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Multiple life stages with multiple replicated density levels are required to estimate density dependence for plants

Satu Ramula and Yvonne M. Buckley

S. Ramula (satu_ramula@hotmail.com) and Y. M. Buckley, Univ. of Queensland, School of Biological Sciences, Queensland 4072, Australia. YMB also at: CSIRO Sustainable Ecosystems, 306 Carmody Rd, St. Lucia, Queensland 4067, Australia.

Negative density dependence resulting from intraspecific competition can regulate plant populations by limiting demographic rates (survival, growth, fecundity). However, the strength of intraspecific competition can vary within and among populations due to spatial or temporal environmental heterogeneity, or genetic differences. Quantification of variation under a relatively constant environment is needed to assess the inherent potential for density dependence to vary. This knowledge will help adjust data collection effort required for parameterisation of density dependence. Our review of published plant demographic studies revealed that only half of the studies included the whole life-cycle in the analysis of density dependence. Approximately half of the studies manipulated density, while the rest examined density dependence from observed densities in the field. Regardless of the design used, density dependence was estimated from a small number of replicates. To investigate inherent variation in density dependence during the life-cycle, and the effect of low replication on density dependence estimates, we combined an experimental approach with simulations for an invasive herb *Senecio madagascariensis*. We found significant negative density dependence for five out of six examined demographic rates in a constant environment, with the strength of density dependence increasing during the life-cycle. An exception was plant growth, in which the direction of density dependence varied from positive to negative depending on the life stage. Simulations showed substantial deviation for density dependence parameterised from a small number of replicates even when environmental variation was minimal. This suggests that data collection procedures currently used to assess the effect of density on plant demographic rates may produce inaccurate estimates, increasing uncertainty in demographic models. Due to variation in the direction and strength of density dependence during the life-cycle, multiple life stages with multiple replicated density levels are required to parameterise density dependence for demographic rates.

In populations regulated by intraspecific competition, low density populations generally grow faster than high density populations (Sibly and Hone 2002). Therefore, populations of invasive plant species tend to grow at a faster rate than those of native species during the rapid expansion phase (Ramula et al. 2008). However, continuous rapid growth of populations over time is rare as density is likely to start limiting population growth. For the invasive shrub *Cytisus scoparius*, population growth rates were considerably lower in interior parts of populations than at the invasion front (Parker 2000), indicating that the role of plant density in population dynamics for invaders increases over time due to intraspecific competition. Density-dependent population dynamics may also explain why established invaders that have passed the rapid expansion phase exhibit similar population growth rates to those of native species (Meiners 2007). The importance of density dependence for determining demographic rates and management options for invasive plants has been acknowledged (Lintell Smith et al. 1999, Buckley et al. 2001, 2005, Buckley and Metcalf 2006, Freckleton et al. 2008). However, variation in the strength of intraspecific competition and hence density dependence of demographic rates throughout a plant's life-cycle is rarely assessed. Quantification of this variation in a constant environment with replicated density levels is the first step towards a greater understanding of how inherent variation in density dependence affects plant population dynamics. This understanding will also help adjust data collection effort required for parameterisation of density dependence in the field.

Density affects plant population dynamics via underlying demographic rates, i.e. survival, growth and fecundity, and the impact may vary from positive to negative. Positive density dependence, also known as an Allee effect, refers to a situation where one or more demographic rates are facilitated by high density and therefore, individuals perform better at higher than lower densities (e.g. for invasive plants see Taylor and Hastings 2005, Buckley et al. 2007). For instance, pollen limitation may lead to a reduction in seed production at low densities (Davis et al. 2004), preventing rapid growth of the population. The more common form of density dependence is negative density dependence, in which intraspecific competition reduces demographic rate(s) at high densities. These

reductions are expected to slow down population growth rate if they occur in demographic rates critical to population dynamics (Tanner 1999), or if the additive impact of reductions in demographic rates is large enough (Fowler et al. 2006).

The inclusion of density dependence in population models is essential for managing invasive species as management outcomes may vary depending on which life stage(s) density dependence is operating and its magnitude (Buckley et al. 2001, Freckleton et al. 2003, 2008). However, density dependence is difficult to detect from observational data sets even if they contain a large number of observations (Murdoch 1994, Fowler et al. 2006). Analysis of observational data may also lead to detection of spurious effects of density, caused by environmental factors rather than density (Shima and Osenberg 2003). Experimentally manipulated densities are therefore preferable to observational studies for assessing the effect of density on demographic rates (Goldberg et al. 2001, Fowler et al. 2006).

Parameterisation of density dependence for only some life stages ignores the fact that the direction and strength of density dependence may change over the life-cycle. High density may facilitate plant survival at early life stages (Goldberg et al. 2001), while it often reduces survival and fecundity at later life stages (Buckley et al. 2001, Goldberg et al. 2001, Sletvold 2005). Therefore, density dependence of different demographic rates may cancel each other out within a season, leading to an overall neutral effect of density (Elmberg et al. 2005). Further, the direction and strength of density dependence may vary between years (Goldberg et al. 2001) or among genotypes (Morrison and Molofsky 1998). Due to this nonlinear and complex nature of density dependence, detailed examination of different life stages using multiple replicated density levels in a constant environment can be used to explore the effect of density on demographic rates and inherent variation in density dependence.

Here, we investigate if and how density dependence operates during a plant's life-cycle, and produce recommendations for parameterisation of density dependence for demographic rates. Based on published studies on plants, we first review data collection procedures used for estimating the effect of density. We then examine inherent variation in density dependence for demographic rates in a constant environment, using a short-lived invasive herb *Senecio madagascariensis* (Asteraceae) as a model species. The manipulation approach with multiple replicated density levels enables us to determine the direction and strength of density dependence for demographic rates at different life stages from seedling emergence to plant senescence. Finally, using the current data set of *S. madagascariensis*, we conduct simulations to investigate the accuracy of density dependence estimates in relation to the number of individuals sampled and the number of replicates used per density treatment. By combining experimental and modelling approaches, we show that due to variation in the direction and strength of density dependence, data collection procedures currently used for estimating density dependence in plant populations may result in inaccurate estimates.

Methods

Review of data collection procedures used for estimating density dependence

Using published demographic studies, we reviewed data collection procedures used to estimate the effect of density on plant demographic rates. More specifically, our aim was to provide an overview focusing on sampling issues, and discuss future directions for studies on density dependence. We noted the design of a study (observational vs manipulative), the number of replicated densities, the number of populations, length of the study, examined life stages and examined demographic rates. The number of plants observed per density level was also recorded when it was reported in the study. For the database, we only included studies that aimed to examine density dependence in a demographic context and excluded all other studies, such as physiological studies. The final database consisted of 19 studies including 19 individual plant species and one annual plant community (Table 1).

Experimentally replicated densities in a constant environment

To examine the direction and strength of density dependence for demographic rates in a constant environment, we used the invasive *Senecio madagascariensis* as a model species. *Senecio madagascariensis* is an annual or a short-lived perennial herb native to Madagascar and southern Africa, and is invasive in parts of Argentina, Japan and Australia. The species was introduced to Australia in early 1900s and has successfully spread inhabiting pastures and arable fields along the east coast, where it is able to reproduce almost year around. Due to its high reproductive potential and toxicity for cattle and horses, *S. madagascariensis* has been declared as a pest in eastern Australia. An individual plant is 20–50 cm tall, consisting of multiple branches and several yellow flower heads of ca 2 cm in diameter. Flower heads are mainly pollinated by insects and the species is self-incompatible (Radford 1997). The total achene (hereafter denoted as seed) production per plant is a few hundreds (Radford and Cousens 2000). The species reproduces only by seed (Radford and Cousens 2000).

In May–June 2007, we collected ripe seed heads from two *S. madagascariensis* populations situated in road sides ca 80 and 110 km south of Brisbane in Queensland, Australia. We separated seed from pappus and mixed seeds from both populations. We included only dark, filled seeds and discarded small pale seeds that were unlikely to germinate. Seeds were stored in paper bags at room temperature until the end of July, at which time they were transferred to $+6$–8°C for a week to stimulate germination.

Density manipulations

To minimise environmental variation, we conducted a randomised block design in the glasshouse. The glasshouse was used instead of a common garden for biosecurity reasons and to comply with local authority regulation on the keeping of a pest species. We used five density levels

Table 1. Summary of studies included in a review of data collection procedures used for estimating density dependence on plants. o = observational study; m = manipulated densities; a = adult; sdl = seedlings; j = juveniles; s = survival; f = fecundity; rec = recruitment; g = growth; em = emergence; fl = flowering; na = information not available; [p] = all data were pooled. The studies are listed in chronological order.

Species	Status	Study design	Density levels	No. replicates	No. pops	No. years	Life stage(s)	Demogr. rate(s)	Sample/density
Salicornia europaea agg.[1]	annual herb	o	5–6	1	2	2	a	s, f	
Cakile edentula[2]	annual herb	m	7	2–100	3	1	a	s, f	2–100
Vulpia fasciculata[3]	annual grass	m	5	3	1	1	all	s, g, f	4–64
Salvia lyrata[4]	perennial herb	m	8	4	1	1	sdl, j	em, s	≤16
Cirsium vulgare[5]	monocarpic herb	o	8	1	1	na	j, a	f	na
Bouteloua rigidiseta and *Aristida longiseta*[6]	perennial grasses	m	6	6	1	5	all	rec, s, g, f	na
Spartina maritima[7]	perennial grass	o	4	1	2	3	j, a	rec, s, f	na
Anisantha sterilis[8]	annual weed	o	24	1	1	3	sdl, a	em, g	na
Euterpe edulis[9]	tree	o	100	1	1	3	all	s, g, f	na
Tripleurospermum perforatum[10]	short-lived weed	m	5	5	1	1	a	s, fl, f	na
Plant community[11]	annual herbs	m	8	2–4	1–3	2	all	em, s, g	≥50
Sanicula europaea[12]	perennial herb	m	2–5	2–4	1	3	all	rec, s, g, fl	na
Shorea quadrinervis[13]	tree	o	2	8	1	2	j, a	s, g	na
Echium plantagineum[14]	annual herb	o	19	1	na	na	a	f	na
Hornungia petraea[15]	annual herb	o	10	1	many	2–3	all	em, s, f	na
Digitalis purpurea[16]	biennial herb	o	na[p]	1	1	3	all	s, g	59–536[p]
Alliaria petiolata[17]	biennial herb	m	10	6	1	2	a	g, f	≤10
Lobularia maritima[18]	short-lived herb	o	12	1	1	3–4	all	s	na
Ipomopsis aggregata[19]	monocarpic herb	m	7	1	1	7	all	em, s, g, f	na

References: [1]Jefferies et al. 1981, [2]Keddy 1981, [3]Watkinson 1982, [4]Shaw and Antonovics 1986, [5]Gillman et al. 1993, [6]Fowler 1995, [7]Castellanos et al. 1998, [8]Lintell Smith et al. 1999, [9]Silva Matos et al. 1999, [10]Buckley et al. 2001, [11]Goldberg et al. 2001, [12]Gustafsson and Ehrlén 2003, [13]Blundell and Peart 2004, [14]Buckley et al. 2005, [15]Kluth and Bruelheide 2005, [16]Sletvold 2005, [17]Rebek and O'Neil 2006, [18]Picó and Retana 2008, [19]Price et al. 2008.

(25, 50, 100, 150 and 200 plants per 30 cm pot) with 20 replicates for each density level, resulting in a total of 100 pots. The pots were placed into 20 blocks, which were considered random samples, with each block containing one replicate of the five randomly allocated density levels. The pots were re-randomized within the blocks every other week. The five density levels were chosen so that the lowest level enabled multiple plants to be sampled per pot, and density was then usually doubled for the next density level. Since the glasshouse environment provided favourable growing conditions, we used higher densities than observed in the field (a range: 5–13 reproductive plants m^{-2}; Radford and Cousens 2000). We emphasise that our aim is to examine the direction and strength of density dependence in detail, not to extrapolate the results from the glasshouse study to field populations of the study species. We note that since overlapping generations often occur in natural *S. madagascariensis* populations (Radford and Cousens 2000), the results from the single cohort glasshouse study are not directly applicable to field population dynamics, where asymmetric competition is generally stronger (Freckleton and Watkinson 2002).

At the beginning of August 2007, we sowed seeds into 30 cm pots filled with a slightly fertilised potting mix. To ensure the adequacy of plants for the experiment, twice as many seeds were sown per pot as required for the given density level. After sowing seeds were sprayed with 200 ppm gibberrelic acid to improve germination (Radford and Cousens 2000). During the whole experiment, the pots were kept in the unheated glasshouse at the Univ. of Queensland, in which the light rhythm and temperature followed the daily ranges of Brisbane (27°24′S, 153°1′E). The pots were watered daily and no fertiliser was added.

Two weeks after sowing when seedlings were ca 15–20 mm and few new germination events occurred, we thinned seedlings to the five density levels. Four weeks after seed sowing when plants were on average 6 cm, we conducted the first survey and randomly marked 24 plants per pot with colour-coded yarns (2400 plants in total). We then monitored the status of the marked plants every other week until the senescence of flowers, resulting in four surveys in total. These four surveys reflect different life stages during the life-cycle, starting from the juvenile stage at the first survey. At the second survey, the plants were at the vegetative stage with a small proportion producing flower buds. At the third survey, the majority of the plants had flower buds but were not yet flowering, while at the fourth survey the plants were flowering. Due to pest control reasons and the self-incompatibility of the species (Radford 1997), we did not record seed production in the glasshouse and therefore used the number of flower buds as a surrogate for fecundity. After the fourth survey, we cut all flower buds and flower heads from the plants to minimise the risk of seed escape through some pollinated flower heads. At that time flower heads were ripening and plants showed signs of senescence with leaves starting to wither.

We recorded proportional seedling emergence two weeks after seed sowing. At each survey, we then recorded survival, height, flowering status (flowering, not flowering) and the number of flower buds of the marked plants. Since plant height may not necessarily describe investment to growth as it ignores the total volume of the plant, we also measured final shoot mass. To obtain shoot mass, we harvested above ground parts of the marked plants, placed them into paper bags and dried at +50°C for 24 h. Due to some mortality before harvesting, shoot mass was obtained from 2313 plants. Note that shoot mass only includes the aboveground vegetative part of the plant as the flower heads were removed before harvesting.

Data analyses

To enable comparison of the strength of density dependence among different demographic rates to be made, we linearised relationships between response and explanatory variables using natural log-transformed density except for analysis of plant height, where a $\log_{10}$-transformation was more appropriate. A quadratic density term was included in all models to test possible nonlinearity but was always non-significant and therefore removed.

We used proportional pot-wise seedling emergence rates to determine how density affects seedling emergence. We conducted a mixed model ANCOVA with density as a continuous fixed explanatory variable (covariate), and block and a block × density interaction as random explanatory variables.

To examine the effect of density on the binary variables, plant survival and flowering probability, we performed hierarchical mixed model logistic regressions using the GLIMMIX procedure in SAS ver. 9.1 with a binomial distribution and logit link function. Density was included as a fixed continuous explanatory variable in the model, while block, pot and a density × block interaction were included as random factors. Due to high plant survival during the experiment, we only used the final survey measurement. Similarly, flowering probability from the final survey was used in the analysis because a relatively small number of plants flowered at the earlier surveys. We assessed the appropriateness of each model based on a dispersion factor, which in both cases indicated an adequate fit of the models (dispersion factors 0.95 and 1.00). Since the GLIMMIX procedure does not produce a significance test for random factors, we examined their significance by calculating z-scores from the model outputs (i.e. variance estimates divided by their standard errors), and tested if these scores differ from zero (Littell et al. 1996).

The effect of density on final shoot mass was determined using a mixed model ANCOVA with density as a fixed continuous explanatory variable, and block, pot and a density × block interaction as random explanatory variables. Shoot mass was x^2-transformed to normalise the residuals.

We explored the effect of density on plant height and the number of flower buds with hierarchical repeated measures mixed model ANCOVAs. Plant height was log-transformed and the number of flower buds was $\log(x+1)$ transformed to normalise the residuals. For the number of flower buds, the first survey was omitted from the analysis because no plant at that time produced flower buds. Plants that did not produce flower buds during their lifetime were also omitted. In the ANCOVAs, we used density as a continuous explanatory variable, time and a time × density interaction as fixed factors. Since the measurements were taken from multiple plants per pot, plants were considered nested within pots which were further nested within blocks.

Table 2. Effect of density on demographic rates for the invasive *Senecio madagascariensis* estimated from hierarchical mixed model ANCOVAs.

Rate	Effect	F/Z	DF/DDF	p
Plant height	density	3.25	1/66	0.0761
	time	6170.16	3/59	<0.0001
	density × time	828.13	3/62	<0.0001
	block	0.91		0.1817
	pot	5.30		<0.0001
	density × block	0.66		0.2556
	time × block	2.89		0.0119
	density × time × block	3.09		0.0010
Shoot mass	density	16.41	1/2311	<0.0001
	block	33.21		<0.0001
No. flower buds	density	216.95	1/92	<0.0001
	time	793.43	2/1273	<0.0001
	density × time	213.91	2/1889	<0.0001
	block	1.32		0.0941
	pot	2.29		0.0109
	time × block	3.14		0.0009

Note: F-test was used for fixed factors and Z-test for random factors. Numerator degrees of freedom (DF) and denominator degrees of freedom (DDF) are calculated only for fixed factors. Random factors with a negligible effect to which mixed procedure in SAS produced missing estimates are not shown.

All possible interactions between the fixed factors and block were included in the models to examine variation in density effects among the blocks. For each model, a covariance structure was defined by comparing fitted models with different variance structures, and the structure that produced the lowest AIC value according to Akaike's information criterion was used for the final model (Littell et al. 1998). For both the models, unstructured variance was found to be appropriate.

Reproductive output is a key parameter for population dynamics of short-lived plant species (Silvertown 1993, Ramula et al. 2008). We therefore examined which demographic rate best predicts reproductive output for *S. madagascariensis*, and ran regression analyses with final shoot mass and log-transformed plant height as explanatory variables. Final shoot mass predicted the number of flower buds slightly better than final plant height (flower buds $= 0.36 + 14.04 \times$ shoot mass, $r = 0.57$ and log (flower buds $+1) = -9.58 + 2.97 \times$ log plant height, $r = 0.43$). For the number of flower buds, we included shoot mass as an explanatory variable in a repeated measures mixed model ANCOVA to test for effects of density dependence on reproductive output over and above the effects of density on shoot mass.

We conducted all the ANCOVAs using the MIXED procedure in SAS with restricted maximum likelihood (REML). Degrees of freedom were estimated using the Kenwardroger method which adjusts them according to the amount of variation in the data set at hand. Normality of the data and homogeneity of residuals were visually studied from the residuals when necessary.

Simulated sampling effort and density dependence

We used simulated data on *S. madagasacariensis* and first explored how the number of individuals sampled per density level affects the accuracy of estimated demographic rates. We simulated 100 data sets, each consisting of 1000 individuals with shoot mass drawn from a normal distribution based on the observed means and standard deviations of final shoot mass for each density level. Final shoot mass was used as a model rate because it exhibited most variation among the blocks (Table 2) and best predicted reproductive output. We then sub-sampled the simulated data sets with sample sizes of 10, 20, 30 ... 100 individuals, and for each sub-sample calculated the average absolute deviation from the input shoot mass. The sub-sampling was conducted with replacement using 100 replicates for each sample size.

Secondly, we examined how the number of replicates per density level affects the accuracy of density dependence estimates. If there is little spatial variation, estimates of the strength of density dependence are expected to remain quite constant (Benton et al. 2004), which would allow parameterization of density dependence from a small number of replicates. We again used shoot mass as a model rate and sub-sampled blocks without replacement from the full observed data set (20 blocks) with sample sizes of 3, 5, 10, 15 and 19 blocks. For each sample size, we used 20 replicates and included all individuals belonging to the chosen blocks in the sub-sampled data set. For each subsample, we fitted a mixed model ANCOVA as described above, estimated a model intercept and slope for density, and predicted the final shoot mass based on the estimated regression equation using the highest density level of 200 plants per pot.

Results

Review of data collection procedures used for estimating density dependence

Our review revealed that published studies were slightly biased towards annual and short-lived species, with majority of the studies conducted in one population over one to three years (Table 1). Approximately half of the studies (47%) manipulated density and the rest examined

density dependence based on observed densities in the field (Table 1). For manipulative studies, the number of replicated density levels usually varied from one to six, with 2–100 plants sampled per density level (Table 1). For observational studies, within-year replicates were often lacking because all study plots represented different densities (Table 1) and density dependence was then estimated across all the data points. Half of the studies (47%) examined density dependence for several life stages during the whole life-cycle, whereas 53% concentrated on one or two life stages (Table 1). Most of the studies (84%) examined more than one demographic rate (Table 1).

Experimentally replicated densities in a constant environment

Plant density significantly reduced seedling emergence for *S. madacascariensis* ($F_{1,98} = 50.18$, $p < 0.0001$, mixed model ANCOVA; Fig. 1a), and the reduction was similar for all the blocks (density × block interaction not estimable in a mixed model ANCOVA due to its negligible effect). Seedling emergence rates were more variable at the lowest density level than at the highest density level (Fig. 1a).

Plant survival was high during the experiment ranging from 88% to 100% among the pots and was unaffected by density for all the blocks (density: $F_{1,2398} = 2.99$, $p = 0.0839$, density × block interaction: $z = 1.54^{-19}$, $p = 0.5$; mixed model ANCOVA; Fig. 1b).

Flowering probability decreased with increasing plant density ($F_{1,2365} = 100.29$, $p < 0.0001$; mixed model ANCOVA), showing similar negative density dependence across the blocks (density × block interaction: $z = 2.96^{-20}$, $p = 0.5$; Fig. 1c).

Density affected plant growth in a complex way. A significant density × time × block interaction for plant height indicated that the strength of density dependence varied over time and among the blocks (Table 2, Fig. 2). At the first survey, plant height increased with increasing density for all the blocks, suggesting that density facilitated growth (Fig. 2a). At the third and fourth surveys, plant height declined with increasing density but the strength of the decline varied among the blocks (Fig. 2c–d). Shoot mass significantly declined with increasing plant density, and the strength of density dependence was constant among the blocks (a nonsignificant density × block interaction; Table 2, Fig. 1d). Interestingly, the block means for shoot mass were more divergent at low density than at high density (Fig. 1d), indicating that increased density equalised phenotypic differences among individual plants.

The impact of density on flower bud production varied over time (a significant density × time interaction; Table 2), with the strength of negative density dependence increasing as a function of time (Fig. 3). Density dependence was weakest at the second survey when the plants had just started to produce flower buds (Fig. 3a), and strongest at the fourth survey when the plants were completing their life-cycle (Fig. 3c). Flower bud production varied from block to block over time (a significant time × block interaction) but the strength of density dependence was constant among the blocks (a nonsignificant density × block interaction; Table 2, Fig. 3). Even if final shoot mass was included in the model as an explanatory variable, density still significantly reduced flower bud production ($F_{1,125} = 4.43$, $p = 0.0374$; mixed model ANCOVA) and this reduction was similar across the blocks (density × block interaction not estimable in mixed model ANCOVA due to its negligible effect). This confirms that reduced flower bud

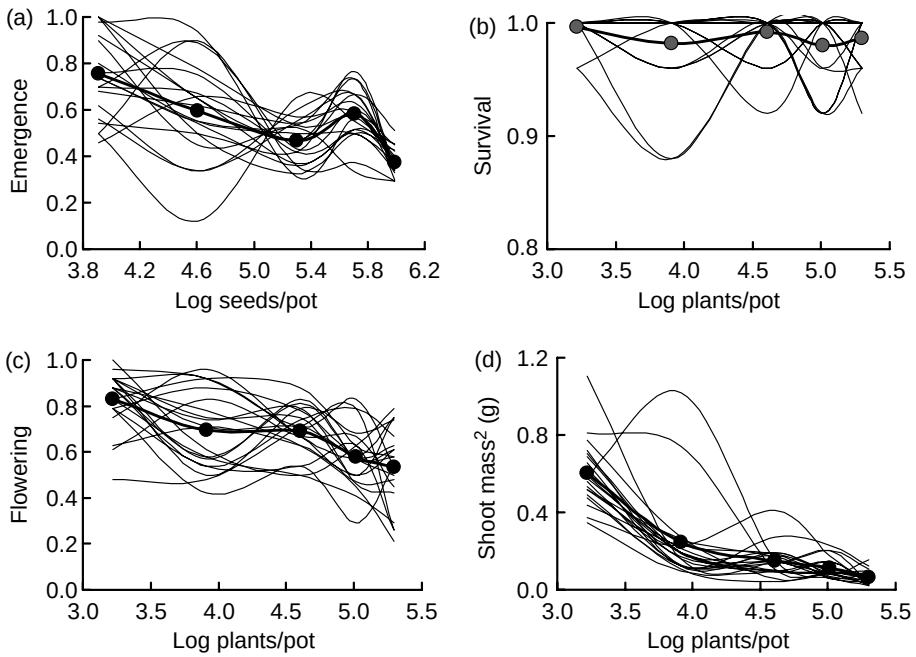

Figure 1. Effect of plant density on (a) seedling emergence, $y = 1.21 - 0.15x$, (b) survival (c) flowering probability, $y = 3.50 - 0.63x$ and (d) shoot mass^2, $y = 2.13 - 0.40x$, for the invasive *Senecio madagascariensis* estimated from 20 blocks. Block estimates are shown separately and the mean value is indicated in bold.

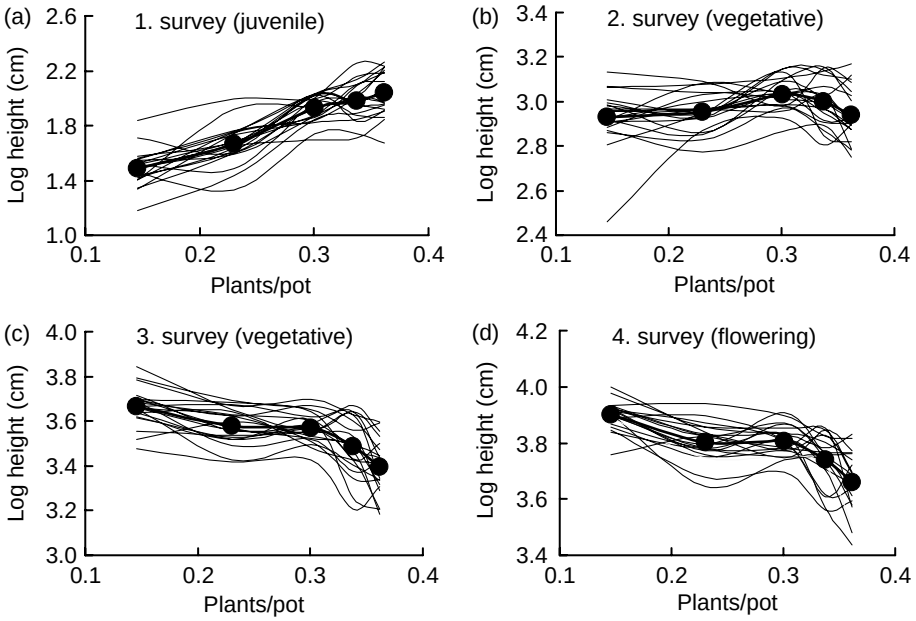

Figure 2. Plant height in relation to plant density for the invasive *Senecio madagascariensis* estimated from 20 blocks during the life-cycle. Block estimates are shown separately and the mean value is indicated in bold, the life stage is in parentheses. Density on the x-axis is $\log_{10}$ ($\log_{10}$(x))-transformed.

production was not just a side effect caused by reduced plant growth with increasing density.

Simulated sampling effort and density dependence

A simulation for *S. madagscariensis* showed that the average deviation for estimates of final shoot mass varied between 1.3–8.5% depending on sampling effort per pot (Fig. 4a). This suggests that the number of individuals sampled per density level in this study (n = 24) produced quite accurate estimates of demographic rates. For the estimates of density dependence, accuracy decreased with the decreasing number of replicates per density level, with the smallest number of replicates producing a deviation of up to 45% for final shoot mass (Fig. 4b).

Discussion

Direction and strength of density dependence

Only half of the 19 examined demographic studies included all life stages from seedlings to adult plants in the analysis of density dependence for plant demographic rates. However, in agreement with findings for both short-lived and long-lived plant species (Goldberg et al. 2001, Howard and Goldberg 2001, Sletvold 2005), our results emphasise the importance of the inclusion of different life stages in the examination of density dependence for demographic rates. Based on observations of multiple life stages during the whole life-cycle for the invasive *Senecio madagascariensis*, we found significant density dependence for seedling emergence, plant growth measured in terms of plant height and shoot mass, flowering probability and fecundity. The only rate unaffected by density was plant survival. A negligible effect on survival may partly be because of favourable growing conditions in the glasshouse, or early harvesting before seed production was complete. On the other hand, high survival has also been detected in the field for early life stages of this species (Radford and Cousens 2000).

Density dependence for demographic rates was negative, except for plant height, in which the direction of density dependence varied from positive to negative during the life-cycle. At the juvenile stage, density facilitated plant growth, whereas at the later life stages density reduced it. This facilitative effect of density on growth may be due to competition for light and space. Avoidance of shadow typically enhances shoot elongation, resulting in taller plants at high than low densities (Dudley and Schmitt 1996).

In the present study, the strength of density dependence tended to increase during the life-cycle, being strongest at the end of the life-cycle when density reduced growth, flowering probability and fecundity. This is not surprising and can be explained by increased competition for space and resources as a large proportion of the plants survived to the adult stage. The strength of density dependence also affected phenotypic differences among individual plants. Among-plant variation in final shoot mass declined with increasing density, suggesting that plants performed equally poorly at high densities due to relative symmetric scramble competition. At lower densities, asymmetric contest competition was more likely as some plants grew considerably better than others. Since asymmetric competition tends to be stronger in natural populations than in experimentally sown populations (Freckleton and Watkinson 2002), our glasshouse experiment may have slightly underestimated the effect of density and variation among individual plants within the density levels. However, the overall impact of density dependence on plant population dynamics depends on the sensitivity of population growth rates to demographic rates in which density dependence operates (Tanner

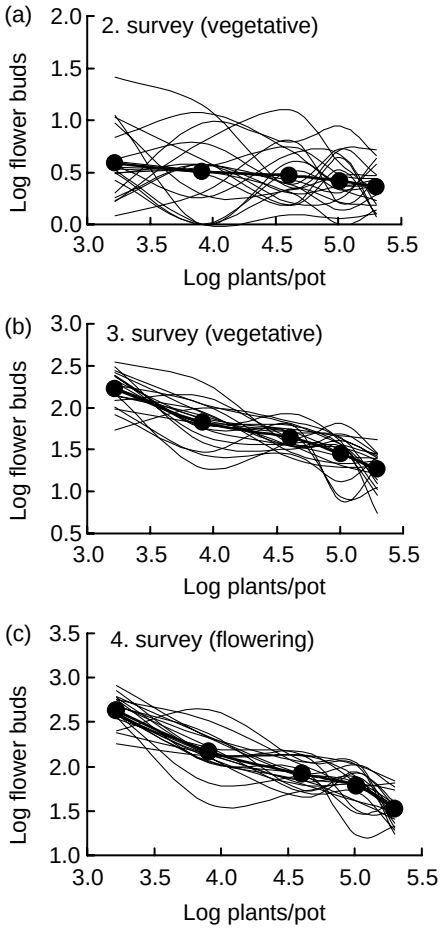

Figure 3. Flower bud production in relation to plant density for the invasive *Senecio madagascariensis* estimated from 20 blocks during the life-cycle. (a) log (buds + 1) = 0.65 − 0.08x, (b) log (buds + 1) = 3.16 − 0.38x, (c) log (buds + 1) = 3.76 − 0.43x. Block estimates are shown separately and the mean value is indicated in bold, the life stage is in parentheses.

1999), and can be examined using simulations with and without density-dependent rates (Tanner 1999, Buckley et al. 2001).

The direction and strength of density dependence both vary in space and time because resources and environmental conditions vary (Goldberg et al. 2001). Density dependence can therefore be expected to remain quite constant if there is little environmental variation (Benton et al. 2004). This was the case for four out of five density-dependent demographic rates here that showed similar direction and strength of density dependence among the blocks. However, the present study also shows that the strength of density dependence may vary even in a constant environment.

Recommendations for parameterisation of density dependence

The literature review revealed that estimates of density dependence were based on a small number of replicated density levels. Moreover, approximately half of the published studies used an observational approach, although such an approach is less effective for detecting density dependence than a manipulative approach (Goldberg et al. 2001, Shima and Osenberg 2003). For instance, Fowler et al. (2006) found that an observational approach failed to detect density dependence for demographic rates of the grass *Bouteloua rigidiseta* despite 14 replicates. Our simulations on the accuracy of density dependence estimates in relation to sampling effort showed that the accuracy of estimates decreased with sampling effort, particularly with the decreasing number of replicates per density level. For the current data set of *S. madagascariensis*, density dependence parameterised from three replicates miss-estimated the final shoot mass by up to 45% even when environmental variation was minimal. Although models of weed population dynamics rarely aim to produce exact predictions of population sizes (Freckleton and Watkinson 2002), accurate estimates of demographic rates are desirable to reduce parameter uncertainty that may have a great qualitative effect on model outcomes (Freckleton et al. 2008). A miss-estimation of a demographic rate by 45% may dramatically affect population growth rate and predictions of future population size, especially when the population is close to zero annual growth (Freckleton et al. 2008). As natural populations may exhibit a considerable amount of environmental variation, small numbers of replicated densities are likely to produce a much greater error for estimates of density dependence than observed in our simulation.

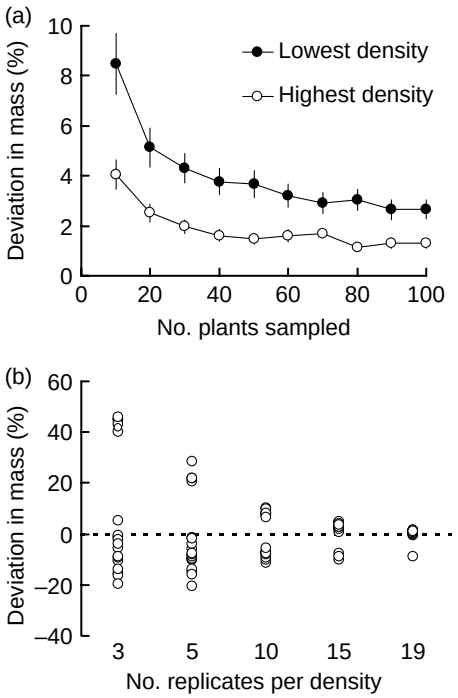

Figure 4. (a) effect of sample size per treatment on the estimates of plant shoot mass for the lowest (25) and highest (200) density levels (mean irrespective of sign ± 95% CI). Deviation is calculated between the sub-sample and the input shoot mass from 100 simulations. (b) effect of the number of replicates on the estimates of density dependence in terms of predicted final shoot mass derived from a regression equation. Deviation is calculated between each sub-sample and the full data set from 20 simulations using the highest density level.

The number of replicated density levels required to reliably estimate the effect of density on plant performance greatly depends on habitat heterogeneity. It is possible for the direction of density dependence to vary within a population depending on, for instance, local water or nutrient availability, or genetic differences among plants. Similarly, the number of individuals required to be sampled for each density level is likely to depend on habitat heterogeneity as well as the size of experimental units (e.g. blocks). The larger the experimental units are, the more individuals probably differ within a density level, increasing sampling effort required per experimental unit. Since larger experimental units are needed for shrubs and trees than herbaceous species, this may mean that shrubs and trees generally require greater sampling effort within experimental units. Due to this strong context-dependence for the effect of density, our aim here is not to provide any numerical recommendations for estimating density dependence but rather to emphasise that attention should be paid to spatial variation within populations and its consequences for parameterisation of density dependence for demographic models. Sampling procedures currently used for estimating the effect of density on plant demographic rates based on one or few replicated density levels are likely to be inadequate, resulting in inaccurate estimates. To better take environmental heterogeneity into account, we recommend distributing replicates across a population to capture a range of density dependence. These multiple and varying estimates of density dependence can then be used for demographic models to examine the sensitivity of model outcomes for the observed range, and potentially presenting outcomes based on the minimum and maximum values.

For plants and other sessile organisms, the strength of density dependence can be expected to increase during the life-cycle because of stronger competition for space and resources as individuals get bigger. This emphasises the importance of observing adult stages for estimates of density dependence. However, other life stages cannot be ignored because density dependence often operates at early stages of the life-cycle (Lintell Smith 1999, Goldberg et al. 2001). To parameterise density dependence for demographic models, observing multiple life stages is essential because density dependence usually has different consequences on population dynamics depending on where in the life-cycle it is operating (Tanner 1999, Buckley et al. 2001). Moreover, multiple replicated density levels are necessary because density estimates derived from unreplicated density manipulations are highly sensitive to the location of each observation on a regression line, and the same is true for observational density studies containing a few density levels.

To conclude, our results indicate that there is no shortcut for parameterisation of density dependence. Therefore, carefully planned data collection including multiple life stages and multiple replicated density levels is required to reduce uncertainty in estimates of density dependence. These estimates can then be incorporated into population models of a given species to predict population performance. By sampling a smaller number of individuals per density level, the number of replicated densities may sometimes be increased without increasing the total sampling effort.

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