ALLEE EFFECT LIMITS COLONIZATION SUCCESS OF SEXUALLY REPRODUCING ZOOPLANKTON

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Abstract. Understanding the dynamics of populations at low density and the role of Allee effects is a priority due to concern about the decline of rare species and interest in colonization/invasion dynamics. Despite well-developed theory and observational support, experimental examinations of the Allee effect in natural systems are rare, partly because of logistical difficulties associated with experiments at low population density. We took advantage of fish introduction and removal in alpine lakes to experimentally test for the Allee effect at the whole-ecosystem scale. The large copepod *Hesperodiaptomus shoshone* is often extirpated from the water column by fish and sometimes fails to recover following fish disappearance, despite the presence of a long-lived egg bank. Population growth rate of this dioecious species may be limited by mate encounter rate, such that below some critical density a colonizing population will fail to establish. We conducted a multi-lake experiment in which *H. shoshone* was stocked at densities that bracketed our hypothesized critical density of 0.5–5 copoepods/m^3. Successful recovery by the copepod was observed only in the lake with the highest initial density (3 copepods/m^3). Copepods stocked into small cages at 3000 copepods/m^3 survived and reproduced at rates comparable to natural populations, confirming that the lakes were suitable habitat for this species. In support of mate limitation as the mechanism underlying recovery failure, we found a significant positive relationship between mating success and density across experimental and natural *H. shoshone* populations. Furthermore, a mesocosm experiment provided evidence of increased per capita population growth rate with increasing population density in another diaptomid species, *Skistodiaptomus pallidus*. Together, these lines of evidence support the importance of the Allee effect to population recovery of *H. shoshone* in the Sierra Nevada, and to diaptomid copepods in general.

Key words: Allee effect; alpine lakes; copepod; Hesperodiaptomus shoshone; *inverse density dependence; mesocosm experiment; reintroduction; Sierra Nevada, California, USA.*

INTRODUCTION

Understanding the dynamics of populations at low density has become a priority, due to concern about the viability of populations of rare and endangered species, and the importance of such dynamics to more general population processes such as colonization. As a result, inverse density dependence, commonly referred to as the Allee effect (Allee et al. 1949, Courchamp et al. 1999), is increasingly recognized as an important phenomenon in natural systems (Courchamp et al. 1999, Stephens and Sutherland 1999). Several mechanisms can result in the Allee effect, including mate limitation and obligate cooperation (Allee et al. 1949, Odum 1959, Courchamp et al. 1999), with two related consequences. The first is a decrease in population growth rate as population density declines to low densities. When severe, this decrease in growth rate can result in the second

consequence, a minimum (critical) density below which the population declines to extinction (Courchamp et al. 1999). Evidence from observational studies supports both decreased growth rate and the existence of critical densities in natural populations (Veit and Lewis 1996, Kuussaari et al. 1998, Morris 2002, Serrano et al. 2005, Stoner and Ray-Culp 2005), and theoretical models have been developed to explore the impact that the Allee effect can have on population persistence (Boukal and Berec 2002, Dennis 2002, Liebhold and Bascompte 2003, Calabrese and Fagan 2004), metapopulation dynamics (Amarasekare 1998, Brassil 2001, Martcheva and Bolker 2007), species invasions (Cruikshank et al. 1999, Taylor and Hastings 2005, Drake and Lodge 2006), and predator–prey dynamics (Kent et al. 2003).

Despite long-standing interest in Allee effects, manipulative experiments in natural systems are rare. A few researchers have examined the Allee effect in plants, finding that low density can threaten population persistence due to insufficient pollination (Lamont et al. 1993, Hackney and McGraw 2001) or reduced competitive ability (Cappuccino 2004). We are aware of only two field experiments on animals that have tested

the existence of a critical density for population establishment, both in insect populations (Campbell 1976, Berggren 2001). Even in the lab, Allee effects in animals have only been demonstrated experimentally a few times (Park 1933, Sakuratani et al. 2001, Noel et al. 2006).

A unique opportunity to experimentally examine Allee effects in natural ecosystems arose in the context of fish introduction to, and removal from, historically fishless alpine lakes. Introduction of nonnative fish to alpine lakes typically results in the local extinction of large-bodied zooplankton. In some cases, cessation of fish stocking or active fish removal has returned lakes to a fishless state (Knapp et al. 2001b, Parker et al. 2001). Following the removal of fish from alpine lakes in California's Sierra Nevada mountains (USA), the diaptomid copepod *Hesperodiaptomus shoshone* sometimes fails to recover despite the recovery of the co-occurring cladoceran, *Daphnia melanica* (Sarnelle and Knapp 2004). Both *Daphnia* and *Hesperodiaptomus* have long-lived egg banks (Hairston and De Stasio 1988, Parker et al. 2001, Sarnelle and Knapp 2004) from which colonists can hatch after the lake reverts to a fishless state, but they differ in their mode of reproduction. Unlike *Daphnia,* which are capable of asexual reproduction, diaptomid copepods are obligately sexual and do not store sperm (Watras 1983). Moreover, as zooplankton, they inhabit a three-dimensional bounded habitat that is very large relative to their body size and mobility. It follows that if densities are low, the probability of male–female encounter may be too low to sustain the population, resulting in an Allee effect via mate limitation. As a result, *H. shoshone* would need a larger number of colonists in a given year, relative to *Daphnia*, to successfully re-establish after extirpation from the water column (Sarnelle and Knapp 2004).

Because copepod populations in some Sierra Nevada alpine lakes have failed to recover following fish removal, these systems are ideal for testing the mate limitation hypothesis at the ecosystem scale, via whole-lake reintroduction experiments. Our study used a four-tiered approach to examine the Allee effect in diaptomid copepods. First, we conducted a series of whole-lake reintroductions, using multiple lakes from which *H. shoshone* had been extirpated, to test the effect of initial density on population recovery. Second, we quantified mate limitation and its relationship to density in both experimental and unmanipulated lakes. Third, we used an in situ caging experiment to determine whether the experimental lakes were suitable habitat for *H. shoshone* survival and reproduction in the absence of mate limitation. Fourth, we conducted a replicated mesocosm manipulation of initial density using *Skistodiaptomus pallidus*, to allow for stronger statistical inference than is possible in whole-lake experiments. The mesocosm experiment also enabled us to better quantify the relationship between population growth rate and density in copepods.
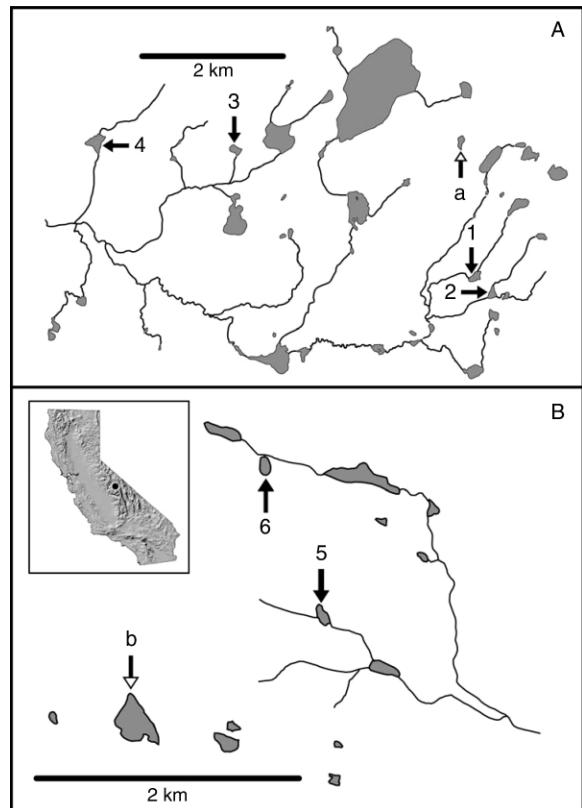

FIG. 1. Map of the lakes used in the whole-lake copepod reintroduction experiment (see inset map for location in California, USA). (A) Humphreys Basin is on the west side of the Sierra Nevada crest in the Sierra National Forest. Locations are: a, Dissertation Lake (source of *H. shoshone* used in reintroductions); 1, No Good Lake; 2, Cony Lake; 3, Square Lake; 4, Knob Lake. (B) Morgan Creek Basin is located on the east side of the crest in the Inyo National Forest and is 12 km north of Humphreys Basin. Locations are: b, Spire Lake (source of *H. shoshone* used in reintroduction); 5, Finch Lake; 6, Middle Morgan Lake.

METHODS

Hesperodiaptomus shoshone is a large (>2 mm body length) pigmented diaptomid copepod that occurs in alpine lakes in the Sierra Nevada and the Rocky Mountains from Colorado to British Columbia. *H. shoshone* is susceptible to local extinction after fish introduction, and is largely restricted to fishless lakes (Knapp et al. 2001b). Using paleolimnological techniques (Knapp et al. 2001a), we identified six historically fishless lakes in two basins (Fig. 1, Table 1) from which *H. shoshone* had been extirpated from the water column by the introduction of nonnative trout (*Oncorhynchus mykiss, Salvelinus fontinalis*) (Knapp et al. 2001a; R. A. Knapp, *unpublished data*). Intensive sampling using vertical tows of a 1 m diameter zooplankton net (250 μm mesh) detected no *H. shoshone* in any of the lakes prior to our reintroductions (detection limit = 0.009–0.015 individuals/m³). Viable *H. shoshone* diapausing eggs were present within 5 cm of the sediment surface in

TABLE 1. Lake morphometry and fish history for the experimental (E) and source (S) lakes.

Lake	Altitude (m)	Surface area (ha)	Volume (m³)	Mean depth (m)	Maximum depth (m)	Fishless since	Fish residence time (yr)
Square (E)	3443	1.71	30 282	1.8	3.5	1997	53
No Good (E)	3516	1.67	30 939	1.9	5	2002	63
Knob (E)	3358	3.39	65 336	1.9	5.5	2001	63
Cony (E)	3492	1.43	17 349	1.2	3.3	2002	63
Finch (E)	3254	1.14	29 543	2.6	7.1	1972	20
M. Morgan (E)	3321	0.57	17 656	3.1	5.5	1976	56
Dissertation (S)	3602	1.08	ND	ND	7	no fish	NA
Spire (S)	3523	6.69	ND	ND	30	fish present	54

Note: Key to abbreviations: ND, no data; NA, not applicable.

only No Good Lake (Fig. 1A), deeper sediments have not been sampled (Sarnelle and Knapp 2004; R. A. Knapp, *unpublished data*). The lakes upstream of Cony and No Good Lakes (Fig. 1A) both contain established populations of *H. shoshone*.

Whole-lake experiment

We stocked lakes with *H. shoshone* at densities (Table 2) that bracketed our estimates of the critical density for population establishment (0.5–5 copepods/m³ [Sarnelle and Knapp 2004]). Estimates of critical density were calculated using Gerritsen's (1980) encounter model and estimates of *H. shoshone* population growth rate, length of reproductive season, encounter radius, and swimming speed. Our estimates of critical density are slightly less than the minimum natural density observed in a survey of ~100 Sierra Nevada lakes harboring *H. shoshone* (6 copepods/m³ [Sarnelle and Knapp 2004]). Given the logistic hurdles associated with stocking whole lakes with up to 300 000 copepods, imprecision in the actual number stocked was expected. Consequently, we chose widely spaced target densities with replicate treatments at the intermediate densities closest to the estimated critical density (Table 2).

Late-stage *H. shoshone* copepodites (pre-adults) were collected from Dissertation and Spire Lakes (Fig. 1, Table 1) using vertical tows of a 1 m diameter zooplankton net with 350 μm mesh. *Hesperodiaptomus shoshone* are univoltine and emerge from the egg bank synchronously (Kramer 2007) so we were able to collect large numbers of animals at a similar life-history stage in a short period of time. The collected animals were transferred to a bucket that was mixed, subsampled to

estimate density, and distributed to a set of plastic containers at a density of ~2000 individuals/L. The containers were kept cold by covering them with snow, and then transported via a combination of helicopter and backpack. Time between collection and release of animals ranged from two to six hours and was unrelated to treatment (Table 2). The longest transport time was for Square Lake, which was stocked at the highest density. Upon delivery, animals were released throughout the central (deepest) part of the lake by emptying the transport container just below the water's surface while slowing paddling in an inflatable raft. Animals were observed swimming in the containers prior to release and in the water following release.

Treatment lakes were intensively sampled for three summers following the reintroductions. Sampling was conducted using vertical tows with the 1 m diameter zooplankton net (350 μm mesh). Detection limits, calculated as the inverse of the total volume of water sampled during two to six visits per lake each summer, were always ≤0.005 copepods/m³ (Table 3). Effort was focused on sampling the deepest area of the lake to minimize disturbance of sediment and enable detection of vertically migrating *H. shoshone*. Horizontal tows and tows in shallow areas were also conducted on at least one occasion in each lake to ensure animals were not aggregating in under-sampled areas. *H. shoshone* were never observed in these samples. Given that individual samples (all tows from a particular lake and date combined) often contained few animals, density is reported as mean annual density, the total number of individuals collected in a lake divided by the total volume sampled in that year. Because of the logistical

TABLE 2. For the six experimental lakes, target stocking density, number of copepods (*H. shoshone*) stocked, source of stocked animals, transport duration, date of stocking, and observed density two weeks following stocking.

Lake	Target density (m⁻³)	No. copepods	Source of stocked animals	Transport duration (h)	Date stocked†	Observed density (m⁻³)
Square	10	300 000	Dissertation	6	14 July	3.9
No Good	1	31 000	Dissertation	2	17 July	0.13
Knob	1	66 000	Dissertation	5	14 July	0.08
Cony	0.1	1800	Dissertation	4.5	14 July	0.05
Finch	0.1	3000	Spire	2.5	24 July	0.02
M. Morgan	0.01	180	Spire	3	24 July	not sampled

† All dates are in 2003.

TABLE 3. The volume of water sampled and the total number of copepods (*H. shoshone*) collected in each lake during the four years of the reintroduction experiment.

Lake	2003		2004		2005		2006	
	Volume (m³)	No. collected	Volume (m³)	No. collected	Volume (m³)	No. collected	Volume (m³)	No. collected
Square	139	358	130	456	90	221	43	275
No Good	550	79	882	81	593	3	446	2
Knob	766	49	413	0	363	0	427	0
Cony	580	15	311	0	284	2	280	3
Finch	368	5	300	0	317	0	232	0
M. Morgan	unsampled	unsampled	198	0	214	0	211	0

challenges of intensively sampling remote lakes, we did not sample Middle Morgan Lake in 2003, and instead focused our sampling effort in the first year on the five lakes where we expected *H. shoshone* to be detectable (Table 3). Additionally, an estimate of *H. shoshone* density in Square Lake in 2007 was calculated from two samples (30 cm diameter net, 64 um mesh, 2 tows/sample) taken in August during regular monitoring of the lake. The total volume sampled was 0.85 m³.

Mating success

Copepods sampled from the experimental lakes, in situ cages (see *Caging experiment*) within the experimental lakes, and eight unmanipulated lakes (Appendix) were examined to assess female mating success in each population. This sampling was conducted in 2003–2005 and included the lakes used as source populations for the experiment. Mating success can be assessed in calanoid copepods by observing the reproductive condition of females (Williamson and Butler 1987). When female copepods are gravid, their ripened ovaries become dark and clearly visible (Watras and Haney 1980). After mating, the fertilized eggs are extruded into an external egg sac that is carried by the female for several days. Thus, gravid females that have not yet mated can be distinguished by their dark ovaries and lack of external eggs, and a high proportion of unmated, gravid females provides an indication of low mating success (i.e., potential mate limitation of reproduction).

Adult female copepods in each sample were scored as non-gravid, non-gravid with external egg sacks, gravid with external egg sacks, or gravid without external egg sacks, and mating success was calculated as the ratio of the number of females with external eggs (i.e., mated females) to the total number of reproductive (i.e., gravid and/or egg-bearing) females. To maximize confidence in our estimates of mating success, each sample was examined live with a hand lens at the time of collection, then preserved and re-examined under the microscope. Live examination was necessary because some females drop their eggs following preservation, making it impossible to determine whether unattached eggs observed in the preserved sample were carried by gravid or non-gravid individuals. Counts from live and preserved animals were compared, and there was a close correspondence between the two (less than 2.5% of individuals mischaracterized).

Relative female mating success was modeled as a logistic function of population density. Because conditions such as food and temperature may influence both the proportion of females ready to mate (Williamson and Butler 1987) and the probability of mating successfully (A. M. Kramer, *unpublished data*), and these conditions will vary among lakes and between years, a random effect of lake by year was included in the model. For lakes sampled multiple times in a single year (i.e., experimental lakes and Dissertation Lake), all data were pooled to give a single per-summer estimate of the proportion of reproductive females bearing eggs. Pooling was necessitated by the small number of copepods in individual samples from the experimental lakes. Adult density was used as the independent variable, because the abundance of immature copepods is irrelevant to mating success.

Caging experiment

In 2004, immature *H. shoshone* were collected from Dissertation Lake and placed in four 33-L mesh cages (400 μm mesh cylinder, 25 cm in diameter, 50 cm long, with 40-cm conical collector), suspended in each of three lakes (Dissertation, Knob, and Cony Lakes). *H. shoshone* had shown no evidence of recovery in Knob and Cony Lakes after the reintroduction in 2003. Animals were subjected to collection and transport conditions that matched the lake-specific conditions in the whole-lake experiment (Table 2). After adding animals, cages were submerged 1.5 m below the surface. Initial density in each cage was approximately 3333 copepods/m³. After two weeks, cages were retrieved and all animals and eggs enumerated on live *H. shoshone*. Animals were then returned to the cages for two additional weeks, after which the copepods in each cage were preserved for microscopic analysis.

Mesocosm experiment

We also manipulated initial copepod density in experimental mesocosms to achieve stronger statistical inference than was possible in the whole-lake experiment. A grid of 38 cattle tanks (1100 L each) was established at Michigan State University's Kellogg Biological Station, Hickory Corners, Michigan in July
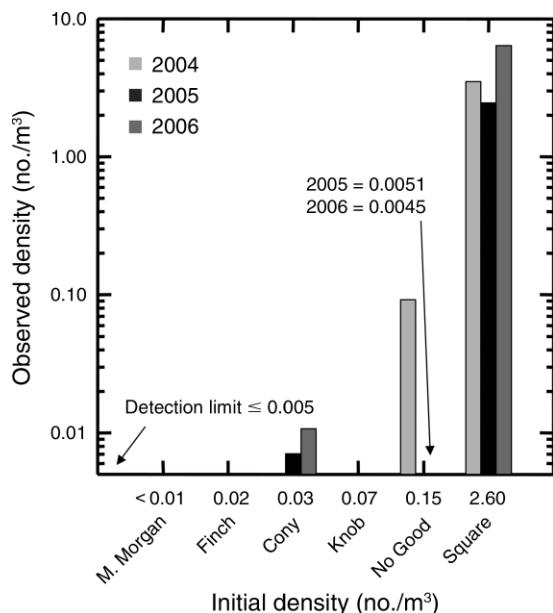
FIG. 2. Density of *H. shoshone* (all life stages combined) in 2004, 2005, and 2006 vs. initial reintroduction density in the experimental lakes. In Square Lake, density was 15 copepods/m³ in 2007.

2006. The tanks were scrubbed with a dilute hydrochloric acid solution and rinsed before use. A thin layer of "play" sand was placed in the bottom of each tank, and tanks were filled with well water to a volume of ~1000 L. Tanks were allowed to equilibrate for four days, after which nutrients were added to establish an initially eutrophic environment with a phosphorus concentration of 100 μg/L and an N:P ratio of 30:1 (by atoms) (Hall et al. 2004). On the same day, an inoculum of filtered (40-μm mesh) lake water from nearby eutrophic Wintergreen Lake (Mittelbach et al. 2006) was added to establish an algal community.

One week after nutrients and algae were added, the small (1.0–1.2 mm) diaptomid copepod *Skistodiaptomus pallidus* was stocked into the tanks. *S. pallidus* is common in eutrophic lakes and ponds of the Midwest (Torke 2001). We collected *S. pallidus* from Wintergreen Lake with vertical tows of a 30 cm zooplankton net. Animals were isolated from the whole sample using a pipette and then separated by sex into two aquaria where they were held overnight. Animals were examined independently by two observers at 10× magnification to confirm sex and eliminate egg-carrying females. Animals were then introduced into the experimental tanks, with males and females released on opposite sides of the tank. Tanks were covered with window screen to reduce transfer of material between tanks by insects, birds, and other animals.

Tanks were stocked at the densities of two ($n = 10$ tanks), four ($n = 10$), eight ($n = 8$), 16 ($n = 5$), and 32 ($n = 5$) individuals per tank. Replication was greater at low stocking numbers because we expected greater variability in population growth rate in the lower density tanks. Sex ratio was always 1:1. The treatments were randomly distributed across the grid. After four weeks, long enough for at least one additional generation to mature and reproduce (Geiling and Campbell 1972), each tank was sampled by pulling a 30-cm zooplankton net vertically through the tank three times, along a transect from one edge of the tank to the center of the tank.

The entire sample was examined for the presence of *S. pallidus* and all nauplii, copepodites, and adults were counted. One tank was contaminated with cyclopoid copepods, but these nauplii and copepodites were easily distinguished from *S. pallidus*. Density was estimated as the total of all life stages. Population growth rate (d⁻¹) was then estimated as

$$\frac{\ln(\text{density}_{\text{final}}) - \ln(\text{density}_{\text{initial}})}{\text{no. days}}.$$

The relationship between growth rate and stocked density was analyzed using linear regression.

RESULTS

Whole-lake experiment

We successfully introduced live *H. shoshone* into the experimental lakes (Table 2). In the two weeks following stocking in 2003, live animals were detected in all but Middle Morgan Lake, which was not sampled in 2003 (Table 2). Observed density after stocking was less than expected density based on lake volume and estimated inoculum size but the intended ranking of treatment densities was maintained (Table 2). This discrepancy presumably was a function of sampling efficiency, mortality due to handling/transport stress, and natural mortality. Given that animals were not sexually mature when initially stocked, we defined initial density as the mean of the 2003 samples, rather than the target density (Fig. 2).

Population recovery occurred only in Square Lake, the lake with the highest initial *H. shoshone* density (3 copepods/m³, Fig. 2). The density of this population remained relatively constant in 2003 and 2004 two years before doubling between 2005 and 2006 to 6.4 copepods/m³, and further increasing to 15.3 copepods/m³ in 2007. The other five populations decreased in density to zero or near-zero after stocking (Fig. 2). In No Good Lake (initial density = 0.14 copepods/m³), the population declined to near detection limits by 2005 and remained at this level in 2006 (Fig. 2). A total of five *H. shoshone* were collected in 2005–2006 in Cony Lake (Table 3, Fig. 2).

Mating success

Relative female mating success was a positive function of population density across stocked and natural populations (Fig. 3). Mating success was variable in established populations such that the lowest values in established populations were similar to the highest
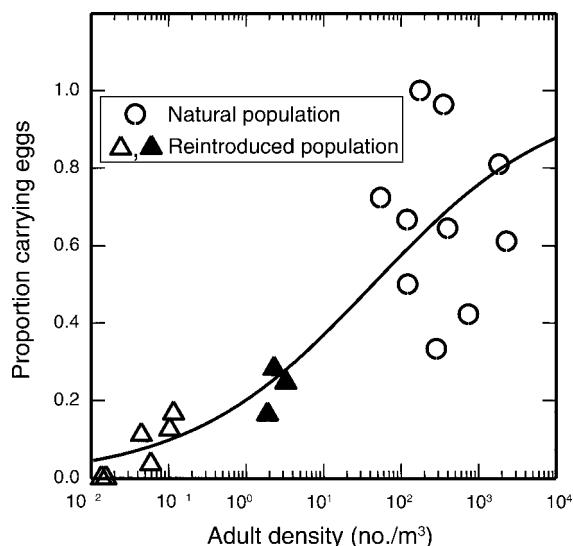
FIG. 3. Proportion of gravid females bearing eggs vs. adult density for *H. shoshone* in reintroduced and natural populations sampled between 2003 and 2005. Each data point represents one lake-year. Solid triangles are for Square Lake, the experimental lake where *H. shoshone* recovered. The equation for the fitted logistic regression is $y = (e^{1.184} + 0.74x)/(1 + e^{1.184} + 0.74x)$ ($P < 0.0005$).

values in the stocked populations, despite the fact that population densities in stocked lakes were more than an order of magnitude lower. Variation in mating success for established populations may in part reflect the added influence of seasonal variation, since these lakes were sampled once in a given year and the sampling date varied among lakes.

Caging experiment

Copepods successfully reproduced when caged at high density (3333 copepods/m^{-3}) in Knob Lake and Cony Lake, at the same time that stocked populations in the lakes declined to non-detectability (Fig. 4, Fig. 2). Clutch size and clutches per female for the caged copepods in Knob Lake and Cony Lake were similar or higher than those observed in caged and uncaged copepods in the source lake (Dissertation) and in Square Lake (Fig. 4). Mortality was also similar to the source lake and substantially lower than the mortality observed following the 2003 reintroduction in Square Lake (Fig. 4), where the population became established.

Mesocosm experiment

Copepods successfully established in most of the mesocosms regardless of initial density. We failed to detect *S. pallidus* in four tanks: three stocked with four individuals, and one stocked with eight. Three of the tanks were immediate neighbors, thus we believe that failure to establish was a function of factors unrelated to initial density. To allow for calculation of growth rate for these four tanks, we added 0.5 copepods/m^3 to the observed density of zero resulting in a negative growth

rate for these units (Fig. 5). The added constant was determined as 0.167×3 copepods/m^3, the detection limit of our sampling regime (Mosteller and Tukey 1977). The significance of the regression slope was insensitive to adding 0.5 or 1, or excluding the four tanks altogether. Initial density had a significantly positive effect on population growth rate ($P = 0.025$, $R^2 = 0.13$; Fig. 5). Mean growth rate at the highest stocking density was
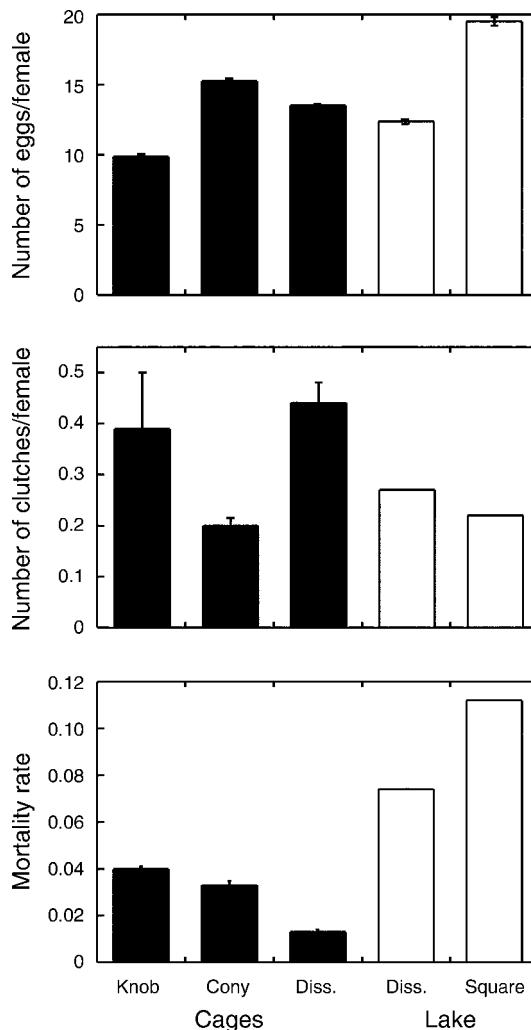

FIG. 4. Comparison of mortality and reproduction in the cages and the lakes. Four cages were placed in each of Knob, Cony, and Dissertation (abbreviated "Diss.") lakes. Mean clutch size is averaged over the course of the experiment (for cages) or the summer of 2004 (for lakes). The number of clutches/female is the number of clutches per reproductive (gravid and/or egg-bearing) female after two weeks in the cages or on the single closest sampling date (lakes). Daily mortality is calculated from regressions of ln(density) vs. time. In cages, mortality is the mean of four cages sampled on three dates from 11 July to 8 August 2004. Dissertation Lake mortality is from three sampling dates from 29 July to 22 August 2004; equivalent data are not available for same time period as the cages. Square Lake mortality data are from the four sample dates following reintroduction. Standard errors (±SE) are shown where available.
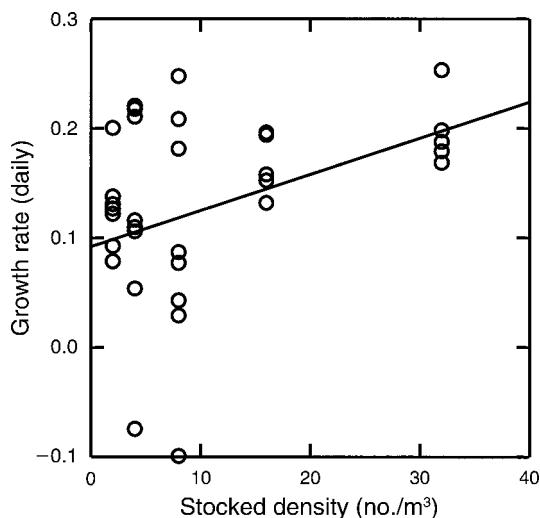
FIG. 5. Daily population growth rate vs. stocked density of *Skistodiaptomus pallidus* in the mesocosm experiment. The population growth rate is calculated as the difference between log-transformed initial and final densities, divided by the number of days. The equation for the linear regression is $y = 0.003x + 0.092$; $P = 0.025$, $R^2 = 0.13$, $n = 38$ mesocosms.

0.21, which is below the reported range of 0.25–0.40 for maximum copepod growth rates (Allan 1976).

DISCUSSION

We found four lines of evidence that the Allee effect operates to reduce establishment success in diaptomid copepods. First, in the whole-lake experiment, population re-establishment occurred only in Square Lake, the lake with the highest initial copepod density. Re-establishment failed in all other lakes, where initial density was <2.6 copepods/m³. Second, copepods caged at high density in two lakes in which the whole-lake reintroductions had failed survived and reproduced at rates comparable to or higher than in natural populations. We thus infer that recovery failure was due to low initial copepod density and not due to these experimental lakes having become unsuitable habitat for *H. shoshone* in the decades following the initial extirpation of these populations. Further, in the experimental lakes cyclopoid copepods are very rare (13 occurrences in ~150 samples, biomass never above 2 μg/L; R. A. Knapp and O. Sarnelle, *unpublished data*) and *Chaoborus* and other potential pelagic predators of *H. shoshone* are absent (Sarnelle and Knapp 2004), so caged animals would not be expected to have higher survival than free-swimming animals. Third, female mating success in the experimental lakes was an increasing function of density, with the highest mating success observed in Square Lake, the only experimental lake showing recovery. We infer from these three lines of evidence that the primary reason for recovery failure of *H. shoshone* in five of the six experimental lakes was negative population growth rate resulting from low mating success (i.e., mate

limitation) at low density. Fourth, the results of the mesocosm experiment with *S. pallidus* were congruent with the whole-lake results in that population growth rate of another diaptomid copepod was a positive function of initial density.

H. shoshone were detected only very rarely in Cony and No Good Lakes (Fig. 2, Table 3), and we suggest that these extremely low densities are indicative of imminent population extinction and not incipient recovery. No mated females were ever detected in Cony Lake, and in No Good Lake mated females were not detected in 2005 or 2006 (Fig. 3). This suggests that the individuals detected by our sampling may have been derived from viable resting eggs that were deposited prior to fish presence or from downstream transport of eggs or live copepods. In No Good Lake, viable diapausing eggs are present in the top 5 cm of sediment (700 eggs/m² [Sarnelle and Knapp 2004]) and although eggs were not detected in the top 5 cm of Cony Lake sediment, our intensive sampling could have disturbed sediments more than 5 cm deep in the lake (A. Kramer, *personal observation*), which may contain older eggs. Lakes upstream of both Cony and No Good Lakes contain *H. shoshone*, and while downstream transport of adults is unlikely (A. Kramer, *unpublished data*), egg transport may be more common. The fact that *H. shoshone* have not recovered in No Good Lake in the five years since fish removal (Sarnelle and Knapp 2004) despite these possible inputs further suggests that these isolated detections are not a prelude to population recovery.

The results of our whole-lake experiments are congruent with studies on *H. arcticus*, an ecologically similar and closely related species in the Canadian Rockies. *H. arcticus* is also extirpated from the water column by the stocking of nonnative fish, followed in some cases by recovery failure in lakes in which egg banks are depleted (Parker et al. 1996). *H. arcticus* was successfully re-established in a single lake through the introduction of reproductive adults at a density of 1.5 copepods/m³ (McNaught et al. 1999), which is within the range of *H. shoshone* critical densities suggested by our experiment. However, the stocking density used for *H. arcticus* is not necessarily comparable to our reintroduction densities because *H. arcticus* females had likely already mated at the time of collection (McNaught et al. 1999).

Although we have considerable evidence supporting the operation of the Allee effect via mate limitation in this study (Figs. 2, 3, and 5), we could not accurately estimate the critical density below which *H. shoshone* populations will fail to establish. Observed densities during the 2003 reproductive season suggest a critical density between ~0.2 copepods/m³ (No Good Lake) and ~3 copepods/m³ (Square Lake) for *H. shoshone* (Fig. 2), or slightly lower than we originally estimated based on Gerritsen's random-encounter model and laboratory data (0.5–5 copepods/m³). However, this

represents more than an order of magnitude of uncertainty.

For *S. pallidus*, the mesocosms were too small to permit an accurate estimate of critical density, since copepods established in all tanks stocked at the minimum of 2 copepods/m^3 (Fig. 5). Based on encounter models (Gerritsen 1980, Kiorboe and Bagioen 2005), *S. pallidus* should have a higher critical density than *H. shoshone* due to its smaller size, which reduces its swimming speed and detection radius. However, *S. pallidus* produces eggs that hatch immediately, in contrast to the production of diapausing eggs by *H. shoshone*, which should contribute to higher population growth, and therefore lower critical density. In cases were the egg bank is fully depleted, such as Square Lake, a portion of the diapausing eggs produced by reintroduced or recolonizing copepods will become buried by sedimentation and bioturbation (Kearns et al. 1996) without concurrent uncovering of eggs already in the egg bank, until the egg bank is re-stocked. This potentially explains the slow population growth observed in Square Lake (Fig. 2), despite our estimate that the Square Lake population was able to produce 5.6 eggs/individual when mating success was 20%. *S. pallidus*' production of immediately hatching eggs avoids this brake on population growth. This difference in life history could be important in colonization success between diaptomid species, and could also cause colonization success to vary seasonally in species that switch between the two egg types (Ellner et al. 1999).

The results in this paper support the hypothesized Allee effect via mate limitation for the frequent recovery failure of *H. shoshone* in alpine lakes following fish disappearance (Sarnelle and Knapp 2004.). *H. shoshone* failed to recover in 46% of 41 Sierra lakes it inhabited before fish introduction, and failure was correlated with fish residence time (Knapp and Sarnelle 2008). During fish presence the egg bank becomes depleted over time due to hatching and egg mortality, so longer fish residence time leads to lower initial copepod density when fish disappear. If initial density is much below ~1 copepod/m^3, we suggest that recovery is unlikely, at least over the time scales of most ecological research. Experimental evidence indicates that calanoid copepods are poor overland dispersers (Jenkins and Buikema 1998, Caceres and Soluk 2002), suggesting *H. shoshone* densities are unlikely to be substantially boosted by colonists from other populations. This was supported by decline of the low-density reintroduced populations some of which had extant populations nearby (see *Methods*), and the failure of *H. shoshone* to recolonize numerous stocked-now-fishless lakes despite the presence of nearby *H. shoshone* populations (R. A. Knapp, *unpublished data*).

If *H. shoshone* is unlikely to recover from depleted, yet viable, egg banks or following colonization events consisting of thousands of viable individuals, how did they become so widespread in the first place? In the Sierra Nevada, *H. shoshone* inhabit at least 60% of never-stocked lakes (Knapp et al. 2001b). Our results suggest that many thousands of individuals need to be present simultaneously for establishment to occur even in the small lakes we manipulated. Here we speculate on three possible explanations for this apparent paradox.

First, population establishment will be enhanced if copepods aggregate to maintain high encounter rates even when few individuals are present. This requires cues acting over long distances. Some copepod species use chemical signaling strategies to increase encounter rate (Doall et al. 1998, Nihongi et al. 2004, Kiørboe and Bagøien 2005), but these strategies act over a limited range, the largest measured range is <150 body lengths in a marine copepod (*Calanus marshallae* [Tsuda and Miller 1998]), and have not been shown to result in local aggregation. Alternatively, aggregations could form at the surface, bottom or sides of a lake in response to an environmental cue such as sunlight (Buskey et al. 1996). We have not observed such aggregations in either the experimental lakes or natural populations. If such mechanisms exist, nothing prevented them from acting in the reintroduced populations, yet these populations failed to recover. Although we did not stock animals in an aggregation, natural colonization by a small number of *H. shoshone* would require aggregations to reform after eggs hatch the following year, even if the initial inoculum was aggregated.

Second, colonization may have occurred more frequently in the past. During the Pleistocene glaciations, the Sierra Nevada was covered with a discontinuous ice cap that underwent several periods of advance and retreat (Gillespie and Zehfuss 2004) before lakes became available for colonization as the glaciers retreated approximately 10 000 years ago. Colonization success may have been facilitated if lakes were initially small, decreasing the necessary number of immigrants to overcome mate limitation, if viable propagules were preserved in glacial ice, or if large numbers of eggs were transferred via vectors that are no longer active, such as formerly abundant duck species. Glacial retreat can also enable spread through surface water at a time when the lakes are more connected, as argued by Stemberger (1995) for lowland lakes, but this explanation seems less likely for high-altitude lakes (Stemberger 1995).

Finally, and we think most likely, perhaps the widespread distribution of *H. shoshone* is the long-term result of a highly stochastic colonization process. Small inocula are unlikely to result in successful colonization, but if lakes receive inocula of *H. shoshone* repeatedly over decades or centuries, there may be enough low probability events (relative to the few trials we can observe today) to result in eventual establishment. Alternatively, some rare events, such as floods that can transport large numbers of eggs, may result in a high probability of colonization. In either case, the Allee effect would operate to greatly delay colonization success, with the egg bank serving to prevent post-

establishment extinction. Long-term delay in the natural recovery of populations, however, is indistinguishable from establishment failure in the context of modern conservation efforts.

We suggest that Allee effects are an important potential force in a range of dynamics, even in species known for large abundances. Natural populations of diaptomid copepods occur by the millions, even in small habitats, yet our data suggest that Allee effects can play an important role in the establishment of such large populations. Our study provides experimental confirmation that theoretical models of the Allee effect have relevance to natural populations. Our results are relevant to other taxa expected to be subject to mate limitation in particular, including gastropods (Stoner and Ray-Culp 2000), and several insects (Hopper and Roush 1993, Berggren 2001, Liebhold and Bascompte 2003) and to the Allee effect in general. Our experimental results reinforce the importance of considering and testing Allee effects in the context of the establishment of populations, but also highlight some of the difficulties associated with the study of populations at very low density.

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APPENDIX

Sample date, location, and lake morphometry for natural populations used in mating success analysis (*Ecological Archives* E089-156-A1).