decay) promote the observed pattern.

The earlier onset of flowering in forests and agricultural areas can be explained by the increasing dominance of trees and cultivated crops in the course of the year. Except for a few tree species and cultivated species, this especially promotes early flowering plants (i.e. before canopy cover closes or crops begin competing for light). We understand the even amplified effect in more urban areas to be a result of the urban heat island effect. Hence, city forests are relatively warmer compared to forests that are distant to cities and, therefore, they lead to an even earlier closure of the canopy and hence to an even earlier onset of flowering. The same applies for arable fields close to cities.

Lastly, there is a common pattern in most observed interactions: Urbanisation leads to later flowering, probably for the reasons discussed above (human preference for late flowering species; most neophytes are introduced for ornamental reasons – Lambdon et al. 2008). Further, late flowering occurs with additional heterogeneity, either the number of geological substrate patches, specific substrates such as sand, or infrastructure such as roads. Again, this might be a confounding effect of sampling from an available species pool: all these additional structures promote species richness. In general, neophytes tend to flower later. Increasing species richness hence increases the probability of drawing a late flowering species from the available species pool of neophytes.

Synthesis

Across all traits, precipitation and temperature were usually the most important predictors as a main effect. In most interaction terms, however, other predictors were included. The functional response of alien species is therefore reliant on climatic variables being independent from urbanisation (e.g. the urban heat island effect). This contrasts with the results of Kühn et al. (2017) for species richness along the rural-urban gradient, for which the length of roads and railroads were important predictors. There is a main difference between a functional and a taxonomic perspective on plant invasion patterns in the rural-urban gradient. While species richness is largely determined by propagule and colonisation pressure (Pyšek et al. 2015), functional traits seem to respond more closely to physiological constraints imposed by climate (Woodward and Williams 1987). There were significant interactions with urbanisation for almost any trait but with varying predictors, suggesting that there is an ‘urban effect’, however much smaller than purely climatic effects. And in addition, an idiosyncratic effect of traits was visible in our study. We therefore argue that the main responses of the studied traits refer to the physiological constraints of climate, but their interactions are rather dependent on the context (Kueffer et al. 2013), i.e. the specific species with their specific environmental preferences at their specific stage of the invasion process being mediated by traits.

Conclusions

We aimed to extend the approach of Kühn et al. (2017) in order to identify changes in the response of traits of neophytes to environmental conditions along the rural-urban gradient. While they did not find conditions that determine neophyte richness in general, being fundamentally different in cities compared to rural areas, we show that considering functional plant traits allows more explicit and more differentiated insights. By modelling the plant traits of neophytes, instead of their richness or their proportion, we could reveal substantial differences between urban and rural environments. In addition, we found a more complex effect of urbanisation in many of the responses of the traits to environmental conditions, suggesting that different ecological processes act within cities compared to rural areas. These differences can mainly be traced back to interferences and alterations that are related to urban development and human activity in general. To enhance our understanding of functional ecological mechanisms in cities, it is not only important to describe how urbanisation affects species composition in relation to species origin, but also to scrutinise the different processes behind these patterns. We argue that studying plant traits is an appropriate way to do so and suggest considering plant traits more explicitly in studies of urban ecology.

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Supplementary material 1

Supplementary tables

Authors: Janis Wolf, Dagmar Haase, Ingolf Kühn

Data type: (measurement/occurrence/multimedia/etc.)

Explanation note: **Table S1:** List of neophyte species included in the study with information on the number of grid cells they occured, how frequently they occured in the 2599 grid cells, pollination type, growth form, diaspore mass and beginning month of flowering. **Table S2:** Overview on minimum, maximum, median and/or mean values across 2599 grid cells in Germany.

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RESEARCH ARTICLE

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Diverse views among scientists on non-native species

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Abstract

Conservation scientists have traditionally viewed non-native species (NNS) as potential threats to native biodiversity. Here, we question whether alternative views of NNS exist in the scientific community that stand in contrast to the dominant narrative that emerges from the literature. We asked researchers from the biological, social, and environmental sciences to participate in an anonymous poll regarding the perceived values and threats of NNS. Some 314 individuals responded, approximately half of whom were biologists and half were social or environmental scientists. We grouped responses into three statistical clusters defined by shared responses. We then analyzed the correlation of responses to individual questions and membership of clusters with predictor variables age, gender, and field of work. Overall, a majority of respondents in our sample supported statements that the species-component of biodiversity should include all species (55%) or some types of non-native species (an additional 32%), which contrasts with the manner in which major biodiversity assessments and indicators are constructed. A majority of respondents in our sample (65%) also supported that measurement of the impact of invasive species should be based on the net biological, social, and economic effects, which also represents a marked departure from current methods that focus only on the adverse effects of a subset of NNS considered as invasive. Field of work and age were correlated with clusters and numerous individual responses. For example, biologists were three-times more likely than non-biologists to support a definition of species richness that included only native species. Two clusters (Cluster 1 and Cluster 3), mainly composed of non-biologists and biologists, respectively, differed in their support for statements that NNS would provide useful ecosystem services in the future (66% and 40%, respectively). Thus, a key result of this study is that a variety of normative stances regarding NNS is present within the scientific community. Current international indicators of progress (e.g., Aichi Targets) capture only a "nativist" set of values, which, if our sample is representative of the scientific community, appears to be a minority view. Therefore, we argue that indicators should be modified to integrate the diversity of views that exist within the scientific community.

Keywords
conservation ethics, exotic species, invasive species, nativism, values

Introduction

There is a consensus among scientists that humanity is experiencing a sixth mass extinction of species, which is resulting in an alarming decline of biodiversity (Barrett et al. 2018; Ceballos and Ehrlich 2018; Ceballos et al. 2015; Ceballos et al. 2017; Mayhew et al. 2007). Species considered as invasive are regarded to be one of the main direct drivers of biodiversity loss and recent extinctions (Bellard et al. 2016; Butchart et al. 2010; McGeoch et al. 2010). Although scientists largely agree on the biodiversity crisis and potential negative effects of invasive species, the debate is just emerging about the potential risks and contributions that should be attributed to the bulk of non-native species (NNS) that are not currently considered to be invasive.

Ambiguities in the definitions and understanding of terms such as "biodiversity" and "invasive species" have contributed to confusion among scientists, policy-makers, and the public. They may also contribute to opposing positions around NNS. Indeed, the term "biodiversity" can have both scientific and cultural meanings. Scientifically, biodiversity (biological diversity) refers to the variability among all living organisms and the ecological systems of which they are a part; this includes variation at the genetic, species, and ecosystem levels [Convention on Biological Diversity (CBD), Article 2]. Culturally, biodiversity refers to the dimensions of nature that we cherish and wish to conserve, wherein individual species have been categorized as more or less desirable in particular places (Fall 2014). For example, a large number of assessments and reports use the word "biodiversity" when in practice only the status, richness and abundance of native species are assessed (Schlaepfer 2018b). In such examples NNS are implicitly - and sometimes explicitly - given either no value or a negative value.

In a similar vein, the term "invasive species" is conceptualized differently across policy contexts. Primarily, species considered as invasive refer to NNS (also referred to as "alien species") whose ancestors were introduced deliberately or unintentionally outside their natural habitats and which become established, proliferate, and spread in ways that cause damage to a range of interests, including biological diversity (Daisie-database; Shine 2007). The term "invasive species" is used in this sense in an international and political context (e.g. CBD, IUCN) and by government and intergovernmental organizations (e.g. the European Commission). A broader definition is used by some researchers and practitioners (landscape managers, botanists, conservationists, horticulturists, etc.) who refer to species "considered as invasive" as native species and NNS that are over abundant, even without any proven adverse effects on the environment (Colautti and MacIsaac 2004). Finally, Richardson et al. (2000) proposed that the term "invasive" should be used without any inference to environmental or economic impact, but rather on the capacity of species to produce reproductive offspring, often in very large numbers, at considerable distances from the parent and thus have the potential to spread over a considerable area.

The versatile meanings of "biodiversity" and "invasive species" highlight the ambiguous thinking on NNS within conservation scientists. This raises some fundamental questions: *What do we wish to conserve? And for whom?* (Mace 2014). Currently, NNS are not considered as (positive) contributions to biodiversity. For example, indices and metrics used to measure progress towards biodiversity conservation and sustainability goals focus solely on the "native" component of biodiversity (Schlaepfer 2018b). Thus, current indicators may capture only a limited range of values that exist in the conservation community and across society.

In practice, the justifications for conserving nature have changed over time, and the current conservation frame is a mix of different conservation models (Mace 2014; Sandbrook et al. 2019), with each model receiving attention from different groups of people depending on their personal values and expertise. These differences in knowledge and values may be contributing factors to the differing views between invasion biologists and the public (Genovesi 2008). Beyond the expertise and influence of the former group (invasion biologists), the importance of the opinion of the latter is increasingly recognized. Indeed, there is a call to include greater input from the public in an attempt to seek greater public support for the conservation goal and reduce the rate of biodiversity loss. In this context, an important aspect that could contribute to a constructive debate surrounding the importance of NNS in conservation is an awareness of the range of views regarding NNS within the conservation community and greater care to consider alternative views in conservation discourse and decision-making (Sandbrook et al. 2019).

Here, we enquired whether a variety of values exists within the scientific community regarding NNS. We also investigated different positions on the debate and to what extent they were associated with the demographic (age, gender) and professional (field of study) factors of participants. The existing perspectives on NNS among scientists (Guiașu 2016; Guiaşu and Tindale 2018; Schlaepfer 2018b; Simberloff 2003), including among invasion biologists (Humair et al. 2014; Young and Larson 2011), suggest the existence of factors (personal values, diverging conceptual understandings, knowledge gaps, etc.) likely to determine or influence attitudes towards NNS. Considering the evolution of conservation paradigms (Mace 2014), and knowing that individual attributes influence people's perception of invasive alien species (Shackleton et al. 2019), we hypothesize that socio-demographic factors and field of work can potentially shape the opinions of scientists. Also, based on our personal experiences at workshops and conferences, we considered age and gender as potential explanatory factors.

This survey differs from previous opinion surveys, which focused mainly on invasive species issues (Andreu et al. 2009; Bardsley and Edwards-Jones 2007; Fischer et al. 2014; Guiaşu and Tindale 2018; Humair et al. 2014; Young and Larson 2011) and contributes to the broader question of how to value and manage NNS (including those considered as invasive). Bringing more diverse voices into our understanding of NNS and the debate on "*conserving what and for whom*" (Mace 2014) can help to inform policy and research directions in the current context of preparation for the post-2020 Strategic Plan for the Convention on Biological Diversity.

Methods

Survey planning and data collection

We designed a poll to query a sample of individuals from the research community on their views and values regarding the role of NNS in the field of conservation science. We designed 13 multiple choice questions (Table 1) that covered the following three areas: (i) individual values associated with biodiversity and NNS, (ii) the perceived threats associated with NNS, and (iii) the most appropriate approaches for assessing the value of NNS. An additional, open-ended question allowed respondents to provide comments and to nuance their responses. The survey was anonymous, although we did ask for age category, gender, and field of work.

The survey used a "snowball" sampling approach, wherein the invitation to complete the survey form was shared through various outlets, including social media platforms (Twitter, Facebook, LinkedIn), the journal of PLOS Biology (in the comments section associated with Schlaepfer (2018b) on the website of PLOS Biology), and five email lists for different fields of research: Eanth-l (official listserv of the Anthropology & Environment Society of the American Anthropological Association; roughly 2000 subscribers), Aliens (researchers interested in biological invasions; 1,505 members); CESG (Cultural and Political Ecology Specialty Group of the Association of American Geographers; no information available on the number of subscribers), the Swiss Biodiversity Forum (researchers and practitioners interested in biodiversity in Switzerland; 1,870 subscribers) and Biodiversity-l (an international list, with 7,970 members). Researchers were also invited to share the invitation within their respective networks.

Using international email lists and social media (Twitter, Facebook, LinkedIn, etc.) with wide global reach, we expected to reach the global community of scientists. We have no information on the country of origin, but we assume that respondents came predominantly from North America and Europe, and we knew through spontaneous feedback that at least a few responses came from Africa. Responses were recorded during 131 days (April 26 – September 04, 2018). The raw results of the survey are available in Suppl. materials 1, 2.

Statistical analyses

Results were analyzed using two approaches. First, responses to questions were analyzed and visualized according to a priori hypotheses regarding independent predictors such as age, gender, and field of work. Sometimes, we pooled together the "strongly support" and "somewhat support" responses to indicate the magnitude of agreement (or, conversely, disagreement). Pearson's Chi-square tests were used to test whether each response variable was independent of the predictor variables. Age, gender, and field of work were used in multiple binary logistic (for binary responses) or ordinal logistic (for ordinal responses) models to test their effect on respondents' opinions and perceptions. After specifying the full model (i.e. including all main effects of each factor), the full model was compared to the null model (a model without any of the factors) to test whether it was globally significant (Crawley 2012). We then identified the most parsimonious model using backward elimination (Crawley 2012). Only the summary of the final model was reported. For binary responses, we reported the odds ratio (OR) and confidence interval. OR expresses the relative chance of an event happening under two different conditions (Szumilas 2010). Here, for a factor with two levels (e.g. gender), it expressed the relative chance of a male respondent agreeing with a statement compared to that of a female respondent. For a factor with more than two levels (e.g. academic background), OR expressed the relative chance of a respondent from a given background (e.g. non-biologist) agreeing with a statement compared to that of a respondent from a reference group (e.g. biologists).

In a second analytical approach, we analyzed responses using a clustering tool, independently of predictor variables. This allowed us to group respondents into relatively homogeneous classes irrespective of their age, gender, and field of work. A multiple correspondence analysis (MCA) was first performed on individual answers, and then the factorial axes from the MCA were used in a hierarchical clustering on principal component analysis (HCPC). HCPC is a multivariate descriptive method that, in addition to assigning individuals into groups, provides information on which of the initial variables submitted to the MCA best describes each cluster (Le et al. 2008). A Chi-square test was used to test independence between clusters and age, gender, and field of work of respondents. Fourteen further independent Chi-square tests were performed to examine the association between clusters and each of the 14 statements. Because conclusions from these multiple independent tests are prone to type I error, a more conservative p-value was obtained using the correction method of Benjamini and Hochberg (1995). This correction method reduces type I error, but also minimizes type II error (Jafari and Ansari-Pour 2019). As an additional step, we reconsidered the dataset without responses to methodological questions (survey questions 10 through 14), which were likely less relevant to the core questions of this study. The reduced and full dataset yielded results that did not differ significantly. For both datasets, the level of three clusters was sufficient to reveal general trends, and retaining a greater number of clusters would have led to complex analyses without any significant added value.

All statistical analyses were performed in R software version 3.5.0 (R Core Team 2018). MCA and HCPC were run using packages *ade4* (Dray and Dufour 2007), *FactomineR* (Lê et al. 2008), and *Factoextra* (Kassambara and Mundt 2016). Binary logistic models and the correction method of Benjamini and Hochberg (1995) were run in R Basics, whereas the ordinal logistic models were implemented in package *ordinal* (Christensen 2018).

Results

Respondents' characteristics

The internet-based survey form was completed by 314 respondents. The majority of responses (251/314) were recorded within the first 30 days following the inception of the survey. The response rate for closed questions ranged from 99% to 100%. For the unique open question, the response rate was 89.17%. The typical time to complete the survey was 8.47 ± 5.49 min.

The reported fields of work included biology (44.05%) social science (31.19%), and environmental science (14.15%). The remaining respondents (10.61%) came from fields of work such as engineering, sustainable development, law, education, and information technology. Hereafter, respondents reporting a field of work other than biology are grouped into the non-biologist category. Respondents were nearly gender balanced (53.07% males and 46.93% females) and covered a wide range of ages, with about half of respondents (50.48%) aged between 34 and 55 years.

Responses to individual questions on places, values, and threats associated with inclusion of non-native species as part of biodiversity, and appropriate methods for measuring their desirability

We found that responses to individual questions/statements were significantly associated with the socio-demographic features (age, gender) and field of work (called professional background on the survey form) in 8 of 14 cases (Table 1). Field of work and age were more likely to be associated with responses than gender (Table 1, Suppl. material 3). In the next section, we illustrate the detailed responses to each group of questions, emphasizing cases with significant associations.

Values associated with non-native species

Responses to the five values-based questions/statements illustrated a range of opinions regarding the current and future values of NNS. Hereafter, all reported results refer to the percentages of respondents in our sample.

More than half of respondents (55.41%) reported that in their view, biodiversity is composed of all species - including NNS, domesticated species, and invasive species (Statement 1, Table 1). The other respondents (44.59%) excluded one or several groups of species based on historical, social, or economic criteria, preconditioning the inclusion of NNS in biodiversity due to their invasiveness, naturalization (being in place for >100 years), or social appreciation. Some rare respondents (8.20%) restricted the notion of species richness to native species only. Respondents were ambivalent about attributing any future value to NNS. Whereas some of the respondents (40.19%) were neutral (Figure 1b), others agreed or disagreed with the statement in roughly equal proportions (Statement 2, Table 1). In addition, about half of respondents (53.55%) felt that it was useful to maintain a distinction between native species and NNS (Statement 3, Table 1). Finally, a majority of respondents (65.07%) agreed that evaluating the invasiveness of NNS should be based on the net impacts (Statement 4, Table 1) or on at least one documented undesirable impact (72.02%, Statement 5, Table 1).

Opinions on the inclusion of NNS as part of biodiversity were primarily associated with the fields of work of the respondents ($\chi^2 = 23.751$, $p < 0.05$) and to a lesser extent with age. Biologists were more likely than non-biologists to restrict biodiversity to native species (Figure 1a) and to attribute no future value to NNS (Figure 1b). Finally, biologists and older respondents were more likely to support maintaining a distinction between native species and NNS than both non-biologists and younger respondents (respectively. $\chi^2 = 27.986$, $p < 0.01$ and $\chi^2 = 16.28$, $p < 0.05$ for field of work and age; Figure 1c, d). Gender, in general, was rarely associated with particular responses ($\chi^2 = 2.7993$, $p > 0.05$; Table 1).

Threats from non-native species

Responses to the four questions/statements related to the perceived threats associated with NNS also showed a large variety of opinions among respondents. All reported results refer to the percentages of respondents in our sample.

More than half of respondents (about 58%) agreed that NNS represent a potential threat to native species (Statement 6, Table 1), whereas about 23% were undecided. About half of respondents (about 49%) disagreed that NNS represent a threat to human well-being (Statement 7, Table 1). Respondents were divided on the statement "*Non-native species should all be considered as potentially invasive species*", with 43% of respondents in agreement and 46% in disagreement (Statement 8, Table 1). Finally, respondents were also divided on the statement "*Including non-native species as part of biodiversity lowers conservation standards (*the *"shifting baseline" effect) for society*", with a quarter of respondents in disagreement with the statement.

Differences in perceptions of the threats from NNS were also associated with the field of work. Non-biologists were more likely than biologists to view NNS as a threat to human well-being (Figure 2a), whereas non-biologists were more likely than biologists to perceive the inclusion of NNS as part of the biodiversity as a lowering of conservation standards (Figure 2b).

Appropriate approaches for measuring whether a non-native species is desirable or not

Statements 10–14 explored different methods for assessing the values and impacts of NNS. The most commonly favored approach was to measure the impact of NNS on ecological functions, followed by approaches that measure the net effect of NNS on native species richness and their net impact on ecosystem services (Table 1).

Field of work influenced the responses on methodological approaches. Biologists generally supported a method in which the impact of NNS on native biodiversity is measured (Table 1). By contrast, non-biologists generally supported an approach in which the net impact of NNS on total species richness is measured (Table 1).

Younger respondents were more likely than older respondents to favor approaches that conduct an evaluation of ecosystem services gained and lost through the addition of the NNS and approaches that measure the effects of the NNS on ecological functions.

Table 1. Survey statements, summary of responses, and statistical tests of correlation with predictor variables.

N°	Statements		Proportion of responses (%)					Logistic regression		
			ASPP	XISP	SASP	LTSP	ONSP	Age	Gender	Field of work
Values associated with non-native species										
1	"Which group(s) of species should be considered when quantifying the "species" dimension of biodiversity?"		55.41	17.70	4.26	14.43	8.20			
		N/A	**Strongly agree**	**Agree**	**Neutral**	**Disagree**	**Strongly disagree**			
2	Non-native species should be conserved because they may provide useful functions ("ecosystem services") in the future.	0	8.04	23.79	40.19	21.22	6.75			*
3	The distinction between native and non-native species is artificial and counter-productive.	0	7.74	19.35	19.35	29.03	24.52	*		*
4	The definition of an invasive species should be based on an evaluation of all its desirable and undesirable impacts[1].	1.28	30.13	34.94	12.82	14.42	6.41			
5	The definition of an invasive species should be based on at least one documented undesirable impact.	2.25	27.97	44.05	8.36	11.58	5.79			
Threats from non-native species										
6	Non-native species, on average, represent a potential threat to the survival of native biodiversity.	10.5	17.25	40.58	14.38	22.04	4.47			
7	Non-native species, on average, represent a potential threat to human well-being.	2.89	6.43	19.29	23.15	31.19	17.04			*
8	Non-native species should all be considered as potentially invasive species.	0.96	10.86	32.27	10.22	28.12	17.57			
9	Inclusion of non-native species as part of biodiversity lowers conservation standards (the "shifting baseline" effect) for society.	0	11.29	28.71	25.81	26.13	8.06			*
What methodological approach is most appropriate for measuring whether a given non-native species is desirable or not?				**Agree**		**Disagree**				
10	Measure the effect of the non-native species on native species richness.	–	–	42.04	–	57.96	–			*
11	Measure the net effect of the non-native species on total species richness.	–	–	22.93	–	77.07	–			*
12	Conduct an economic cost-benefit analysis of the addition of the non-native species.	–	–	15.92	–	84.08	–			
13	Conduct an evaluation of ecosystem services gained and lost through the addition of the non-native species.	–	–	42.68	–	57.32	–		*	
14	Measure the effects of the non-native species on ecological functions.	–	–	53.50	–	46.50	–		*	

[1] on native biodiversity, economy, or human health, social; **All species**=all species, including non-native species, domesticated species, and invasive species; **exclude invasive**=native and most non-native species, but excluding invasive species; **Useful NNS**=native species and socially appreciated non-native species; **exclude recent**=native species and non-native species that have been present for a "long time" (e.g. >100 years); **Native only**=only native. *=significant in determining the opinion.

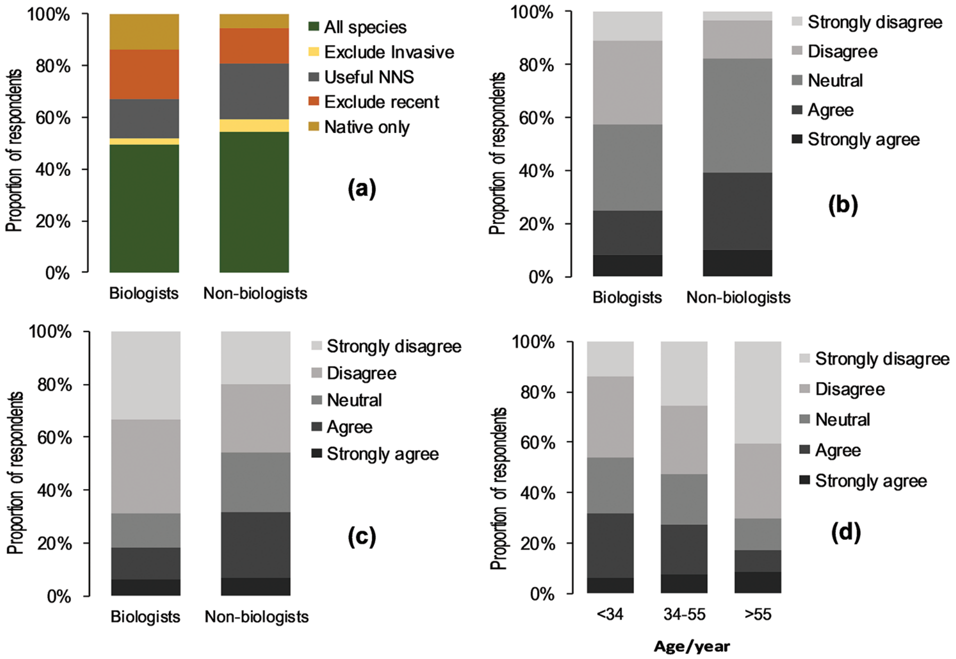

Figure 1. Proportion of responses to statements on values with significant associations to at least one predictor variable. Field of work was associated with the respondents' definitions of biodiversity (Statement 1, panel **a**) and "*NNS should be conserved for potential future value*" (Statement 2, panel **b**), and the distinction between native species and NNS was artificial (Statement 3, panel **c**). Age (years) was associated with responses to Statement 3 (panel **d**). **All species**=all species, including non-native species, domesticated species and invasive species; **Exclude invasive**=native and most non-native species, but excluding invasive species; **Useful NNS**=native species and socially appreciated non-native species; **Exclude recent**=native species and non-native species that have been present for a "long time" (e.g. >100 years); **Native only** = only native. <34 = 34 years and younger, 34–55 = between 34 and 55 years, and >55 = 55 years and older.

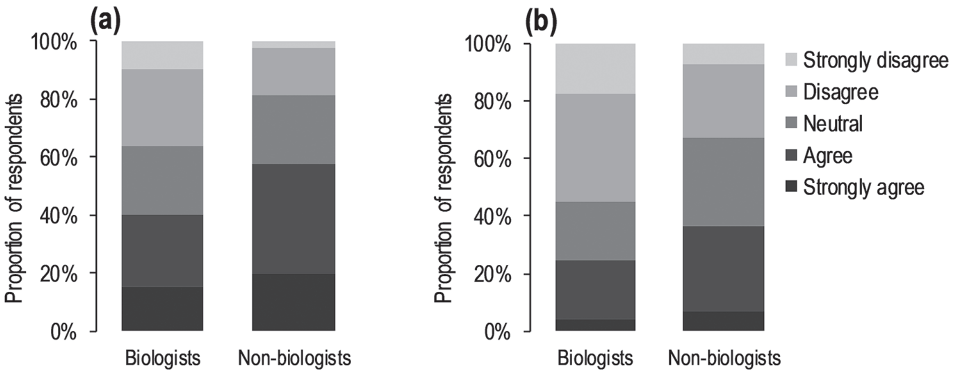

Figure 2. Proportion of responses to statements on perceived threats with significant associations and at least one predictor variable. Field of work was associated with the perception that NNS represent a threat to human-well-being (Statement 7, panel **a**) and that including NNS as part of the biodiversity leads to a lowering of conservation standards (Statement 8, panel **b**).

Statistically-independent groups of opinions on biodiversity and non-native species issues

The MCA performed on the dataset of 314 respondents and 15 variables (which corresponded to the responses to questions) followed by HCPC distinguished three distinct clusters of respondents (Figure 3). Each cluster represents a generally shared opinion across the poll and can be thought of as analogous to political parties. The three clusters captured 18.05%, 66.43%, and 15.52% of respondents, respectively.

Here, we report the statistical associations between predictor variables and clusters. We also describe how clusters were positioned with regard to values associated with NNS, threats perceived from NNS, and the most appropriate methods for evaluating NNS.

A significant association was found between field of work and the clusters ($\chi^2 = 14.889$; $p < 0.05$; Figure 4a). Cluster 1 was composed primarily of non-biologists (66%) whereas Cluster 3 was composed mostly of biologists (62.79%). The three clusters were statistically independent from age and gender ($\chi^2 = 5.5518$; $p > 0.05$), although Cluster 3 was composed of more males and older respondents (Figure 4b, c) than the other two clusters.

As with the individual responses, the three clusters differed with regard to the respondents' opinions on values associated with NNS, perceived threats, and methodologies to measure the impact of NNS (Suppl. material 3). Cluster 1 was generally the most distinct from Cluster 3, with Cluster 2 being intermediary. Differences between Cluster 1 and Cluster 3 were marked for at least one statement or for questions pertaining to values, perceived risks, and methods for evaluating NNS (Table 2; Suppl. material 3).

Relative to the other two clusters, members of Cluster 1 were more inclined to agree that NNS have the potential to become invasive and represent a potential threat to native biodiversity and human well-being. However, they mostly agree that an evaluation of the net impacts of NNS should be undertaken before considering a species as invasive. Similarly, they mostly agreed that the decision to label an NNS as invasive or not should be driven by an evaluation of its net effect on total species richness. Respondents from this cluster mostly agreed that the distinction between native species and NNS is artificial and counter-productive and were likely to accept that NNS should be conserved in the future. However, they mostly thought that inclusion of NNS as part of biodiversity may lower conservation standards (Table 2, Suppl. material 3).

Collectively, these responses showed concern for the potential negative impacts of NNS, but also an open-mindedness towards their potential future contributions and a desire to measure both their positive and negative impacts. We coined this cluster as "liberal".

Relative to the other two clusters, members of Cluster 2 were more inclined to agree that biodiversity should include both native species and NNS but not invasive species. According to those respondents, NNS represent a potential threat to the survival of native biodiversity. They are mostly undecided on the potential invasiveness of NNS and were likely to think that the net impact of an NNS should be evaluated before considering a species as invasive. Similarly, they were undecided on whether NNS could potentially threaten human well-being and whether their inclusion as part of biodiversity

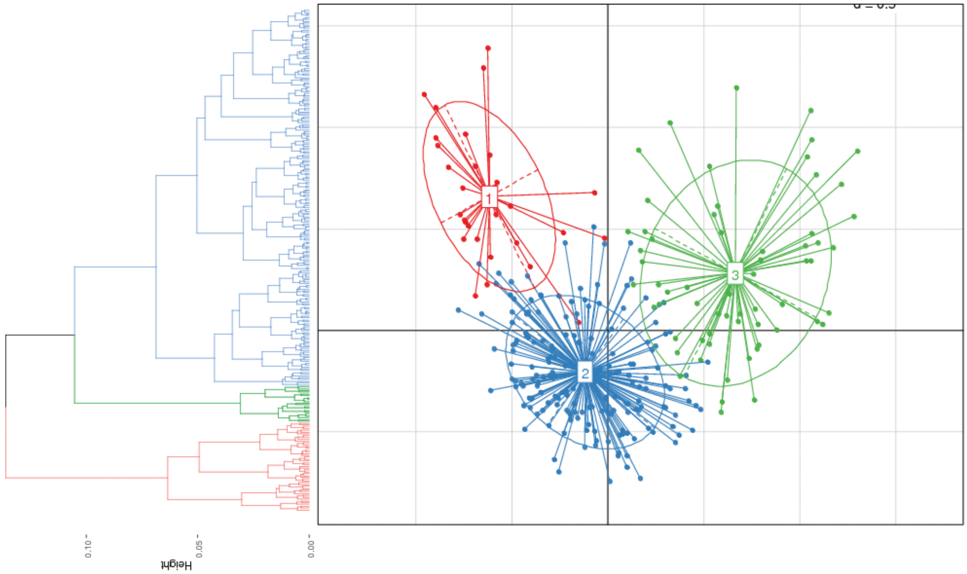

Figure 3. Clustergram showing the three independent groups of respondents.

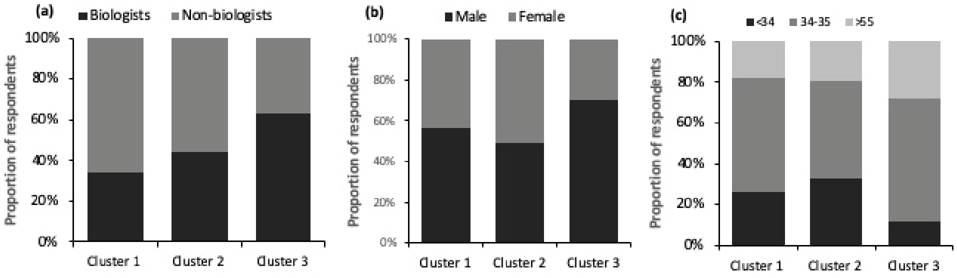

Figure 4. Distribution of the fields work (**a**), gender (**b**), and age (**c**) of respondents across the three clusters.

would lower the conservation standards. They also remained undecided on whether the distinction between native species and NNS is artificial and counter-productive and on the need to conserve NNS in the future (Table 2, Suppl. material 3).

Collectively, these responses were ambivalent and intermediate between Cluster 1 and Cluster 3. As a result, we coined this cluster as "agnostic" based on the assumption that as more data become available (e.g. about the dangers of NNS or their positive contributions) that values of this group could change.

Finally, relative to the other two clusters, members of Cluster 3 were more inclined to support the idea of restricting biodiversity to only native species even though they strongly disagreed that all NNS are potentially invasive and that there is no risk of lowering the conservation standards if NNS are included as part of the biodiversity.

Table 2. Statistically significant associations (+ positive; - negative) between the responses to questions/statements and the three clusters.

N°	Statements	Opinions				
Values associated with non-native species		**ASPP**	**XISP**	**SASP**	**LTSP**	**ONSP**
1	“Which group(s) of species should be considered when quantifying the “species” dimension of biodiversity?”	C1(+)	C2(+)			C2(-), C3(+)
		Strongly agree	**Agree**	**Neutral**	**Disagree**	**Strongly disagree**
2	Non-native species should be conserved because they may provide useful functions (“ecosystem services”) in the future.	C1(+), C2(-)	C1(+), C2(+) C3(-)	C1(-), C2(+) C3(-)	C1(-), C3(+)	C2(-), C3(+)
3	The distinction between native and non-native species is artificial and counter-productive.	C1(+), C2(-)	C2(+), C3(-)	C2(+), C3(-)	C1(-), C2(+) C3(-)	C1(-), C2(-) C3(+)
4	The definition of an invasive species should be based on an evaluation of all its desirable and undesirable impacts[2].	C1(+), C2(-)	C2(+), C3(-)			C2(-), C3(+)
5	The definition of an invasive species should be based on at least one documented undesirable impact.	C2(-), C3(+)	C2(+)	C3(-)		C2(-), C3(+)
Threats from non-native species						
6	Non-native species, on average, represent a potential threat to the survival of native biodiversity.	C1(+)	C1(+), C2(-)	C1(-), C2(+), C3(-)	C1(-), C2(+), C3(-)	C1(-), C2(-), C3(+)
7	Non-native species, on average, represent a potential threat to human well-being.	C1(+), C2(-), C3(-)	C2(+), C3(-)	C1(-), C2(+), C3(-)	C1(-), C2(-), C3(+)	C2(-), C3(+)
8	Non-native species should all be considered as potentially invasive species	C1(+), C2(-), C3(-)	C1(-), C2(+), C3(-)	C2(+), C3(-)	C1(-)	C2(-), C3(+)
9	Including non-native species as part of biodiversity lowers conservation standards (“the shifting baseline” effect) for society.	C1(+), C2(-)	C2(+), C3(-)	C2(+), C3(-)	C1(-), C3(+)	C1(-), C2(-), C3(+)
What methodological approach is most appropriate for measuring whether a given NNS is desirable or not?						
10	Measure the effect of the non-native species on native species richness.		C1(-)		C1(+), C3(-)	
11	Measure the net effect of the non-native species on total species richness.		C1(+)		C1(-), C3(+)	
12	Conduct an economic cost-benefit analysis of the addition of the non-native species.					
13	Conduct an evaluation of ecosystem services gained and lost through the addition of NNS					
14	Measure the effects of the non-native species on ecological functions.					

[2] on native biodiversity, economy, or human health, social

All species=all species, including non-native species, domesticated species, and invasive species; **exclude invasive_**=native and most non-native species, but excluding invasive species; **Useful NNS**=native species and socially appreciated non-native species; **exclude recent**=native species and non-native species that have been present for a “long time” (e.g. >100 years); **Native only**=only native; C1=Cluster 1; C2=Cluster 2; C3=Cluster 3. (+)=Highly represented; (-)=Less represented.

They mostly agreed that the distinction between native species and NNS is useful. According to those respondents, there is no need to evaluate the net impact of NNS nor to document at least one undesirable impact before considering a species as invasive. They mostly disagreed that NNS can potentially threaten native biodiversity and human well-being. They also disagreed that NNS may provide useful functions in the future (Table 2, Suppl. material 3).

Collectively, these responses matched the current dominant value system, whereby native species are valued and NNS are viewed as potential threats with little or no potential value. We, therefore, coined this cluster as "nativist" to capture the strong preference for "native" species.

Discussion

The primary aim of this study was to investigate whether a variety of perceptions and values regarding NNS exists amongst scientists. Further, we investigated whether a person's opinions were correlated with their age, gender, or field of work (e.g. biologist or non-biologist). An important result of this survey is the demonstration that a great variety of normative positions exist beyond the nativist position, which currently dominates the scientific literature and the operationalization of biodiversity programs.

From the perspective of the scientific community, should NNS be considered as part of biodiversity along with native species? A key result from our study is that in our sample there was a poor consensus on which types of species belong to what is called "biodiversity" (Table 1, Statement 1). First, the fact that some respondents excluded NNS from biodiversity or conditioned its inclusion to some features stands in contradiction to the definition of biodiversity stated in Article 2 of the CBD agreement (CBD 1992). Second, this may indicate that some scientists perceive NNS species to have less value outside of their historic ranges. Yet, recent studies have documented that some NNS do have a positive contribution to biodiversity and human-well-being (Guiașu 2016; Schlaepfer 2018a; Schlaepfer 2018b; Schlaepfer et al. 2011). According to some respondents, the risk of accepting NNS as part of biodiversity will be that "*Non-native species will be seen as normal. This is not the case.*" (*Respondent #45, Biologist, male, 45–54 years old*) or "*People turn their backs on native species if both native and non-native are seen as of equal value*" (*Respondent #26, Biologist, female, 35–44 years*). Such attitudes of re-conceptualization and operationalization of the term "biodiversity" at the level of the scientific community may reflect a shift from a scientific to more vernacular or cultural meaning of the term biodiversity that increasingly takes into account the desirability of the public and the political vision. This attempt to re-conceptualize and operationalize the term biodiversity may also reflect the different aspects of biodiversity being emphasized (Russell and Kueffer 2019).

Nevertheless, we were surprised to note that responses in our sample tended to be at odds with the current practices of assessing progress in biodiversity conservation, which use metrics focused only on native species. For example, the vast majority of respondents did not support the statement that species richness is defined by only native species. Instead, they favor notions of species richness that also included some other NNS and – more surprisingly – a notion of species richness based on all species, including invasive species (Figure 1, Table 1). Scientists in our sample mostly find the distinction between NNS and native species as artificial and counterproductive. In practice, the distinction between which species are native to a region or not is a matter of uncertainty on the origin of the species and therefore impacts management options. Species whose geographic origins cannot be determined based on the available evidence are now referred to as "cryptogenic" species, a much less stigmatizing expression than "invasive" (Carlton 1996; Guiaşu and Tindale 2018).

Does the origin of a species really matter? In the field, both native species and NNS are identified as drivers of species' extinction and, therefore, the theoretical or practical usefulness and importance of a distinction between native species and NNS to conservation may be questioned (Davis et al. 2011; Guiașu 2016; Venter et al. 2006). However, recent evidence indicated that the biogeographic origin of species is likely to worsen the ecological impact (Blackburn et al. 2019), suggesting that the distinction may be justified, especially in an island context.

Does the scientific community consider NNS as a threat to native species and human well-being? Respondents in our sample mostly believed that NNS represent a threat to the survival of native biodiversity and to human well-being and should be considered as potentially invasive, which is congruent with the position of many invasion biologists (Richardson and Ricciardi 2013; Simberloff 2003). This opinion indicates a leeriness towards NNS and may reflect the magnitude of an "a priori" negative association to NNS in the collective consciousness (Guiașu 2016), even though evidence shows that only a small percent of established NNS ever become problematic (IUCN 2019; Moore 2005; Primack 2012; Seebens et al. 2018; Vander Zanden 2005). Interestingly, the fact that some respondents thought that NNS do not, on average, represent a threat to native species reveals a shift in how NNS are viewed within the field of conservation.

The views of respondents in our sample on how to define an invasive species were also at odds with current practices. An introduced species for instance can be considered as invasive if "at least" one undesirable economic, biological, social, or human-health effect is reported (Daisie-database; Shine 2007). By contrast, Schlaepfer (2018a) defined a species as "invasive" if the negative effects exceed the positive effects. The finding that respondents mostly supported such an approach acknowledges a need to account for the net impact of NNS, including invasive ones (Young and Larson 2011) and to call for more realism and transparency in communication on NNS and invasive species to curb the social anxiety regarding NNS (Ernwein and Fall 2015).

How best to decide whether an NNS is desirable or not? Most of the respondents in our sample agreed on the importance of taking into account the effects of NNS on ecological functions, evaluating the net ecosystem services provided by NNS, and taking into account the effect of NNS on native species richness. This observation suggests that the scientists surveyed were therefore aware of the variety of positive and negative effects of NNS, similar to those of native species (Davis et al. 2011). However, they were frequently neutral on the opportunities to conserve NNS that may provide useful functions ("ecosystem services") in the future. Such an attitude confirms the hesitancy about NNS but also suggests the versatility of this position, which may evolve with more information about the importance and contribution of NNS to biodiversity and sustainable development.

The scientific community is heterogeneous and our results indicate that the perceptions of NNS may be influenced by the demographic features and professional training of scientists, among others factors established to influence people's perceptions of NNS, especially the invasive subset (Shackleton et al. 2019). More generally, the opinions on NNS were not significantly associated with gender. However, unlike non-biologists, biologists (mainly older ones) were more likely to distinguish NNS from native species and were more likely to exclude NNS from biodiversity. However, both groups (biologists and non-biologists) showed a great deal of heterogeneity, generating the lack of consensus on some aspects of NNS even among biologists (Young and Larson 2011). Curiously, non-biologists were more likely than biologists to associate NNS with potential threats to the survival of native biodiversity and to human well-being, and thus – by extension – to consider them as invasive. This trend in the opinion of non-biologists may have reflected a "fear of the unknown" maintained by communication around NNS to influence the opinions, perceptions, and actions of the public (McNeeley 2012; Larson 2008). Beyond demographic and professional factors, scientists' perceptions of NNS may also be influenced by other individual factors (i.e., knowledge and value systems, relationship), the species itself, the effect of the species, the socio-cultural context, the landscape context, the institutional and policy context (Shackleton et al. 2019).

Overall, the mapping of opinions on NNS within our sample indicated three main groups of scientists in our sample corresponding to three leading opinions: two minority, extreme positions and one dominant, intermediate position. Scientists, practitioners, and policymakers within the "nativist" position are likely to focus excessively on the potential negative effects of NNS, declaring them as harmful or potentially harmful even in the absence of evidence (Ricciardi and Simberloff 2009). In opposition to this group, the group that we characterized as "liberal" was represented mainly by non-biologists, and its respondents were more open to the potential benefits of NNS. This position reflects the belief that NNS should be considered "innocent until proven guilty". Between these two opposing opinions was the group characterized as "agnostic", in which scientists were undecided towards the values associated with and perceived threats from NNS as well as the opportunities for their conservation. Their prevalence may be symptomatic of a transition process, suggesting a possible shift in opinions on NNS issues in the future. This observed trend of the debate is reflexive of the middle–ground approach on the perception and management of NNS proposed by Shackelford et al. (2013), in which the authors suggested a stepwise consideration when analyzing the situation of NNS. Although a cautious approach is recommended especially in the initial stages of the introduction and establishment of NNS (Shackelford et al. 2013), our results indicate that the net impact and the desirability of a species, not its origin, were what mattered most to scientists in our sample (see also Lindemann-Matthies 2016; Van Der Wal et al. 2015).

This study also aimed to describe and explain the views of scientists on the importance and contribution of NNS to biodiversity. We speculate that the above-mentioned diverse views indicated an evolution of the views of biodiversity and the values associated with NNS. This diversity of views may reflect a transitionary period during which the primary motives for protecting nature are called into question (Mace 2014; Sandbrook et al. 2019). In this sense, instead of the simplifying and leveling of values to match the views of a single group of biologists, the policy commitments and indicators used to measure progress in conservation may need to account for this diversity of views. Because NNS are also of importance and interest to the public, their conservation value should be captured by indicators of progress in biodiversity conservation.

A second important implication of this study derives from the observed "leading opinions groups" and points to a need to question the use of an apparent consensus to define policy instruments and biodiversity indicators. While being consistent with the traditional positions of invasion biologists, our results revealed the emergence of two other groups of opinions, reflecting a lack of consensus on the concepts, opportunities, and risks associated with NNS. This lack of consensus in values observed both among experts and non-experts (Humair et al. 2014; Young and Larson 2011) clearly showed that positions on the debate were not just a matter of diverging conceptual understandings and knowledge gaps but also reflected underlying personal values and/or alignments to social norms and group ideologies (Essl et al. 2017; Humair et al. 2014). Although divergence in concepts could be fixed and knowledge gaps bridged with more education, convergence of personal values, motivations, and emotions would be a much greater task.

This study was based on the opinions of 314 scientists, whom we presume were located primarily in Western countries (Europe, USA, and Canada) based on participation patterns in other studies. We do not know to what extent our sample was representative of the global community. Thus, as in any study of this nature, the precise percentage of respondents that we reported should be treated with caution and should not be extrapolated to represent the wider scientific community. Nevertheless, our study provides the best estimate to date of the relative importance of different value systems found within the scientific community and our findings clearly revealed a diversity of views regarding NNS. As such, results from our study call for a shift in how we collectively think about and define priorities for the conservation of biodiversity in its broadest sense. Here, we addressed a small subset of the interdisciplinary challenges that are linked to NNS and we look forward to other researchers exploring these questions in alternative ways and from different angles (importance of ethics, different types of intrinsic values, a regional focus, etc.). We believe that such efforts will be both timely and welcome in the context of the preparation for the post-2020 Strategic Plan for the Convention on Biological Diversity.

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Supplementary material 1

Raw data of the survey on perception and valuation of non-native species

Authors: Rodrigue C. Gbedomon, Valère K. Salako, Martin A. Schlaepfer

Explanation note: This material is the raw data file as downloaded from surveymonkey.

Link: https://doi.org/10.3897/neobiota.54.38741.suppl1

Supplementary material 2

Perception and valuation of non-native species

Authors: Rodrigue C. Gbedomon, Valère K. Salako, Martin A. Schlaepfer

Data type: Opinion

Explanation note: This material is the survey report as generated by surveymonkey.

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Supplementary material 3

Distribution of respondents across the clusters regarding their opinions on values associated to non-native species

Authors: Rodrigue C. Gbedomon, Valère K. Salako, Martin A. Schlaepfer

Data type: Multimedia

Explanation note: This figure shows the distribution of respondents across the cluster clusters regarding their opinions on values associated to NNS, threats perceived from NNS and most appropriate methods for measuring whether a given non-native species is desirable or not.

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Context-dependent differences in the functional responses of conspecific native and non-native crayfishes

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Abstract

Invasive species are proliferating globally and cause a range of impacts, necessitating risk assessment and prioritization prior to management action. Experimentally derived estimates of *per capita* effects (e.g. functional responses) have been advocated as predictors of field impacts of potential invaders. However, risk assessments based on estimates from single populations can be misleading if *per capita* effects vary greatly across space and time. Here, we present a large-scale, multi-population comparison of *per capita* effects of the American spinycheek crayfish, *Faxonius* (formerly *Orconectes*) *limosus*—a species with an extensive invasion history in eastern North America and Europe. Functional responses were measured on individuals from six geographically disparate populations of *F. limosus* in its native and invaded ranges on two continents. These revealed inter-population differences in both the maximum feeding rate and functional response type that could not be explained by the biogeographic origin of the population nor by time since the invasion. We propose that other differences in source communities (including the presence of competitors) impose selective pressures for phenotypic traits that result in dissimilar *per capita* effects. We also compared functional responses of the congeners *F. limosus* and *F. virilis* in the presence and absence of potential competitors to examine indirect competitive effects on feeding behaviour. The maximum feeding rate of *F. limosus*, but not *F. virilis*, was suppressed in the presence of heterospecific and conspecific competitors, demonstrating how the *per capita* effects of these species can differ across biotic contexts. In the competitor-presence experiments, individuals from the invasive population of *F. limosus* consistently had a higher maximum feeding rate than those of the native *F. virilis*, regardless of treatment. Our results caution against invasion risk assessments that use information from only one (or a few) populations or that do not consider the biotic context of target habitats. We conclude that comparative functional responses offer a rapid assessment tool for invader ecological impacts under context dependencies when multiple populations are analyzed.

Keywords

competition, context dependence, impact, invasive species, maximum feeding rate, risk assessment

Introduction

Invasive species risk assessment is hampered by a lack of quantitative methods for predicting ecological impact (Jeschke et al. 2014). Moreover, predictions are challenged by context-dependent variation in the invader's *per capita* effects and abundance (Ricciardi et al. 2013). Resource consumption is considered a determining factor affecting an invader's success and ecological impact (Catford et al. 2009; Ricciardi et al. 2013), and high-impact invaders appear more efficient at using key resources than their non-invasive counterparts (Carlsson et al. 2004; Rehage et al. 2005; Morrison and Hay 2011; Dick et al. 2013). Therefore, the relationship between an invader's consumption rate and prey density – i.e. its functional response (FR) – has been proposed as a universal measurable trait that predicts key elements of invasibility and impact (Dick et al. 2017a; but see Vonesh et al. 2017 and Dick et al. 2017b). A comparison of FR offers a standardized estimate of the *per capita* effect (e.g. maximum feeding rate) of study populations to help explain and predict differences in impacts across populations, species, and environment contexts (Haddaway et al. 2012; Dick et al. 2013; Alexander et al. 2014; Barrios-O'Neill et al. 2014; Iacarella et al. 2015a, 2015b). Further information is derived from the shape of plotted FR data, most often characterized by so-called Type II or Type III curves, which indicate whether the predator-prey dynamic is likely to be destabilizing or stabilizing, respectively (Alexander et al. 2012; Dick et al. 2013); Type I curves are mainly observed in filter feeders and rarely in other consumers, as such a relationship only exists when handling does not interfere with searching for resources such as prey (Holling 1959; Jeschke et al. 2014). Previous experiments using a comparative FR approach have demonstrated higher *per capita* effects for invaders compared to trophically or taxonomically similar native or non-invasive species and were also able to predict impacts on prey populations observed in the field (Dick et al. 2013; Alexander et al. 2014).

Few studies have tested whether *per capita* effects are conserved across populations of congeners or conspecifics (e.g. Boets et al. 2019; see review by Jeschke et al. 2014). Although conspecific individuals share traits that contribute to their success and impact as invaders, population traits could differentiate after periods of isolation and, as a result, produce different impacts (Tsutsui et al. 2003; Dlugosch and Parker 2008; Vellend et al. 2009; Lockwood et al. 2013; Evangelista et al. 2019). Important insights into such variation can be derived from distributed experiments, in which standardized, controlled protocols are employed across a wide geographic range (Fraser et al. 2013; Borer et al. 2014). For example, a study by Dick et al. (2013) examined the FR of invasive bloody red mysid shrimp (*Hemimysis anomala*) in invaded ranges in Quebec and Northern Ireland. While the invader's *per capita* effects were consistently greater than native mysids, they differed between study populations (maximum feeding rate of 1.82 *Daphnia pulex*/hour in Northern Ireland versus 3.39 *D. pulex*/hour in Quebec) (Dick et al. 2013), possibly reflecting differences in experimental handling, animal care, or population traits. This finding raises the question of how variable FR is across conspecific populations and ecological contexts. It has been hypothesized that sources of variation in *per capita* effects include biogeographic (native/non-native) origin (Rehage et al. 2005; Paolucci et al. 2013), time since invasion (Iacarella et al. 2015a, 2015c; Evangelista et al. 2019), and community structure (Hayes et al. 2009).

Here, in two sets of experiments we measured the *per capita* effects of the American spinycheek crayfish *Faxonius* (formerly *Orconectes*) *limosus* and the virile crayfish *F. virilis*, both of which have extensive invasion histories (Henttonen and Huner 1999; Souty-Grosset et al. 2006; Taylor et al. 2007) but are also threatened in parts of their native ranges by introduced congeners (Dubé and Desroches 2007; Swecker et al. 2010). We compared the FR of geographically disparate populations of *F. limosus* in its native and invaded ranges. To assess the role of biotic context in mediating impacts, we also compared the maximum feeding rates of *F. limosus* and *F. virilis* in the presence of heterospecific and conspecific competitor signals. We predicted that the FR (curve type and maximum feeding rates) would differ significantly between conspecific populations of *F. limosus*, owing to divergence of population traits. Additionally, we tested the hypothesis that invasive populations have a greater maximum feeding rate, and thus impact (Dick et al. 2013), based on the premise that crayfish have increased growth rates – and thus greater energetic demands – in their invaded range than native conspecifics (Pintor and Sih 2009; Sargent and Lodge 2014). Finally, we predicted that the *per capita* effects would be reduced in the presence of a perceived competitor, owing to feeding activity being inhibited by agonistic interactions.

Methods

Animal collection and care

Experiments were conducted in climate-controlled facilities at Queens University Belfast (UK) and McGill University (Canada) to ensure environmental conditions were constant throughout trials. In the summers of 2016 and 2017, *F. limosus* were collected from two native populations (hereafter designated by *N*; Quinebaug River, Massachusetts: 42°06'32"N, 72°07'25"W; Panther Pond, Maine: 43°54'04"N, 70°27'55"W) and four invasive populations (hereafter designated by *I*; St Lawrence River, Quebec: 46°09'22.81"N, 72°59'54.85"W; St Croix River, New Brunswick: 45°37'01"N, 67°25'35"W; Lake Müggelsee, Germany: 52°26'54"N, 13°38'55"E; Albert Canal, Belgium: 50°56'34"N, 5°29'27"E). Crayfish collected from European sites were transported overnight by courier to Queen's University Belfast. North American populations were transported by research vehicle from the field site to McGill University within 2–48 h of collection. The population of *F. limosus* from the St Lawrence River (*I*) was used first in distributed experiments and then in competitor-signal experiments three months later. Individuals of *F. virilis* used in competitor-signal experiments were collected from Blue Chalk Lake (*N*) in Dorset, Ontario (45°11'55"N, 78°56'20"W). For competitor-signal experiments, subjects were held in communal aquaria with up to five other individuals for three months prior to experiments. All crayfish collected from their invasive range were done so in areas where no other crayfish species currently co-exist. In contrast, those collected in their native ranges were from sites with sympatric crayfish species.

Crayfish were introduced to holding tanks at 18 °C immediately upon arrival and allowed to acclimate for at least one week prior to the start of experiments. Individuals were housed at low densities with ample shelter to mitigate territorial and aggressive behaviour (Reynolds et al. 2013) and they were fed a diet of sinking shrimp pellets. Aquaria maintenance included weekly water changes (25–50%, depending on tank size and crayfish density) and biweekly water quality testing. Given that *Faxonius* spp. are more active at night (personal observation), experimental animals were acclimated to a reversal of their usual 12:12 hour light:dark regime over a period of 96 h, following Alexander et al. (2012).

Low sample sizes of *F. limosus* obtained in the St Lawrence River (*I*) required that some individuals from this population be used in more than one trial, but each individual was tested only once at each density and in a maximum of three trials. To track individual identity, each crayfish was tagged with visible implant elastomer tags – a method that has been shown to have high tag retention rates and no influence on crayfish growth rates or mortality (Clark and Kershner 2006; Buřič et al. 2008).

Experimental designs

Distributed experiments

All experiments were completed by the same researcher to minimize handler variation that often occurs in spatially distributed experiments coordinated among multiple research groups (Fraser et al. 2013; Dick et al. 2013). FR trials were conducted at ambient temperatures of 18 °C in opaque experimental tanks (61 cm × 35.5 cm filled with 10.8 L of water, and no substrate), and provided with a single PVC pipe as shelter (~12 cm length, 5 cm diameter). Experiments allowed for prey depletion, potentially underestimating the attack rate. However, the type of FR curve, handling time and maximum feeding rates remain uninfluenced by this procedure (Alexander et al. 2012).

Owing to natural variation in body size (carapace length) among populations of *F. limosus* crayfish and the low sample sizes available, no attempt was made to size-match individuals; instead, crayfish representing the estimated median size of individuals within each source population were used (see Suppl. material 1). Crayfish were introduced into experimental tanks 24 h prior to the beginning of experiments and were not fed during this period to standardize hunger levels. Following acclimation, each individual was randomly assigned one of 11 prey density treatments (3, 4, 5, 7, 10, 15, 20, 25, 30, 40 or 50 gammarid amphipod individuals of *Gammarus pulex* in the UK and *Gammarus fasciatus* in North America). In addition, one control treatment at each prey density was conducted in the absence of a crayfish to account for potential mechanisms of prey death other than crayfish predation. Experiments were run for 6 h in the dark, after which the crayfish were removed from experimental tanks and the remaining gammarids counted to determine the number of prey consumed. This procedure was replicated three times for each crayfish population (*n* = 33, plus controls for each population). Following each trial, crayfish were blot-dried and weighed, and their carapace length was measured.

Amphipod prey activity levels

We did not have access to a gammarid prey species common to both the UK and eastern North America (NA); therefore, it was necessary to account for differences in body size (length) and activity levels of a subsample (*n* = 30) of *Gammarus* spp. from each region. Activity level was measured at 18 °C by placing an individual amphipod into a petri dish filled with 1 cm of dechlorinated tap water, allowing the individual to acclimate for 90 s, and then counting the number of times it crossed the center of the dish in 60 s (Maynard et al. 1998).

Competitor-signal experiments

This second set of FR experiments took place between February 10 and April 18, 2017, and consisted of six experimental treatments using the two crayfish species in a full factorial design, plus predator-free controls (Table 2). Experiments featured 'focal' and 'competitor' crayfish(es) that occupied a shared experimental arena but had minimal physical contact. Experimental conditions were identical to those of the distributed FR experiments, except that the addition of a lidded container with 1cm holes in each side was included in the experimental arena. The focal crayfish wandered freely in the experimental chamber and could access the shelter, while the competitor crayfish was confined to the secondary container. The container holes were large enough to allow crayfish antennae and claws to pass through, but the competitor was unable to access the prey.

The focal and competitor crayfish were introduced to the experimental chamber simultaneously, 24 h before the beginning of the trial. The beginning of the trial was signalled by the introduction of defrosted bloodworms (Diptera, Chironomidae, *Chironomus*) to the experimental chamber, in each of the following prey densities: 15, 20, 25, 30, 40, 50, 60, 70, 80, 100, and 120 individuals. Trials lasted 6 h in the dark and allowed for prey depletion. Each of the 11 densities in each treatment was replicated in triplicate (*n* = 33 for each of the six experimental treatments, plus one replicate at each density as a predator-free control). After each trial, crayfish were blot-dried and weighed, and their carapace length measured. The remaining prey were counted to determine the number attacked during the trial. Prey were scored as 'attacked' if at least part of the worm had been eaten (determined by fragmented worms and loss of colour caused by draining of hemolymph). Owing to insufficient numbers of experimental animals, individual crayfish were re-used in trials up to 10 times, but never re-used twice at the same density, regardless of treatment.

Statistical analysis

Model selection and fitting

All analyses were completed using R (version 3.2.4). As is appropriate for prey non-replacement designs, FR was modeled using the Random Predator Equation (Rogers 1972) for Type II curves and Hassell's equation (Hassell et al. 1977) for Type III curves, following Alexander et al. (2012) and Iacarella et al. (2015a). Model selection was conducted using three methods outlined by Pritchard et al. (2017). The best fit model was selected for each population (distributed experiments) and experimental treatment (competitor-signal experiments), based on consensus of selection methods. Best-fit models were fit to FR data via maximum likelihood estimation (see Suppl. material 2).

Model comparisons

To compare the fitted FR curves among populations and experimental treatments, the data were bootstrapped (n = 999) to produce 95% confidence intervals (CI) on the fit. Using this method, we may statistically compare models between populations by simply observing the overlap, or lack of, between model CIs (Pritchard et al. 2017). In addition, the *frair_compare* function of the FRAIR package was used to compare the difference in handling time (h) estimates between populations, but is limited to comparing those that are fit by the same model type (Pritchard et al. 2017). An overall assessment of differences between model parameters can also be achieved by observing overlap between 95% CIs, as with full model comparisons. The effect of crayfish size (carapace length and weight) on maximum feeding rate and proportion of prey killed was tested using linear models (linear regression for each population). Sex-related effects were also assessed for each population using t-tests to compare the proportion of prey consumed between sexes.

Results

Distributed experiments

Functional responses differed among populations by maximum feeding rate and curve type. The responses of populations from Lake Müggelsee (*I*), Albert Canal (*I*), and the

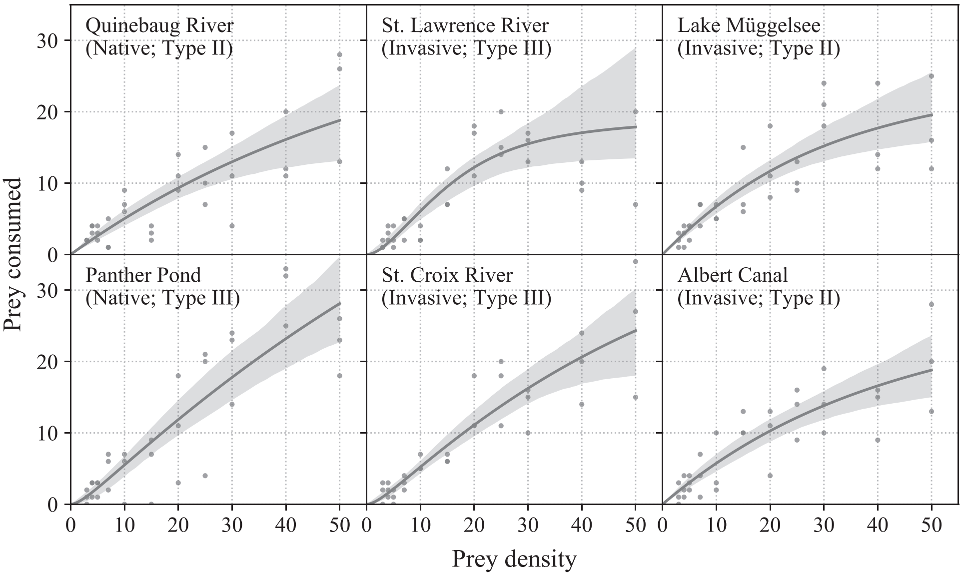

Figure 1. Functional response curves with bootstrapped 95% confidence intervals (shaded regions) for *F. limosus* from native and invasive populations. Lines represent the best fit model for each population (Type II or Type III). *n* = 33 for each population.

Table 1. **(a)** Comparisons between handling time (*h*) parameters for populations fit with Type II curves. *Δh* represents the difference (*Δ*) in *h* between the two populations' model fits. (*) represents a significant difference to the standard α = 0.05. **(b)** Comparison between *h* parameters for populations fit with Type III curves.

	Fit 1	Fit 2	***Δh* (h)**	***p*-value**
(a)	Lake Müggelsee (*I*)	Albert Canal (*I*)	0.016	0.76
	Lake Müggelsee (*I*)	St. Croix River (*I*)	0.17	0.0012*
	Lake Müggelsee (*I*)	Quinebaug River (*N*)	0.075	0.24
	Albert Canal (*I*)	St. Croix River (*I*)	0.15	0.0087*
	Albert Canal (*I*)	Quinebaug River (*N*)	0.058	0.39
	St. Croix River (*I*)	Quinebaug River (*N*)	-0.096	0.16
(b)	St. Lawrence River (*I*)	Panther Pond (*N*)	0.21	0.02*

Quinebaug River (*N*) were best fitted by a Type II curve, while those of remaining populations were best fitted by a Type III curve (Fig. 1). Bootstrapped 95% confidence intervals indicated significant overlap in model fits and maximum feeding rates among the populations, regardless of whether the population was native or invasive (Fig. 1); however, parameter comparisons using the *frair_compare* function indicate a significantly greater maximum feeding rate (lower handling time, *h*) for the population of *F. limosus* from the St Croix River (*I*) than either the Lake Müggelsee (*I*) or Albert Canal (*I*) populations. Similarly, the Panther Pond (*N*) population exhibited a greater maximum feeding rate than the St Lawrence River (*I*) population (Table 1).

Crayfish predation was the principal source of prey death in experimental trials, as indicated by high survival rates in control treatments (across all populations, controls exhibited

Table 2. Treatments for functional response experiments in which the focal crayfish was allowed to roam freely in the experimental chamber with access to prey and shelter, while the perceived competitor crayfish was confined to a porous container within the experimental chamber. L = *F. limosus* alone, LL= *F. limosus* with an *F. limosus* competitor, LV = *F. limosus* with an *F. virilis* competitor, V = *F. virilis* alone, VL= *F. virilis* with an *F. limosus* competitor, and VV = *F. virilis* with an *F. virilis* competitor. Sample size, *n* = 33 in the first six experimental treatments and *n* = 11 for the control treatment.

Treatment	Focal species	Competitor species
V	*F. virilis*	–
L	*F. limosus*	–
VV	*F. virilis*	*F. virilis*
LL	*F. limosus*	*F. limosus*
LV	*F. limosus*	*F. virilis*
VL	*F. virilis*	*F. limosus*
Control	–	–

99.98% survival of prey during 6-hour experimental trials). Overall, maximum feeding rates declined with mean crayfish size and weight (linear models; carapace length: $F_{1,4}$ = 10.83, p = 0.030, weight: $F_{1,4}$= 10.40, p = 0.032), but the size effects on prey consumption varied among populations. The proportion of prey consumed increased with crayfish size for the Panther Pond population (*N*) (linear models; carapace length: $F_{1,31}$ = 4.36, p=0.048, adjusted r^2 = 0.09, weight: $F_{1,31}$ = 6.91, p = 0.013, adjusted r^2 = 0.16), but decreased with crayfish weight for the Albert Canal population (*I*) (linear model; $F_{1,31}$ = 4.68, p = 0.038, adjusted r^2 = 0.10). Female crayfish from the Quinebaug River population consumed a marginally greater proportion of prey (*t*-test; t_{28} = 2.45, p = 0.021) than males. No differences in prey consumption were detected between crayfish sexes in other populations.

Amphipod prey activity levels

Amphipods used as prey in FR experiments in the UK (*G. pulex*; mean size ± SE = 6.67 mm ± 0.50) and North America (*G. fasciatus*; 6.10 mm ± 0.48) did not differ in size (Mann-Whitney U Test; *W* = 560.5, p = 0.091). However, North American prey were significantly more active than UK prey, crossing the centre of the disk an average of 5.7 times per minute (SE = 1.8) while *G. pulex* in the UK crossed an average of 3.7 times per minute (SE = 1.1) (Mann-Whitney U Test; *W*= 624, p = 0.0098).

Competitor-signal experiments

The maximum feeding rate of *F. limosus* was suppressed in the presence of conspecific and heterospecific competitors, whereas the handling time (and thus, maximum feeding rate) of *F. virilis* was unaffected (Fig. 2, Table 3). However, despite apparent inhibition in the presence of competitors, *F. limosus* consistently exhibited a significantly higher maximum feeding rate than that of *F. virilis* (Fig. 2). All treatments were best fitted by Type II curves.

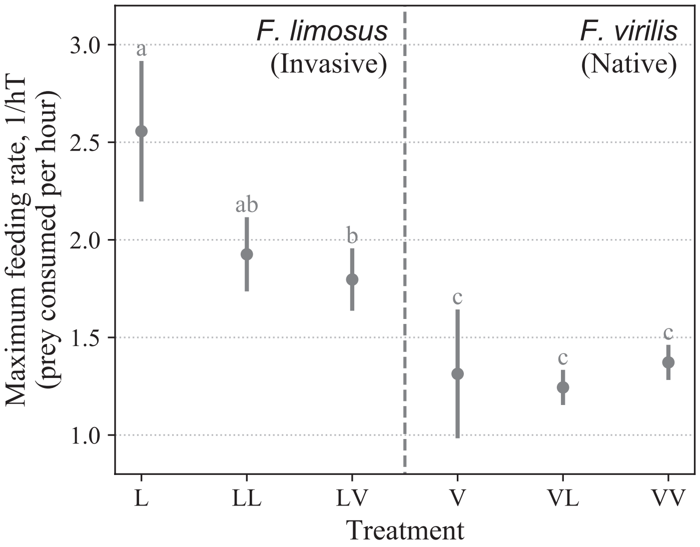

Figure 2. Maximum feeding rate (MFR) calculated for each treatment in the competitor-signal experiments ($1/hT$, where h is estimated handling time and T is experimental duration). Treatment codes represent the focal and competitor crayfish species in each treatment – L = *F. limosus* alone, LL = *F. limosus* with an *F. limosus* competitor, LV = *F. limosus* with an *F. virilis* competitor, V = *F. virilis* alone, VL = *F. virilis* with an *F. limosus* competitor, and VV = *F. virilis* with an *F. virilis* competitor. *F. limosus* used in these experiments came from an invasive population, whereas *F. virilis* came from a native population. Bars indicate the standard errors of the MFR calculated by propagating the model fit standard error given for h for each treatment. Differences in letters above error bars indicate significant differences (α = 0.05) between treatments. n = 33 for each treatment.

Table 3. Comparisons between handling time (h) parameters for populations fit with Type II curves. Δh represents the difference (Δ) in h between the two treatments' model fits. (*) represents a significant difference to the standard α = 0.05.

Fit 1	Fit 2	Δh (h)	*p*-value
L	VV	0.012	< 0.0001*
L	VL	-0.069	< 0.0001*
V	L	0.062	0.00024*
LL	VL	-0.048	0.00038*
VL	LV	0.041	0.0015*
VV	LL	0.035	0.0028*
VV	LV	0.029	0.011*
V	LL	0.040	0.015*
L	LV	-0.028	0.025*
V	LV	0.034	0.036*
L	LL	-0.021	0.095
VV	VL	-0.013	0.32
LL	LV	-0.0063	0.60
V	VL	-0.0072	0.68
V	VV	0.0054	0.74

Despite significant size differences between *F. limosus* and *F. virilis*, carapace length and crayfish weight were not significant predictors of maximum feeding rate (linear models; carapace length: $F_{1,4}$ = 0.055, p = 0.83; weight: $F_{1,4}$ = 0.059, p = 0.82).

Discussion

Our study demonstrates intraspecific variation in the *per capita* effects of conspecific populations. The *per capita* effects of *Faxonius* crayfishes differed across geographically disparate populations and different biotic contexts. Despite large confidence intervals on model fits, we detected differences in both FR curve type and maximum feeding rates between conspecific populations of *F. limosus* (Fig. 1, Table 1). The source of differences remains to be determined, but we are confident that it is not an artifact of our experimental design, because in addition to the implementation of standardized protocols and controls in experimental procedures, handler variation was minimized by having the same individual researcher responsible for all animal care and experiments. In addition, crayfish were selected to represent the median population size and therefore representative of true population differences. Nevertheless, there was no consistent relationship between crayfish body size and maximum feeding rates, nor between crayfish sex and the proportion of prey consumed. Finally, differences in amphipod activity level did not explain variation in maximum feeding rates, as demonstrated by remarkably similar maximum feeding rates found between the European (*I*) and St Lawrence River (*I*) populations which were supplied different gammarid prey species. The most discordant maximum feeding rates were amongst populations within North America, which were supplied with a common prey species.

Our prediction that invasive populations of *F. limosus* would have greater maximum feeding rates than native populations was not supported, perhaps owing to an insufficient number of populations studied. There are numerous potential explanations for differences among *per capita* effects of populations, including differences in resident community composition (Barrios-O'Neill et al. 2014; Médoc et al. 2015; Wasserman et al. 2016), time since invasion (Iacarella et al. 2015a, 2015c; Yokomizo et al. 2017; Evangelista et al. 2019) and abiotic conditions (Eggleston 1990; Alexander et al. 2012; Iacarella et al. 2015b), but we were unable to determine the cause in our distributed experiments. In mesocosm experiments, Evangelista et al. (2019) found contrasting ecological impacts amongst individuals from four invasive populations of red swamp crayfish (*Procambarus clarkii*) and thus demonstrated that even geographically-close populations can vary in intraspecific trait variability, consistent with studies showing trait differences amongst individuals across a single expanding population (e.g. Iacarella et al. 2015a). They found that crayfish from the most recently invaded lakes in their study had the greatest impacts on ecosystem processes.

Our populations were sourced from locations with differing biotic contexts: all invasive populations from Europe and Canada were collected from sites where no other crayfish species were detected, whereas native populations collected from the USA were found in sympatry with congeners. Hayes et al. (2009) demonstrated that *F. virilis* from populations sympatric with *F. rusticus* (30 years of coexistence) grew faster and were more aggressive towards the congener than naïve populations of *F virilis*. Conversely, Pintor et al. (2008) found that invaders from sites without a congeneric competitor were bolder, more voracious and more aggressive towards congeners relative to those from sites with competitor crayfish; they postulated that this trend was due to differences in prey densities between sample sites, with more aggressive crayfish hailing from low prey density sites. However, trends in our data could not be explained by sympatry with congeners and, owing to the correlation of biotic context and invasive status (all invasive populations were allopatric to other crayfish species) in our study, we are unable to disentangle possible effects. Increasing the scope of conspecific populations studied should allow for more power to test for hypothesized trends and improve predictions.

Biotic context mediates *per capita* effects

Our competitor-signal experiments tested the influence of biotic context on *per capita* effects of invasive species and found that closely related species differ in their response to the presence of competitor signals. Invasive *F. limosus* adjusted its feeding behaviour in the presence of conspecific and heterospecific competitor signals, whereas native *F. virilis* did not (Fig. 2). However, even where the maximum feeding rate of *F. limosus* is suppressed, it still exceeds that of the native, consistent with the resource consumption hypothesis (Dick et al. 2013; Ricciardi et al. 2013). This result suggests that *F. limosus* can have a greater impact on prey populations than *F. virilis* where the two species are sympatric; but as demonstrated, impact differs between *F. limosus* populations. Therefore, conclusions on the potential species impacts cannot be reliably drawn from estimates from only one population. We tested individuals from a population of *F. virilis* that was sympatric with a native congener (*F. propinquus*), whereas our *F. limosus* population was allopatric. We hypothesize that the suppression of feeding rates resulted from intimidation by adversarial crayfish, the effect of which could dampen over time as dominance hierarchies are established and agonistic interactions decline (Goessmann et al. 2000). Thus, a likely explanation for the unaffected feeding rate of *F. virilis* in the presence of a competitor is that individuals from this population are accustomed to the presence of other crayfishes.

For *F. limosus*, the presence of congeners can trigger individuals to trade off foraging effort with shelter protection. Access to suitable shelters is crucial for crayfish survival by enhancing predator avoidance, facilitating successful moulting, and reducing the frequency and intensity of agnostic interactions with other individuals (Nyström 2005, Hirsh et al. 2016, Lele and Pârvulescu 2017). Previous studies have demonstrated the superior ability of invasive crayfishes to evict natives and co-opt shelters for themselves (Lele and Pârvulescu 2017), aided by increased aggression (Klocker and Strayer 2004). Therefore, a reduced maximum feeding rate could have been the result of *F. limosus* sacrificing foraging opportunities to maintain possession of shelter in the presence of a perceived competitor. A competitor signal might have triggered increased aggressive or territorial behaviour in

F. limosus at the expense of sacrificing its foraging effort. We did not make observations throughout the trial and so cannot confirm these behaviours. In contrast, the lack of a behavioural change in *F. virilis* in the presence of competitors might also be explained by shelter usage; Garvey et al. (1994) examined interspecific competition for shelter among congeneric *Faxonius* crayfishes and determined that among the three species studied, *F. virilis* was most often found actively mobile outside of shelters even in the presence of predators. Bold behaviour and naivety to competition from non-native species could prevent crayfishes from trading off feeding intensity for shelter or territorial protection.

Conclusions

We conclude that *per capita* effects, and thus possibly overall field impacts, of crayfishes are mediated by context dependencies including indirect species interactions. Although we were unable to detect trends explaining the sources of variation in our distributed experiments, the observed differences in *per capita* effects indicate the need to conduct broader comparisons of conspecific populations separated by a range of geographic distances, in order to test the generality of hypotheses related to invasion impact (Ricciardi et al. 2013). We advocate the development and testing of hypotheses that explicitly incorporate context dependencies from both the source and recipient communities of invasive species.

Crayfish populations are declining worldwide; nearly half of all species in North America are considered endangered or vulnerable (Taylor et al. 2007), owing in part to the spread of invasive species (Crandall and Buhay 2008). Those in the genus *Faxonius* (formerly *Orconectes*) are not exempt from this trend, but remarkably are also among the most disruptive invaders. Our results suggest a mechanism (feeding efficiency) by which the success and impact of these crayfishes vary with different environmental contexts. Understanding the causes of variation in their *per capita* effects would not only inform the prioritization and management of invasive populations but could also be valuable to the conservation of threatened populations. Most risk assessments of invader impact rely upon either expert opinion or invasion history (Ricciardi 2003; Kulhanek et al. 2011; Dick et al. 2013), whereas comparative experimental approaches that consider both species traits and environmental context offer standardized, objective, and informative measures of *per capita* effects that could be used in prioritizing emerging and potential future invaders. However, our results caution against deriving risk assessment information from studies of a single population.

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Supplementary material 1

Crayfish size and weight

Authors: Jaime Grimm, Jaimie T.A. Dick, Hugo Verreycken, Jonathan M. Jeschke, Stefan Linzmaier, Anthony Ricciardi

Data type: measurement

Explanation note: Body weights and carapace lengths of individuals.

Link: https://doi.org/10.3897/neobiota.54.38668.suppl1

Supplementary material 2

Statistical techniques

Authors: Jaime Grimm, Jaimie T.A. Dick, Hugo Verreycken, Jonathan M. Jeschke, Stefan Linzmaier, Anthony Ricciardi

Data type: statistical data

Explanation note: Details of statistical methods.

Link: https://doi.org/10.3897/neobiota.54.38668.suppl2

Supplementary material 3

Locations of crayfish populations

Authors: Jaime Grimm, Jaimie T.A. Dick, Hugo Verreycken, Jonathan M. Jeschke, Stefan Linzmaier, Anthony Ricciardi

Data type: occurrence

Explanation note: Geographical coordinates of populations.

Link: https://doi.org/10.3897/neobiota.54.38668.suppl3

Supplementary material 4

Amphipod sizes

Authors: Jaime Grimm, Jaimie T.A. Dick, Hugo Verreycken, Jonathan M. Jeschke, Stefan Linzmaier, Anthony Ricciardi

Data type: measurement

Explanation note: Body lengths of amphipod prey used in the experiments.

Link: https://doi.org/10.3897/neobiota.54.38668.suppl4

Supplementary material 5

Data from functional response experiments

Authors: Jaime Grimm, Jaimie T.A. Dick, Hugo Verreycken, Jonathan M. Jeschke, Stefan Linzmaier, Anthony Ricciardi

Data type: measurement

Explanation note: Functional response parameters from experimental trials.

Link: https://doi.org/10.3897/neobiota.54.38668.suppl5

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REVIEW ARTICLE

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Prevalence and management of *Solenopsis invicta* in China

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Abstract

Red imported fire ant, *Solenopsis invicta*, was first found in Taiwan province of China in 2003. To prevent transboundary expansion, the Chinese government has formulated various control strategies in the last 15 years to slow down the spread of *S. invicta*. Despite all efforts, *S. invicta* invasion has reached more than 390 counties of 13 provinces and two special administrative regions in China; at present, *S. invicta* remains at the stage of rapid expansion. The transnational entry of *S. invicta* is linked to imported logs and wastepaper coming mostly from the United States. In domestic settings, long-distance expansion of *S. invicta* relied on potted plant and turf transportation. Both monogyne and polygyne social forms of *S. invicta* were present in China with polygyne colonies as the dominant one. Data on population and breeding dynamics of *S. invicta* reveal the presence of two peaks annually with nuptial flights occurring throughout the year. Arthropods, plant seeds, and honeydew are important food sources of *S. invicta*, thereby causing negative impacts on the abundance, diversity, and richness of native arthropod communities. Fire ants are threats not only to agriculture and power facilities, but also to human health, with more than 30% of people having suffered from the sting and 10% having experienced an allergic reaction. To address the expansion of *S. invicta*, the National Fire Ant Detection and Management Union was established and the formulation and implementation of management policies were drawn. Plant quarantine becomes an essential step in fire ant management, whereas the two-step method of combing toxic baits and contact dust emerges as the forefront method crucial in managing *S. invicta*. The experience and lessons learned from fire ant management in China could benefit other countries when facing similar challenges.

Keywords

Control, fire ant, invasive ant, management policies, quarantine

Introduction

China suffers huge economic and biodiversity losses from biological invasions. The complex topography, diverse native ecosystems, and increasing international trade of China with other countries provide numerous opportunities for the entry and colonization of invasive species. In fact, more than 750 invasive species were confirmed in China (http://www.chinaias.cn, accessed on 2019-6-5). Among these pests, almost half of the 100 World's Worst Invasive Alien Species were identified in China, including the notorious pest, the red imported fire ant, *Solenopsis invicta*. *Solenopsis invicta* is an ant species native to tropical regions of South America, and its original homeland was thought to be the Pantanal region of Brazil. The *S. invicta* invasion caused many problems with human health, public safety, agriculture, economy, and ecology around the world (Vinson 1997; 2012; Wojcik et al. 2001; Wang et al. 2019). For example, an investigation in 1999 showed that *S. invicta* infestation causes $38 million loss on crops and livestock productions in Texas, USA (Lard et al. 2002). Moreover, the sting of *S. invicta* can cause skin redness, swelling, urticaria, allergic shock, or in the worst case, death (deShazo et al. 1984; deShazo and Banks 1994). In the United States, almost 40% of the population may be stung by fire ants each year in cities of the southeastern states (deShazo and Williams 1995). Investigation in four provinces of China showed more than a third of people in fire ant-infested areas have suffered stings, and about 10% of them have experienced fever and allergic reactions (Xu et al. 2012).

Solenopsis invicta was introduced to the United States in 1930s and infested more than 320 million acres of land in southern United States (Williams et al. 2003; Wetterer 2013). In the Americas, *S. invicta* was also introduced to Mexico and the West Indies (Wetterer 2013). In Oceania, *S. invicta* was found in Australia and New Zealand (Pascoe 2002; Henshaw et al. 2014), and New Zealand claimed that the *S. invicta* population there was eradicated successfully several years after invasion (Moloney and Vanderwoude 2010). In Asian countries, *S. invicta* was found in Taiwan province of China in 2003, then appeared in mainland China in 2004 (Zeng et al. 2005a). As a consequence of its continuous expansion in China, the Chinese government invested efforts in controlling *S. invicta*, however, this invasive species continued to expand its territory throughout the country. In 2017, Japan and South Korea, *S. invicta* was found in containers and within container yards in several ports, but populations did not become established (Lyu and Lee 2017; Ujiyama and Tsuji 2018). Bertelsmeier et al. (2018) indicated that invaded areas act as a bridgehead of alien ants and the recurrent bridgehead effects increased the global rates of alien ant invasions. With increasing international communication and newly infested areas of *S. invicta*, the opportunity of *S. invicta* invasion is also increasing for tropical and subtropical regions.

The emergency response and management against the invasive fire ants have been carried out since *S. invicta* were found in China. Technical officers from government agencies and entomologists from universities and research institutions were sent to the USA and Australia by the Ministry of Agriculture and Rural Affairs to learn how to deal with the *S. invicta* invasion. These activities helped Chinese experts realize that prevention and control of *S. invicta* is long-term work. After comparison and analysis of the situation of *S. invicta* at home and abroad, the aim of plant quarantine and management of *S. invicta* became clearer in China. It is impossible to eradicate *S. invicta* in most infested regions in some provinces and several isolated infested places in China where it has already become established. Here, we review the Chinese experiences in coping with the *S. invicta* invasion. We also put forward the challenges in managing *S. invicta* in China by offering suggestions to potentially solve the problem. Our focus is to discuss experiences and reflect on the lessons learned from *S. invicta* management in the context of potential fire ant invasion in tropical and subtropical countries.

1. History, current situation and trend of *S. invicta* infestation in China

The initial entry of *S. invicta* was firstly discovered in Taoyuan County and Chiayi County of Taiwan province between September to October 2003, where thorough investigation revealed that 4000 ha land were infested. A decade later, 10 counties with more than 60000 ha land were occupied by *S. invicta* in Taiwan province (http://www.fireant.tw, accessed on 2019-6-5). In mainland China, *S. invicta* was first found in Wuchuan, Guangdong province in September 2004, where, at the end of 2014, 12680 ha land were infested (Lu 2014). Wang et al. (2018b) predicated that 32°N may be the northern limit boundary of the certain infestation zone and 34°N may be the northern limit boundary of possible infestation zones of *S. invicta*. In 2018, *S. invicta* were detected in over 390 counties of 15 provinces and regions including Zhejiang, Fujian, Jiangxi, Hunan, Hubei, Guangdong, Guangxi, Hainan, Chongqing, Sichuan, Guizhou, Yunnan, Taiwan, Hongkong, and Macau (Figs 1, 2) (Lu and Zeng 2015; Ministry of Agriculture and Rural Affairs of the People's Republic of China 2018). Investigation showed that the density of fire ant was approximately 0.9–11.3 mound per 100 m^2 (average 7.26) when first found in newly infected areas (Lu et al. 2008). Based on its geographical distribution, Lu (2014) estimated that the speed of invasion via human-assisted transport in mainland China was about 26.5 km/a using the area sqrt method. Lu and Zeng (2015) estimated that *S. invicta* invasion remained at the stage of rapid expansion, through local and long-distance expansion, so that 20–30 counties may be infested per year in the future if appropriate actions will not be taken.

2. The mechanism of *Solenopsis invicta* invasion in China

2.1 Invasive routine and expanded speed in China

The origins of populations of *S. invicta* in China are important in the terms of tracing the source of the species and instigating future preventive measures. Several studies suggested that *S. invicta* populations in China may have originated from the USA (Ascunce et al. 2011; Yang et al. 2012; Zeng et al. 2005a; Zhang and Hou 2014). However, He

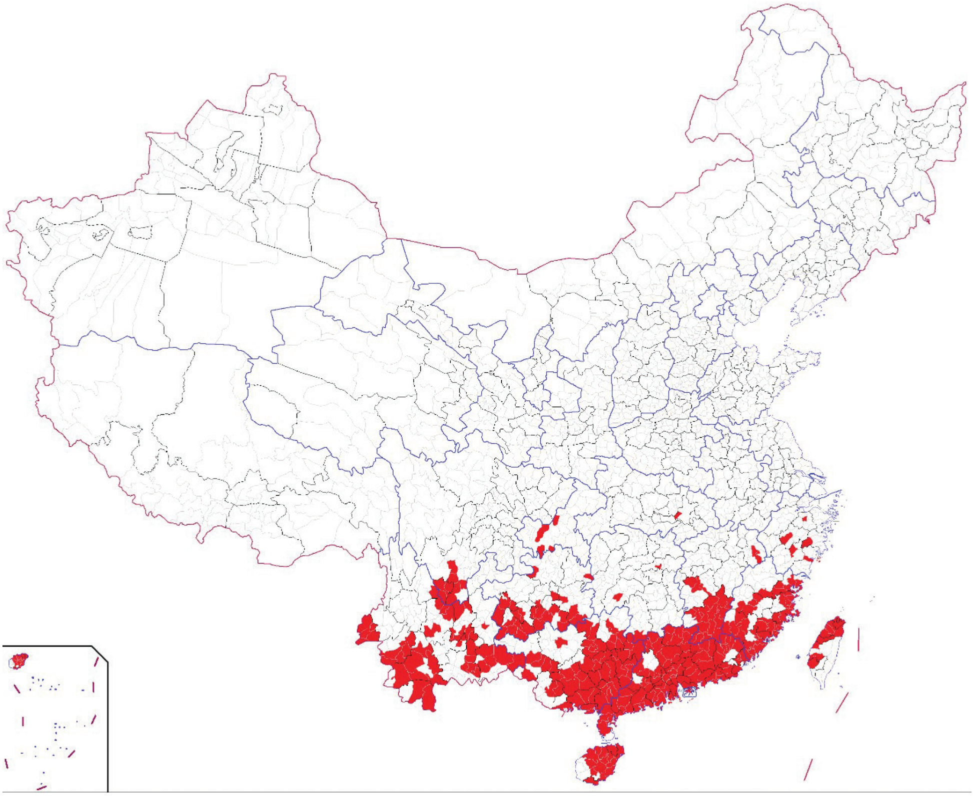

Figure 1. Distribution of *Solenopsis invicta* in China in May, 2019. The red areas represent fire ant infested territories at a national level.

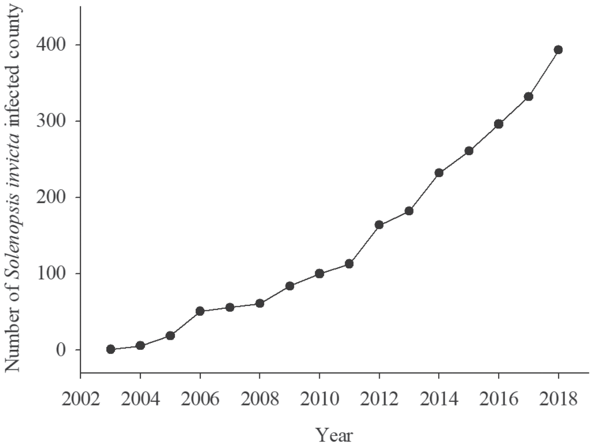

Figure 2. Growth dynamics of *S. invicta* infected counties from 2003 to 2018. The data for infected counties in China quoted from Lu (2014), http://www.moa.gov.cn/, and http://www.fireant.tw.

et al. (2006) indicated that South America may also have been a source of Chinese populations of this species. This conclusion was based on the analysis of mitochondrial cytochrome oxidase I (COI) of the 13 *S. invicta* populations in newly invaded areas in mainland China. These findings indicated that the invasion history of *S. invicta* in China is complex with multiple invasions routes from other regions infected by *S. invicta*.

The growth in global trade and travel accelerated the spread of *S. invicta* from one infected region to another (Floerl et al. 2009; Ascunce et al. 2011). Investigation of Chinese quarantine agencies revealed that the interceptions of *S. invicta* from imported goods increased rapidly during 2005–2017, where importation of logs, wastepaper, and wood packing materials showed the greatest risk to introduce the invasive fire ants to China (Ma et al. 2010; Xian et al. 2019). On the other hand, in Chinese domestic settings, long-distance expansion of *S. invicta* relied mainly on potted plants, turf, recyclable waste, and transport of dairy cattle forage (Lu et al. 2008; Fig. 3). A previous study showed that the transportation of contaminated turf and nursery stock resulted in about 85% of new infections (Lu and Zeng 2015). Investigation of horticulture farms in Guangzhou and Shenzhen showed that several plant species have high infection rate of *S. invicta* compared to other garden plants, including *Chamaedorea erumpens*, *Pachira macrocarpa*, *Rohdea japonica*, *Murraya exotica*, *Magnolia denudate*, *Fortunella japonica*, *Eucalyptus robusta* seedlings, *Phoenix dactylifera*, *Ficus benjamina*, *F. microcarpa*, *Melaleuca bracteate*, *Setaria viridis*, and *Podocarpus macrophyllus* (Huang et al. 2007; Zhou 2009). Investigation on turf plantations and lawns in Guangzhou,

Figure 3. Sod and nursery stock transportation is the main pathway of *Solenopsis invicta* expansion in China (photo by Lei Wang).

Shenzhen, and Huizhou also showed very similar results (Li et al. 2014). Furthermore, the same study recorded that the average fire ant colony density was 0.4 nest per 100 m^2 in turf plantations (Li et al. 2014). *Solenopsis invicta* is most likely to spread in other regions through turf transportation with inappropriate quarantine treatment. Zhang et al. (2015b) also found that potted plant and turf transportation, and recyclable waste transportation were the main routes of *S. invicta* invasion in Fujian province.

3. The impact of *Solenopsis invicta* invasion in China

Solenopsis invicta is a major threat to biodiversity, human health, and public safety in regions where it is introduced (Morrison et al. 1997; Vinson 1997; Wojcik et al. 2001). Here we summarized the impact of *S. invicta* in China.

3.1 Risk in ecology

In China, fire ants invasion has caused a severe reduction of diversity and abundance of arthropods (Wu et al. 2008; Wu et al. 2009; Song et al. 2010b; Xi et al. 2010a, 2010b; Huang et al. 2012a).

Investigation in China showed that *S. invicta* invasion presented a serious negative impact on the abundance, diversity, and richness of native ants in *Litchi chinensis* and *Dimocarpus longan* orchards, lawns, abandoned land, grassland, and residential areas. Ant species richness was reduced by more than 33% in several habitats (Tsai et al. 2009; Jiang et al. 2010; Song et al. 2010; Wu et al. 2010; Lu et al. 2012a; Qi et al. 2015). As an aggressive species, *S. invicta* have a significant negative impact on the diversity index and evenness index of the ant communities within the 3 m of its mound (Wu et al. 2009). Similar to the findings of Porter and Savignano (1990), simple habitats, including abandoned lands and lawn, lost more native ant richness than complex habitats, including *L. chinensis* and *D. longan* orchards (Wu et al. 2008). As resource competition is the main driving mechanism for species replacement, the invasive *S. invicta* disturbs the mutualistic balance between native ant species and honeydew-producing hemipterans by monopolizing the honeydew. Since *S. invicta* can find food more rapidly than the native ant *Tapinoma melanocephalum*, this behavior can therefore cause a negative impact on the foraging behavior of the native species (Lu et al. 2012b; Wu et al. 2014a). In a few cases, however, some native ants can co-exist with *S. invicta*, including *Pachycondyla luteipes*, *Pheidole piel*, *P. yeensi*, *Crematogaster macaoensis*, *Monomorium concolor*, *T. melanocephalum*, *Paratrechina flavipes*, and *Camponotus dolendus* (Wang et al. 2019). Previous studies showed that some of these ants change their foraging and aggression behavior, as well as food preferences, in response to *S. invicta* invasion (Huang 2016; Cheng et al. 2019).

Solenopsis invicta invasion has a negative impact on other arthropods. For example, Huang et al. (2012b) found that *S. invicta* invasion decreased the diversity of spider communities in a cornfield, with a significant effect on the density of *Oxyopex* species. Also, *S. invicta* has a negative impact on the diversity and abundance of the arthropod communities in *L. chinensis* orchards, not only in the canopy of *L. chinensis* trees but also on the ground (Xi et al. 2010b). In a one-year investigation on abandoned land in Guangxi province, Zhang (2008) found that *S. invicta* invasion decreased the diversity of insect communities no matter how low the density of *S. invicta* mounds was. In Fujian province, Chen (2008) reported that *S. invicta* has a negative impact on the diversity of insect communities of grassland, bushes, bamboo groves, orchards, and roadsides dominated by insects belonging to Collembola, Labiduridae, Gryllidae, and Carabidae. These four groups of insects dominated non-infected area but *S. invicta* displaced three of the four groups, leaving only Collembola in the fire ant-infected area.

Plant seeds are important food sources of *S. invicta*. Zhang et al. (2015a) found that plant seeds consisted of 4.6–68% of solid foraging materials for *S. invicta* in a mulberry orchard and on barren land in South China. Xu et al. (2009e) found plant seeds make up to 12% of refuse in middens of *S. invicta*. *Solenopsis invicta* also decreased plant seeds germination by moving and scarifying them. For example, Huang et al. (2010) found that germination rates of *Sesamum indicum* (sesame), *Ageratum conyzoides* (goatweed), and *Pennisetum purpureum* (napier grass) seeds decreased by 63%, 56%, and 50%, respectively.

Solenopsis invicta invasion also changes the physical and chemical properties of soil. The organic matter, alkalihydrolysable nitrogen, and available phosphorus decreased over time while the available potassium and soil acidity was raised after fire ant infestation in an orchard of *L. chinensis* (Xi et al. 2010b). These changes may cause a chain of reaction on the communities of organisms living in the soil.

3.2 Loss in agriculture

Using the plant seeds preference test, Huang et al. (2010) found that *S. invicta* preferred 100% of *S. indicum* (sesame) seeds, and so 82.4% and 64% of the seeds observed are scarified and not able to germinate, respectively. *Sesamum indicum* seeds are rich in oil, which might be a reason why *S. invicta* prefer to consume these seeds. In another field test, the number of seeds which failed to germinate increased 2.86 times and 1.21 times under heavy infection with *S. invicta* (5 mounds per 100 m^2) (Huang et al. 2014). Another field test showed that *S. invicta* and its mutualism with aphids disturb the flower-visiting behavior of insects on crops, causing approximately 35% of yield loss in mungbean (Wu et al. 2014b). Figure 4 shows workers of *S. invicta* attacking a *Vigna unguiculata* plant.

Although we did not get any authoritative data to show how much losses were attributed to *S. invicta* invasion in Chinese agriculture, a loss assessment in Fujian province showed that *S. invicta* has caused 744.70 Yuan/ha direct loss and 2756.48 Yuan/ha indirect loss in its agriculture sector (Hu 2008).

Figure 4. *Solenopsis invicta* workers attack a *Vigna unguiculata* plant (photo by Lei Wang).

3.3 Threat in human health

Solenopsis invicta is an aggressive species that may attack people upon contact. Humans who suffer from the sting of *S. invicta* can experience itchiness and redness, and most of the time, a white pimple may appear several hours after the sting (Fig. 5). Some people also experience serious hypersensitivity, including fever, urticaria, shock, and even death (Fig. 6). In China, greenbelts, farmland, and parks are the top three places where fire ant sting occurs (Zhao and Xu 2015). An investigation in 2004 showed that more than 30% of people in four provinces of China suffered from fire ant stings, and 10% of them experienced fever, with a few individuals experiencing systemic allergic reaction (Xu et al. 2012), and death (Zhang et al. 2006; Zhao and Xu 2015) (http://www.jinciwei.cn/i239583.html, accessed on 2019-7-15). Hu (2008) indicated that one person could lose 83.80 Yuan after being stung by *S. invicta*; this would include charges for treatment and loss of working time.

3.4 Threat in public safety

In China, researchers found fire ant mounds in power facilities and transformer stations (Luo 2005; Zhao et al. 2008). In Boluo county, Guangdong province, 12 of 19 power facilities and transformer stations were infected by *S. invicta*, causing damage on wire and cable, cable boxes, power transformer boxes, and other facilities of power

Figure 5. The typical symptom after *Solenopsis invicta* sting. A white pimple will appear in the place *S. invicta* sting several hours later (photo by Lei Wang).

Figure 6. A person experienced a serious allergic reaction after *S. invicta* sting (photo by Shenlei Li).

stations (Luo 2005). Since *S. invicta* can be attracted to the electric field (Cui et al. 2018), people are concerned that fire ant may induce a short circuit that will result in power system failure. Figure 7 shows *S. invicta* workers attacking a cable box.

Figure 7. *Solenopsis invicta* workers attack a cable box (photo provided by Guangzhou Metro Group Co., Ltd).

Dikes and dams are among the places with *S. invicta* mounds. An investigation in 2013 showed that 28 of 64 dikes and dams were infected by *S. invicta* in Guangdong province (Pan 2013). *Solenopsis invicta* can destroy electric facilities, sting the staff, and may threaten the stability of river banks (Pan 2013). Also, Chen et al. (2017) reported that a destroyed polyethylene gas tube caused a gas leak (Fig. 8). We do not have any reliable data on the economic loss associate with public safety concerns caused by *S. invicta*.

4. Biology of *S. invicta* in China

The creation of a suitable strategy for management of *S. invicta* would depend on the deep understanding of its biology and ecology and must be tailored to the regions where the invasive species is newly introduced. In China, many research institutions have focused their work on fire ant biology and ecology since the first reports of the *S. invicta* invasion.

Solenopsis invicta has two social forms, monogyne (having a single queen within a colony) and polygyne (having multiple queens within a colony), which both possess several distinct differences in terms of biology and dispersal abilities (Porter and Savi-

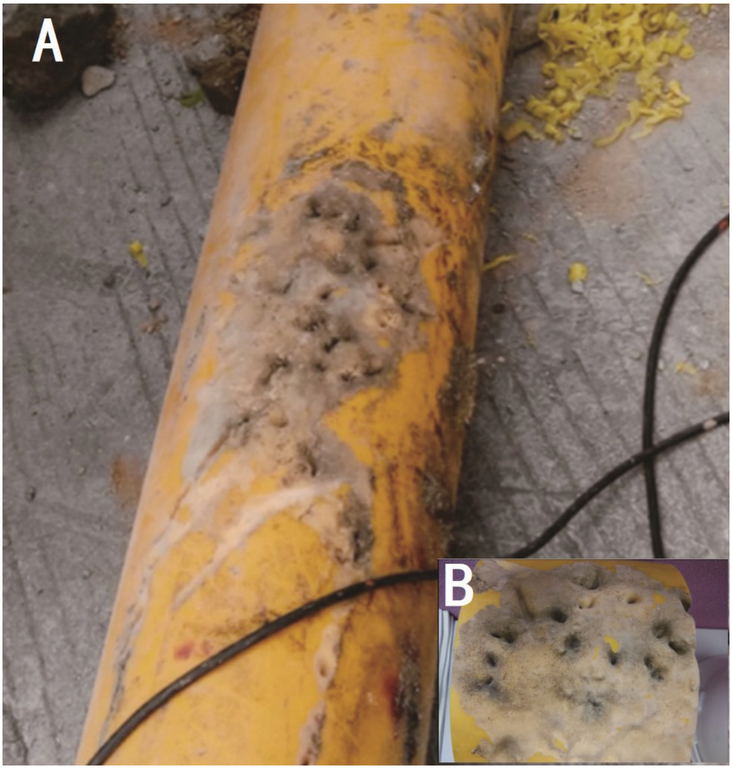

Figure 8. The PE gas tube was destroyed by *Solenopsis invicta*. **A** The position of destroyed position **B** The detail of the destroyed position) (photo from Chen et al. 2017).

gnano 1990; Ross and Keller 2003). Although both monogyne and polygyne colonies have invaded China, it is the polygyne type that retains the dominant social form (Chen et al. 2006; Zeng et al. 2005b; Shao et al. 2008).

Previous studies have shown that the population and breeding dynamics of *S. invicta* consist of two peaks in a year, i.e., spring and autumn (Xu et al. 2009d); however, their nuptial flights occur throughout the year (Xu et al. 2009a). The foraging activity of *S. invicta* also consists of more than two peaks in a year, but the daily foraging activity and the number of foraging workers vary among seasons in south China (Li et al. 2008; Chen et al. 2010; Jiang et al. 2011).

Wang et al. (2016) found that small workers serve as the main food transporters and that the cooperation of *S. invicta* was not well-coordinated in dealing with food transportation. Using sugar water to observe feeding behavior, Wang et al. (2018a) identified five foraging patterns and revealed that the feeding pattern is performed most frequently in the field. Another study on feeding behavior found that *S. invicta* prefers to collect liquid food in the field (Qiu et al. 2014c). Qin et al. (2019b) observed how fire ants transport food on vertical surfaces, leading to the basis of developing novel fire ant baiting systems placed on tree trunks. Qin et al. (2019a) also found that certain groups of workers have a burying behavior and that this behavior may suppress foraging activity. Research has also shown that the foraging gene of *S. invicta* (*sifor*) mediates environmental signals associated with temperature gradient and food-seeking behavior; hence, *sifor* may play an important role in the photoperiod response of this species (Lei et al. 2019; Zhou et al. 2019).

Honeydew is an important food resource of *S. invicta* (Zhou et al. 2012a, 2012b, 2012c). To acquire the honeydew resource, *S. invicta* excludes native ants from the honeydew-producing hemipterans and builds a stronger mutualistic interaction with the hemipterans (Zhou et al. 2014, 2017). Also, *S. invicta* can protect honeydew-producing hemipterans against their natural enemies by attacking their predators (Huang et al. 2011; Zhou et al. 2013; Cheng et al. 2015) and hide hemipterans in shelters from parasitic wasps (Zhou et al. 2012d). As ant-tending behavior produces positive effects on the physiological and behavioral performance of the mealybug *Phenacoccus solenopsis*, this hemipteran tends to excrete honeydew more frequently with lighter droplets rich in melezitose to attract more fire ant workers (Zhou et al. 2015). Therefore, mutualistic interactions between ants and hemipterans are mediated by the honeydew produced by the hemipterans (Zhou et al. 2012b).

The main chemical components of *S. invicta* venom are 2-methyl-6-alkyl or alkenylpiperidines (Chen et al. 2009). However, other pyridine alkaloids were also isolated and identified, including, 2-methyl-6-undecylpyridine, 2-methyl-6-tridecenylpyridine-isomer, 2-methyl-6-tridecenylpyridine-isomer, 2-methyl-6-tridecylpyridine, 2-methyl-6-pentadecenylpyridine-isomer, and 2-methyl-6-pentadecylpyridine (Chen et al. 2019a). Liu et al. (2017) suggested that by using the whole body solvent-soaking method, an adequate amount of fire ant venom alkaloids can be extracted for chemical analyses and bioassays. Fox et al. (2018) also developed a straightforward method for extracting within minutes aculeate *S. invicta* venoms by centrifugation.

The venom alkaloids of *S. invicta* have many functions. For example, an alkaloid isosolenopsin A from *S. invicta* queens could knockdown its competitors rapidly and plays a role in successful nest founding (Fox et al. 2019). Venom alkaloids can also inhibit germination and hyphal growth of entomopathogenic fungi (Obin and Vander Meer 1985). Research has shown that venom alkaloids inhibited the plant pathogen *Pythium ultimum* and bacterial canker of tomato, and thus there is the possibility for developing venom alkaloids as novel fungicides and bactericides (Li et al. 2012, 2013). Cuticular hydrocarbons (CHCs) are a type of chemicals that prevent desiccation, aside from playing a role in signal communication (Hu et al. 2017). Xu et al. (2018a) found that CHCs of *S. invicta* can retain more water under relatively higher temperature, which helps *S. invicta* in occupying warmer environment, and they concluded that CHCs played an important role in shaping the distribution of fire ants in the USA.

Aggressiveness is a very important weapon of fire ants in food competition and territory defense in the introduced regions. The aggressive activity of *S. invicta* can be affected by mound size, colony structure, habitat, temperature, humidity, degree of disturbance, and the use of pesticides (Gao 2007; Wang 2010). However, aggressiveness seemed unaffected by starvation and rafting (Cao et al. 2011; Huang et al. 2016c).

Resistance to adversity is an important trait for successful adaption of the invasive species in a particular region. Xu et al. (2009f) found that *S. invicta* improved their drought tolerance after a short period of non-lethal drought by maintaining low-level water consumption during the acclimation process. *Solenopsis invicta* also improves its cold and heat tolerance after a short period of non-lethal exposure to high- or low-temperatures (Xu et al. 2009b, 2009c). These results imply that *S. invicta* can strongly adapt to extreme weather.

Necrophoric behavior is an important behavior aspect of *S. invicta* to avoid horizontal transmission of pathogens in the colony. Qiu et al. (2015) found that the presence of fungus-infected pupal corpses will accelerate necrophoric behavior. *Solenopsis invicta* receive linoleic and oleic acids at a minimum concentration of approximately 1 μg/μL as a chemical signal to aid necrophoric behavior (Qiu et al. 2015; Xu et al. 2018b). Fire ants sense the presence of oleic and linoleic acids by using the chemosensory protein gene Si-CSP1 (Qiu and Cheng 2017).

Understanding the mechanisms of insecticide metabolism will provide a theoretical basis for the rational usage of insecticides in controlling *S. invicta*. Research has shown that larvae and pupae were less sensitive to insecticides than adults (Guo et al. 2015; Xiong et al. 2019) as a result of the high activity of their metabolic enzymes (Yan et al. 2011). Miao et al. (2009) showed that acetylcholinesterase did not correlate with phoxim detoxification. Zhang et al. (2016b) indicated that the cytochrome P450 genes of *S. invicta*, including *SinvCYP6B1*, *SinvCYP6A1*, *CYP4AB2*, and *SinvCYP4G15*, play an important role in enhanced metabolic detoxification of insecticides. After chlorpyrifos and fipronil exposure, increased transcription levels of cytochrome P450 cause increased O-demethylase activity, a process related to the detoxification of insecticides in *S. invicta* (Zhang et al. 2016a).

5. The responses of China on *Solenopsis invicta* invasion

5.1 Management agencies and research institutions

Strong leadership, good organization, and coordination skills provide a guarantee on preventing *S. invicta* invasion. Specific research institutions are needed for strong scientific and technological support in combatting the spread of this species. For example, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) is responsible in laying down operational responses against imported fire ants (i.e. *S. invicta*, *S. richteri*, and hybrids of these species) through strict quarantine activities (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/imported-fire-ants, accessed on 2018-8-27). In Australia, the Australian Quarantine and Inspection Service (AQIS) of Department of Agriculture and Water Resources is the government agency responsible for the quarantine of *S. invicta* (https://www.daf.qld.gov.au/business-priorities/plants/weeds-pest-animals-ants/invasive-ants/fire-ants/general-information-about-fire-ants, accessed on 2018-8-27).

Several attempts were also made in China to make further exploration in this field. In Taiwan province, the Fire Ant Control Organization was established through multi-agency cooperation involving universities and government agencies of agriculture, environmental protection, health, education, economy, and traffic. The functions of the organization included the collaborative work of various agencies, creation of control plans and strategies, funds allocation, research, fire ant management, and the holding of meetings. In mainland China, specific organizations and research institutions were also established for fire ant management. The provincial government of every infected province established a leading group for fire ant control. The Red Imported Fire Ant Research Center of South China Agricultural University was established to provide scientific and technological support and counseling and services on fire ant management for the government, enterprises, and the public. The National Fire Ant Detection and Management Union was founded by the Ministry of Agriculture and Rural Affairs in September 2006, and the union was comprised of provincial plant quarantine agencies of Guangdong, Guangxi, Hunan, Fujian, Hainan, and research institutions, including Institute of Zoology, Chinese Academy of Science, and South China Agricultural University, among others. The aim and missions of the union are to provide guidelines for fire ant management, control training, detection, specimen identification, etc. The National Fire Ant Detection and Management Union gives strong technical support to the government to cope with the fire ant invasion, holds management training meetings for the public which teach ways to appropriately deal with fire ants.

5.2 The formulation and implementation of management policies and technical plans

In countries infested with *S. invicta*, such as the United States and Australia, there are strict quarantine regulations drawn up for pest control and management (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/

pests-and-diseases/imported-fire-ants, accessed on 2019-6-5; https://www.daf.qld.gov.au/business-priorities/plants/weeds-pest-animals-ants/invasive-ants/fire-ants/general-information-about-fire-ants, accessed on 2018-8-28). With reference to the experience and lessons learned from the United States and Australia, *S. invicta* was put on the list of quarantine pests in China, and the strictest quarantine management was implemented immediately after the first report of *S. invicta*. In response to *S. invicta* invasion, many rules and plans were formulated by administration departments and enforcement agencies, including the Ministry of Agriculture and Rural Affairs, General Administration of Quality Supervision, Inspection and Quarantine, National Forestry and Grassland Administration, and General Administrative Office of National Health and Family Planning Commission (http://www.moa.gov.cn/xw/zwdt/200603/t20060306_563900.htm; http://www.moa.gov.cn/xw/zwdt/200412/t20041208_283440.htm; http://www.forestry.gov.cn/main/4818/content-797166.html). In addition, in provinces infected by *S. invicta* provincial governments issued a work program for red imported fire ant investigation, surveillance, and management, following the policies and rules of the Ministry of Agriculture and Rural Affairs. These policies and rules provided the basis and guarantee on red imported fire ant management.

5.3 Emergency response for *S. invicta* infestation

In China, epidemics of *S. invicta* are ranked as Level 1, 2, 3, or 4 following the "*Contingency plans for emergency response on red imported fire ant*" (http://www.moa.gov.cn/nybgb/2005/derq/201806/t20180617_6152397.htm). Level 4 means that the invasive fire ant is in one county and that the county government or its agricultural agency must start a contingency plan to control its spread. Level 3 means that *S. invicta* is in two or more than two counties but these infected counties belong to the same prefecture; hence, the contingency plan for *S. invicta* control must start through the prefectural government or its agricultural agency. Level 2 means that *S. invicta* invasion is in different prefectures but in the same province, and thus the provincial government or its agricultural agency must start the contingency plan. Level 1 means *S. invicta* invasion is in different provinces, the Ministry of Agriculture and Rural Affairs starts the contingency plan. This emergency response system plays a key role in preventing the rapid expansion of *S. invicta* in China. This model could also become the blueprint in managing other new invasive pests in China.

5.4 Framework of fire ant routine management

Expansions of highly effective mechanisms and models designed for controlling the spread of *S. invicta* and preventing its entry to new regions are arduous tasks but need to be established urgently. In response to the invasion of *S. invicta*, China has dramatically launched several programs since 2004. There are three steps for the management of *S. invicta*, i.e., confirming the epidemic, formulating a scheme, and planning and organizing its implementation. In China, government agencies lead and are actively involved in all three management steps.

The current principle of routine fire ant management is dependent on the administration, i.e., fire ant management was launched by local county government where fire ant was invaded (Fig. 9). There are three government-levels for fire ant management: (1) the central government (mainly in charged by Ministry of Agriculture and Rural Affairs of China), (2) the provincial government (mainly in charged by Department of Agriculture and Rural Affairs of a province), and (3) the county government (mainly in charged by Bureau of Agriculture and Rural Affairs). The duty of the Ministry of Agriculture and Rural Affairs of the People's Republic of China is to draw policy, standards, and guidelines for fire ant management. Limited financial support was given in key regions or national demonstration areas for fire ant management. The duty of the Department of Agriculture and Rural Affairs, a provincial agency, includes the provision of financial support for management of key regions of the province, giving technical support and supervising and inspecting control efficiency of fire ant management in the province as a whole. The duty of the Bureau of Agriculture and Rural Affairs, a county agency, includes responses for fire ant management for the whole county by following the policy of the central government and provincial government. Other duties include providing financial support and organizing the management plan.

5.5 Financial support

Financial support is one of the key factors on fire ant eradication and management. For example, New Zealand spent three years and ca 7.41 million USD to eradicate *S. invicta* successfully from a 12.56 km^2 area of Whirinaki, which means that New Zealand spent 0.20 million USD per km^2 in one year (http://www.scoop.co.nz/stories/PA0611/S00286.htm, accessed on 2019-6-5; http://b3.net.nz/gerda/refs/14.pdf, accessed on 2019-6-5). In China, Jiahe county of Hunan province spent three years and ca 82000 USD on an isolated infested area (ca 0.4511 km^2) to successfully eradicate *S. invicta*, which translates to ca 0.06 million USD per km^2 spent in a year just for eradicating an isolated infested area (data was collected from Bureau of Agriculture and Rural Affairs, Jiahe county). At present, Australia is planning to spend ca. 300 million USD over 10 years to eradicate red imported fire ants from a 7800 km^2 region in south-east Queensland (https://www.daf.qld.gov.au/business-priorities/biosecurity/invasive-plants-animals/ants/fire-ants/eradication/10-year-plan, accessed on 2019-6-5).

For many regions infested by fire ants, eradication is becoming impossible, and the local government instead runs a long-term management program that potentially consumes considerable financial resources. Shenzhen City is one of the best models in China. Shenzhen City spent ca 0.32 million USD to keep the fire ant under control in a 2000 km^2 region in 2018, costing approximately 160 USD per km^2 per year (data was collected from Shenzhen Agricultural Science and Technology Promotion Centre, http://www.szcast.com/index.html, accessed on 2019-6-5).

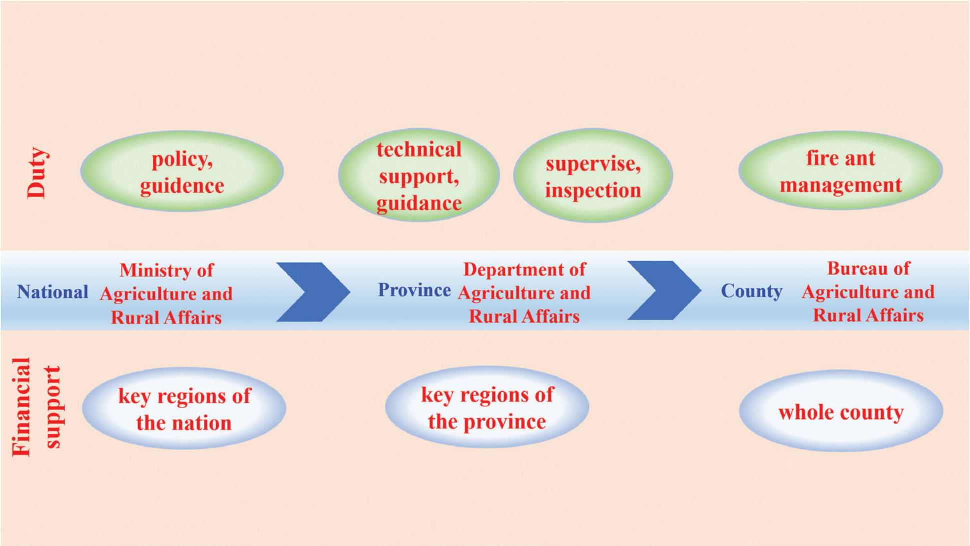

Figure 9. Framework of fire ant routine management in China. Different governmental levels have different duties.

6. Current strategy for *S. invicta* management in China

6.1 Exploration for control methods of *S. invicta*

Although 40 products were registered for management of *S. invicta* in China, the main active ingredients were only 3 or 4 types, i.e. indoxacarb, fipronil, hydramethylnon, and beta cypermethrin. Among these four, the application of fipronil was forbidden in the agricultural system (http://www.icama.org.cn/hysj/index.jhtml, accessed on 2019-12-10). The selection of effective ingredients was done in China. Previous reports have suggested that other insecticides have great potential for the management of *S. invicta*, including sulfoxaflor, dinotefuran, clothianidin, nitenpyram, thiamethoxam, ethiprole, chlorfenapyr, cyantraniliprole (Pan 2017; Pan et al. 2017; Du 2018; Zhou et al. 2018). Green insecticides were also evaluated for their effect on *S. invicta*. The toxicities of plant extracts derived from *Lantana camara*, *Pronephrium megacuspe*, *Cinnamonum camphora*, *Murraya exotica*, *Viburnum odoratissimum*, *Michelia alba*, and *Periploca sepium* were also tested (Zhong et al. 2008; Fu et al. 2015; Huang et al. 2016a, 2016b; Hui et al. 2014; Qin et al. 2018; Zhang et al. 2017b; Huang et al. 2019). Many of these plant extracts exhibited excellent fumigation and contact toxicity under laboratory condition. For example, an ethanol extract of the shrub *L. camara* exhibited 100% killing effect against *S. invicta* workers in five days in an indoor experiment (Zhong et al. 2008). Volatiles from *M. alba* leaves caused 100% mortality of *S. invicta* workers in 16 h in a fumigation bioassay (Qin et al. 2018). Decylamine from *Monomorium minimum* show toxicity by causing a knockdown effect and suppressed the digging behavior in *S. invicta* workers (Wang and Chen 2015). Effects of flavoring agents were also evaluated by Zhang et al. (2017a), who found that 0.1 or 0.2g/mL erythritol caused 80%

mortality among *S. invicta* workers after 72 h treatment. Furthermore, glycine and Guanosine 5'-monophosphate (GMP) exhibited high toxicities to *S. invicta* with LC_{50} values of 0.004 g/mL and 0.02 g/mL, respectively (Huang et al. 2018).

Some research has also been done on repellence selection. Wang et al. (2012) found that 1000 µl/mL of *Capsicum annuum* (sweet pepper) oil showed >90% repellent activity against *S. invicta* workers under laboratory conditions. Essential balm strongly repelled *S. invicta* workers even at the concentration of 0.5µL/cm^2 (Wen et al. 2016). The repellence effect of two food additives, ethyl anthranilate and butyl anthranilate, were even better than diethyltoluamide (DEET) against *S. invicta* (Chen et al. 2019b).

Studies on the biological control of *S. invicta* have been ongoing in China where entomopathogenic nematodes and fungi were evaluated. Zhang et al. (2010) found that *S. invicta* workers can protect the queen and its colony from the entomopathogenic nematodes *Steinernema carpocapsae* (strain AII) and *S. scapterisci* by decreasing the effect these nematodes on *S. invicta*. Many entomopathogenic fungi strains were evaluated for control of *S. invicta* under laboratory conditions, including *Paecilomyces lilacinus*, *Matarhizium anisopliae*, *M. flavoviride*, and *Beauveria bassiana*, among others (Li et al. 2007; Yang et al. 2009; Liu et al. 2010; Xu et al. 2013; Wang et al. 2018c). Some of them showed a good impact on *S. invicta* in the laboratory but had poor effect under field conditions, indicating that the social immunity of *S. invicta* may play a modulating role. Wang et al. (2015) reported that *S. invicta* may use the antimicrobial property of volatiles to fumigate their nest. Qiu et al. (2016) found that *S. invicta* workers exposed with *M. anisopliae* showed self-medication and increased interaction with their nestmates in response to infection by *M. anisopliae*. Also, these *M. anisopliae*-exposed *S. invicta* workers spent less time in the pupal chamber so that the healthy workers could perform more grooming behavior; thus, increased duration of grooming counteracted infection of pupae by *M. anisopliae* (Qiu et al. 2014a). Qiu et al. (2015) also found that *M. anisopliae*-infected pupal corpses accelerated the necrophoric behavior of *S. invicta* workers. Qiu et al. (2014b) showed that *S. invicta* avoids food contaminated with entomopathogenic fungi and speculated that *S. invicta* may discriminate entomopathogenic fungi based on the profile of their volatile organic compounds. To enhance the efficiency of controlling *S. invicta* using entomopathogenic fungi, Li et al. (2016) designed an injector to deliver entomopathogenic fungi spores into the nest; this strategy resulted in control effect in both laboratory and field conditions. A new microencapsulation method of *M. anisopliae* was obtained with improved stress resistance and insecticidal activity on *S. invicta* (Qiu et al. 2019). Fan et al. (2012) presented a strain of *B. bassiana* that expressed pyrokinin β -neuropeptide (β-NP) and thereby increased its fungal virulence towards *S. invicta*. Also, the combination of entomopathogenic fungi with pesticides seem to be one of the best control strategies against *S. invicta*. Wang et al. (2016) found that *M. flavoviride* SM076 spores can be mixed with 0.1 mg/mL indoxacarb, 0.1 mg/mL pleocidin, and 1.0 mg/mL methoprene without any negative effect. Moreover, *M. anisopliae* M09 can mixed with 0.5 mg/mL indoxacarb, 0.1 mg/mL abamectin, 0.01 mg/mL fenoxycarb, 0.5 mg/mL methoprene, and 0.05 mg/mL fipronil without any negative effect (Wang et al. 2016). Li et al. (2018) showed that the combination of *B. bassiana* spores and thiacloprid at a mass ratio of 8:2 demonstrated a very significant synergistic effect in laboratory and field conditions.

6.2 Education

Education is very important for invasive species management. After the pest was found in mainland China, the Ministry of Agriculture and Rural Affairs immediately announced the notice for the management of *S. invicta*. Then, several campaigns to inform the public about *S. invicta* were launched through various television commercials and leaflets. For example, several programs that introduce the biology and control methods of *S. invicta* were recorded and broadcast by China Central Television. These campaigns help the Chinese government to deliver messages to avoid panic by the public and to spread knowledge of the biology, behavior, and control of *S. invicta*. For instance, an intervention questionnaire (1715 valid questionnaires) in Shenzhen city showed that TV, internet, newspaper, and leaflets were the main ways residents received knowledge of *S. invicta* (Li et al. 2011). The intervention indicated that public education was an important way to distribute information about this species. In an 2013 investigation in Nansha district of Guangzhou, 65.2% of the total participants (2638 valid questionnaires) heard of *S. invicta* but 47.65% had no knowledge on the correct method for fire ant management (Wang et al. 2014). These investigations showed that the pubic was aware of *S. invicta* but had no correct skills in controlling infestations. The next step, therefore, should be to strengthen public education on fire ant management using both traditional and modern media. For instance, books, including “Fire Ant Monitor and Management”, “Fire Ant Management”, and “Red Demon: Red Imported Fire Ant Invasion”, and a film, “Stopping Fire Ant Invasion”, were published and distributed freely since 2016 in infested regions. The three books and the film will help people learn how to deal with fire ants.

Meanwhile, fire ant management training activities were also lunched in infested regions, where entomologists who specifically study *S. invicta* were invited to give educational seminars and field training to residents, staff of agricultural agencies, and pest control companies. The national fire ant management training also held an anniversary for the staff of the agricultural agencies. The training normally has two parts where the attendees received educational presentations and fact sheets about fire ants and then field demonstrations of the methods for fire ant control.

In China, *S. invicta* can spread a long distance by the transportation of contaminated turf and nursery stock (Lu et al. 2008). Hence, it is hard to control the fire ant expansion without the cooperation of stakeholders. Thus, quarantine agencies provide professional support and training on management of *S. invicta* to owners of turf and nursery stock farms. Meanwhile, a responsibility agreement was also signed with stakeholders for preventing the spread of fire ants.

6.3 Fire ant management

6.3.1 Plant quarantine

Surveillance at Chinese ports of logs, wastepaper, and wood packing materials from *S. invicta*-infected regions have shown that these objects were high-risk for the entry of *S. invicta* into China (Ma et al. 2010). Huang and Sun (2006) indicated traditional quarantine management for imported goods are inadequate and resulted in the introduction of *S. invicta* into China. The constraints of the current quarantine requirements are increasingly massive due to interconnected global trade and travel. Zhang et al. (2007) suggested that quarantine procedures should not only give more attention to high-risk goods but also undertake proactive surveillance at ports of entry.

Domestically, transportation of infected garden plants and turf are considered the main vehicle the spread of fire ants (Lu et al. 2008). For example, an investigation in Guangdong showed that 28.4% of garden plant nurseries and 48.8% of turf farms were infested by *S. invicta* (Huang et al. 2007). Li et al. (2014) found that 85.2% of 122 newly introduced cases of *S. invicta* were caused due to garden plants and turf. It is important to kill the red imported fire ant in contaminated garden plants before they are planted; hence, the use of contact insecticides for quarantine treatment is suggested (Wang et al. 2011). Cooperation from stakeholders is also very important, including both customers and personnel from the garden plant nurseries.

6.3.2 Population assessment

Understanding the density, degree of damage, and boundary of occurrence is the first step for management of *S. invicta*. Populations can be estimated by scoring active *S. invicta* mounds, the number of workers per bait, and the percentage of bait with workers (Lofgren and Williams 1982; Vander Meer et al. 2007). In China, the protocol "*Guidelines for quarantine surveillance of* Solenopsis invicta *Buren (GB/T 23626-2009)*" was formulated and announced in 2009. The guidelines give criteria on regional surveillance, temporal suitability, surveillance tools, surveillance methods, specimen identification and preservation, surveillance record table, etc. These guidelines also provide standards to rank occurrence levels of *S. invicta* populations based on the number of workers per bait and the active mounds in a unit area. In Guangdong province, the local "*Guideline for Delimiting and Management of* Solenopsis invicta *Infected Site, Area of Surveillance Regions*" was also formulated and announced in 2013 (http://www.gdagri.gov.cn/zwgk/zcfg/zhfg/201708/t20170811_602860.html, accessed on 2019-6-5). In this guideline, specific regulations were given on how to delimit sites infected by *S. invicta* and the area of surveillance regions, control measures of infected sites, and how to evaluate control effect.

In China, "Solenopsis invicta *Occurrence Notification System*" was also applied. A total of 2488 surveillance sites were set up in more than 230 counties nationally where the area under surveillance reached more than 0.6 million hectares (Lu and Zeng 2015; Ministry of Agriculture and Rural Affairs of the People's Republic of China 2018). Therefore, the system provides a favorable guarantee for the alerting of new introductions of *S. invicta* in China.

6.3.3 Chemicals application

Chemical application is the main method in managing the expansion of fire ants, especially in newly invaded regions. Many chemicals are effective for control, including bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fluvalinate, lambda-cyhalothrin, permethrin, s-bioallethrin, es-fenvalerate, tefluthrin, tralomethrin, carbaryl, and acephate (Wang and Zong 2006). In China, the guiding protocol "*Pesticide-guidelines for the field efficacy trials (2)–Part 149: insecticides against* Solenopsis invicta *Buren (GB/T 17980.149-2009)*" was announced in 2009. This guideline gives criteria to evaluate the field efficacy of insecticides against *S. invicta*, which helps Chinese insecticide companies to develop effective insecticide products against *S. invicta*. An industry standard for the control of *S. invicta* is the "*Guidelines for chemical prevention and control of Solenopsis invicta Buren (NY/T 2415-2013)*", which was announced at 2013 by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (Ministry of Agriculture and Rural Affairs of the People's Republic of China 2013). These guidelines not only give criteria on strategy, optimum control period, control techniques, and matters needing attention in chemical control, but also recommend active ingredients for the control of the species, including bifenthrin, cyfluthrin, avermectin, spinosad, indoxacarb, sulfluramid, fipronil, and rotenone.

In the first few years after *S. invicta* was found in mainland China, pesticides for *S. invicta* control were classified as special-need pesticides by the Ministry of Agriculture and Rural Affairs so that companies could put a certain number of specific pesticides on the market with a temporary registration certification (Wang and Zong 2006). This policy not only minimized the hazards caused by *S. invicta* but also prevented its quick spread at the beginning of its invasion in China. Currently, 36 products from 22 companies are registered and available for managing *S. invicta* in mainland China. The active ingredients of these products include sulfluramid, indoxacarb, fipronil, hydramethylnon, beta-cypermethrin, spinosad, imidacloprid, and chlorpyrifos (www.icama.org.cn, accessed on 2019-6-5).

Toxic bait and surface treatments, including liquid mound drench and application of granules or dusts, are the main methods for control of *S. invicta* (Drees et al. 2013). Surface treatments are used as contact insecticides to eliminate *S. invicta* quickly, but most of them are used as individual mound treatment (Drees et al. 2013). Toxic bait works slowly, but it is effective and is also suitable for applications over large areas (Vogt et al. 2003). Soon after the initial invasion by *S. invicta*, Chinese scientists realized that not all methods used in other countries in the control of *S. invicta* were well suited in China. For example, the current use of individual mound drench is not recommended in China because previous experiences showed that residents had difficulty in learning the correct application of mound drench, which, in effect, eventually helped in the spread of *S. invicta*. Mound drench also needs too much water (about 10–20 L water for a mound) (Liu et al. 2006), contributes to environmental pollution, and causes harmful effects to the native arthropods (Liu et al. 2006; Yu et al. 2015). The most important limitation of this method is it cannot be used over wide areas. Currently, baits and contact dust are the main products for managing *S. invicta* in China.

The two-step method developed in the Unites States is one of the main and most effective methods for management of *S. invicta*. It involves a combination of contact dust and baits. i.e., "*Step 1. broadcast a fire ant bait once or twice a year to reduce fire ant colonies by 80 to 90 percent. Step 2. Treat nuisance mounds or colonies that move into the bait-treated areas. Step 2 may not be needed.*" (http://articles.extension.org/pages/14345/fire-ant-control:-the-two-step-method-and-other-approaches). Following the example used in the United States, a modified two-step method was developed in China. Step 1: whole area control. Broadcast a bait in areas with high densities of *S. invicta* or apply bait on individual mounds in areas with low densities. Meanwhile, contact dust is used to treat individual mounds that are easy to handle. Step 2: Key area control. After 4–6weeks, the population of *S. invicta* is assessed. Then, the remaining parts of the area with a high population of *S. invicta* is treated by bait and the individual mounds is treated by contact dust. After the two steps, fire ant colonies can be reduced by as much as 90%. The cycle of the two-step method can run again and again until *S. invicta* population is under control or eradicated in the treated area.

6.3.4 Model for fire ant management in China

The main system for managing *S. invicta* in China is hierarchical from top to down, i.e., the local government provides the financial support and the agricultural sectors (such as the Department of Plant Protection and Plant Quarantine, Agricultural Agency of county government), purchases the pesticides and management tools, and distributes these to the organizations of mass self-government (such as resident committees). However, this system is not very suitable for the management of *S. invicta*. Here are the main shortcomings: 1) basic-level agricultural agencies have a hard time comprehensively and effectively managing *S. invicta* because these agricultural agencies have limited human resources to oversee the huge and complicated job of managing all pests. This may result in a scenario where some staff may lack the professional ability to involve, guide, and supervise on management of *S. invicta*. 2) The field workers involved in the management of this species are always temporary employees in the basic-level government. Even with professional training, most workers still cannot fully master the technique and, as a result, pesticide use has always been a waste due to improper application. For instance, an investigation found that the control efficacy was only 20–65% when control was organized and operated by resident committees (personal communication).

Currently, professional pest control organizations are involved in the management of *S. invicta* in some provinces. In this system, the government agencies must create control plans and goals, review and approve the implementation plans that are provided by pest control organizations, inspect and supervise the implementation process, and check the control efficiency. The professional pest control organizations must make implementation plans, implement the management plans, and follow the goals of the government agencies. A third party, which is also the professional organization, was introduced by government agencies to inspect the implementation process and check the control efficiency. The professional pest control organizations have the professional skills to run an effective measure against *S. invicta*. This system does not only easily achieve the aim of managing *S. invicta* but also saves manpower, pesticides, and financial resources. The model is already running in several provinces and shows good effectiveness (http://www.hnjh.gov.cn/jhzf/2/3723/3757/3995/content_280184.html, http://www.ccgp.gov.cn/cggg/dfgg/jzxcs/201812/t20181213_11335311.htm; http://www.sx.gov.cn/art/2018/7/2/art_1463355_19027725.html, http://www.changsha.gov.cn/xxgk/szfxxgkml/ztbd/2014ndzldbzjxkhgzmbgs/zssnyj/201505/t20150518_722617.html, accessed on 2019-6-5).

The model was also designed and implemented for area-wide suppression or eradication of fire ants in China. When the control strategies for *S. invicta* are formulated, several factors are taken into consideration, including the distribution pattern of the infestation (whether widely distributed or localized), the environmental characteristics of infested area (complexity of habitat), and the characteristics of the occurrence of *S. invicta* (mounds are easy or difficult to find). Inspections of the whole given area are conducted at the onset of the program to identify unreported infestations. Then, plant quarantine is strictly implemented to prevent dispersal of *S. invicta* in the given areas. Education of residents about the control measure follows. The new two-step method is then applied to the area of infestation. After one cycle of the new two-step method, the inspections of the whole given area are conducted again to evaluate the efficiency of the control and the situation of the infestations. These control steps can be applied in the infected area over and over until the aim is achieved. The model has been conducted in fire ant management programs and has been successful. For example, in the Tianhe District of Guangzhou after using the model for five months, from June to October 2013, the active mound of *S. invicta* reduced by 99.3% and the number of active fire ant workers reduced by 98.4% (unpublished data). In Hunan province, the agricultural agency of Jiahe county used the model and successfully eradicated *S. invicta* from a 45-ha region (http://www.hnjh.gov.cn/jhzf/2/3723/3757/3995/content_280184.html, access on 2019-6-5).

7. Challenges

Since the discovery of *S. invicta* in China, great efforts have been made to control and prevent its invasion and expansion. To date, safe and effective technology systems designed for monitoring, quarantine, emergency response, and eradication has been developed. These technology systems give strong support to the management of *S. invicta*, although fire ants still expand their territory within China. Based on past experiences of fire ant management programs in China, the strategies below might help the country in better responding to the threat of invasion by *S. invicta*.

First, plant quarantine should be put in place. Poor fire ant management and lack of plant quarantine for seedlings remain one of the important reasons why *S. invicta* continues to quickly expand its territory in China. Long-distance domestic transportation of potted flowers, turf, nursery stock, and waste materials is the main means of dispersal for this species. Urbanization in China has increased the requirement for potted flowers, turf, and nursery stock and has accelerated the spread of fire ants. Meanwhile, more than 10 million landscaping seedlings are transferred from *S. invicta*-infested regions to uninfested regions every year in China, and many of these plants may not get the appropriate quarantine treatment because of lack of funds and a shortage of human resources. Also, interceptions of *S. invicta* in many Chinese ports has increased in cargo originating from other countries and regions. Therefore, the management of fire ant population in farms producing seedlings, effective quarantine technology system, and quarantine model should be strengthened and developed, as good management and quarantine can slow down the speed of *S. invicta*.

Second, the fire ant management system of government agencies should be updated. Various government agencies, for example agricultural and forestry, are involved in the management of *S. invicta*. However, cooperation among different local government agencies is not well executed, which has resulted in poor control of *S. invicta* in some regions. For fire ants to be eradicated at all points and in all regions, local government agencies should update the monitoring system, as well as its management, to avoid fire ants from reinfecting regions.

Third, developing more effective pesticides and applied methods is important to curb the spread of *S. invicta*. Traditional management methods are time consuming and laborious, especially in large areas with a high density of *S. invicta*. The development of rapid detection of nests is important for assessing control efforts and monitoring dispersal. Satellite aerial imagery could be used to assess the distribution of *S. invicta* mounds on a large scale (Vogt 2004a, 2004b), in addition to the deployment of trained dogs and spectrum analysis technology to inspect and count mounds (Lin et al. 2011; Wu et al. 2014c). Other new technologies are needed for rapid and accurate detection of mounds, thus facilitating precise, targeted insecticide treatments. Meanwhile, to reduce both environmental and economic losses related to chemical use, new management strategies and environmentally friendly insecticides are needed, especially for aquafarm, drinking water source areas, organic farms, and natural reserves.

Fourth, Korzukhin et al. (2001) predicted that the range of *S. invicta* in the eastern United States will expand over the next century due to climate change. A significant warming trend also has occurred throughout China over the past 20 years (Yu et al. 2011). With changes in the global weather, different types of ecological regions may be invaded by *S. invicta*, and *S. invicta* will likely adapt to new habitats and increase its impact on ecosystems.

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