Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores

Bart M. C. Grutters*,[1], Yvonne O. A. Roijendijk[1], Wilco C. E. P. Verberk[2] and Elisabeth S. Bakker[1]

[1]*Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB, Wageningen, The Netherlands; and* [2]*Department of Animal Ecology and Ecophysiology, Institute for Water and Wetland Research, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands*

Summary

1. Globalization and climate change trigger species invasions and range shifts, which reshuffle communities at an exceptional rate and expose plant migrants to unfamiliar herbivores. Dominant hypotheses to predict plant success are based on evolutionary novelty: either herbivores are maladapted to consume novel plants (enemy release hypothesis), or novel plants are maladapted to deter herbivores (biotic resistance hypothesis). Since novelty can work both ways, it fails to consistently predict when herbivores will consume novel over non-novel plants. Surprisingly, the value of using plant traits to predict herbivore consumption of novel plants remains largely unexplored. We hypothesized that (i) plant traits explain generalist herbivore consumption rates of novel and non-novel plants, and (ii) any effect of novelty will be grounded in consistent trait differences between native and novel plants. Lastly, we expected to find (iii) differences in plant traits and plant consumption rates across latitude.

2. To test these hypotheses, we measured the consumption rate of plant species for a tropical and a temperate generalist herbivore in controlled feeding trials by offering them a large variety of 40 plant species from different geographical origins. Therefore, whether a plant was novel depended on the herbivore used, allowing us to disentangle plant identity from plant novelty. We also measured plant chemical traits and determined whether traits, geographical origin or novelty best explained herbivore consumption rates.

3. Both generalist herbivores consumed more of plants with a high nitrogen-to-phenolic compounds ratio, irrespective of the plant's novelty to the herbivore. A pattern of increasing plant's nitrogen-to-phenolics ratio with latitude could explain why both the tropical and temperate herbivore consumed more of plants from temperate regions. Plant novelty and its geographical origin no longer explained consumption rates once differences in nitrogen-to-phenolic compounds ratio were taken into account.

4. We show that differences in plant traits along a latitudinal cline determine herbivore consumption rates, irrespective of whether plants are novel or familiar. Therefore, we propose that integrating evolutionary novelty theory with plant traits and biogeography will increase our understanding of the consequences of plant species migration beyond biogeographical barriers.

Key-words: biotic resistance hypothesis, enemy release hypothesis, exotic species, food quality, invasive species, latitude, trait-based approach

Introduction

Plant species increasingly cross biogeographical dispersal barriers due to globalization and climate change (van Kleunen *et al.* 2015). The entry of new species into communities can greatly impact ecosystem functioning and biodiversity (Simberloff 2011) and the mitigation of these impacts costs billions of dollars annually (Vilà *et al.* 2009; van Kleunen *et al.* 2015). Native herbivores can potentially prevent these impacts by consuming non-native plants and thus providing biotic resistance to non-native plants (Parker, Burkepile & Hay 2006; Alofs & Jackson 2014). Generalist herbivores are more likely to consume non-native plants as they are less constrained in their selection of food

plants than specialist herbivores (Parker & Hay 2005; Parker *et al.* 2012). While this makes generalist herbivores more likely to provide biotic resistance, it also makes their food preference hard to predict. This is clearly indicated by opposing reports on herbivore preference: meta-analyses document both successful and unsuccessful biotic resistance of herbivores to plant invaders (Keane & Crawley 2002; Levine, Adler & Yelenik 2004; Parker & Hay 2005; Parker, Burkepile & Hay 2006; Jeschke *et al.* 2012). An urgent question is therefore: when do native herbivores provide biotic resistance to non-native plants?

The likelihood of biotic resistance by consumers is traditionally predicted from the evolutionarity novelty of the plant to the herbivore (Levine, Adler & Yelenik 2004). As species migrate beyond biogeographical barriers, they encounter species that they have not coevolved with. Evolutionary novelty theory predicts that without co-evolution, species will be maladapted. This yields two alternative hypotheses: either native herbivores are maladapted to consume non-native plants (enemy release hypothesis), or non-native plants are maladapted to deter consumption by herbivores (biotic resistance hypothesis). To resolve the generated opposing effects on whether generalist herbivores prefer native (Keane & Crawley 2002; Liu & Stiling 2006; Xiong *et al.* 2008) or non-natives plants (Parker & Hay 2005; Parker, Burkepile & Hay 2006; Morrison & Hay 2011; Jeschke *et al.* 2012), novelty-based hypotheses may need to be extended by including mechanisms underlying plant palatability (Verhoeven *et al.* 2009). The opposing effects can be confounded by known differences in plant palatability over latitude, as many novel plants have a tropical origin. Ultimately, however, plant palatability depends on plant traits related to plant nutritional value and anti-herbivore defences. Surprisingly, the value of plant traits and biogeography in resolving the inconsistent results of novelty has not been thoroughly investigated.

Plant palatability depends on multiple plant traits and their interaction (Hay 1996; Elger & Lemoine 2005; Agrawal 2011). Traits such as nitrogen content, phosphorus content and total phenolic content have been found to correlate with the plant preferences and consumption rates of generalist herbivores in native communities (Mattson 1980; Cronin *et al.* 2002; Elger & Lemoine 2005; Dorenbosch & Bakker 2011), but see (Steinberg & van Altena 1992; Targett *et al.* 1995). However, few studies have tested whether plant traits underlie differences in herbivore consumption of native and non-native plants (but see Lind & Parker 2010 and Morrison & Hay 2011).

The opposing results for evolutionary novelty can also derive from biogeography. Biogeographical research shows that plant palatability increases with latitude (Bolser & Hay 1996; Siska *et al.* 2002; Salgado & Pennings 2005; Pennings *et al.* 2007; Moles *et al.* 2011; Morrison & Hay 2012), with support for increasing nutritional value and decreasing anti-herbivore defences with latitude (Reich & Oleksyn 2004; Schemske *et al.* 2009), but see Moles *et al.*

(2011). Hence, the geographical origin of non-native plants may affect herbivore preference via its effect on plant traits.

We hypothesized that (i) plant traits correlate with the consumption rates of plant species by generalist herbivores, so that (ii) any effect of novelty of the plant to the herbivore will be grounded in consistent trait differences between native and novel plants. We also expected that (iii) herbivore consumption rates and plant traits including nitrogen, phosphorus and phenolic content will show biogeographical patterns, with increased consumption of high latitude plants.

We performed controlled feeding trials to test the herbivore consumption rate of 40 aquatic plant species, including species native and non-native to Northwestern Europe, to two generalist gastropods that likewise differed in their geographical origin. Novelty in plant-herbivore pairs was based on the presence or absence of overlap in the native ranges of species. We measured plant traits, tested which ones best correlated with herbivore consumption rates, and subsequently assessed whether plant traits and patterns therein help predict generalist herbivore consumption of novel and known plants.

Materials and methods

STUDY SYSTEM

The main herbivores on submerged aquatic vascular plants are generalists such as waterfowl, crayfish and snails (Lodge *et al.* 1998; Parker & Hay 2005; Wood *et al.* 2016) and they strongly regulate plant abundance and ecosystem processes (Lodge *et al.* 1998; Veen *et al.* 2013; van der Wal *et al.* 2013; Bakker *et al.* 2016). Unsurprisingly therefore, consumers provide most biotic resistance to plant invasions in aquatic ecosystems (Kimbro, Cheng & Grosholz 2013; Alofs & Jackson 2014). Because generalist herbivory is the dominant form of plant consumption and because of the huge ecological and economic impacts that freshwater invaders cause in aquatic ecosystems (Dudgeon *et al.* 2006; Vilà *et al.* 2009), we used freshwater plants and herbivores as a model system.

We performed no-choice feeding trials in which we tested 40 vascular freshwater plant species, of which 20 are non-native to Northwestern Europe (see Supporting Information Table S1). We used non-native plant species that are native to North America, South America, Africa and Australia. We fed the aquatic plants to two generalist consumers that have been frequently used in no-choice feeding trials (Elger & Willby 2003; Elger & Lemoine 2005; Xiong *et al.* 2008; Burlakova *et al.* 2009) and that originate from contrasting biogeographical regions: the South American freshwater snail *Pomacea canaliculata* (Lamarck 1819) and the Eurasian freshwater snail *Lymnaea stagnalis* (Linnaeus 1758).

AQUATIC PLANTS

The non-native plant species used in this study include those most widely spread in Europe (Hussner 2012), as well as a few less common non-natives. Native and non-native species from 12 different plant orders were tested to cover a broad phylogenetic plant range (see Table S1). We collected 23 vascular aquatic plant species from 12 field sites in the Netherlands from late September to early October 2012. The other 17 species were unavailable at field

locations and were ordered from an aquatic plant supplier, these groups were equally consumed (Appendix S1). All plants were green and looked healthy at the time of collection and during their use in the feeding trials. Plant species that were not used immediately in the feeding trials were either stored at 4 °C and used within a week (11 species) or kept in cattle tanks in the greenhouse on commercial pond sediment and filled with groundwater for later tests (29 species).

GENERALIST HERBIVORES

The invasive herbivore species *P. canaliculata* (Channeled apple snail), a native of South America, is listed among the 100 worst invasive species because of its impact on ecosystems and agriculture (Lowe *et al.* 2000; Carlsson, Bronmark & Hansson 2004). The *P. canaliculata* used in the experiment originated from a lab culture with snails imported from the Philippines. Besides *P. canaliculata*, we tested the non-invasive *L. stagnalis* (Great pond snail), a native of Europe and Asia, because of its contrasting origin to *P. canaliculata* and its inclination to feed on freshwater plants. *L. stagnalis* individuals were collected from plant-free or *Chara* dominated experimental ponds in the Netherlands (located at 52·2116 N, 5·0384 E) on three occasions between September and November 2012.

Both species were separately kept in aerated aquaria at 20 °C (pH 7·8; EC 0·2 mS cm^{-1}; hardness 5·6 °D), supplemented with chalk, and fed butterhead lettuce five times a week and fish food pellets (Velda, Enschede, The Netherlands) once a week.

FEEDING TRIALS

We performed 48 h no-choice feeding trials following established protocols (Elger & Barrat-Segretain 2002; Burlakova *et al.* 2009), with three simultaneous treatments ($n = 12$ per treatment): grazing by *L. stagnalis*, grazing by *P. canaliculata* and a no-grazing control (for pros and cons of no-choice trials see Appendix S1). Herbivores were collected from aquaria, blotted dry, had their shell length (Velleman digital calliper; Gavere, Belgium) and wet mass (Sartorius BL60S, Goettingen, Germany) recorded (details in Appendix S1), and were then starved for 48 h prior to the trials. Non-apical plant fragments or leaves collected from the tested plant species were blotted dry, weighed (FW$_{plant,initial}$) and offered to the starved snails in unlimited availability (see Appendix S1). Snails grazed individually on plant parts in plastic beakers filled with water (19 to 20 °C, pH 8, conductivity 200 µS cm^{-1}; WTW 350i Multimeter, Weilheim, Germany) and covered with 1 mm mesh. After 48 h, we removed each snail from its experimental unit, collected the remaining plant material and dried the plants in a stove (60 °C for at least 72 h) before reweighing (DW$_{plant,end}$). Snails were frozen individually after each trial and later on dried to measure their dry shell mass, which allowed us to express consumption standardized for snail tissue mass (FW$_{snail,tissue}$). Following Elger & Barrat-Segretain (2004), we used the mean initial fresh-to-final dry mass ratio of the no-plant controls to calculate DW$_{plant,initial}$ from FW$_{plant,initial}$. Using this method, possible autogenic changes in plant mass in the control treatment are included in the calculation of the initial dry mass ratio of the fresh plants fed to the snails. In a previous experiment, no autogenic changes in plant fresh mass were detected at 20 °C in 24 h trials (Zhang *et al.* 2016).

The relative consumption rate (RCR) was calculated as:

$$\mathrm{DW}_{plant.initial} - \mathrm{DW}_{plant.final}/\mathrm{FW}_{snail.tissue}/\mathrm{time}_{days}.$$

This yielded several slightly negative mean RCRs (three plant species for *P. canaliculata* and seven species for *L. stagnalis*), likely because initial dry weights were calculated, which may have induced extra variation. As no consumption was observed in these trials, the mean consumption of plant species with negative RCRs was set to 0·01 mg g^{-1} day^{-1} (i.e. 0·2% of mean consumption rate) instead of 0 or negative numbers to ease data transformation.

PLANT TRAIT ANALYSES

We measured the following plant traits: dry matter content (DMC), carbon content, nitrogen content, phosphorus content and total phenolic content, because of their correlation to herbivore consumption rates of freshwater plants (Lodge 1991; Cronin *et al.* 2002; Dorenbosch & Bakker 2011). Phenolics are an ubiquitous class of chemical compounds in aquatic plants and are hypothesized to be a deterrent to herbivores (Lodge 1991). The evidence is predominantly correlational (Qiu & Kwong 2009; Dorenbosch & Bakker 2011), and sometimes no correlation is found Wong *et al.* (2010) and Cronin & Lodge (2003). Total phenolics measurements do not always capture anti-herbivore defences, because not all phenolics are active deterrents (Boettcher & Targett 1993; Gross & Bakker 2012), some stimulate herbivory (Rowell & Blinn 2003) and many are likely neutral. Chemical defences are best tested using feeding assays with plant extracts incorporated into an agar-based food matrix (methods described in Cronin *et al.* 2002; Morrison & Hay 2011). Yet, given the logistic constraints of testing extracts of all plant species, we resorted to the total phenolic content as a common currency (Agrawal & Weber 2015) across aquatic plant species, although we are aware of its limitations.

Non-apical parts of each plant species were collected, weighed and dried (60 °C until constant weight) and weighed again to calculate their DMC using five replicates (Elger & Willby 2003). We determined the total carbon, nitrogen, phosphorus and phenolic content of finely ground dried material (ball grinder; Retch MM301, Haan, Germany). To analyse the carbon and nitrogen content, we weighed in c. 1·5 mg of sample in tin cups for analysis on an organic elemental analyser (FLASH 2000; Thermo Scientific, Waltham, MA, USA). Total phosphorus content was determined by incinerating 1 mg of each sample at 500 °C for 30 min and digesting the remainder with 5 mL of 2·5% persulphate in an autoclave (30 min at 121 °C). These samples were then centrifuged (30 min at 2500 rpm) and the P content of their supernatant analysed on an Auto Analyzer (QuAAtro method Q-037-05; Seal Analytical, Fareham, UK).

For the analysis of total phenolic compounds (subsequently referred to as phenolics), 10 mg of plant material was extracted with 5 mL of 80% ethanol for 10 min at 80 °C before adding sodium dodecyl sulphate solution and FeCl$_3$ reagent (Mole & Waterman 1987a; Hagerman & Butler 1989; Smolders *et al.* 2000). The change in color due to reduction in Fe^{3+} to Fe^{2+} was measured at 510 nm on a spectrophotometer (Synergy HT Microplate Reader; BioTek, Winooski, VT, USA) against a tannic acid calibration curve (tannic acid ACS reagent 403040; Sigma-Aldrich, St. Louis, Missouri, USA). The phenolics content was expressed as mg tannic acid equivalents per gram plant dry weight. This method, based on metal complexation is less sensitive to non-phenolic oxidizing agents than Folin-Denis (Hagerman & Butler 1989), and these agents are likely present in varying concentrations in the selected plant species. The method quantifies tannins and other phenolic compounds that produce violet iron complexes, but not specifically biologically active compounds (Mole & Waterman 1987a, b; Bernays, Driver & Bilgener 1989) and it can fail to capture active compounds (Bernays, Driver & Bilgener 1989). These phenolics assays do not measure oxidized phenolics, which are important in plant-insect interactions (Appel 1993; Barbehenn & Constabel 2011), although their relevance for snails has not been tested.

DOCUMENTATION OF NATIVE RANGE AND EVOLUTIONARY NOVELTY

We documented the geographical origin of all species based on literature (Aiken 1981; Les & Mehrhoff 1999; Hussner 2012) and the USDA ARS GRIN database (http://www.ars-grin.gov) (Table 1). We classified plant-herbivore pairs as evolutionary novel to each other (Morrison & Hay 2011) if species occurred on different continents (North and South America, and Europe and Asia are considered as separate continents). Although species distributions are dynamic, we expected that this classification at least indicates whether plants and herbivores have encountered each other in the last 10 000 years. The invaded ranges were excluded from analysis as the resultant interactions are limited on an evolutionary scale.

To identify the relevance of biogeography, we assessed whether the native plant range mainly lies in frost-free (warm temperate, subtropical or tropical: referred to as tropical) or frost-prone (cold temperate: referred to as temperate) regions, see Table S1 (Morrison & Hay 2012). *Ceratophyllum demersum* and *Pontederia cordata* could not be classified according to biogeography given their broad latitudinal ranges. The latitudinal classification likely captures variation in the length of growing season and leaf longevity of plant species and the abundance and diversity of herbivores that plants are exposed to, which can yield differences in plant traits (Morrison & Hay 2012).

DATA ANALYSIS

Herbivore consumption rate was expressed as a fraction of the mean herbivore consumption rate for each herbivore averaged across all plants, to compare the relative plant consumption by both herbivores. We compared the consumption rate of *P. canaliculata* and *L. stagnalis* using Pearson correlation (correlations among traits in Table S2). We selected the plant trait best explaining herbivore consumption rate using stepwise multiple regression (forward and backward) from all single plant traits and their ratios (see Table S3) and used this trait in the subsequent analysis using linear models (see Appendix S2 for details on trait ratios and the stepwise selection procedure).

We constructed a linear model to test which parameters best related to the herbivore consumption rate. It included four fixed effects: the nitrogen-to-phenolics ratio, novelty to the herbivore, biogeography as a dichotomous classification: temperate

Table 1. Results of the linear model testing which factors best explain generalist consumption of native and novel plant species

Fixed effects	F	Numerator d.f.	P value
Herbivore species	1·00	1	0·32
Evolutionary novelty	0·25	1	0·62
Biogeography	0·21	1	0·30
Nitrogen-to-phenolics ratio	16·5	1	**<0·001**

	Denominator d.f.	R^2-adjusted
	70	0·23

Results of the linear model of the normalized relative consumption rate (square-root transformed) of plant species ($n = 38$, excluding *Ceratophyllum demersum* and *Pontederia cordata* which could not be classified according to biogeography). Insignificant interaction terms were dropped following a stepwise approach, at each step dropping the most insignificant interaction term. Bold value indicates statistical significance ($P < 0.01$).

or tropical, and herbivore species (Table S1). All possible interactions of fixed effects were added to the initial model. We then simplified the model by eliminating insignificant interaction terms. F tests were used to test significance of the fixed effects (Table 1).

In addition, we extended the model to test for and include possible phylogenetic constraints in plant consumption by herbivores (see Appendix S3 for details). Furthermore, we assessed the phylogenetic signal in plant traits and herbivore consumption rate using Blomberg's K (Kembel *et al.* 2010).

All data were analysed in R version 3.2.3 using the PICANTE, APE, NLME and CAR packages. Model assumptions were verified through residual analysis, e.g. for non-normality, heteroscedasticity, and when necessary to meet assumptions data were square-root transformed. T-tests assuming unequal variance were used if groups were heteroscedastic.

Results

Both herbivores showed a strong positively correlated consumption rate (Fig. 1a; Pearson's $r = 0.69$; $P < 0.001$), with the non-standardized mean consumption rate of *P. canaliculata* almost three times higher (7·8 mg g^{-1} day^{-1}) than the consumption rate of *L. stagnalis* (2·7 mg g^{-1} day^{-1}; $t_{df=72.4} = -4.34$; $P < 0.001$). In stepwise multiple regression, we found that the ratio between plant nitrogen and phenolics content best correlated with the consumption rates of both herbivores (Fig. 1b; regression analysis: *L. stagnalis*: $R^2 = 0.18$; $P = 0.004$, *P. canaliculata*: $R^2 = 0.26$; $P < 0.001$; Appendix Table S3). For *L. stagnalis*, the DMC also explained variation in consumption, but three times less than the nitrogen-to-phenolics ratio as indicated by their F values, which here are indicative of the relative variance explained (see Table S3; model with dry matter and nitrogen-to-phenolics ratio: $R^2 = 0.33$; $P < 0.001$). Plant consumption rates of both snails were positively, marginally significant, related to the plant nitrogen concentration (linear regression, *L. stagnalis*: $F_{1,38} = 4.30$; $P = 0.046$, *P. canaliculata*: $F_{1,38} = 3.67$; $P = 0.063$) and significantly negatively to the plant phenolic content (linear regression, *L. stagnalis*: $F_{1,38} = 4.7$; $P = 0.0036$, *P. canaliculata*: $F_{1,38} = 10.3$; $P = 0.0027$, see Fig. S1).

A consequence of the correlated consumption rates of both herbivores is that the herbivore species differed in their consumption rate of novel plants (Two-way ANOVA: evolutionary novelty x herbivore species $F_{d.f. = 1,76} = 11.3$, $P = 0.001$) as the Eurasian *L. stagnalis* consumed more of native European than novel plants (Fig. 2; One-way ANOVA: $F_{d.f. = 1,38} = 4.6$, $P = 0.039$), whereas the South American *P. canaliculata* consumed more of novel than native South American plants (Fig. 2; One-way ANOVA: $F_{d.f. = 1,38} = 7.5$; $P = 0.009$). In addition, consumption rates differed with geographical origin: both herbivores consumed more of high latitude than low latitude plants (Fig. 2; Two-way ANOVA: $F_{d.f. = 1,73} = 4.57$, $P = 0.036$). Importantly, differences in the herbivore consumption rate between plant origin and across latitude were mirrored by differences in plant traits (compare upper and lower panels in Fig. 2b). Plant species from the tropics had lower nitrogen-to-phenolics ratios than species from temperate regions
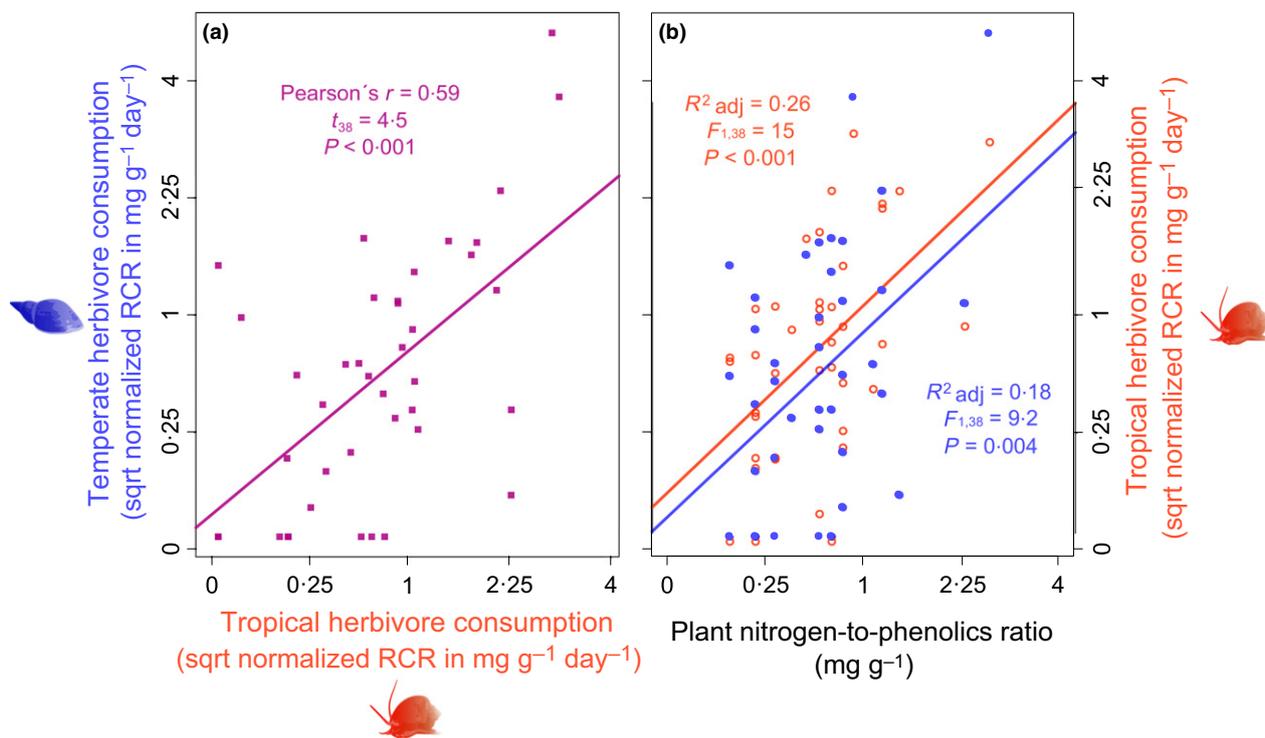
Fig. 1. Comparison of generalist herbivore consumption for plant species (a) and the relation to the plant's nitrogen–to-phenolics ratio (b). Scatterplot showing the normalized plant preference of the temperate herbivore (*Lymnaea stagnalis*) vs. the tropical herbivore (*Pomacea canaliculata)* (a). The normalized plant preference of both herbivores in relation to the plant nitrogen-to-phenolics ratio (b). The data points are square-root transformed, but the axis labels show non-transformed values.

(Fig. 2; *t*-test: $t_{d.f. = 33.4} = 2.24$, $P = 0.032$) and the plants novel to, and consumed more by *P. canaliculata*, had higher nitrogen-to-phenolics ratios (*t*-test: $t_{d.f. = 38} = -2.25$; $P = 0.030$). As a result, when including all three factors (geographical origin, novelty and the nitrogen-to-phenolics ratio) in a single model of herbivore consumption rates, only the nitrogen-to-phenolics ratio was significant (Table 1) and accounted best for the documented patterns in herbivore consumption rates across evolutionary novelty and biogeography. This model accounted for 23% of the variance in consumption rates (R^2-adjusted, Table 1), so 77% is left unaccounted for.

Of the 15 tropical species, four were novel to *P. canaliculata*, and of the 23 temperate species, four were novel to *L. stagnalis*. In these two subsets of tropical plants for the tropical herbivore and temperate plants for the temperate herbivore, plant novelty did not affect consumption rate, although it should be noted that the power of these analyses was low (*t*-tests: temperate *L. stagnalis*, $t_{22} = 1.22$, $P = 0.24$ and tropical *P. canaliculata*, $t_{14} = -0.94$, $P = 0.36$; Fig. S2). In contrast, for both the tropical and temperate subset of plants, the nitrogen-to-phenolics ratio was still positively correlated with consumption rates (linear regression, temperate *L. stagnalis*, $F_{1,22} = 7.81$, $P = 0.011$ and tropical *P. canaliculata*, $F_{1,14} = 4.95$, $P = 0.043$).

Patterns in the nitrogen-to-phenolics ratio, the plant trait that correlated best to consumption rates, showed no phylogenetic signal ($K = 0.059$; $P = 0.86$; if traits show a strong phylogenetic signal, K is close to 1). The consumption rate of *L. stagnalis* showed a small phylogenetic signal (Fig. S3; $K = 0.36$; $P = 0.009$) but not the consumption rate of *P. canaliculata* (Fig. S3; $K = 0.10$; $P = 0.56$). The results of the phylogenetic analyses were qualitatively identical to those of the linear models (Table 2): The nitrogen-to-phenolics ratio explained variance in the consumption rates for both herbivores, whereas evolutionary novelty and biogeography did not explain extra variance (Table 2).

Discussion

The feeding trials showed inconsistent results for evolutionary novelty: the South American *P. canaliculata* consumed more of novel than non-novel plants, whereas this was reversed for the Eurasian *L. stagnalis*. Our study thus finds support for both the biotic resistance hypothesis for *P. canaliculata* (Parker & Hay 2005; Parker, Burkepile & Hay 2006; Morrison & Hay 2011), and the enemy release hypothesis for *L. stagnalis* (Keane & Crawley 2002; Xiong *et al.* 2008). In this respect, our results mirror the contrasting results reported by other studies. However, contrary to most previous studies, we could explain this inconsistency by including plant traits, where differences in the nitrogen-to-phenolics ratio were in line with the higher generalist herbivore consumption rates on native or non-native plants, both across and within latitudinal ranges.
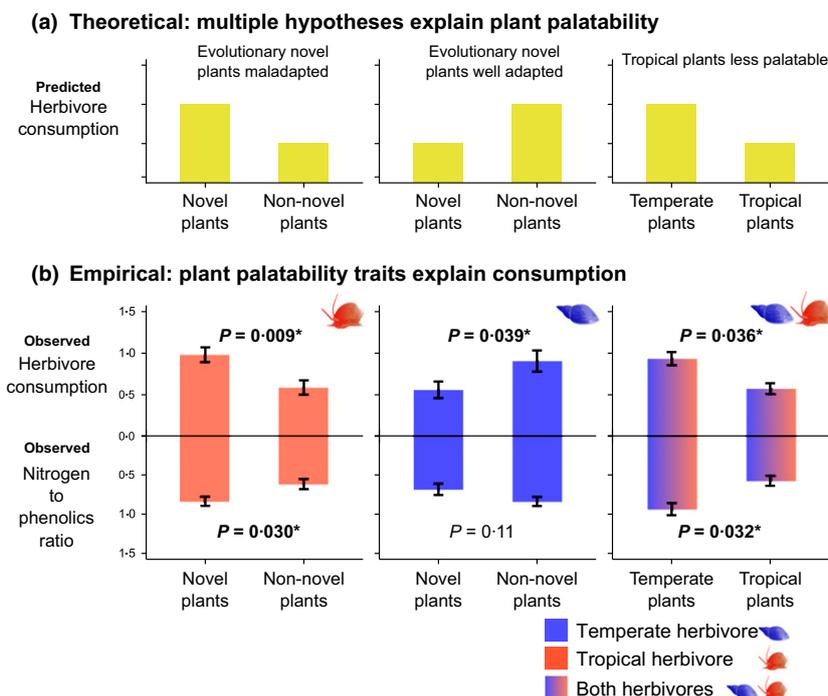
Fig. 2. Plant traits underpin novelty-based hypotheses that predict generalist consumption. (a) Predicted herbivore consumption rates of plant species based on three existing hypotheses (yellow bars): evolutionary novel plants are maladapted to generalist herbivores (Parker, Burkepile & Hay 2006), evolutionary novel plants are well adapted to generalist herbivores (Keane & Crawley 2002), and plants originating from low latitudes are less palatable than plants from higher latitudes (Schemske *et al.* 2009). (b) Observed normalized consumption (square root of mean ± SEM) for the Eurasian temperate (blue bars; *Lymnaea stagnalis*) and South American tropical freshwater snail (red bars; *Pomacea canaliculata*) or for both (blue-red bars). Mirroring consumption is the mean nitrogen-to-phenolics ratio of plant groups (square root of mean ± SEM; mg g^{-1}). Significant differences in two-sided t-tests in mean herbivore consumption or mean nitrogen-to-phenolics ratio are shown in bold and with an asterisk. Table 1 shows the output of a single statistical model to test all fixed effects simultaneously.

Table 2. Results of phylogenetic generalized least squares (PGLS)

Fixed effects	Likelihood ratio	Difference d.f.	P value
Lymnaea stagnalis			
Evolutionary novelty	3·25	1	0·072
Biogeography	0·94	1	0·33
Nitrogen-to-phenolics ratio	8·61	1	**0·003**
Pomacea canaliculata			
Evolutionary novelty	0·38	1	0·54
Biogeography	0·21	1	0·65
Nitrogen-to-phenolics ratio	8·33	1	**0·004**

Likelihood ratio tests were used to test fixed effects of PGLS. The likelihood ratio indicates the difference in likelihood of full and reduced models, difference d.f. denotes the difference in degrees of freedom. The consumption of both herbivore species was analysed in separate PGLS models. Bold value indicates statistical significance ($P < 0.01$).

Interestingly, despite the fact that herbivores came from different biogeographical regions, their consumption rates of the 40 tested aquatic plants were positively correlated. Both herbivores consumed more of plants that contained high levels of nitrogen and low levels of phenolics, resulting in an even stronger positive correlation between herbivore consumption rates and the plant nitrogen-to-phenolics ratio, reflecting the general principle that organism performance is governed by multiple, interacting traits (Cruz-Rivera & Hay 2003; Verberk, Van Noordwijk & Hildrew 2013). Hence we can confirm our first hypothesis that plant traits correlate with the consumption rates of generalist herbivores of the tested native and non-native plant species. In our study with *L. stagnalis* and *P. canaliculata*, the nitrogen-to-phenolics ratio was the plant trait that best predicted herbivore consumption rate, explaining 23% of the variance. This result is in line with studies on food preference in generalist herbivores, which commonly prefer plants high in protein or nitrogen, and low in chemical or structural components that may act as anti-herbivore defences (Mattson 1980; Lodge 1991; Cruz-Rivera & Hay 2003; Wong *et al.* 2010; Dorenbosch & Bakker 2011). However, whereas the positive relationship between nitrogen or protein content of plants and increased food quality for herbivores has often been demonstrated (Bakker & Nolet 2014 and references therein; Grutters, Gross & Bakker 2016), the role of plant defences is less clear. In particular the unit in which to express the strength of plant defences across unrelated plant species is uncertain. To express plant toughness we used dry matter concentration as a proxy (Elger & Willby 2003; Elger & Lemoine 2005; Burlakova *et al.* 2009), but other proxies including specific

leaf area and punching resistance have also been used. Also total phenolics may correlate with lignin and fibre content and thus link to plant toughness. Total phenolics is an imperfect proxy for chemical plant defences: in aquatic plants, several active deterrent single phenolic compounds have been identified (see Table S5 for an overview), but many phenolics are neutral (Bernays, Driver & Bilgener 1989; Boettcher & Targett 1993) and some are feeding stimulants (Bernays, Driver & Bilgener 1989; Rowell & Blinn 2003). Because total phenolics do not accurately measure active deterrent compounds, the amount of total phenolics correlates with low palatability in some studies (Boettcher & Targett 1993; Vergés *et al.* 2007), but not in others (Steinberg & van Altena 1992; Targett *et al.* 1995), which can be explained by the differing structures and function of the total phenolics involved (Bernays, Driver & Bilgener 1989). Nevertheless, the fact that the nitrogen-to-phenolics ratio explained more variance in consumption rates than novelty and latitude shows that involving plant traits has much potential in understanding patterns in consumption rates.

Plant phylogeny can explain differences in plant traits and consumption, but as expected for generalist herbivores, in our feeding trials we detected no strong phylogenetic signal in herbivore consumption rates or in the nitrogen-to-phenolics ratio. However, consistent with literature, certain plant species were generally consumed less. Many eudicots such as *Myriophyllum* spp., *Hydrocotyle* spp. and *Crassula helmsii* were hardly consumed, which matches previously conducted feeding assays (Parker & Hay 2005; Qiu & Kwong 2009; Wong *et al.* 2010; Morrison & Hay 2011). Most importantly, the nitrogen-to-phenolics ratio captured a portion of the variance in herbivore consumption rates irrespective of plant phylogeny.

Our results confirm the second hypothesis that an effect of evolutionary novelty (comprising both the enemy release hypothesis and its converse, the biotic resistance hypothesis) could in fact be due to consistent differences in plant traits between novel and non-novel plants. Indeed, whether a given herbivore consumed more (*P. canaliculata*) or less (*L. stagnalis*) of evolutionary novel plants than non-novel plants was found to be best correlated with the nitrogen-to-phenolics ratio, which was lower in tropical plants than in temperate plants. Hence, no effect of either biogeographical region or novelty was found after taking the plant's nitrogen-to-phenolics ratio into account (Table 1; Fig. 2).

We also found evidence for our third hypothesis that latitudinal differences in plant traits affect the outcome of whether feeding trials support the enemy release hypothesis or its converse, the biotic resistance hypothesis. In our study, both herbivore consumption rates and the plant nitrogen-to-phenolics ratio showed a significant latitudinal signature. Plant species from the tropics, i.e. low latitudes, were consumed less by both herbivores and had a lower nitrogen-to-phenolics ratio than plant species from temperate regions. These results match the

increasing palatability of plants with increasing latitude (Bolser & Hay 1996; Siska *et al.* 2002; Salgado & Pennings 2005; Schemske *et al.* 2009; Moles *et al.* 2011; Morrison & Hay 2012) and the currently debated patterns of increasing foliar nitrogen content with latitude (Siska *et al.* 2002; Reich & Oleksyn 2004; Borer *et al.* 2013) and decreasing plant defences with latitude (Bolser & Hay 1996; Pennings *et al.* 2007; Schemske *et al.* 2009; but see Moles *et al.* 2011). The snails that we tested thus responded similarly in their higher consumption of high latitude compared to low latitude plants as was previously reported for crayfish, crabs, sea urchins and other snail species (Bolser & Hay 1996; Siska *et al.* 2002; Morrison & Hay 2012). Our study furthermore shows that these latitudinal patterns in plant consumption are underpinned by plant traits related to consumption; both across temperate and tropical regions (Table 1, Fig. 2) and within each region (Fig. S2). The results also indicate that temperate herbivores have a reduced capacity to provide biotic resistance against non-native plants from low latitudes, assuming that feeding trials are generally predictive for impacts in plant communities (Lubchenco & Gaines 1981; Hay 1997; Parker & Hay 2005; Parker, Burkepile & Hay 2006; Alofs & Jackson 2014). This notion fits the general pattern of most invasions occurring towards higher latitudes (Guo *et al.* 2012), increasing tropicalization of temperate marine ecosystems (Vergés *et al.* 2014) and the stronger impact of temperate herbivores on native than non-native plants in field studies (Wood *et al.* 2016).

Conclusions

We show that plant traits determine whether generalist herbivores consume plants, irrespective of plant novelty. The plant nitrogen-to-phenolics ratio was positively correlated with herbivore consumption rates and increased with latitude. Hence, whether novel plants are consumed more than natives depends on their biogeographical origin, with biotic resistance likely being lower in temperate regions. Nevertheless, the trait used in this study could only explain 23% of the variance in consumption, so there is much to be gained in finding additional plant traits. Our results challenge the generality of the enemy release hypothesis and the biotic resistance hypothesis and hence their value in predicting plant success based on evolutionary novelty. Instead, to understand the potential for biotic resistance better, we propose that an integration of evolutionary novelty theory with plant traits and biogeography will increase the predictability of the consequences of plant migration beyond biogeographical barriers.

Authors' contributions

B.M.C.G., Y.O.A.R. and E.S.B. designed the experiment; B.M.C.G. and Y.O.A.R. performed the experiment and data analysis; B.M.C.G. wrote the first draft of the manuscript and all authors subsequently improved and finalized the manuscript.

Acknowledgements

Data accessibility

All data files are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.d4k51 (Grutters *et al.* 2017).

References

Agrawal, A.A. (2011) Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, **25**, 420–432.

Agrawal, A.A. & Weber, M.G. (2015) On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. *Ecology Letters*, **18**, 985–991.

Aiken, S.G. (1981) A conspectus of myriophyllum (haloragaceae) in North America. *Brittonia*, **33**, 57–69.

Alofs, K.M. & Jackson, D. (2014) Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology*, **95**, 3259–3270.

Appel, H.M. (1993) Phenolics in ecological interactions: the importance of oxidation. *Journal of Chemical Ecology*, **19**, 1521–1552.

Bakker, E.S. & Nolet, B.A. (2014) Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. *Oecologia*, **176**, 825–836.

Bakker, E.S., Wood, K.A., Pagès, J.F., Veen, G.C., Christianen, M.J., Santamaría, L., Nolet, B.A. & Hilt, S. (2016) Herbivory on freshwater and marine macrophytes: a review and perspective. *Aquatic Botany*, **135**, 18–36.

Barbehenn, R.V. & Constabel, C.P. (2011) Tannins in plant–herbivore interactions. *Phytochemistry*, **72**, 1551–1565.

Bernays, E.A., Driver, G.C. & Bilgener, M. (1989) Herbivores and plant tannins. *Advances in Ecological Research*, **19**, 263–302.

Boettcher, A.A. & Targett, N.M. (1993) Role of polyphenolic molecular size in reduction of assimilation efficiency in Xiphister mucosus. *Ecology*, **74**, 891–903.

Bolser, R.C. & Hay, M.E. (1996) Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. *Ecology*, **77**, 2269–2286.

Borer, E.T., Bracken, M.E., Seabloom, E.W. *et al.* (2013) Global biogeography of autotroph chemistry: is insolation a driving force? *Oikos*, **122**, 1121–1130.

Burlakova, L.E., Karatayev, A.Y., Padilla, D.K., Cartwright, L.D. & Hollas, D.N. (2009) Wetland restoration and invasive species: apple snail (*Pomacea insularum*) feeding on native and invasive aquatic plants. *Restoration Ecology*, **17**, 433–440.

Carlsson, N.O.L., Bronmark, C. & Hansson, L.A. (2004) Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology*, **85**, 1575–1580.

Cronin, G. & Lodge, D.M. (2003) Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia*, **137**, 32–41.

Cronin, G., Lodge, D.M., Hay, M.E., Miller, M., Hill, A.M., Horvath, T., Bolser, R.C., Lindquist, N. & Wahl, M. (2002) Crayfish feeding preferences for freshwater macrophytes: the influence of plant structure and chemistry. *Journal of Crustacean Biology*, **22**, 708–718.

Cruz-Rivera, E. & Hay, M.E. (2003) Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. *Ecological Monographs*, **73**, 483–506.

Dorenbosch, M. & Bakker, E.S. (2011) Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. *Freshwater Biology*, **56**, 1783–1797.

Dudgeon, D., Arthington, A.H., Gessner, M.O. *et al.* (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**, 163–182.

Elger, A. & Barrat-Segretain, M.-H. (2002) Use of the pond snail *Lymnaea stagnalis* (L.) in laboratory experiments for evaluating macrophyte palatability. *Archiv für Hydrobiologie*, **153**, 669–683.

Elger, A. & Barrat-Segretain, M.-H. (2004) Plant palatability can be inferred from a single-date feeding trial. *Functional Ecology*, **18**, 483–488.

Elger, A. & Lemoine, D. (2005) Determinants of macrophyte palatability to the pond snail *Lymnaea stagnalis*. *Freshwater Biology*, **50**, 86–95.

Elger, A. & Willby, N.J. (2003) Leaf dry matter content as an integrative expression of plant palatability: the case of freshwater macrophytes. *Functional Ecology*, **17**, 58–65.

Gross, E.M. & Bakker, E.S. (2012) The role of plant secondary metabolites in freshwater macrophyte-herbivore interactions: limited or unexplored chemical defences. *The Integrative Role of Plant Secondary Metabolites in Ecological Systems* (eds G.R. Iason, M. Dicke & S.E. Hartley), pp. 154–169. British Ecological Society/Cambridge University Press, Sussex, UK.

Grutters, B.M., Gross, E.M. & Bakker, E.S. (2016) Insect herbivory on native and exotic aquatic plants: phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia*, **778**, 209–220.

Grutters, B., Roijendijk, Y., Verberk, W. & Bakker, E. (2017) Data from: Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.d4k51.

Guo, Q., Sax, D.F., Qian, H. & Early, R. (2012) Latitudinal shifts of introduced species: possible causes and implications. *Biological Invasions*, **14**, 547–556.

Hagerman, A.E. & Butler, L.G. (1989) Choosing appropriate methods and standards for assaying tannin. *Journal of Chemical Ecology*, **15**, 1795–1810.

Hay, M.E. (1996) Marine chemical ecology: what's known and what's next? *Journal of Experimental Marine Biology and Ecology*, **200**, 103–134.

Hay, M. (1997) The ecology and evolution of seaweed-herbivore interactions on coral reefs. *Coral Reefs*, **16**, S67–S76.

Hussner, A. (2012) Alien aquatic plant species in European countries. *Weed Research*, **52**, 297–306.

Jeschke, J., Gómez Aparicio, L., Haider, S., Heger, T., Lortie, C., Pyšek, P. & Strayer, D. (2012) Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota*, **14**, 1–20.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164–170.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P. & Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.

Kimbro, D.L., Cheng, B.S. & Grosholz, E.D. (2013) Biotic resistance in marine environments. *Ecology Letters*, **16**, 821–833.

van Kleunen, M., Dawson, W., Essl, F. *et al.* (2015) Global exchange and accumulation of non-native plants. *Nature*, **525**, 100–103.

Les, D.H. & Mehrhoff, L.J. (1999) Introduction of nonindigenous aquatic vascular plants in southern New England: a historical perspective. *Biological Invasions*, **1**, 281–300.

Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, **7**, 975–989.

Lind, E.M. & Parker, J.D. (2010) Novel weapons testing: are invasive plants more chemically defended than native plants? *PLoS ONE*, **5**, e10429.

Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8**, 1535–1545.

Lodge, D.M. (1991) Herbivory on freshwater macrophytes. *Aquatic Botany*, **41**, 195–224.

Lodge, D.M., Cronin, G., vanDonk, E. & Froelich, A.J. (1998) Impact of herbivory on plant standing crop: comparisons among biomes, between vascular and nonvascular plants, and among freshwater herbivore taxa. *Structuring Role of Submerged Macrophytes in Lakes* (eds E. Jeppesen, M. Sondergaard & K. Christofferson), pp. 149–174. Springer-Verlag, New York, NY, USA.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. (2000) *100 of the World's Worst Invasive Alien Species: A Selection From the Global Invasive Species Database*. Invasive Species Specialist Group, Auckland, New Zealand.

Lubchenco, J. & Gaines, S.D. (1981) A unified approach to marine plant-herbivore interactions. I. Populations and communities. *Annual Review of Ecology and Systematics*, **12**, 405–437.

Mattson, W.J. Jr (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.

Mole, S. & Waterman, P. (1987a) A critical analysis of techniques for measuring tannins in ecological studies: I. Techniques for chemically defining tannins. *Oecologia*, **72**, 137–147.

Mole, S. & Waterman, P. (1987b) A critical analysis of techniques for measuring tannins in ecological studies: II. Techniques for biochemically defining tannins. *Oecologia*, **72**, 148–156.

Moles, A.T., Bonser, S.P., Poore, A.G.B., Wallis, I.R. & Foley, W.J. (2011) Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology*, **25**, 380–388.

Morrison, W.E. & Hay, M.E. (2011) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naive. *PLoS ONE*, **6**, e17227.

Morrison, W.E. & Hay, M.E. (2012) Are lower-latitude plants better defended? Palatability of freshwater macrophytes. *Ecology*, **93**, 65–74.

Parker, J.D., Burkepile, D.E. & Hay, M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, **311**, 1459–1461.

Parker, J.D. & Hay, M.E. (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters*, **8**, 959–967.

Parker, J.D., Burkepile, D.E., Lajeunesse, M.J. & Lind, E.M. (2012) Phylogenetic isolation increases plant success despite increasing susceptibility to generalist herbivores. *Diversity and Distributions*, **18**, 1–9.

Pennings, S.C., Zimmer, M., Dias, N. *et al.* (2007) Latitudinal variation in plant–herbivore interactions in European salt marshes. *Oikos*, **116**, 543–549.

Qiu, J.-W. & Kwong, K.-L. (2009) Effects of macrophytes on feeding and life-history traits of the invasive apple snail *Pomacea canaliculata*. *Freshwater Biology*, **54**, 1720–1730.

Reich, P.B. & Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 11001–11006.

Rowell, K. & Blinn, D.W. (2003) Herbivory on a chemically defended plant as a predation deterrent in Hyalella azteca. *Freshwater Biology*, **48**, 247–254.

Salgado, C.S. & Pennings, S.C. (2005) Latitudinal variation in palatability of salt-marsh plants: are differences constitutive? *Ecology*, **86**, 1571–1579.

Schemske, D.W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M. & Roy, K. (2009) Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, **40**, 245–269.

Simberloff, D. (2011) How common are invasion-induced ecosystem impacts? *Biological Invasions*, **13**, 1255–1268.

Siska, E.L., Pennings, S.C., Buck, T.L. & Hanisak, M.D. (2002) Latitudinal variation in palatability of salt-marsh plants: which traits are responsible? *Ecology*, **83**, 3369–3381.

Smolders, A.J.P., Vergeer, L.H.T., van der Velde, G. & Roelofs, J.G.M. (2000) Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: why do they differ? *Oikos*, **91**, 307–310.

Steinberg, P.D. & van Altena, I. (1992) Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australasia. *Ecological Monographs*, **62**, 189–222.

Targett, N.M., Boettcher, A.A., Targett, T.E. & Vrolijk, N.H. (1995) Tropical marine herbivore assimilation of phenolic-rich plants. *Oecologia*, **103**, 170–179.

Veen, G., Sarneel, J.M., Ravensbergen, L., Huig, N., Paassen, J., Rip, W. & Bakker, E.S. (2013) Aquatic grazers reduce the establishment and growth of riparian plants along an environmental gradient. *Freshwater Biology*, **58**, 1794–1803.

Verberk, W., Van Noordwijk, C. & Hildrew, A. (2013) Delivering on a promise: integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science*, **32**, 531–547.

Vergés, A., Becerro, M., Alcoverro, T. & Romero, J. (2007) Experimental evidence of chemical deterrence against multiple herbivores in the seagrass Posidonia oceanica. *Marine Ecology Progress Series*, **343**, 107–114.

Vergés, A., Steinberg, P.D., Hay, M.E. *et al.* (2014) The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20140846.

Verhoeven, K.J.F., Biere, A., Harvey, J.A. & Van Der Putten, W.H. (2009) Plant invaders and their novel natural enemies: who is naïve? *Ecology Letters*, **12**, 107–117.

Vilà, M., Basnou, C., Pyšek, P. *et al.* (2009) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, **8**, 135–144.

van der Wal, J.E., Dorenbosch, M., Immers, A.K., Vidal Forteza, C., Geurts, J.J., Peeters, E., Koese, B. & Bakker, E.S. (2013) Invasive crayfish threaten the development of submerged macrophytes in lake restoration. *PLoS ONE*, **8**, e78579.

Wong, P.K., Liang, Y., Liu, N.Y. & Qiu, J.-W. (2010) Palatability of macrophytes to the invasive freshwater snail *Pomacea canaliculata*: differential effects of multiple plant traits. *Freshwater Biology*, **55**, 2023–2031.

Wood, K., O'Hare, M., McDonald, C., Searle, K., Daunt, F. & Stillman, R. (2016) Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews*. doi: 10.1111/brv.12272

Xiong, W., Yu, D., Wang, Q., Liu, C.H. & Wang, L.G. (2008) A snail prefers native over exotic freshwater plants: implications for the enemy release hypotheses. *Freshwater Biology*, **53**, 2256–2263.

Zhang, P., Blonk, B.A., van den Berg, R.F. & Bakker, E.S. (2016) The effect of temperature on herbivory by the omnivorous ectotherm snail *Lymnaea stagnalis*. *Hydrobiologia*, doi: 10.1007/s10750-0016-12891-10757.

Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Details on no-choice feeding trials.

Appendix S2. Details on the use of ratios and selecting the best predictor trait.

Appendix S3. Details on phylogenetic analyses.

Table S1. Plant species metadata.

Table S2. Correlation matrix of plant chemical traits.

Table S3. Multiple stepwise regression of plant traits on herbivore consumption.

Table S4. Significance tests of mean plant trait composition.

Fig. S1. Herbivore consumption vs. nitrogen and vs. phenolics.

Fig. S2. Novelty and plant trait tests within subsets of temperate and tropical plants.

Fig. S3. Herbivore consumption shown in relation to plant phylogeny.