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Preference, performance, and impact of the water-lily aphid on multiple species of duckweed

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Abstract. 1. The role of herbivores in driving the structure of freshwater macrophyte communities remains poorly understood in comparison with terrestrial ecosystems. For instance, although duckweed (subfamily Lemnoideae) are globally distributed, can be locally highly abundant and ecologically dominant, and are of growing economic importance, their interactions with herbivores remain understudied.

2. To address how herbivores may impact duckweed species composition, we here experimentally quantify the preference and performance of a common duckweed herbivore, the water-lily aphid (*Rhopalosiphum nymphaeae*) on four widespread duckweed species.

3. Our two-way choice experiments reveal that aphids display a preference for *Spirodela polyrhiza* > *Landoltia punctata* = *Lemna minor* >> *Wolffia brasiliensis*. These results are rarely influenced by natal host species.

4. By evaluating the growth of aphid populations on each duckweed species, we find that preference may be adaptive in certain ecological conditions.

5. Quantifying the population growth rate of duckweed growing in the presence and absence of aphids revealed differential tolerance of herbivory across duckweed species.

6. This study shows that aphids, through preferential feeding and significant differential effects on duckweed growth, can have a significant impact on duckweed population dynamics and potentially community composition.

Key words. aquatic herbivory, *Lemnaceae*, plant-herbivore interactions, preference-performance hypothesis, resistance-tolerance tradeoff.

Introduction

Herbivory in aquatic systems

The consumption of plants by herbivores fundamentally structures ecosystems (Schmitz, 2008) and impacts agricultural production (Holland *et al.*, 1992; Hidding *et al.*, 2016). Although there have been extensive studies quantifying herbivory in terrestrial plants (summarised in Turcotte *et al.*, 2014a; Turcotte *et al.*, 2014b), herbivory in aquatic ecosystems has historically been dismissed as having little to no effect in regulating vascular plant abundance. This view began to change with Lodge's (1991) review, which inspired numerous studies showing that herbivores can significantly impact macrophyte biomass resulting in community and ecosystem effects (Jacobsen & Sand-Jensen, 1992; Bolser & Hay, 1998; Carlsson *et al.*, 2004; Reeves & Lorch, 2012; Bakker *et al.*, 2016; Wood *et al.*, 2017; O'Hare *et al.*, 2018).

Interactions between aquatic plants and their herbivores may differ from terrestrial counterparts (Lodge *et al.*, 1998). First, primary production and mass-specific herbivory rates are greater in aquatic compared to terrestrial ecosystems (Cyr and Pace, 1993). Biomass removal by herbivores in marine and freshwater ecosystems is considerably higher (40–48%) than in terrestrial ecosystems (4–8%; Turcotte *et al.*, 2014a; Bakker *et al.*, 2016; Wood *et al.*, 2017). Second, aquatic macrophytes have diverse growth forms, including submerged, emergent, and floating. Herbivore access to some forms could be limited without aquatic life-stages or adaptations that allow feeding underwater, such as water beetles that create an air bubble (Gaevskaya, 1969). Third, certain floating freshwater macrophytes can reproduce clonally within less than 1 week, much faster than most of their herbivores. This may have important implications for their ability to resist and/or tolerate

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herbivory. Although the extent of research on freshwater herbivory is growing, experimental quantification of the effects of macrophyte-herbivore interactions remains undertested (O'Hare *et al.*, 2018).

Herbivore preference and performance and plant tolerance

An effective way of gaining insight into plant-herbivore interactions is by testing the preference-performance hypothesis, which states that herbivores preferentially oviposit on host species that result in higher offspring performance (Gripenberg *et al.*, 2010). Yet, preference is often stronger for hosts that result in lower offspring performance. This may still be adaptive if the best host species is less abundant (optimal foraging) or if a poorer quality host plant has less predators (Gripenberg *et al.*, 2010; Clark *et al.*, 2011). Maladaptive host choice may be more common in polyphagous than oligophagous insects due to limited insect neural capacity when faced with many concurrent choices. Proximate mechanisms of preference include various host plant traits. For instance, herbivores may prefer larger vigorous plants, which may produce stronger visual or olfactory feeding cues or provide more resource for offspring (Price, 1991; Cornelissen *et al.*, 2008). In addition, an adult insect's preference may be biased towards hosts on which they developed as larvae or early adults (natal effect), or the host used by their mother (maternal effect) (Barron, 2001; Cahenzli & Erhardt, 2013; Thöming *et al.*, 2013).

While there have been many studies supporting the preference-performance hypotheses in terrestrial systems (Gripenberg *et al.*, 2010; Clark *et al.*, 2011), studies in freshwater systems remain less common, limiting our understanding of the generality of the hypothesis. Studies using emergent macrophytes find support for the preference-performance hypothesis (Dorn *et al.*, 2001; Solarz & Newman, 2001; Ding & Blossey, 2009), and in certain cases, preference is mediated by the natal host (Solarz and Newman, 2001).

Herbivore driven changes in plant community composition can be driven by herbivore preference and host resistance, which is the inverse of herbivore performance. An additional key determinant is whether plant species vary in their tolerance of herbivore damage (Carlsson & Lacoursierre, 2005; Kempel *et al.*, 2015). Moreover, there could be trade-offs between resistance and tolerance (Agrawal, 2007). Interspecific variation in tolerance has been shown in some freshwater ecosystems (Hidding *et al.*, 2010).

Duckweed herbivory

A prominent group of floating freshwater macrophytes that has received little empirical attention is duckweed (Landolt, 1986), which are among the fastest growing and most productive higher plants (Ziegler *et al.*, 2015; Laird & Barks, 2018). This subfamily of 37 species are found in lentic and slow-moving freshwater systems worldwide (Landolt, 1986). Due to their high reproductive rate, they can quickly blanket entire water bodies, causing wide ecological impacts (Scheffer *et al.*, 2003; Tezanos Pinto & O'Farrell, 2014; Driscoll *et al.*, 2016). Their rapid growth makes them attractive for applications such as bioremediation, agricultural feed, and biofuel production (Cui & Cheng, 2015; Ziegler *et al.*, 2015). They are also an emerging experimental evolutionary-ecology model system (Scheffer *et al.*, 2003; Laird & Barks, 2018; Armitage & Jones, 2019; Hart *et al.*, 2019; Xu *et al.*, 2019). Though duckweed is economically and ecologically important, their interactions with enemies remain poorly quantified.

Beyond descriptive studies of herbivores associated with duckweed (Scotland, 1940), experimental studies of duckweed herbivory remain rare (Bjorndal & Bolten, 1993). It has been demonstrated that *Lemna minor* is preferred over other species of duckweed and floating aquatic macrophytes by the golden apple snail (*Pomacea canaliculata*; Carlsson & Lacoursierre, 2005) and the leaf-mining duckweed shore fly (*Lemnaphila scotlandae*; Mansor and Buckingham, 1989). Moreover, studies on the China-mark moth larvae show that they feed without preference on *L. minor* and the locally invasive *Lemna minuta* (Mariani *et al.*, 2020), and their grazing pressure on duckweed decrease with increasing temperature (Heide *et al.*, 2006).

Interactions between herbivores and rapidly clonally reproducing taxa such as duckweed may differ from those with other macrophytes or terrestrial plants. We hypothesise that preference among duckweed species may be weaker than in other macrophyte growth forms or ecosystems because there is relatively little morphological differentiation among species. A duckweed individual's shoot is a single frond (similar in appearance to a leaf) and they have simple roots if any and few physical defences (Landolt, 1986). On the other hand, preference and host performance may differ strongly because of known differences in total phenolic content (Smolders *et al.*, 2000). The rapid generation times of duckweed may allow greater herbivory tolerance through fast population recovery due to density-dependent population growth (Hart *et al.*, 2019). Tolerance may also differ among duckweed species due to known differences in growth rates as well as body size, which may lower the per capita impact of herbivory.

To provide insight into freshwater herbivore interactions, we here test herbivory by a common generalist herbivore, the water-lily aphid (*Ropaloshiphum nymphaeae)* on four species of duckweed. Research on this herbivore remains limited despite its widespread abundance (Storey, 2007). We conducted three separate experiments in growth chambers to quantify (1) aphid preference and the impact of the natal host using two-way choice trials, (2) aphid performance by quantifying population growth on each duckweed species, and finally (3) duckweed tolerance by quantifying reductions in duckweed population growth rate.

Materials and methods

Study system

The water-lily aphid is a globally distributed herbivore known to feed on 12 genera of aquatic plants and numerous terrestrial plants (Center *et al.*, 2002). It has been proposed as a biological control of aquatic weeds (Oraze & Grigarick, 1992). These aphids reproduce parthenogenetically and through live birth on aquatic macrophytes as their secondary hosts (Hance

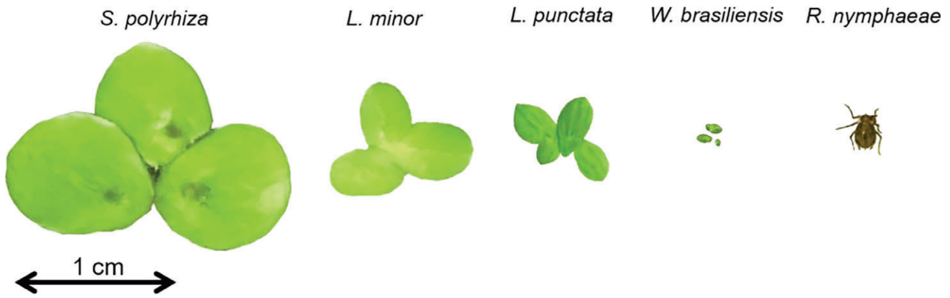

Fig 1. Top-down view of the relative size of four species of duckweed. Each frond (leaf-like structure) is an individual that grows in clusters before separating. All fronds within a cluster are of the same genotype. In addition, we present the water-lily aphid (*Rhopalosiphum nymphaeae)*. [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 1994). In Western Pennsylvania, USA, we have observed aphids feeding on various duckweed species in many locations. Given that duckweed communities are often composed of multiple genera (Pers. Obs.) and that aphids can easily walk on the water surface implies that they may have the ability to select their hosts.

Monospecific colonies of four duckweed species were established in 2017 from a single individual (i.e., a frond, Fig. 1). *Lemna minor*, *Spirodela polyrhiza*, and *Wolffia brasiliensis* were collected from Twin Lakes Park Westmoreland County, Pennsylvania, USA (40.323, −79.472). *Landoltia punctata* was collected from Panhandle Trail, Allegheny County, PA, USA (40.394, −80.136). Colonies were maintained in laboratory conditions, free of herbivores, at room temperature. Preceding experiments, they were kept under 24-h lighting on 50% concentration growth media (Kuehdorf *et al.* 2014).

We established aphid colonies in September 2017 from a single aphid individual reproducing clonally collected from a duckweed community composed of a mixture of *L. minor*, *S. polyrhiza*, *and W. brasiliensis* from Twin Lakes Park. The initial colony was split and grown on three species of duckweed to account for maternal and natal experience (henceforth referred to as "natal host") 5 weeks before experiments. We could not test the natal impact of *W. brasiliensis* because the aphids could not survive on this host alone. These colonies were kept in a growth chamber at 23.5 °C, 50% humidity, 50 μmol/m^2/s light, and 16:8 light/dark cycle.

Aphid preference

To quantify aphid preference, we conducted two-way choice trials consisting of every possible combination of the four duckweed species. To avoid confounding plant size differences among species (Fig. 1), we used approximately equal surface area instead of equal abundances. To do so, we placed duckweed in six-well plates, with each well being 7 cm^2, so there was a single dense but not overlapping layer of duckweed floating on the surface. This represented approximately 38, 62, 100, and 841 individuals of *S. polyrhiza*, *L. minor*, *L. punctata*, and *W. brasiliensis*, respectively. The two species in each trial were then transferred into a 59.1 ml jar with an area of 22.82 cm^2 filled with 40 ml of 25% concentration growth media and mixed to allow the aphid in the trial equal access to both species.

Each choice trial was conducted with a single third instar aphid. Aphids in their third instar were used because aphids are relatively immobile when younger and thus limit their ability to select host species. The aphid was placed onto a small (0.5 cm^2) floating plastic platform placed in the middle of the duckweed mat, as to not bias their initial choices. We factorially manipulated the current host choice pair and aphid natal host. Each combination was replicated 10 times for a total of 180 trials. Trials were conducted under 20 μmol m^{-2} s^{-1} of 16:8 lighting at room temperature. The experimental jar position was block randomised at the beginning of the experiment. Each aphid individual and duckweed frond were tested only once.

Preference was determined by observing if the aphid's stylet was inserted into the duckweed. If the aphid was not feeding no choice was recorded. To account for changes in aphid preference we recorded choice at 5 min, 10 min, 1 h, 4 h, 24 h, and 4 days. If the aphid died or crawled out of the jar, no choice was recorded.

Generalised linear mixed models were fit to each pair of species separately using the R package "lme4" (Bates *et al.*, 2015). The analysis used a logit transformation on the binomial response variable (host preference). First, we fit a model with natal host and time as fixed factors, and experimental jar as a random effect to account for repeated measures. When the time was non-significant, which was the case in all but two species pairs (see results), we removed it from the model. To test the importance of natal host, we compared models with natal host specified or not using AIC in the "bbmle" package (Bolker & R Development Team, 2017). When natal host improved model fit, analyses were run on the trial data separated by the natal host.

Aphid performance

We tested aphid performance on three species of duckweed (*L. punctata*, *L. minor*, and *S. polyrhiza*). Seven cm^2 area of a single species of duckweed was placed into a 236.6 ml jar with an area of 43.7 cm^2 filled with 175 ml of 50% concentration growth media. It was replenished with 30 ml of 25% concentration media every other week. In each jar, we placed five third instar aphids. We began with a low ratio of aphids to duckweed to ensure aphids were not limited by hosts, and thus the only variable determining their performance was the species of duckweed. We tested the importance of natal host by having separate treatments for aphids grown on each species of duckweed. Each treatment was replicated eight times and conducted in growth chambers at 23.5 °C, 50% humidity, and 16:8 lighting. Jars were covered with fine mesh. Jar position was block randomised by replicate and re-randomised weekly.

We quantified aphid performance at the population level by measuring population size twice per week over approximately four generations (42 days). Under experimental conditions, aphid generation time (birth to first reproduction) is approximately 10.2 days (Hance *et al.*, 1994). Using multigenerational population dynamics integrates various unmeasured changes in individual fitness proxies (i.e., survival, reproductive timing, and fecundity) and can reveal density-dependent growth. Using these time-series, we parameterised exponential and logistic population growth models using the R package "nlme" (Pinheiro *et al.*, 2018). We compared their fits using AIC values and likelihood ratio tests (LRT) with the R package "bbmle" (Bolker & R Development Team, 2017). Current duckweed host species, natal host, and their interaction were included as fixed effects, and the individual experimental jar was a random effect. We included an autoregressive correlation error structure to account for repeated measures. Natal host and its interaction were tested by comparing models with and without their inclusion using LRTs. Then we tested for differences in both per capita aphid intrinsic growth rate (r) and carrying capacity (K) among current host species by comparing models that allowed these parameters to vary or not among treatments using AIC and LRTs.

Duckweed tolerance

We tested the tolerance of three species of duckweed (*L. punctata*, *L. minor*, and *S. polyrhiza*) to aphid herbivory by quantifying duckweed population growth in the presence and absence of aphids over multiple generations. Unlike the performance experiment, we used a standard initial abundance of duckweed. Ten individuals of a single species of duckweed were placed into a 236.6 ml jar filled with 175 ml of 25% concentration growth media and replenished with 30 ml of 25% concentration media every other week. We added five third instar aphids in the treatment with aphids. Control treatments received no aphids. All aphids were from a colony grown on *S. polyrhiza*. Each treatment was replicated eight times, and the experiment was conducted in growth chambers at 23.5 °C, 50% humidity, 16:8 lighting, and were covered with fine mesh. The experimental jar position was block randomised by replication at the beginning of the experiment and weekly thereafter.

Duckweed performance was measured as population size and population dry weight measured after 32 days of growth or approximately five generations. Under experimental conditions duckweed generation time (birth to first reproduction) is under one week. Aphid impact on each species of duckweed was tested with linear models using the R package "lme4" on abundance data and ANOVA models on dry biomass data (Bates *et al.*, 2015). Fixed effects in models were aphid presence, duckweed species, and their interaction. Tolerance of each duckweed species was calculated as final abundance or biomass of duckweed in the treatment with aphid herbivory divided by final abundance or biomass in the treatment without aphid herbivory (Strauss & Agrawal, 1999). Replicates were randomly paired to calculate tolerance values.

Results

Aphid preference

Our results showed that aphids have a preference for specific duckweed species (Fig. 2) that were highly consistent over four days (Fig. S1). The only choice trials in which time was a significant predictor were *S. polyrhiza* versus *W. brasiliensis* and *L. minor* versus *W. brasiliensis*. This is because *W. brasiliensis* was only rarely selected in the early timepoints and never beyond 24 h. Thus, time was removed as a fixed factor from these analyses. Natal hosts had a limited impact on preference and will be discussed when it improved model fit.

Aphids preferred *S. polyrhiza* over *L. punctata* and *L. minor* (which are preferred equally) and never preferred *W. brasiliensis* (Fig. 2). *Spirodela polyrhiza* was preferred over all other species (Table 1). Natal hosts improved model fit in the *S. polyrhiza versus L. punctata* trial (model comparison, $\Delta\text{AIC} = 3.6$). When analysing natal host separately, aphids reared on *S. polyrhiza* significantly preferred it over *L. punctata* (Fig. 2, 96% of trials, $P < 0.0001$). Aphid preference for *S. polyrhiza* was non-significant when the natal host was *L. punctata* (74%, $P = 0.0654$) or *L. minor* (63%, $P = 0.36$). In the *L. punctata* versus *L. minor* trial, aphids showed no significant preference between the species ($P = 0.407$). Although the model with natal host identity fit better (model comparison, $\Delta\text{AIC} = 3.4$), no specific natal trial was significant on its own (Table 1).

Aphid performance

Aphid performance depends on the duckweed species identity (Fig. 3). The logistic growth models fit aphid population dynamic data better than the exponential models (model comparison, $\Delta\text{AIC} = 1043.1$; LRT, $P < 0.0001$). Natal host and its interaction with the current host did not significantly impact aphid performance on any species (LRT, $P < 0.0001$). Model comparisons showed intrinsic per capita growth rate (model comparison, $\Delta\text{AIC} = 88.4$, LRT, $P < 0.0001$) and carrying capacity ($\Delta\text{AIC} = 99.3$, LRT, $P < 0.0001$) to be significantly different among host species. Aphids reached the highest abundance at the end of the experiment on *L. punctata*. However, the models show that aphids have the highest intrinsic per capita growth rate on *S. polyrhiza* and the highest potential carrying capacity on *L. minor* (Fig. 3 and Table 2).

Duckweed tolerance

All duckweed species were severely negatively affected by the presence of aphids but to different extents (Fig. 4). Duckweed species, aphid presence, and their interaction significantly influenced duckweed abundance and biomass (ANOVA, $P < 0.001$).

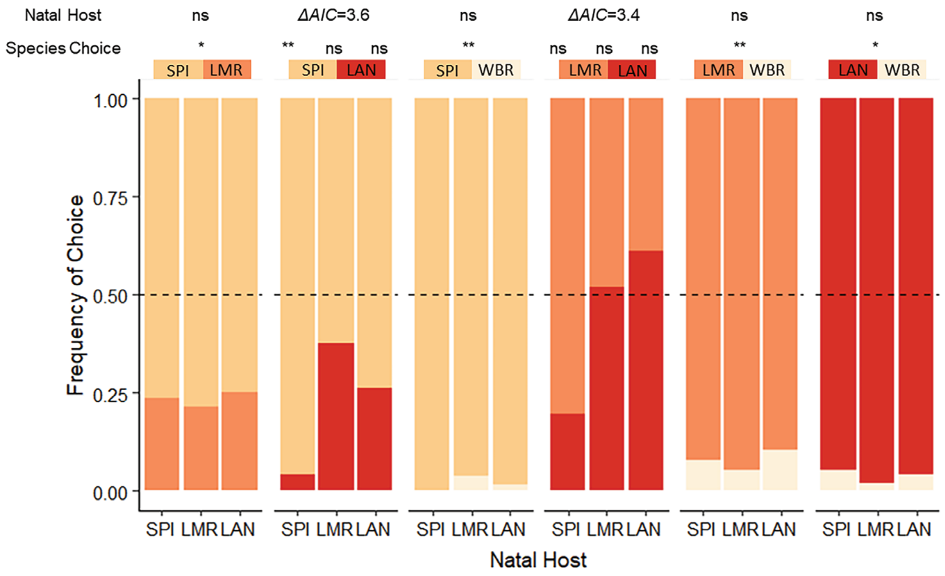

Fig 2. Preference of aphids among four species of duckweed (SPI: *Spirodela polyrhiza*, LMR: *Lemna minor*, LAN: *Landoltia punctata*, WBR: *Wolffia brasiliensis*) in two-way choice trials averaged across six-time points (see Fig. S1 for time-series). When a model with natal host had the best fit, analyses were conducted using natal hosts separately. The statistical analyses (shown in the table above bars) test the null hypothesis of equal preference, denoted by the dotted line at 0.50. Significant preference for one species over another is denoted $*P<0.05$ and $**P<0.0001$. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. GLMM results of preference of water-lily aphids between four species of duckweed in two-way choice trials

	Preference				Preference by natal host				
Choice trial	Species preferred	*n*	*Z*	*P*	Natal host	Species preferred	*n*	*Z*	*P*
S. polyrhiza v. L. minor	*S. polyrhiza*	175	2.086	0.037	Did not improve model fit				
S. polyrhiza v. L. punctata	*S. polyrhiza*	163	3.24	0.0012	*S. polyrhiza*	*S. polyrhiza*	50	4.404	<0.0001
					L. minor	Neither	56	0.912	0.362
					L. punctata	Neither	57	1.843	0.0654
S. polyrhiza v. W. brasiliensis	*S. polyrhiza*	159	−6.779	<0.0001	Did not improve model fit				
L. punctata v. L. minor	Neither	157	0.83	0.407	*S. polyrhiza*	Neither	56	1.239	0.215
					L. minor	Neither	52	−0.105	0.917
					L. punctata	Neither	49	−0.912	0.362
L. minor v. W. brasiliensis	*L. minor*	157	−4.064	<0.0001	Did not improve model fit				
L. punctata v. W. brasiliensis	*L. punctata*	156	2.086	0.037	Did not improve model fit				

Note: Natal and maternal effects were tested through pre-experimental rearing on three plant hosts referred to as a natal host. When natal host improved model fit, analyses were run on the trial data separated by the natal host. The sample size summed across all time points is represented as '*n*', but models accounted for repeated measures.

To better visualise and interpret the interaction, we calculated duckweed tolerance to herbivory (Fig. 4). *Spirodela polyrhiza*'s performance was the least impacted by aphids, followed by *L. minor* and *L. punctata*. *S. polyrhiza* maintained 64.5% and 42.2% abundance and biomass, respectively, compared to the no-aphid controls. *L. minor* maintained 49.3% abundance and 29.6% biomass. *L. punctata* was affected the most with a tolerance of 29.1% and 29% in abundance and biomass, respectively.

Discussion

Our results show that water-lily aphids have clear preferences for certain duckweed species. We additionally find that the aphid population growth rate is maximised on one species but reaches higher carrying capacity on another. Finally, duckweed species vary significantly in their tolerance to aphid damage. We here discuss these results, possible mechanisms, and implications for macrophyte communities.

Aphid preference

Many aphid species show a strong preference for plant species based on visual cues such as colour (Archetti & Leather, 2005), olfactory cues (Hori, 1999), gustatory cues (Powell *et al.*, 2006), and natal/maternal experience (Barro *et al.*, 1995; Nikolakakis *et al.* 2003). Similarly, we find that water-lily aphids have a

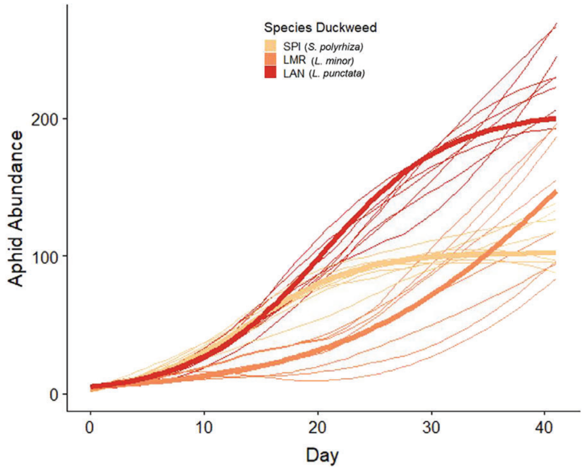

Fig 3. Performance of water-lily aphids on three species of duckweed over 42 days. Each thin line is one of eight replicates illustrated with a spline fit. The bold lines represent the best logistic model fits. [Colour figure can be viewed at wileyonlinelibrary.com]

strong preference for different co-occurring duckweed species. They prefer *S. polyrhiza* over *L. punctata* and *L. minor* (which are preferred equally) and strongly disfavour *W. brasiliensis*. Our preference ranking is similar to Storey (2007), except that they found *L. punctata* was preferred over *L. minor*, and *W. brasiliensis* was not tested. Although Solarz and Newman (2001) showed an increased preference for a natal host in aquatic herbivores, our results suggest that natal and/or maternal experience plays a minimal role.

Our study was not meant to identify the proximate mechanism of preference, but one corollary is plant size, which approximately matches preference (Fig. 1). This is consistent with the hypothesis that herbivore preference is dependent on plant size or vigour (Price, 1991; Cornelissen *et al.*, 2008). Here, we control for that possibility at the population level, but aphids may still prefer larger individual plants. Other preference cues are possible as is suggested by Storey's (2007) slightly different results. Phloem feeding aphids may prefer species with greater phloem volume or vasculature, which is also correlated with frond size (Segovia & Brown, 1978). In addition to morphological traits, preference may be driven by differences in nutrient composition and secondary chemicals. Appenroth *et al.* (2018) found differences in nutrient content in the duckweed genus *Wolffia*, but differences among species evaluated here remain unknown. In terms of chemical defences, Smolders *et al.* (2000) found that *S. polyrhiza* had four times higher total phenolic concentration than *L. minor*. *Landoltia punctata* and *W. brasiliensis* were not measured in that study. Given that certain phenolic compounds can deter aphids (Zucker, 1982; Leszczyński *et al.*, 1985), these results do not help explain the preference for *S. polyrhiza*. Further research into secondary chemicals especially in the salicylic acid pathway may help identify the drivers of aphid preference (Cao *et al.*, 2016). Thus, aphids preferentially feed on certain duckweed species suggesting they could impact species composition. This preference may also be adaptive.

Aphid performance

Similarly to preference, variation in aphid performance on different species of host plants can be caused by variation in the amino acid composition of phloem sap (Sandström & Pettersson, 1994), presence of secondary chemicals, and local adaptation to hosts (Nikolakakis *et al.*, 2003). We found that aphids perform differently on each species of duckweed and that natal and maternal experience have no discernable effect on performance. Which host maximises aphid performance depends on the measure of performance. Intrinsic per capita growth rate (r) was highest on *S. polyrhiza*, which could be due to this species having greater vasculature and large frond size that can support multiple aphids. In contrast, aphids have the lowest intrinsic per capita growth on *L. minor* but have a much higher carrying capacity (about three times that of *S. polyrhiza*). Whether this difference in carrying capacity is a consequence of decreasing the health of individual *S. polyrhiza* or due to differences in host plant population size over time remains untested. How these results compare to other macrophyte herbivore data is unknown because most studies quantify herbivore performance at a single time point.

Implications for the performance-preference hypothesis

Whether our results support the preference-performance hypothesis is contingent on the metric of performance. Many studies on the preference-performance hypothesis measure insect performance using offspring survival, offspring size, and developmental time (Dorn *et al.*, 2001; Solarz & Newman, 2001; Ding & Blossey, 2009). However, with aphids, one can measure multigenerational population dynamics. Our results support the preference-performance hypothesis when

Table 2. Parameters of logistic models for aphid performance on three species of duckweed.

	Intrinsic per capita growth rate (individual/individual/day), $P < 0.0001$				Carrying capacity (individuals), $P < 0.0001$			
Duckweed species	r	SE	df	t	K	SE	df	t
Spirodela polyrhiza	0.213	0.0137	764	37.44	101.83	20.34	764	12.49
Lemna minor	0.0954	0.0128	764	22.20	335.19	51.48	764	24.90
Landoltia punctata	0.1767	0.0053	764	33.16	203.78	9.36	764	21.78

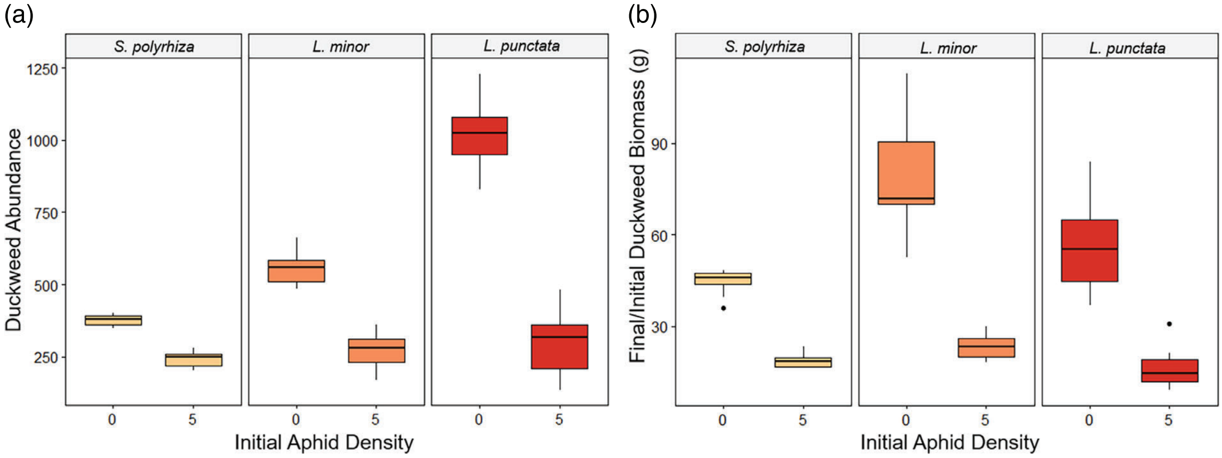

Fig 4. Performance of duckweed populations (initiated with 10 individuals) with and without aphids after 32 days. (a) Final duckweed abundance. (b) Total duckweed dry biomass. [Colour figure can be viewed at wileyonlinelibrary.com]

using an intrinsic per capita growth rate. On the contrary, preference is negatively related to performance when measured as carrying capacity. The relevancy of each measure is dependent on ecological conditions. Jacobsen and Sand-Jensen (1992) found that the effect of invertebrate herbivory on freshwater macrophytes in natural communities is significant, but that there is strong seasonal variation in these dynamics. Extrapolating our lab results to the field, we suggest that in early summer, when duckweed population size increases rapidly in eutrophic ponds, aphid intrinsic growth rate (*r*) may be the best measure of performance due to abundant resources (hosts). However later in the season, duckweed population growth slows or is in decline. At this time, the ability of aphids to maintain high density may be a better measure of performance. Thus selection on host preference may be seasonal.

Duckweed tolerance

Understanding the impact of herbivores on macrophyte communities requires quantifying changes in host performance (Hidding *et al.*, 2009; Kempel *et al.*, 2015). Herbivores can have large and species-specific impacts on their aquatic host plants (Carlsson & Lacoursierre, 2005). We show that five water-lily aphids added to 10 duckweed individuals can reduce abundance by as much as 70% within 32 days, but this impact varies among duckweed species.

Differences in tolerance between duckweed species could be driven by a number of factors. The fastest reproducing species may be more tolerant to damage by being able to compensate for losses and re-grow in abundance rapidly. Faster growing species are predicted by the Resource Availability Hypothesis to be less defended but more tolerant of herbivory (Endara & Coley, 2011). Alternatively, faster growing but poorly defended species may be less tolerant because early losses to herbivory could have compounding effects on exponential population growth. Our results suggest the latter since *S. polyrhiza* has the slowest growth rate and is much more tolerant of aphid damage than the faster growing species (Fig. 4). *Spirodela polyrhiza*'s higher tolerance could be due to its larger body size that can support multiple aphid individuals. The ability to tolerate aphids could also be adaptive since *S. polyrhiza* is also the species most preferred by aphids.

The ecology and evolution of plant-herbivore interactions can also be influenced by a trade-off between resistance and tolerance (Agrawal, 2007). As with the performance-preference hypothesis, our results depend on which measure of resistance we use. A tradeoff is supported if the measure of resistance is one minus aphid intrinsic growth rate, which implies that *S. polyrhiza* is the least resistant but most tolerant species. However, there does not seem to be a tradeoff if we consider resistance based on carrying capacity or total phenolic concentration, where *S. polyrhiza* has the highest resistance (Smolders *et al.*, 2000). *Lemna minor* has intermediate tolerance and the highest resistance when considering aphid intrinsic growth rate, but the lowest resistance when considering carrying capacity and phenolic concentration. *Landoltia punctata* has the lowest tolerance to aphids and has intermediate resistance when looking at both measures of aphid performance. Thus resistance-tolerance tradeoffs are partially supported for duckweed and may vary with season, suggesting possible selective maintenance of both strategies in these species (Núñez-Farfán *et al.*, 2007).

Limitations and conclusions

Some limitations of our study should be considered. First, by using one genotype per species we assume that they represent species differences. For instance, the aphid genotype chosen may be specialised on one duckweed species. This is unlikely given that *R. nymphaeae* is highly polyphagous and macrophyte communities are speciose on small scales. To address such concerns, future studies should quantify intraspecific variation in duckweed-herbivore interactions. Second, our study was conducted in controlled lab conditions. To truly understand the impact of herbivores on macrophyte population dynamics and community composition may require manipulative field experiments.

Our study provides critical experimental plant-herbivore data on a widespread group of common macrophytes. The knowledge that aphids preferentially feed on some species over others and affect species differently implies duckweed community composition could be driven by herbivory, as is demonstrated in terrestrial (Kempel *et al.*, 2015) and aquatic systems (Bakker *et al.*, 2016). Furthermore, we show that a phloem-feeding herbivore can quickly and severely reduce the abundance and biomass of some of the fastest-growing plants in the world. Such herbivory, by altering the penetration of light into freshwaters, may cause widespread ecosystem impacts and possible regime shifts (Scheffer *et al.*, 2003; Stenberg & Stenberg, 2012). Finally, understanding the potential devastating impact of herbivores will be critical for the commercial use of duckweed.

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Authors Contributions

Swapna K. Subramanian and Martin M. Turcotte conceived and designed the experiments. SKS performed the experiments. SKS and MMT analysed the data. SKS wrote the first draft, and MMT provided substantially to revisions.

Data availability statement

Data is available on request from the authors.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Preference of water-lily aphids between four species of duckweed in two-way choice trials over five-time points.

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