

# Propagule pressure, Allee effects and the probability of establishment of an invasive species (*Bythotrephes longimanus*)

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**Abstract.** Predicting establishment of exotic species is a central goal of invasion biology, and is dependent upon propagule pressure and population processes. We introduced invading spiny water fleas, *Bythotrephes longimanus* at different propagule pressures into 19 experimental enclosures, following populations over asexual generations, resting egg production, and emergence in the following year. We integrated experimental results with field data to generate a stochastic population model, predicting establishment in relation to propagule pressure and introduction date. Our results suggested that Allee effects are operational at higher densities or smaller volumes than previously predicted, that stochasticity plays an important role in establishment, and demographic stochasticity may be correlated between individuals. Further, our novel theoretical derivations suggest that organisms should modify their sex ratios to reduce Allee effects. The functional form using adaptive sex-ratios was consistent with both mesocosm and field data. Despite the occurrence of Allee effects and stochasticity, there was still no date during the growing season where we predict lakes to be entirely safe from *Bythotrephes* invasions. A single propagule had approximately a 0.15 establishment probability in our mesocosms, if introduced early in the season; propagule pressures of 10 had > 0.50 probability, regardless of introduction date.

**Key words:** adaptation; Allee effects; exotic species; mesocosm experiment; non-indigenous species; optimality; population dynamics; probability; sex-ratio.

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## INTRODUCTION

Estimating the probability of establishment of non-indigenous species (NIS) is an important component of risk assessments aimed at slowing the spread of biological invaders (Leung et al. 2004, Stohlgren and Schnase 2006). The probability of establishment should be influenced by propagule pressure (number of individuals introduced) (Kolar and Lodge 2001, Lockwood et al. 2005). Therefore, managing the spread of NIS often focuses on reducing propagule pressure to locations through the control of vectors (Lodge et

al. 2006). Understanding how propagule pressure relates to establishment is a logical focus for analysis.

Despite the assumed importance of propagule pressure in invasion biology, there are few experimental tests of the relationship between propagule pressure and the probability of establishment for aquatic invasive species. There is mounting observational evidence that propagule pressure relates to invasions (e.g., Leung et al. 2004, Lockwood et al. 2005, Simberloff 2009). However, propagule pressure is often correlated with other factors such as invader characteristics

and susceptible habitats (Colautti et al. 2006). Large-scale observational studies are useful, but experiments are also needed to control for external factors and examine the important underlying processes.

Only a handful of experiments have investigated the role of propagule pressure on the population growth rates of plants (Von Holle and Simberloff 2005, Edward et al. 2009) and insects (Grevstad 1999, Fauvergue et al. 2007); however, it remains unclear as to how these population growth rates translate into probability of establishment estimates (but see Grevstad 1999). There are a few experimental studies that have examined the role of propagule pressure on population growth rates (Drake et al. 2005) and critical densities for establishment (Bailey et al. 2009) in aquatic systems. However, these studies typically have not followed the entire life cycle of the organisms, and have not examined mating success or recruitment/emergence of offspring (but see Kramer et al. 2008). Recruitment or emergence may be low and mating success could be reduced by Allee effects, which cause small populations to experience reduced or negative per capita growth rates (Courchamp et al. 2008), reducing the probability of establishment. Thus, arguably, it is critical to incorporate stages that act as bottlenecks to establishment into risk assessments, and to augment existing work by explicitly examining the entire life cycle of organisms.

Particularly, dose-response curves describing the relationship between propagule pressure (the “dose”) and the probability of establishment (the “response”), while accounting for all stages of the life cycle, are needed. Such curves are conceptually straightforward, but rarely quantified (Kolar and Lodge 2001), particularly in aquatic systems. With quantified dose-response curves, policy makers could begin to make informed decisions on the benefits of management options that reduce propagule pressure. While several models have evaluated the relationship using parameters from field data for well established populations (Drake et al. 2006, Jerde et al. 2009), the dynamics early in an invasion need to be assessed, because well established populations have clearly overcome any initial dose-response limitation (i.e., Allee effect thresholds).

A powerful approach to understanding and

extrapolating establishment probabilities to different scenarios (e.g., timing of introduction), would be to combine mathematical models with experiments of early dynamics that measure the entire life cycle of an organism. In addition, such analyses could provide insight into fundamental processes of interest to ecologists. Specifically, processes such as Allee effects (Courchamp et al. 2008) and stochasticity, where random population fluctuations may cause local extinctions (Dennis 2002), are of particular interest in invasion biology. As with the relationship between propagule pressure and establishment, there are a growing number of correlational studies demonstrating the Allee effect, but the numbers of experimental ones are rarer (Kramer et al. 2009). Moreover, there is a need to link component Allee effects (i.e., in one phase of the life cycle) with demographic Allee effects, which ultimately affect establishment (Kramer et al. 2009).

Finally, if Allee effects are important, one might expect evolutionary adaptations to help minimize them. Behavioral ecologists have considered aggregation as a method to reduce impacts of Allee impacts (Stephens et al. 1999). However, for mating systems such as polygyny, the primary equations which underlie theoretical models of mate-limited Allee effects have as their central component the numbers of males versus females (Courchamp et al. 2008). While a number of researchers have considered stochastic fluctuations in sex-ratios as a cause of Allee effects (e.g., Bessa-Gomes et al. 2004), here we consider the possibility that organisms modify their sex-ratios in response to changing population densities to reduce Allee effects. Indeed, evidence exists for modified sex-ratio allocations given different environmental conditions and male quality (Trivers and Willard 1973, West et al. 2002), and it seems plausible that some organisms have the capacity to adapt their sex-ratios to population density.

In this manuscript, we used the spiny water flea, *Bythotrephes longimanus* (henceforth *Bythotrephes*), a harmful NIS, as our case study. We investigated the relationship between propagule pressure and probability of establishment. To gain an in depth understanding of the processes underlying this relationship, we examined the following elements: we experimentally controlled

propagule pressure using different stocking rates in mesocosm enclosures; we followed *Bythotrephes* through their entire life cycle—asexual reproduction, sexual mating and the resulting overwintering egg production, and hatching success in the following year; we built a population model and derived novel optimality relationships between sex-ratio and population density, and tested for these relationships using mesocosm and field data; we integrated our population model with results from our enclosure experiments and field data to quantify Allee effects as well as the magnitude and form of stochasticity; finally, we brought together all the above components to estimate the probability of establishment given propagule pressure and introduction date.

## MATERIALS AND METHODS

### Study system

*Bythotrephes* is a harmful aquatic NIS that invaded all the Laurentian Great Lakes of North America in the 1970s and 1980s (Bur et al. 1986), dramatically lowering the biodiversity of pelagic zooplankton (Barbiero and Tuchman 2004, Yan et al. 2002). It has since spread to >150 inland lakes in, and beyond the Great Lakes watershed in Canada and the USA (Therriault et al. 2002, Branstrator et al. 2006, Weisz and Yan 2010). *Bythotrephes* is a cyclic parthenogen, and is therefore of particular concern (Bailey et al. 2009). When species adopt this strategy, they reproduce asexually during favorable periods, thereby increasing density and reducing Allee effects. During periods of stress, they begin to produce males and switch to sexual reproduction, normally as environmental conditions deteriorate in autumn. The animals typically disappear from the water column in the winter, but the resting eggs that were the product of fall sexual reproduction repopulate the water column in the spring. Hence, populations are sustained via these resting eggs (Yurista 1992). While Allee effects are reduced due to parthenogenetic reproduction, they may operate during the sexual phase, and thus the overall consequence of Allee effects on establishment remains unknown. In some invaded areas with high boating traffic, *Bythotrephes* has spread to only roughly 20% of lakes (Weisz and Yan 2010), and

it is not clear why this is the case, especially given that a previous study omitting Allee effects (Drake et al. 2006) has suggested that even 2 propagules should be sufficient for a 90% probability of establishment. Thus, to estimate the establishment risk, researchers should consider the entire life cycle of the organism, the time spent in each mode of reproduction, and the dynamics affecting each mode.

### Data collection

*Propagule pressure experiment.*—The experiment was conducted from August 2008 to June 2009. Enclosures were placed in Fletcher Lake (45°20'56 N, 78°47'51 W), located in Haliburton County of south-central Ontario, Canada. Fletcher Lake is a 266 hectare oligotrophic lake with a maximum depth of 23 m. *Bythotrephes* is established in the lake so our experiments posed no additional risk to the lake.

We used an enclosure experiment because it allowed us to investigate our specific research questions while maintaining real-world environmental conditions and controlling for potential confounding factors (Strecker and Arnott 2005). We employed 6 levels of propagule pressure (1, 2, 4, 8, 16, 32 individuals) with replication at each dose and multiple replicates at low introduction doses to account for the greater effect of stochasticity at low density (1 × 6; 2 × 4; 4 × 2, 8 × 2, 16 × 2; 32 × 3). In total, we analyzed 19 mesocosm populations.

Experimental enclosures were made of clear polyethylene plastic (FilmTech Plastics, Brampton, ON), and were 1 m in diameter by 9 m long, sealed at the bottom, with a total volume of 7068 L. The enclosures extended into the thermocline to allow *Bythotrephes* and other zooplankton to migrate vertically (Young and Yan 2008). The enclosures were raised 30 cm above the water surface to avoid mixing with outside lake water and were covered with 2 mm mesh to avoid colonization by *Chaoborus* spp. larvae and other insects. *Chaoborus* spp. are predatory and may consume *Bythotrephes*. Although predators may influence the establishment of *Bythotrephes*, we restricted our experiments to effects of propagule pressure and mate limitations to isolate potential Allee mechanisms and remain logistically feasible.

Enclosures were filled with water from Fletch-

er Lake that was filtered through a 100- $\mu\text{m}$  mesh to remove all but the smallest zooplankton. The enclosures were subsequently filled with a naïve zooplankton community—one not previously exposed to *Bythotrephes*—collected using a 100- $\mu\text{m}$  mesh net from Troutspaw Lake (45°23'47 N, 78°45'19 W), a nearby uninvaded lake that has similar water chemistry to Fletcher Lake. Because early invasion dynamics are likely important in establishment, using a naïve community, rather than an invaded community, should most similarly mimic a realistic NIS introduction.

Zooplankton community densities were monitored throughout the experiment and additional zooplankton from Troutspaw Lake were added fortnightly to all enclosures, as required to ensure that population levels were maintained at field levels through the experiment; this simulated immigration from other portions of a lake, a normal wind-induced process, as *Bythotrephes* would not decimate a lake zooplankton community early in an invasion.

*Bythotrephes* collected from Fletcher Lake were introduced into the enclosures at prescribed propagule pressures. A representative sample of the population age was used in the experiment to most accurately simulate an introduction event—individuals from the field collection were randomly introduced across treatments. Individuals were first examined for resting eggs. We found none. Thus, all resting eggs produced during the experiment were a result of population dynamics and mating within the enclosures.

In late October, we collected any *Bythotrephes* overwintering resting eggs that had been produced by filtering the entire contents of each enclosure through a 100- $\mu\text{m}$  mesh filter bag and retaining all contents of this as well as the bottom portion of the enclosure. By mid-late October, active populations of *Bythotrephes* have mated and/or died in area lakes, and overwintering resting eggs have been deposited in the sediments (Young 2007).

All *Bythotrephes* tail spines (i.e., caudal processes, Martin and Cash-Clark 1995) and resting eggs that had accumulated in the enclosures were counted. Each individual *Bythotrephes* produces a single tail spine that remains in the sediment after death (Hall and Yan 1997). Thus, the number of spines collected in each enclosure represented the cumulative population size over

the course of the experiment, which was used to derive population growth in the model. Once counted, resting eggs were transferred into environmental growth chambers. Hatching experiments were conducted in the laboratory in order to facilitate monitoring, using established methodology for hatching *Bythotrephes* resting eggs (Brown 2008). Resting eggs were placed in jars covered by 100  $\mu\text{m}$  mesh in groups of 10. The jars were placed in aquaria filled with filtered lake water and were oxygenated throughout the experiment. The aquaria were stored at 4°C in the dark for four months to simulate overwintering conditions in lakes. After this period, the temperature in the chamber was raised to 6.5°C to promote hatching (Brown 2008). Hatching was monitored weekly for four months and the percentage of eggs hatched from each enclosure that had produced resting eggs was calculated.

The following environmental variables did not differ between enclosures: water temperature and dissolved oxygen (profiles taken weekly) chlorophyll-*a* (measured at the beginning, middle and end of the experiment using an 8 m integrated tube sampler); and dissolved organic carbon, phosphorus, pH and conductivity (measured at the beginning and end of the experiment).

#### Population model

*Population dynamical equations.*—We modeled parthenogenetic population dynamics as follows:

$$N_{t+1} = N_t \left( 1 + \frac{b}{m(Z_t)} - d + \varepsilon_{At} \right) \quad (1)$$

where  $N$  is the population size on day  $t$ ,  $d$  is the death rate,  $b$  is the average asexual clutch size,  $\varepsilon_{At}$  causes stochasticity,  $m(Z_t)$  is the maturation rate as a function of  $Z_t$ , the water temperature at time  $t$  (Lehman et al. 1997); thus,  $b/m(Z_t)$  is the asexual reproductive rate. We considered two forms of stochasticity for  $\varepsilon_{At}$ —where each individual within the population varied independently (uncorrelated) and where variation was correlated between individuals. For correlated stochasticity, the random variate  $\varepsilon_{At}$  is chosen from a distribution with variance  $\sigma_A^2$ . In contrast, for uncorrelated stochasticity, the variance was inversely related to population size and  $\varepsilon_{At}$  is chosen using variance  $\sigma_A^2/N_t$ . This is how demographic stochasticity is typically treated

(Lande 1993). We used the empirical pattern of variance in population reproduction from our mesocosm experiment to determine which form of stochasticity dominated.

We assumed that organisms produced asexually to increase their numbers while environmental conditions were favorable. We modeled a switch to sexual reproduction when environmental conditions deteriorated and the asexual population size no longer increased. While this is a simplification given seasonal uncertainties and variation in phenology, it is a reasonable approximation, based on logic and given that we know that sexual reproduction primarily occurs late in the season in well established populations (Young 2007). We used the population size produced from the asexual model (Eq. 1) in an Allee effect equation for polygamous mating systems (Courchamp et al. 2008) to model the sexual production of resting eggs ( $E$ ) at the end of the growing season:

$$E = (c + \varepsilon_s)N_f(1 - e^{-\frac{N_m}{\theta}}) \quad (2)$$

where  $\theta$  is the Allee effect parameter,  $N_f$  and  $N_m$  are the population sizes of males and females at time of reproduction,  $c$  is the average resting egg clutch size, and  $\varepsilon_s$  is a random noise term.  $1 - e^{-\frac{N_m}{\theta}}$  is the probability of each female being fertilized and is dependent on number of males. We examined forms of stochasticity of  $\varepsilon_s$  in a similar way to Eq. 1, using the empirical patterns in egg production to determine if correlated or uncorrelated stochasticity dominated.

*Adaptive response of sex ratios to Allee effects.*—To derive our “adaptive sex-ratio” hypothesis, we began with the Allee model, using the expected dynamics ( $\hat{E}$ ), without stochasticity

$$\hat{E} = cN_f(1 - e^{-\frac{N_m}{\theta}}) \quad (3)$$

The number of eggs produced depends upon the number of females, but the probability of fertilization depends on the number of males. Thus, one can modify the equation to consider the proportion of a population that should be female ( $P_f$ ) (or male:  $P_m = 1 - P_f$ ) to maximize the number of eggs produced.

$$\begin{aligned} \hat{E} &= cNP_f(1 - e^{-\frac{N(1-P_f)}{\theta}}) \\ \frac{d\hat{E}}{dP_f} &= dcNP_f(1 - e^{-\frac{N(1-P_f)}{\theta}})/dP_f = 0 \end{aligned} \quad (4)$$

Solving for  $dE/dP_f$  yields the following optimality expression:

$$e^{-\frac{N}{\theta}}e^{\frac{N}{\theta}P_f} \left(1 + P_f \frac{N}{\theta}\right) = 1 \quad (5)$$

After algebraic manipulation, the optimal ratio of males  $P_m$  to females  $P_f$  is:

$$\frac{N}{\theta}P_m = \ln\left(1 + P_f \frac{N}{\theta}\right) \quad (6)$$

Now, we can numerically determine the sex ratio given population size ( $N$ ) which maximizes egg production. Generally, the optimal proportion of males is a log function of the proportion of females. As  $N/\theta$  approaches zero,  $P_m$  approaches  $P_f$  approaches 0.5, since a general property of the natural logarithm is that  $\ln(1 + x)/x = 1$  as  $x$  approaches zero. As  $N/\theta$  becomes larger,  $P_m$  must grow smaller and  $P_f$  must grow larger to maintain the equality in Eq. 6.

*Model parameterization.*—All individuals produce a single tail spine that sinks to the sediment after death. Thus, we used the number of spines to estimate the total population accumulation and related this to population growth parameters (Eq. 1). Spine accumulation is given by:

$$\begin{aligned} \text{Spines} &= N_T + dN_0 \sum_{i=1}^{T-1} \prod_{j=1}^i \left(1 + \frac{b}{m(Z_j)} - d + \varepsilon_{A_j}\right) \\ N_T &= N_0 \prod_{j=1}^T \left(1 + \frac{b}{m(Z_j)} - d + \varepsilon_{A_j}\right) \end{aligned} \quad (7)$$

The population accumulation equals the population size in the last time interval  $N_T$  plus all individuals that died before the last time interval (death rate  $d$  times the individuals at each time interval) (see Eq. 1 and Table 1 for parameters).  $m(Z_t)$  was calculated daily using  $\ln(1/D) = 0.101Z_t - 5.547$  and  $m(Z_t) = 6.705D \times 1\text{day}/24\text{hr}$ , describing *Bythotrephes* development (Lehman et al. 1997). We used weighted average temperature  $Z$  by depth recorded in the encl-

Table 1. Summary of parameter values used to parameterize asexual and sexual population growth models for *Bythotrephes* introductions.

Parameter	Definition	Value or Equation	Source
$P_m$	Probability of being male	Constant sex-ratio model: $P_m = 0.1$ ; Adaptive sex ratio model: See Eq. 6	Constant sex-ratio model: Harp Lake <i>Bythotrephes</i> data; Adaptive sex-ratio model: Population model
$m(Z_t)$	Maturation rate	see Lehman et al. 1997 for equation details	Temperature data recorded in enclosures per time, using weighted average by depth
$b$	Average asexual clutch size	2.75	Harp Lake <i>Bythotrephes</i> data
$c$	Average sexual clutch size	3.75	Harp Lake <i>Bythotrephes</i> data
$d$	death rate	0.202	Fitted to mesocosm data
$\varepsilon_A$	Stochasticity in asexual growth	$\sigma_A^2 = 0.195$ , uncorrelated stochasticity	Fitted to mesocosm data
$\varepsilon_S$	Stochasticity in egg production	Adaptive sex-ratio model: $\sigma_S^2 = 23.13$ , correlated stochasticity; Constant sex-ratio model: $\sigma_S^2 = 26.11$ , correlated stochasticity	Fitted to mesocosm data
$\theta$	Allee effect term	Adaptive sex-ratio model: $\theta = 11.81$ ; Constant sex-ratio model: $\theta = 4.71$	Fitted to mesocosm data
$\kappa$	Constant scalar for estimate of probability of establishment	See Fig. 3	Fitted to simulated establishments
$\alpha$	Shape parameter for estimate of probability of establishment	See Fig. 3	Fitted to simulated establishments
$\gamma$	Demographic Allee parameter for estimate of probability of establishment.	See Fig. 3	Fitted to simulated establishments

tures.  $b$  was based on *Bythotrephes* field data (Table 1; Young 2007). We simulated 1000 times for each parameter set to fit  $d$  and variance term ( $\sigma_A^2$ ). We used Multiresponse Permutation Procedure (MRPP) (Mielke and Berry 2000) as our metric of fit, which is simply the absolute distance from every observed data point to every simulated point, within each propagule pressure level.

Next, we parameterized the sexual growth model (Eqs. 2–6).  $c$  was based on field data (Table 1; Young 2007). To estimate population size at time of sexual reproduction, we used the best-fitting asexual growth model to generate 1000 estimates that matched our observed spine accumulations closely for each data point (within 5% of observed value). This minimized the unwanted deviations due to dynamics in the asexual phase. For each of the 1000 simulations, we calculated the expected number of resting eggs (Eq. 3). We used maximum likelihood to find the best fitting parameter set ( $\vartheta, \sigma_S^2$ ) based on our model, comparing expected to observed number of resting eggs. We repeated the entire set of simulations for 1) our adaptive sex-ratio model (using Eq. 6 to estimate optimal proportions of males and females) and 2) a fixed sex-ratio (based on *Bythotrephes* field data, Table 1).

### Hypotheses

We hypothesized that 1) Allee effects would be present for sexual reproduction of eggs, as mate limitations could occur; 2) Allee effects would be absent for parthenogenetic reproduction; 3) stochasticity would be uncorrelated between individuals, which is typically how demographic stochasticity is modeled; and 4) the relationship between proportion of males and population size would follow the functional form described by Eq. 6, if sex ratios were adaptive.

We tested for Allee effects and the form of stochasticity using our data on spine and egg production. As mentioned, spine production reflected the cumulative number of individuals that had lived during the experiment. We used per capita growth in our analysis because demographic stochasticity could generate apparent Allee effects in absolute growth (Lande 1993). Thus, for our measures of per capita growth we used number of spines/propagule pressure for parthenogenetic growth and number of eggs/number of spines for sexual reproduction. Because we had data on spines rather than population size per se, we simulated predicted relationships between per capita egg production and spine number with and without Allee effects, and with correlated and uncorrelated stochastic-

ity (Appendix A). We do not present simulations for per capita spine production versus propagule pressure, as these are simply analogous to increasing the time frame of population growth and thus followed general theoretical relationships exactly (see Dennis 2002, Taylor and Hastings 2005). Optimizing sex ratios (Eq. 6) did not change the qualitative patterns (although it affected the quantitative production), and therefore also was not presented.

Generally, if Allee effects are present, we expect per capita growth rate to have a positive slope as population size increases (see Appendix A: Fig. A1 for details; Taylor and Hastings 2005). Our theoretical analysis suggested that in the absence of Allee effects, uncorrelated and correlated stochasticity result in decreasing and equal variance in per capita growth as population size increases, respectively. In the presence of Allee effects, uncorrelated and correlated stochasticity result in equal and increasing variance in per capita growth, respectively (Appendix A).

For our empirical data, we used regression to assess changes in per capita growth versus population size. We used Levene's test to assess changes in variance (Snedecor and Cochran 1989), modified for regression (e.g., Leung and Forbes 1997). Levene's test compares differences in the absolute value of the residuals, using t-test for comparison between groups and regression for continuous variables. It is robust to assumption violations such as deviations from normality.

We examined the relationship between proportion males ( $P_m$ ) versus population size, using regression to compare model predictions (Eq. 6) to observed levels of  $P_m$  in field data (Young 2007). For completeness, we also examined relationships between proportion of males and time (seasonality) to see whether temporal trends could account for the variation in proportion of males.

#### *Probability of establishment model*

We estimated the probability of establishment, operationally defined here as the successful emergence of *Bythotrephes* in the following year, using the population models (Eqs. 1 and 2, parameterized as above) and hatching success from our mesocosm experiments. We simulated introduction events at different times through the season, running 1000 simulations for each intro-

duction date for each propagule pressure between 1 and 32. We described the resultant dose-response curve by fitting a similar functional form suggested by Dennis (2002) and used in Leung et al. (2004):

$$P = \kappa_t [1 - \exp(-\alpha_t N_0^{\gamma_t})] \quad (8)$$

where  $P$  is the probability of establishment,  $N_0$  is the propagule pressure, the subscript  $t$  denotes the date of introduction, and  $\alpha_t$ ,  $\kappa_t$  and  $\gamma_t$  are fitted coefficients.  $\kappa$  allows the possibility that the probability of establishment reaches an asymptote lower than unity.  $\gamma$  describes the demographic Allee effect: no critical threshold exists when  $\gamma = 1$ , and Allee effects become increasingly strong as  $\gamma$  increases. Thus, we can concisely describe changes in the probability of establishment by seeing how the fitted coefficients change with date of introduction.

## RESULTS

### *Enclosure experiment*

Propagule pressure and the number of tail spines produced were positively related ( $r = 0.88$ ,  $df = 17$ ,  $p < 0.001$ , Fig. 1A). Per capita growth rate did not increase with propagule pressure, providing no evidence of Allee effects during parthenogenetic reproduction ( $r = -0.25$ ,  $df = 16$ ,  $p > 0.2$ , Fig. 1B). The per capita relationship was analyzed with the removal of one outlying data point (propagule pressure = 1, #spines = 266; inclusion of outlier:  $r = -0.23$ ,  $df = 17$ ,  $p > 0.2$ ), which did not change the conclusions. The variance in per capita growth rate decreased significantly with propagule pressure ( $r = -0.54$ ,  $df = 16$ ,  $p < 0.02$ , Fig. 1B), indicating that stochasticity was uncorrelated, which we therefore used in our parthenogenetic sub-model. The best fitting parameters were  $\sigma_A^2 = 0.195$ ; the death rate  $d = 0.202$ .

The number of resting eggs increased nonlinearly with spine numbers, with an initial lag phase at low spine numbers (exponential regression:  $r = 0.83$ ,  $df = 17$ ,  $p < 0.001$ , Fig. 1C). Further, the per-capita egg production increased with spine numbers ( $r = 0.51$ ,  $df = 16$ ,  $p = 0.025$ , Fig. 1D). The per capita relationship was analyzed with the removal of one outlying data point (spines = 3, eggs = 4, per capita egg production = 1.33; inclusion of outlier:  $r = 0.15$ ,  $df = 17$ ,  $p >$

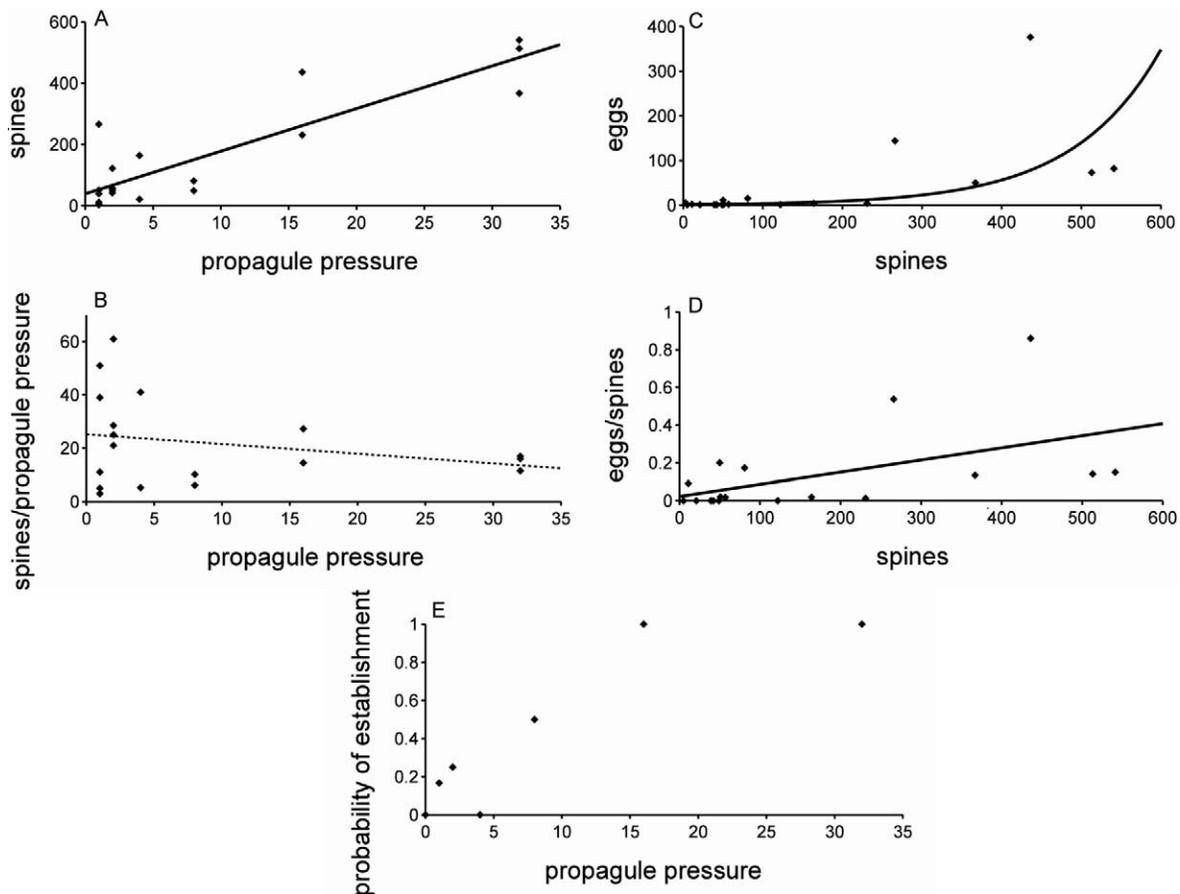


Fig. 1. (A) propagule pressure vs spines ( $r = 0.88$ ,  $p < 0.001$ ). (B) Propagule pressure vs per capita spines ( $r = -0.25$ ,  $p > 0.2$ ). The flat line indicates absence of Allee effects, and the decreasing variance indicates uncorrelated stochasticity. (C) Spines vs eggs ( $r = 0.83$ ,  $p < 0.001$ ), (D) per capita egg production (eggs/spines) versus number of spines ( $r = 0.51$ ,  $p = 0.025$ ). The increasing slope in combination with increasing variance and uncorrelated stochasticity in the parthenogenetic reproduction indicates that Allee effects and correlated stochasticity are present in egg production (compare with panel c in Appendix A: Fig. A1). (E) empirical estimate of probability of establishment (fraction of mesocosms with one of more successfully hatching *Bythotrephes* in the following spring) versus propagule pressure.

0.5). This outlier occurred with our smallest spine count, and could occur if a single female successfully mated (average clutch size = 3.75, Young 2007). The pattern was robust to removal of other data points. We note that it is theoretically possible to obtain a positive relationship for per capita egg production in the absence of Allee effects, if stochasticity was correlated for spine production *but* uncorrelated for egg production (see Appendix A: Fig. A1F). However, in this case, we would expect decreasing variance in per capita egg production as spine number

increased (Appendix A: Fig. A1F). Instead, we found an increasing pattern of variance in per capita egg production (Fig. 1D,  $r = 0.66$ ,  $df = 16$ ,  $p = 0.005$ ). Moreover, the evidence suggested that stochasticity was uncorrelated in spine production, given the decreasing variance in per capita spine production (Fig. 1B). Given these patterns of stochasticity, the increasing slope of per capita egg production, and the accelerating slope in absolute egg production, our results indicated Allee effects were present and that stochasticity was correlated in the sexual phase of *Bythotrephes*

(compare Fig. 1D with Appendix A: Fig. A1C).

Using our model of adaptive sex-ratios, we determined that the best-fitting value for the Allee effects term was  $\theta = 11.81$  and for the correlated stochasticity term was  $\sigma_s^2 = 23.13$ . For constant sex-ratio based on field data, the best fitting parameters were  $\theta = 4.71$  and  $\sigma_s^2 = 26.11$ . The fit of the adaptive sex-ratio model to the observed egg production was better than the constant sex-ratio model, based on Akaike Information Criterion ( $\Delta AIC = 2.86$ ). However, the major consequence of adaptive sex-ratios was to reduce the Allee effect by a factor of 2.5. Both models performed substantially better than excluding the Allee effect (i.e., setting  $\theta$  to a low value) ( $\Delta AIC = 39.69$ ).

Hatching was observed in 8/14 enclosures that produced resting eggs, with 22.8% of eggs hatching overall. The probability of a given egg hatching was not related to propagule pressure ( $r = 0.002$ ,  $df = 12$ ,  $p = 0.99$ ). Overall, 11/19 (58%) of enclosures did not establish; this was strongly related to propagule pressure (Fig. 1E).

#### Population model

The optimal proportion of males ( $P_m$ ) and population size theoretically followed an inverse relationship with population size (Eq. 6; Fig. 2). This functional form was observed in the empirical field data ( $r = 0.43$ ,  $df = 36$ ,  $p < 0.01$ , Fig. 2). The variation in  $P_m$  in the field data could not be explained by time ( $r = 0.22$ ,  $df = 36$ ,  $p > 0.1$ ).

Next, we predicted probability of establishment. Using the optimal sex-ratio model, at propagule pressure of 1, there was a  $\sim 15\%$  chance of establishment, which was fairly con-

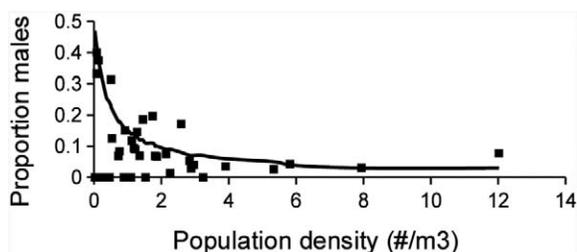


Fig. 2. Proportion of males versus population size. Dots represent empirical data points. The line represents the best-fit theoretical relationship that maximizes egg production using Eq. 6.

stant for introduction before mid-August (Fig. 3). This probability declined to  $\sim 3\%$  later in the season. At propagule pressures above 20, the probability of establishment reached an asymptote at  $\sim 75\%$ – $80\%$  across all simulations (Fig. 3A). Strong Allee effects (inflection points) were detectable later in the season, but were weak early in the season (Fig. 3A). The parameter values that describe the probability of establishment (Eq. 8) changed over the season. Specifically,  $\gamma_t = 0.0122t + 0.99$  (Fig. 3B),  $\alpha_t = -0.0041t + 0.1942$  (Fig. 3C),  $\kappa_t = -0.0008t + 0.7783$  (Fig. 3D), where  $t = 1$  corresponds to August 15. Relations were fit between  $t = 0$  and  $t = 35$  days. Relationships before  $t = 0$  were close to flat and the probability of establishment was nearly constant for a given propagule pressure introduced before August 15 (Fig. 3B–D). After approximately  $t = 35$ , parthenogenetic population growth should no longer be favorable, and mating should begin. The demographic Allee effect parameter ( $\gamma$ ) increased from 1 (no critical threshold) to 1.5, alpha decreased from 0.2 to 0.05, and  $\kappa$  was relatively constant decreasing from 0.78 to 0.75. The probabilities of establishment were very similar between the optimal sex-ratio model and the constant sex-ratio model, due to compensation by a lower  $\theta$  value (Eqs. 2–6). Therefore, we do not present additional figures for the constant sex-ratio model.

#### DISCUSSION

To date, no empirical studies have assessed the relationship between propagule pressure and probability of establishment for *Bythotrephes*, even though it is the world's best studied planktonic invader in freshwaters. Further, despite the relationship being a central assumption in invasion biology, across all invasive species, experiments measuring the entire life cycle of an organism to determine establishment are sparse (but see Grevstad 1999, Kramer et al. 2008), although a handful of studies have conducted insightful experiments using portions of species life cycles (e.g., Von Holle and Simberloff 2005, Bailey et al. 2009). Thus, it is not surprising that dose-response relationships have rarely been quantified (Kolar and Lodge 2001). Yet, such dose-response relationships are critical for invasive species policy, to estimate the benefits of

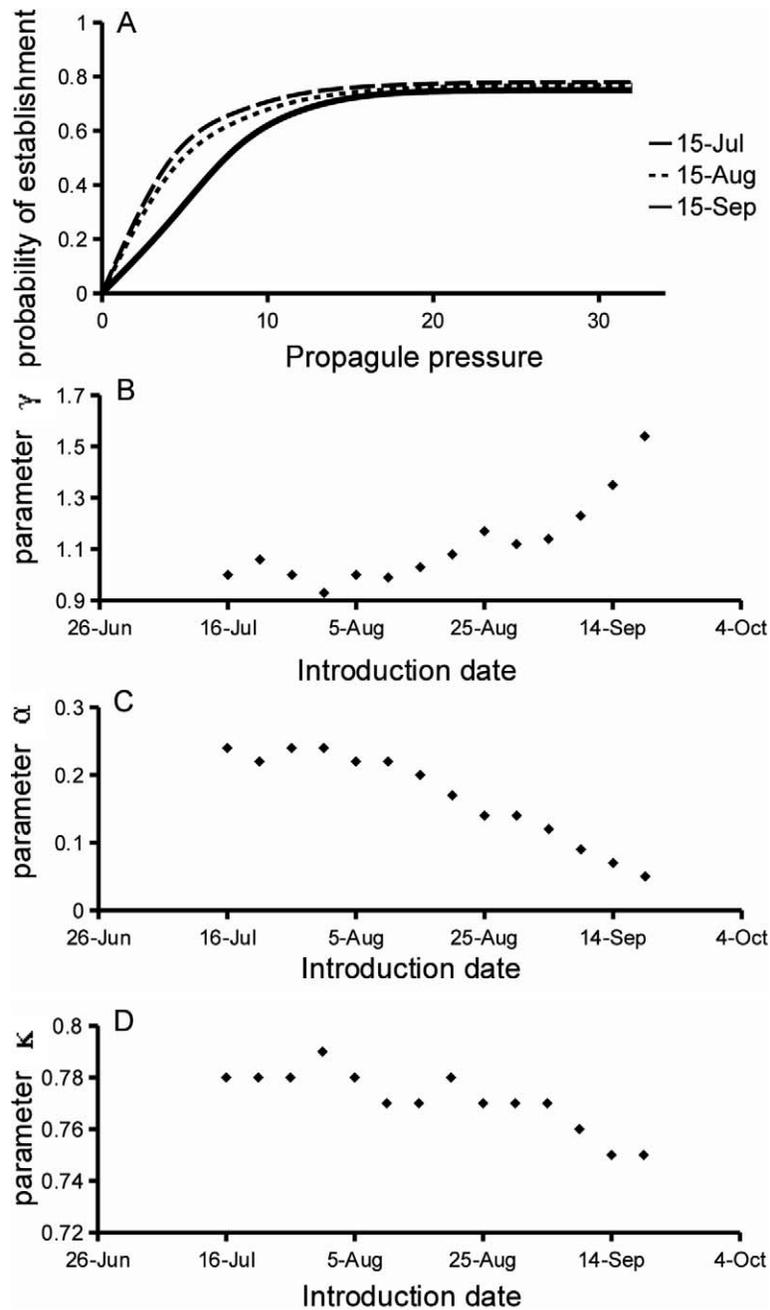


Fig. 3. Probability of establishment over time based on population model. (A) probability of establishment versus propagule pressure are shown for three time intervals. The curve does not change much before Aug 15. (B–D) changes over time in parameter values that describe the relationship between probability of establishment and propagule pressure (Eq. 8).  $\gamma$  (B) shows the change in the Allee effect parameter, and is therefore the most interesting parameter for the purposes of our study, where  $\gamma$  values greater than 1 denote a critical threshold and an increasingly strong Allee effect. Parameter  $\alpha$  (C) is a scaling parameter affecting the rate of increase in the probability of establishment, and  $\kappa$  (D) permits an asymptote.

management that reduces propagule pressure. As a field, we need to begin to develop a baseline of dose-response relationships across species and conditions, using complementary approaches such as experiments, correlational studies, and models to truly develop a quantitative predictive science, and to better understand the important processes underlying establishment.

Allee effects are putatively one of the most important processes determining biological invasions, and there is interest in determining when they should occur and how strong they should be (Taylor and Hastings 2005). However, empirical evidence is currently lacking for organisms such as *Bythotrephes*, despite the noted ecological relevance of such invaders. Intuitively, it may not be expected that *Bythotrephes* would experience Allee effects because they reproduce parthenogenetically through most of the season, a strategy which should reduce the magnitude of an Allee effect. However, given that sexual reproduction is a required part of *Bythotrephes*' life cycle, and that without it, an invasion cannot be sustained, an examination of the occurrence and magnitude of Allee effects is merited. We showed that Allee effects were detectable in our mesocosms and influenced the probability of establishment. For comparison, the only other model of *Bythotrephes* that also considered Allee effects suggested that a critical threshold density of  $1.0 \times 10^{-3}$  ind/m<sup>3</sup> were required for establishment (Drake 2004). Drake (2004) extended previous models to incorporate parthenogenetic life cycles, and estimated probability of mating theoretically using factors such as swimming speed, assumed detection radius and duration of the mating phase. In our study, we found Allee effects even though the propagule pressure density introduced into our mesocosms ranged from 0.14 ind/m<sup>3</sup> (1 animal) to 4.5 ind/m<sup>3</sup> (32 animals) and were orders of magnitude higher than the theoretical critical threshold density. Given parthenogenetic population growth, densities were even higher during the sexual mating phase. Thus, our experiments demonstrated that Allee effects were operational at much higher densities and/or much smaller volumes than previously expected.

We argue that the mechanism underlying the Allee effect was mate finding and that this component Allee effect would result in a demo-

graphic Allee effect. Other factors such as predation, which could cause an Allee effect by itself (Courchamp et al. 2008) should not reduce the Allee effect we observed but could increase it. Population factors such as carrying capacity could negate component Allee effects; however, this is unlikely to do so in our study given densities of *Bythotrephes* in established population are higher than those observed in our study (Young 2007), and given that we were primarily interested in the early establishment phase when densities are low. Finally, we examined the entire life cycle of *Bythotrephes*, such that we know how mate limitations translate into demographics in the following generation.

Mate limitation was the most plausible explanation for the Allee effects observed. Predators were excluded, and Allee effects were unlikely to be due to simple demographic stochasticity (Lande 1993), given the patterns in per capita egg production observed (Dennis 2002), and given that fitting the Allee parameter yielded a substantially stronger fit to the egg production data than the stochasticity term alone ( $\Delta AIC = 39.69$ ). As another mechanism, authors have argued that stochasticity in sex ratios could cause Allee effect (Bessa-Gomes et al. 2004). Although this process could result in Allee effects, based on simulations it was unlikely to have produced the magnitude of the Allee parameter ( $\theta$ ) observed in our study (see Appendix B for details). Thus, of the known processes causing Allee effects (Stephens et al. 1999), difficulty in mate finding appears to be the most parsimonious option explaining our results.

Given the occurrence of mate-finding Allee effects in *Bythotrephes*, it is logical that *Bythotrephes* might have life history adaptations to minimize them. Although we do not understand the physiological mechanism, we know that *Bythotrephes* have some capacity for sex-determination. Thus, we argue that organisms may modify their sex-ratios to lessen Allee effects, although removing Allee effects entirely may not be possible. This sex-ratio/density relationship was derived theoretically, resulted in a better fitting model to the egg production data in the mesocosm experiments, and was consistent with empirical field data on proportion of males (which could not be explained by seasonality). However, researchers should conduct additional

experimental studies to further explore this relationship. Yet, if our hypothesis is true, this process could be important for population dynamics and the probability of establishment. Such adaptive mechanisms are also interesting from an evolutionary perspective, affecting invasion dynamics over time (Sakai et al. 2001). For instance, in new habitats, environments might differ, changing the optimal sex-ratio (e.g., differences in visibility affecting the probability of finding a mate). Assuming spatially correlated environments, if organisms can adapt appropriate sex-ratio allocations to the new habitat, Allee effects would lessen and the rate of invasion would accelerate.

Another important consideration was the form of stochasticity. Previous researchers have shown that stochasticity should affect the probability of establishment, and have made distinctions between environmental and demographic stochasticity (Dennis 2002). Demographic stochasticity is often modeled as uncorrelated noise between individuals and environmental stochasticity is modeled as correlated noise, i.e., environmental fluctuations affect the entire population (Dennis 2002). However, in our system, stochasticity was uncorrelated for parthenogenetic reproduction but was correlated for egg production, even though we controlled for environmental differences in our mesocosm experiment. Thus, both correlated and uncorrelated stochasticity most likely arose due to demographic processes. We hypothesize that this occurred because individuals were strongly related to one another—populations were founded by relatively few individuals and they reproduced parthenogenically. Thus, large portions of the population shared similar traits. This effect could be generally important for invasive species, where populations are founded by relatively few individuals. However, while the inference of correlated stochasticity was consistent with theoretic simulations, was statistically significant, and is the most parsimonious interpretation, we note that it was driven by relatively few data points and that the hypothesis was generated a posteriori, and therefore requires further experimental study.

In combination, we found that both Allee effects and stochasticity were important for establishment. A previous model of *Bythotrephes*,

based on demographics of established populations, suggested that stochasticity was likely not important, and that even 2 individuals would be sufficient for 0.90 probability of establishment (Drake et al. 2006). In contrast, we found a substantially lower probability of establishment of 0.25 for 2 introduced individuals, in both the empirical results and the population model, depending on the time of introduction. The Allee effect accounted for part of this discrepancy, but even in its absence, our estimated probability of establishment was substantially lower, even under the permissive conditions of the mesocosm experiments. As for the Allee effect, our model predicted a demographic Allee parameter ( $\gamma$ , Eq. 8) ranging from 1 (no critical threshold) to 1.5 later in the season. We note that in nature, Allee effects could be even stronger, as there are many additional factors that might be important. Particularly, clearly our mesocosms were much smaller in volume than entire lakes. It is unknown what metric of density or population size is the most relevant. On the one hand, dispersal can be important (Taylor and Hastings 2005), arguing for some average lake-wide density. On the other hand, organisms often aggregate such that the effective density is much higher than expected (Stephens et al. 1999). For *Bythotrephes*, we know that aggregation occurs in the field (Petruniak 2009), due to physical forcings (e.g., wind and waves) and potentially also organism behavior. As such, while clearly density is relevant, absolute numbers may be equally relevant, particularly early in an invasion when a number of individuals may have been released in a localized area. Regardless, regional correlational models have used similar functional forms (Eq. 8) to examine the probability of establishment (e.g., Leung et al. 2004, Leung and Mandrak 2007), and it would be interesting to compare the strength of the Allee effects estimated using different approaches at different scales.

More specifically for our study system, before mid-August, the probability of establishment was largely constant with respect to introduction date, and was dominated by stochasticity and extinction during the parthenogenetic phase rather than by mate limitations. Roughly around September 15, mortality rates should outpace maturation and asexual reproduction and *Bytho-*

*trephes* should switch predominantly to sexual reproduction. Of course, this is a simplification in our model; in reality, there is variance in phenology and from an evolutionary standpoint, it could be interesting to consider adaptation in novel environments and the optimality of this variance, given environmental uncertainty. Nevertheless, later in the season, the probability of establishment will likely be determined by the chance that introduced females already carry resting eggs, rather than by the population dynamics in the introduced system. Thus, the seasonal changes in probability of establishment due to population dynamics occur in a relatively short window between August 15 and September 15, in our study system. Researchers interested in studying phenomenon such as Allee effects should consider their time interval of analysis.

#### Conclusions and ramifications

We conducted one of the only experimental manipulations across the entire life cycle of an invasive species, to estimate the relationship between propagule pressure and probability of establishment, and explore important mechanisms underlying the relationship. First, we observed evidence of Allee effects, and suggest that mate limitations occur at higher densities/smaller volumes than previously calculated. We expect that the strength of Allee effects should strengthen over the season, and a critical threshold may only be detectable late in the season. Second, the available evidence suggests that *Bythotrephes* may adaptively determine their sex ratios to minimize Allee effects, affecting population dynamics and increasing the probability of establishment. Third, our results suggest that stochasticity can be correlated for purely demographic reasons, and we cannot necessarily assume that demographic stochasticity becomes unimportant as population size increases. This may be especially important for biological invasions and endangered species, where founder effects are strong and individuals are genetically related. Thus, our work suggests potentially important processes that should be considered to estimate the probability of establishment and invasion success.

Although Allee effects and stochasticity both had a greater effect than expected and reduced

the probability of establishment, there was still no time period during the growing season that would be obviously safe from invasion by *Bythotrephes*. A single introduced propagule ( $=0.14$  individuals/m<sup>3</sup>) could be sufficient to result in an invasion 15% of the time. With a propagule pressure density of 1 individual/m<sup>3</sup> (or 7 individuals), both the empirical results and model simulations indicated 40–60% establishment success, depending on the timing of introduction. We note however, that other factors such as predation, environmental conditions, dispersal and propagule viability could be important in natural systems. Further experiments could assess the contribution of these elements.

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## APPENDIX A

### *Theoretical patterns in per-capita egg production*

To generate theoretically expected patterns in per-capita egg production versus spines production (a measure of the cumulative population size, or number of individuals that had lived), we varied parameters randomly - net asexual reproductive rate between 0 and 0.2, death rate between 0% to 100%, stochasticity ( $\sigma^2_A$  and  $\sigma^2_s$ ) between 0 and  $2 \times$  net reproductive rate,

proportion of females between 0.5 and 0.99, the Allee term ( $\theta$ ) between 0 and 50, and propagule pressure introduction between 1 and 32 (as in our experiment). We ran the parthenogenetic reproduction for 10 time intervals. We examined 100 simulations for each scenario to assess the general patterns produced, and we presented typical patterns for visualization (Fig. A1).

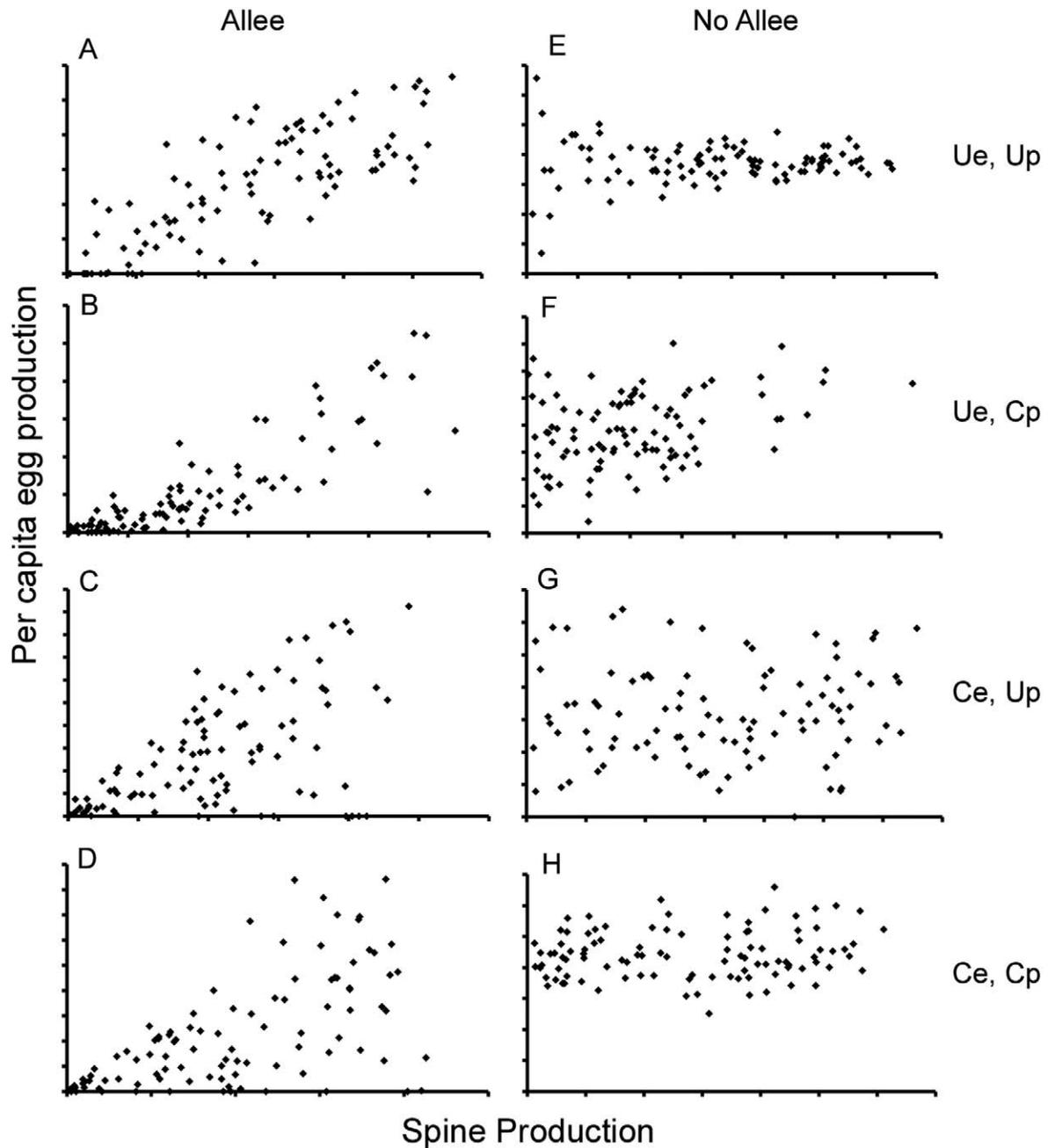


Fig. A1. Examples of expected patterns for the relationship between per capita egg production (estimated by eggs/spines) versus number of spines, for different scenarios: all combinations of Allee effect absent and present, uncorrelated stochasticity for parthenogenetic reproduction and for egg production, and correlated stochasticity for parthenogenetic reproduction and egg production. Generally, Allee effects result in a positive relationship (panels A–D). With any correlated stochasticity in either parthenogenetic reproduction or egg production, there is a tendency to have increasing variance (B–D). When all stochasticity is uncorrelated, variance does not change (A). In contrast, when Allee effects are absent, uncorrelated stochasticity in egg production results in reduced variance with spine number (E, F), whereas correlated stochasticity in egg production results in equal variance in egg production (G, H). Generally, per capita growth does not increase (E, G, H); however, with correlated stochasticity in

←Fig. A1. continued.

parthenogenetic reproduction and uncorrelated stochasticity in egg production, there is a tendency for per capita egg production to increase with spine number (F). This is because spines measure cumulative population size. A positive slope in per capita egg production due to Allee effects (A–D) can be distinguished from non-Allee scenario (F) by considering the stochasticity—with Allee effects, variance is equal or increasing (panels A–D), whereas it is decreasing in panel F.  $U_e$  = uncorrelated variance in egg production,  $C_e$  = correlated variance in egg production,  $U_p$  = uncorrelated parthenogenetic variance,  $C_p$  = correlated parthenogenetic variance.

## APPENDIX B

### Sex-ratio models

Our results suggested that Allee effects were present ( $\theta = 11.81$  for the optimal sex ratio model, and  $\theta = 4.71$  for the constant sex-ratio model). While implicitly this was due to mate finding, it was conceivable that stochasticity in sex-ratios, another mechanism of Allee effects, might result in similar patterns. We evaluated whether stochasticity in sex ratios could account for the strength of Allee effect observed in our mesocosm study, using a simulation approach.

If mate finding were not a problem in our mesocosms (volume =  $7\text{m}^3$ ),  $\theta$  would approach zero. However, Allee effects could occur if, due to

random chance, there was only a single sex or if there were few females. We generated stochasticity in sex ratios using a Bernoulli process, with an underlying probability of being female ( $P_f$ ). *Bythotrephes* are typically female biased, with average  $P_f$  values of 0.9 ( $P_m = 0.1$ , Table 1). We examined a range of  $P_f$  values from 0.7 to 0.95, and a range of our stochasticity parameter from  $\sigma^2 = 5$  to  $\sigma^2 = 30$  for a total of 2400 simulations. We found that stochasticity in sex ratios could not account for the strength of Allee effects observed ( $\theta$ , Fig. B1). Thus, of the known processes causing Allee effects (Stephens et al. 1999), difficulty in mate finding appears to be the most parsimonious option.

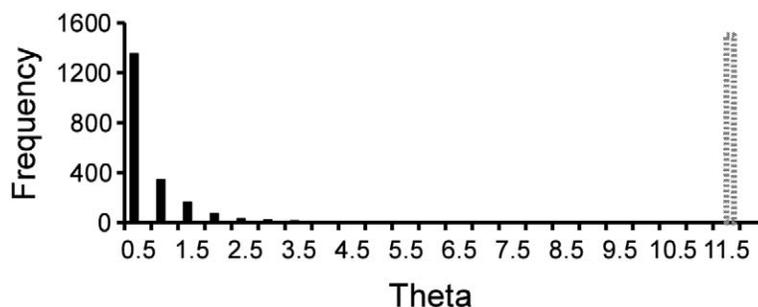


Fig. B1. The magnitude of Allee effect terms ( $\theta$ ) that can be generated from stochasticity in sex-ratios alone. Solid bars represent frequency of  $\theta$  values generated from simulations with stochasticity in sex-ratios, but no mate finding limitations. Dotted line represents  $\theta$  estimated from empirical data ( $\theta = 11.8$ ). Allee parameter estimated from empirical data did not overlap with any of the simulations, indicating that the observed Allee effects were not due to sex-ratio alone.