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SHORT COMMUNICATION

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Asymmetric density-dependent competition does not contribute to the maintenance of sex in a mixed population of sexual and asexual *Potamopyrgus antipodarum*

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Abstract

Asexual reproduction is expected to have a twofold reproductive advantage over sexual reproduction, owing to the cost of producing males in sexual subpopulations. The persistence of sexual females, thus, requires an advantage to sexual reproduction, at least periodically. Here, we tested the hypothesis that asexual females are more sensitive to limited resources. Under this idea, fluctuations in the availability of resources (per capita) could periodically favour sexual females when resources become limited. We combined sexual and asexual freshwater snails (*Potamopyrgus antipodarum*) together in nylon mesh enclosures at three different densities in an outdoor mesocosm. After 1 month, we counted the brood size of fertile female snails. We found that fecundity declined significantly with increasing density. However, sexual females did not produce more offspring than asexual females at any of the experimental densities. Our results, thus, suggest that the cost of sexual reproduction in *P. antipodarum* is not ameliorated by periods of intense resource competition.

KEYWORDS
adaptation, artificial selection, life history evolution, natural selection, trade-offs

1 | INTRODUCTION

The maintenance of sexual reproduction remains an important question in evolutionary biology (Neiman et al., 2018). The question arises in part because sexual females produce male offspring, which do not bear young, whereas asexual females produce only daughters. This gives asexuals an intrinsic twofold advantage over their sexual counterparts, which is called the 'cost of males' (Lively & Lloyd, 1990; Maynard Smith, 1978). The cost of males has been demonstrated in several systems, including Amazon Mollies (Schlupp et al., 2010) and *Daphnia pulex* (Wolinska & Lively, 2008). In addition to this intrinsic cost, there may be additional ecological costs of sex, such as the lower overwinter survival observed in sexual *Boechera* wildflowers compared to asexuals (Rushworth et al., 2020). The long-term persistence of sexual reproduction in the face of these costs implies that sexual reproduction must confer significant short-term fitness advantages (Maynard Smith, 1978). The ecological hypotheses for the source of such advantages have tended to focus on the potential value of producing genetically variable offspring in the face of temporal changes in the abiotic environment [Lottery Model (e.g. Williams)], competition for variable resources [Tangled Bank Model (e.g. Bell, 1982)] and coevolving parasites [The Red Queen Hypothesis (e.g. Hamilton, 1980)].

The New Zealand freshwater snail, *Potamopyrgus antipodarum*, is a suitable system for testing the alternative ecological hypotheses for the maintenance of sex. These snails are found in mixed populations of sexual and asexual females in nature (Dybdahl & Lively, 1995), making direct comparisons possible, both within and between populations. In addition, both experiments and field data have shown that *P. antipodarum* exhibit a twofold cost of males (Gibson et al., 2017; Jokela et al., 1997), as originally predicted by Maynard Smith (1971, 1978). Thus far, the snail system has been used to test the ecological hypotheses for the maintenance of sex, of which the Red Queen hypothesis is the best supported. The Red Queen hypothesis suggests that the cost of males in sexuals is mitigated by the benefits of

JOURNAL OF Evolutionary Biology

producing genetically variable progeny in the face of highly virulent, coevolving parasites (Bell, 1982; Hamilton, 1980; Jaenike, 1978). Laboratory and field data both suggest that sterilizing trematode parasites drive strong selection against common clones, thus allowing sexuals to persist (Gibson et al., 2016; King et al., 2009; Koskella & Lively, 2009; Vergara et al., 2014). These results, however, do not rule out other ecological factors that could contribute to the selection for sex, especially when infection levels are low (see Gibson et al., 2018). Here, we tested a new hypothesis: sexual females are less sensitive than asexual females to resource limitation, and that fluctuations in resource availability could favour sexual females during periods of intense resource competition. The idea is similar to the Tangled Bank hypothesis (Bell, 1982) in that it relies on competition for limited resources. However, the Tangled Bank model assumes negative frequency-dependent selection in competitive environments, whereas the hypothesis presented here assumes density-dependent selection, that is, frequency independent.

There are some data to suggest that asexuals may, in fact, be more sensitive to resource limitation. For example, brood size in asexual *P. antipodarum* was shown to be positively correlated with phosphorus availability (McKenzie et al., 2013). Because asexual *P. antipodarum* has higher ploidy, they may require more phosphorous (a key component of DNA) to grow and develop. As such, asexuals may be more sensitive to phosphorous limitation than sexual snails (Neiman et al., 2013). The lower minimum resource requirement might favour sexuals, until nutrients increase or until the population declines to a point where individuals are not resource limited. Alternatively, asexuals might be more sensitive to low nutrients as a result of mutation accumulation in non-recombining genomes (i.e. Muller's ratchet; see Muller, 1964; Haigh, 1978; Howard & Lively, 1994). This could be the case if mutations decrease starvation tolerance.

Resources might fluctuate due to extrinsically driven cycles in nutrient availability, such as those associated with changes in nutrient-associated runoffs following increased precipitation. Alternatively, per capita resources could fluctuate due to intrinsically driven dynamics within the system. The coexistence of two species (here, two reproductive modes) on a single resource is predicted to be possible with intrinsically generated cycles (Armstrong & McGehee, 1980). Intrinsically generated cycles are predicted to occur in clonal populations that overshoot their carrying capacity and then subsequently decline (Doebeli & de Jong, 1999; Greischar & Lively, 2011). These oscillations can be chaotic, and they could potentially lead to the extinction of a clonal population (Doebeli & de Jong, 1999). Sexuals have lower per capita intrinsic birth rates than asexuals, leading to more stable population dynamics (Doebeli & de Jong, 1999). Periods of rapid population growth could cause periods of extreme resource limitation, resulting in reduced fecundity. If sexuals are less sensitive to resource limitation than asexuals, they could have periodic advantages (higher fecundity) when resources are low (Figure 1).

We hypothesize that oscillatory dynamics in resources in which sexuals are periodically favoured could contribute to the selective advantage of sexual reproduction in nature. This hypothesis predicts that asexuals would have a lower proportion of brooding females and smaller brood sizes at high densities. Here, we measured brooding of sexual and asexual female snails after they had been kept at three density treatments for 1 month. We found that fecundity decreased with increasing density, but the two reproductive modes were not significantly different at any density treatment. Our results, thus, suggest that asymmetric competition does not contribute to the maintenance of sex in field populations of *P. antipodarum*.

2 | METHODS

2.1 | Natural history

All *P. antipodarum* snails were collected from Lake Alexandrina, a mesotrophic high-country lake on the South Island of New Zealand. This lake experiences rapid plant growth in the spring, which corresponds to a rapid increase in *P. antipodarum* populations (Talbot & Ward, 1987). This growth in snail population size was especially apparent after algal blooms (Talbot & Ward, 1987), suggesting an increase in resources correlates with higher brooding. This pattern has been seen in other species, including the freshwater snail *Helisoma trivolvis* (Hoverman et al., 2005), and several species of *Daphnia* (Walls et al., 1991; Wu & Culver, 1994).

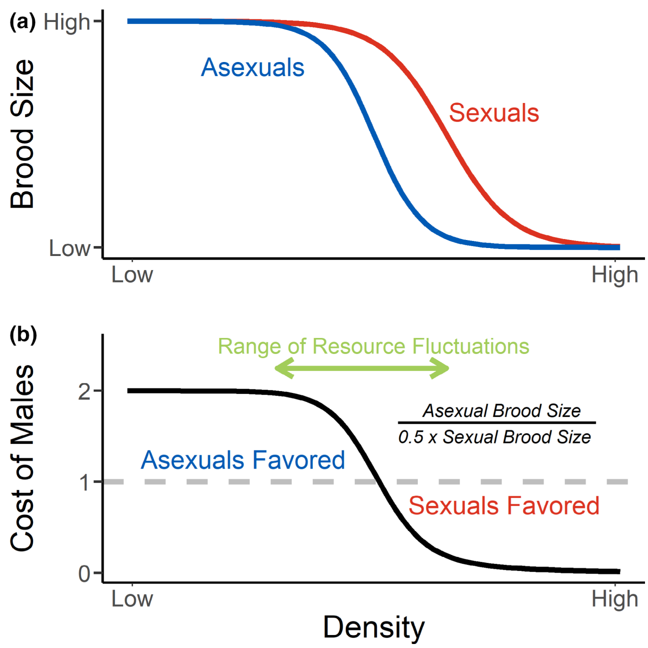

FIGURE 1 Example of how asymmetric sensitivities to limited resources might periodically favour sexual reproduction. The top graph (a) indicates that asexuals (blue line) are more sensitive to resource limitation at high density than sexuals (red line). The bottom graph (b) shows the net cost of sexual reproduction (in terms of a relative number of eggs per capita) as represented by the equation in the lower graph. Per capita birth in the asexual population is equal to that for the sexual population when at 1 (grey dashed line). Sexuals would be periodically favoured if resources fluctuated around the inflection point of the cost (indicated by the green arrow)

Female snails in Lake Alexandrina reach sexual maturity at about 3-mm shell length. Females are ovoviviparous with the lower oviduct forming a brood pouch (Winterbourn, 1970a). Asexuals reproduce by apomictic parthenogenesis. Mated sexual female snails will store sperm (Fretter & Graham, 1962; Wallace, 1992), indicating that male limitation is not an issue for sexually mature females. In both reproductive modes, offspring are retained within the brood pouch until they reach a 'crawl-away' stage (Jokela et al., 1997; Winterbourn, 1970a).

2.2 | Collection and density

In January 2020, a large ($N > 10\,000$), a random sample of *P. antipodarum* was collected near site JMS at Lake Alexandrina, NZ (GPS coordinates: −43°56′12.1″S, 170°27′36.7″E; or see JMS on Figure 3 in Million et al., 2021). Snails were collected by pushing a kick net through a bed of the macrophyte, *Isoetes kirki* at a depth of 1.5–3.0 m. We focused on this site at this depth because it contains a large population, with mixtures of sexual and asexual snails (Jokela & Lively, 1995). In addition to snails, six water samples (125 ml each) were collected at sites around Lake Alexandrina to condition the water in the experiment with local microflora and fauna. This water would also seed the experimental tank with natural algal and diatom propagules that would contribute to resources available to the snails. Six days prior to the experimental start, these water samples and 2.25 mL of dried *Spirulina* powder were combined and mixed into an 800-L tank of aged tap water in Kaikoura, NZ. Experimental enclosures were also placed within the tank and allow for algal growth on the mesh (Movie S1). Each enclosure consisted of a 2-L plastic bottle with eight cut-out holes (four circles approximately 5.5 cm in diameter and four rectangles approximately 12 ×5.5 cm) to ensure water exchange and a tagged float to keep the enclosures upright (Figure S1a). The bottle and float were sewn into 500-µm nylon mesh bags, a mesh size which allows newborn snails to escape.

Adult snails were separated with a 1.7-mm sieve, which allows juvenile snails (<3 mm) through, but retains adults. Adult snails were randomly selected (males, sexual females and asexual females), counted and assigned to eight replicates each of sample sizes of 100, 400 and 900 snails for a total of 11 200 snails. An additional sample of 500 snails was frozen to determine the brooding status before the experiment. Each group was placed within an experimental enclosure and returned to the tank with pre-conditioned water. A submersible pump was added to the tank to gently circulate the enclosures during the day, which also ensured that the food was well mixed in the water column. Density was maintained at experimental levels throughout the experiment because newborn snails in the crawl-away stage were small enough to pass through the enclosure mesh. We did not measure the production of crawl-away offspring, as we were instead interested in the brooding following a period of resource deprivation.

The water in the tank was replaced halfway through the experiment with aged tap water held in a separate tank. After 30 days in these conditions, all enclosures were photographed (Figure S1b–d; representative photographs for each density), and the snails were removed to 20-L containers. The experiment was constrained to 30 days due to the length of the field season, however the visual differences between enclosures indicated that this time was sufficient to induce resource differences, and the later dissection results indicated that the resource difference was sufficient to reduce brooding.

2.3 | Resource quantification

Each of the enclosure photographs was analysed in ImageJ (Schneider et al., 2012). The background of the image was excluded from analysis using the polygon selection tool. For each image, the mean grey value of the selection was measured and recorded. Higher mean values for the greyscale were indicative of more resources per snail.

2.4 | Tissue preservation, transport and dissection

Immediately following the removal of snails from the experimental enclosures, 10 snails per replicate per density were sexed and dissected. The pilot samples from the 100-snail treatment were preserved by freezing for further analysis. An additional sample of N = 40 for each 100-snail replicate and N = 100 for each 400- and 900-snail replicates were then frozen for transport to Indiana University in Bloomington, IN, USA. The frozen snails were thawed, sexed and dissected in Bloomington, IN, USA, to determine trematode infection status. Because trematode infection is sterilizing, infected snails were excluded from further analysis. Brooding status was defined as the presence or absence of eggs in any stage in the brooding pouch of the snail. Brood size was measured as the number of eggs present in the brooding pouch—these eggs are identifiable even after freezing. For all snail samples, head tissue was preserved by freezing at -80C and reserved for DNA extraction and further analysis. Body tissue was preserved by freezing at -80C and reserved for flow cytometric-based determination of DNA content, to estimate ploidy as a proxy for reproductive mode.

2.5 | Determination of DNA content through flow cytometry

We used flow cytometry to quantify DNA content (following Osnas & Lively, 2006). Each sample was homogenized in 200 µl of DMSO using a microtube homogenizer (Benchmark Scientific) with 1.5-mm zirconium homogenizer beads. After homogenizing, each sample was incubated at 4C for 30 min in a staining solution of 200ug/mL propidium iodide, 3.4-mM trisodium citrate dihydrate, 0.1% nonidet P-40, 0.5-mM Tris, 466.7-µg/ml spermine, 693.3-µg/ml spermine tetrachloride and 200-µg/ml Rnase A. Each sample was filtered through Celltrix 50-µm mesh into a flat-bottomed Falcon plate to remove cell aggregates, homogenizer beads and debris. These samples were kept at 4°C until they were analysed, at which point they were transferred to a u-bottomed Falcon plate at a final volume of 200 µl per sample.

We recorded the fluorescence intensity of a maximum of 5000 single-cell nuclei per sample, using the Y1-A channel on the MACSQuant VYB cytometer running MACSQuantify software (Miltenyi Biotec). The mean fluorescence intensity was recorded for the snail tissue in each sample. We visually inspected the sample peak and used gating to create subpopulations of cells that corresponded to cell nuclei in the G1 phase. We excluded from the data set any samples that yielded ambiguous or low-quality fluorescence peaks because we were unable to estimate ploidy for these individuals. Each sample was initially analysed using MACSQuantify software, then later processed using the R flowCore package (Ellis et al., 2019). To estimate ploidy and thus reproductive mode for each sample, we used males (assumed to be diploid), which were collected from the same enclosures as the females, as an internal reference, and we used lab-reared triploid snails as an external reference. Because triploids have three chromosomes for every two chromosomes in diploids, asexual snails were assumed to have 1.5 times the DNA of diploid sexual females. However, recent work has shown that asexual triploid snails vary in DNA content (Million et al., 2021; Neiman et al., 2011). Despite this variation, asexuals still tend to have higher DNA content than sexuals. In addition, the fluorescent peak of asexual nuclei tends to be wider than sexual nuclei (see Figure 2 in Neiman et al., 2011; Figure 3 in Soper et al., 2013). Therefore, we have adapted our analysis of flow cytometry files to conservatively identify diploids and triploids, using the intensity and standard deviation of the fluorescence peak for each snail. This analysis identified two populations: one sexual and one asexual, with minor overlap (Figure S2). The overlapping snails were excluded from further analysis as a conservative estimate of ploidy. We confirmed that including these snails did not affect the statistical results or overall conclusions of this experiment.

2.6 | Statistical analyses

All statistical analyses were performed in R version 3.6.3 (R Core Team, 2020). We used the dplyr package to organize the data and the GGPLOT2 package for all graphs (Wickham, 2016; Wickham et al., 2020). The mean grey values (generated by IMAGEJ) for all enclosure photographs were analysed using a generalized linear model with a density as the independent variable. We also analysed a pairwise comparison of all densities with linear contrasts. To analyse snail brooding, we created a generalized linear model, with brooding/non-brooding as a binomial response variable, and density treatment, reproductive mode and the interaction between the two variables as independent factors. Further, we created a generalized linear model with a Gaussian distribution of brood size (excluding non-brooders) as the dependent variable, and with density treatment, reproductive mode and the interaction between the two variables as independent effects. We tested the normality of residuals for this model using the Shapiro–Wilk normality test and the assumption of equal variance using the Studentized Breusch–Pagan test. We calculated estimated marginal means with 95% confidence intervals for each model, using the emmeans package (Lenth, 2021), and the fixed effect *F* statistics for each independent variable using the rstatix package (Kassambara, 2020).

To generate a combined metric for fitness, including brooding and non-brooding snails, which creates a data set that is not normally distributed, we also computed a non-parametric test. We performed a Kruskal–Wallis test for brood size on group. The group variable was a factor which combined the factor variables of density and reproductive mode. Finally, we performed the Dunn test (FSA package; Ogle et al., 2021) with the Bonferroni correction for multiple comparisons as a post hoc analysis to determine which groups significantly differed from each other group. Finally, a post hoc power analysis was performed using the pwr package (Champely, 2020).

3 | RESULTS

Algal growth on the mesh of the experimental enclosures indicated that density had a visible effect on food availability (Figure S1b–d). Linear contrasts of the mean grey value by density indicate that 100-snail bags were significantly darker than either the 400-snail bags ($p = 0.013$) or the 900-snail bags ($p < 0.001$) (Figure 3). The 400 bags were not significantly darker than the 900 bags ($p = 0.226$). The highest mortality rate was 9.7% in one 900 snails/bag replicate. All other replicates had <3% mortality. This indicates that even the lowest food availability was sufficient for the survival of most snails, and thus the results below are due to differences in brooding rather than mortality.

Our results suggest that the lowest density treatment (100 snails per cage) presented an ecologically relevant per capita resource availability as reflected by the mean brooding. The Dunn test indicated that the mean fecundity of asexuals in the initial field sample was not significantly different than the mean fecundity of asexuals in the 100-snail density ($p = 0.709$). However, sexuals had a significantly lower brood size in the initial field sample than the 100-snail density ($p = 0.004$). Linear contrasts from the brood-frequency and brood-size results (generalized linear models) indicate that this difference is due to a lower frequency of brooding snails ($p = 0.003$) rather than the number of eggs per brooding snail ($p = 0.284$). Together, these results suggest that snails in the field were brooding at rates similar to or less than the lowest experimental density in the cages. The lowest density may be ecologically relevant based on brooding relative to the initial field sample; data on food availability in the field are sparse. Therefore, rather than reflecting ecological relevance, the higher density treatments represent strong selection treatments to test the hypothesis that periodically limited resources would disproportionately reduce reproduction in asexual snails.

The density treatments were highly significant for both brooding frequency ($F_{3,1855} = 17.819$, $p < 0.001$) and brood size ($F_{3,409} = 32.659$, $p < 0.001$; Figure 2). However, the reproductive mode was not significant for either brooding frequency ($F_{1,1855} = 2.232$, $p = 0.135$) or brood size ($F_{1,409} = 1.911$, $p = 0.168$; Figure 2). There was a marginally significant interaction between reproductive mode and density

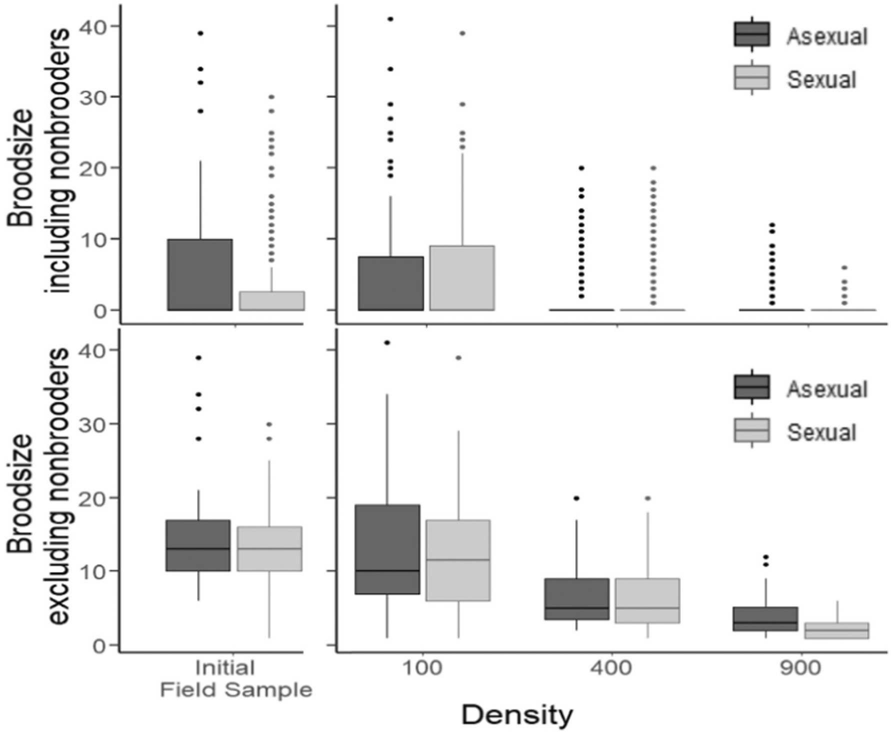

FIGURE 2 The mean number of eggs in brooding female snails excluding (top) and including nonbrooding snails (bottom). The centre line indicates the median value; the lower and upper hinges correspond to the first and third quartiles (25th and 75th percentiles), respectively. The lower whisker extends to the lowest value. The highest whisker extends to the highest value within 1.5* the interquartile range. Finally, each dot represents a single outlier observation. Linear contrasts showed no significant differences between sexuals and asexuals within each of the lower two density treatments for brooding frequency (100-snail density $p = 0.853$, 400-snail density $p = 0.137$ or any density treatment for brood size (100-snail density $p = 0.365$, 400-snail density $p = 0.782$, 900-snail density $p = 0.420$) or in the initial field sample (brooding frequency: $p = 0.135$; mean brood size: $p = 0.167$). Asexuals had marginally higher brooding frequency ($p = 0.075$) in the 900-snail density treatment

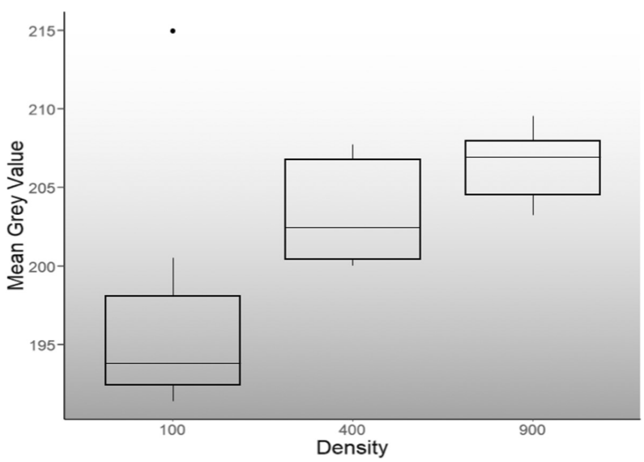

FIGURE 3 Box plots of the mean grey value of the enclosures. Lower mean grey values indicate a darker colour. Given that the bags were white to start with, we assume that a darker colour indicates more algal growth, and therefore higher food availability for the snails

treatment for brooding frequency ($F_{3,1855} = 2.393$, $p = 0.067$), but not for brood size ($F_{3,409} = 0.276$, $p = 0.843$; Figure 2). The significant interaction in brooding frequency was likely seen because sexuals were brooding less than asexuals in the initial field sample and the highest density treatment, but the opposite pattern holds in the 400 snails/bag density treatment. However, there was no significant difference between sexual and asexual brooding frequency in the 400 snails/bag density treatment ($p = 0.782$).

A Kruskal–Wallis test was used as a combined fitness metric and to account for the non-normally distributed data when non-brooders were included in the model. Whilst a zero-inflated negative binomial could account for non-normally distributed data, we preferred a method, which gave a single metric for fitness, rather than one test for zero-inflation, and a second metric for the negative binomial. This test indicated that there was a significant difference ($\chi^2 = 152.55$, $df = 7$, $p < 0.001$) amongst the eight groups (the initial field sample and three density treatments, each with two reproductive modes). The Dunn test post hoc analysis indicated that there were no significant differences in mean fecundity between reproductive modes in the initial field sample ($p = 0.084$), the 100-snail density ($p = 0.902$) or the 400-snail density ($p = 0.200$). In the 900-snail density, asexuals had marginally higher brood (combined brood size and brooding frequency) than sexuals ($p = 0.097$). These results further support the conclusions of the GLMs that sexual snails did not gain an advantage over their asexual counterparts at high densities. Given the non-significant result, we performed a post hoc power analysis. Based on our sample size, alpha = 0.05 and a small effect size using Cohen's (1988) criteria, we found that we had a power of 1.000 to detect differences in brooding frequency between reproductive modes at each density. We had a power of 0.999 to detect differences in brood size between sexuals and asexuals at each density (Table 1).

4 | DISCUSSION

We hypothesized that fluctuations in resources over time could give a periodic advantage to sexual reproduction; provided asexuals are more sensitive to resource limitation than the sexuals. We, thus, predicted that asexual snails would be more negatively affected by intense resource competition. This prediction was not upheld. We found that, overall, fewer snails were brooding at higher densities, indicating that the competition led to reduced fecundity. However, sexual and asexual snails did not significantly differ for fecundity or brooding frequency within the 100- and 400-snail-density treatments. In the highest density treatment (900 snails/bag), asexuals were brooding significantly *more* than sexual females, both in terms of frequency of brooding females and the number of eggs per brooding female. Taken together, the results indicate that asexual snails are not more negatively affected by density than sexual snails. As such, the results do not support our hypothesis that resource fluctuations could contribute to the persistence of sexual reproduction in *P. antipodarum*.

The relatively high fecundity of asexuals in the 900-snail density treatment could indicate that they are *less* sensitive to resource limitation than sexual snails. This could support the idea that these asexual snails have evolved a General Purpose Genotype (GPG; Baker, 1965). Clones with a GPG should have broad tolerance ranges and low fitness variance under temporally variable environments (Lynch, 1984), allowing them to persist in times of stress. However, this should result in reduced clonal diversity, as only the GPG should be maintained. Despite this prediction, recent field surveys suggest high genetic diversity of *P. antipodarum* in the field (Million et al., 2021; Paczesniak et al., 2014).

TABLE 1 ANOVA table of all statistical tests performed

	Sum Sq	*df*	*F* values	*p*-values
Brooding frequency density and reproductive mode as fixed effects				
Density	8.703	3	17.819	<0.001
Mode	0.363	1	2.232	0.135
Interaction	1.169	3	2.393	0.067
Residuals	301.998	1855	Power: 1	
Brood size: Density and reproductive mode as fixed effects				
Density	3877.8	3	32.659	<0.001
Mode	75.6	1	1.911	0.168
Interaction	32.8	3	0.276	0.843
Residuals	16,188.0	409	Power: 0.999	

Note: The power listed was calculated from a post hoc power analysis of reproductive mode for the initial field sample or of the interaction for experimental enclosures. This post hoc power analysis is based on an effect size of 0.1 and an alpha of 0.05.

Fecundity is not the only fitness metric that could be affected by resource fluctuations. Our hypothesis might be supported by other metrics, such as growth rate, time to reproductive maturity or mortality. Fecundity and age at reproductive maturity are correlated with body size in *P. antipodarum* (Tibbets et al., 2010; Verhaegen et al., 2018; Winterbourn, 1970b). Therefore, different growth rates and development times could contribute to differences in fecundity over the lifetime of a snail, leading to the maintenance of sex. Whilst sexual and asexual snails do not differ in size at reproductive maturity, sexuals can have significantly lower growth rates than single clones (Jokela et al., 1997). Because we used adult snails for this experiment, we could not observe these differences. Prior work indicates that density can reduce growth rates in *P. antipodarum* (Zachar & Neiman, 2013), though direct comparisons between sexual and asexual snails are still needed. If sexuals have higher growth rates than asexuals when resources are limited, they can reach sexual maturity earlier, and therefore might reproduce more over their lifetime.

Higher mortality in asexuals than sexuals at high densities could also contribute to the maintenance of sexual reproduction in nature. In this case, asexual snails would face a direct cost to limited resources, favouring sexuals which could survive to reproduce when resources increase. Mortality in this experiment was too low to determine whether asexuals were more affected than sexuals. However, previous work suggests that competition does not increase mortality in asexuals relative to sexuals in *P. antipodarum* (Lively et al., 1998). Interestingly, differential mortality due to limited resources is found in Amazon mollies (Tobler & Schlupp, 2010), indicating that the hypothesis tested in this experiment could contribute to the maintenance of sex in other systems. This is further supported by studies in *Daphnia carinata*, which show increased investment in sex when food resources are low, since sexual eggs can remain dormant until favourable conditions arise (Lever et al., 2021). Additionally, *D. magna* shows increased allocation to sexual reproduction as population growth rates decline (as the population reaches carrying capacity), indicating density itself may be a cue that sexual reproduction will be favoured (Gerber et al., 2018).

Simulations show that in species with facultative sexual reproduction, unstable fluctuating resources lead to increased sexual reproduction (MacPherson et al., 2022). Resource fluctuations are also suggested to favour sexual morphs of aphids, which are more prevalent in annual crops, in which resources are periodically destroyed by harvest. Asexual aphids dominate perennial crops, which are relatively stable (Frantz et al., 2006). Resource limitation due to habitat disruption also favours sexual taxa of oribatid mites in marine and freshwater habitats (Krause et al., 2016). High frequencies of asexual oribatid mite taxa are also correlated with high mite densities (as a proxy for habitats with high resource availability), further supporting the idea that asexuals might be more sensitive to limited resources than sexuals in this system (Maraun et al., 2012; Mauran et al., 2019). This finding is further supported by laboratory experiments, which found that in resource-limited conditions, asexual taxa carry fewer eggs than sexual taxa (Domes et al., 2007).

In addition to resource quantity, resource quality might differentially affect sexuals and asexuals. Laboratory experiments indicate that sexual reproduction can be induced by poor-quality food in *Daphnia*, and asexual reproduction is rescued by the addition of additional lipids and proteins (Koch et al., 2009). In *D. pulcaria*, low food quality reduced fecundity and population growth rates regardless of food quantity (Kilham et al., 1997). For *P. antipodarum*, resource quality may be dependent on phosphorous content; asexuals are triploid or polyploid, and thus may require more phosphorous than diploid sexuals (Neiman et al., 2013).

Overall, our results suggest that whilst density has a significant effect on brooding in *P. antipodarum*, asexuals are not disproportionately affected by resource limitation. Thus, fluctuations in overall resource availability would not be likely to contribute to the continued persistence of sexual reproduction in natural populations of *P. antipodarum*.

AUTHOR CONTRIBUTIONS

Z.M.D. and C.M.L. conceived the original idea and carried out the experiments. Z.M.D performed the data analysis and designed the Figures. C.M.L. and Z.M.D. wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in Dryad at https://doi.org/10.5061/dryad.5tb2rbp69.

PEER REVIEW

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