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**COPEPODOLOGY IN ALPINE LAKES: LIMITATIONS
TO RECOVERY OF *HESPERODIAPTOMUS*
SHOSHONE AFTER EXOTIC FISH ERADICATION**

presented by

ANDREW M. KRAMER

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Fisheries and Wildlife and
Program in Ecology,
Evolutionary Biology and
Behavior



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COPEPODOLOGY IN ALPINE LAKES: LIMITATIONS TO RECOVERY OF
HESPERODIAPTOMUS SHOSHONE AFTER EXOTIC FISH ERADICATION

By

Andrew M. Kramer

A DISSERTATION

Submitted to
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Department of Fisheries and Wildlife and Program in Ecology, Evolutionary Biology and
Behavior

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ABSTRACT

COPEPODOLOGY IN ALPINE LAKES: LIMITATIONS TO RECOVERY OF *HESPERODIAPTOMUS SHOSHONE* AFTER EXOTIC FISH ERADICATION

By

Andrew M. Kramer

In Chapter 1, I took advantage of fish introduction and removal in alpine lakes to experimentally test for the Allee effect at the whole-ecosystem scale. We conducted a multi-lake experiment in which the copepod *Hesperodiaptomus shoshone* was stocked into lakes from which it had been extirpated by the introduction of non-native fish. The stocked densities bracketed a hypothesized critical density ($0.5\text{-}5\text{m}^{-3}$). Successful recovery by the copepod was observed in only the lake that was stocked at the highest density. Copepods stocked into small cages at high density 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, I found a significant positive relationship between mating success and density across experimental and natural *H. shoshone* populations. Further evidence for Allee effects was obtained from a mesocosm experiment with a related 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.

In Chapter 2, I examine the possibility that the Allee effect influences population genetics. By setting a lower limit on population size, Allee effects can constrain the loss of genetic variability due to genetic drift. In *H. shoshone*, models, surveys and

experimental data suggest populations at densities less than 0.5 - 5 individuals/m³ are unable to persist due to mate limitation. Combining this range of critical density with reproductive characteristics and the distribution of habitat sizes in nature, I estimated minimum effective population sizes for *H. shoshone*. Our calculations suggest that >90% of *H. shoshone* populations in the Sierra Nevada are resistant to significant changes in heterozygosity or genetic distance, and 70-75% of populations will lose <10% of allelic richness, during bottlenecks or founder events. By setting a lower limit on population size, Allee effects constrain the loss of genetic variability due to genetic drift.

In Chapter 3, I combine 3-dimensional video analysis from laboratory experiments with life history data to improve the precision and accuracy of existing estimates of *H. shoshone* critical density, and to increase the general understanding of copepod mating behavior. I also investigate the impact of temperature variation on mating behavior. I estimate *H. shoshone* critical density to be 0.56 – 1.3 m⁻³ and to be highly dependent on body size, primarily due to changes in swimming speed. *H. shoshone* swimming speed increased >25% as temperature increased from 5°C to 16°C. The corresponding decrease in critical density was roughly equivalent to the decrease resulting from the six-fold variation in net reproductive rate observed for large-bodied alpine copepod species. The average swimming speeds measured for *H. shoshone* (1.7 - 2.4 cm/s) are dramatically faster than those previously reported for similar sized calanoid copepods. Rapid swimming and the ability to follow pheromone trails greatly improve the ability of *H. shoshone* to find mates, and the relationship between temperature and critical density suggests that recovery or colonization events may be more likely to succeed in warmer lakes and/or warmer years.

ACKNOWLEDGEMENTS

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INTRODUCTION

The ability of a population to recover or re-establish following severe reduction in size has important influences on species persistence, resistance to disturbance, and, often, colonization success. Inverse density dependence, or the Allee effect, has been suggested as an important force in the dynamics of low-density populations of an increasing number of species. Following a severe reduction in population size, species subject to the Allee effect experience increasing population growth rate with increasing density. This means smaller populations are less likely to recover, and the Allee effect can produce a minimum (critical) density below which population growth and persistence is extremely unlikely. A group of zooplankton, calanoid copepods, are a taxa where the Allee effect is predicted to occur, based on the mechanism of mate limitation. Because calanoid copepods occur in three-dimensional habitats, i.e. ponds, lakes and oceans, with dimensions much greater than their detection and movement capabilities, and reproduce sexually, individuals in a low-density population may not encounter each other at a high enough rate to result in population growth. This constitutes mate limitation and creates a critical density below which the population will decline to extinction.

The theoretical prediction of the Allee effect in calanoid copepods is supported by observations in nature. The copepod *Hesperodiaptomus shoshone* has been extirpated from shallow alpine lakes following the introduction of non-native fish. Surveys and fish removal experiments show that following fish removal, this copepod often fails to re-establish despite recovery of other zooplankton and potential for hatching from a long-lived egg bank. We hypothesized that this failure to recover is a result of small numbers of individuals hatching from the egg bank, resulting in a population density below a

critical density established by the Allee effect. Chapter 1 of this dissertation uses population-level experiments to detect for the existence of a critical density due to the Allee effect in the copepod *H. shoshone* and calanoid copepods in general. Chapter 2 combines population genetic theory and the Allee effect to establish important consequences of critical density on population genetics, using *H. shoshone* as a concrete example. Chapter 3 uses individual-scale experiments to predict the critical density based on physiological and behavioral characteristics of *H. shoshone*.

Chapter 1 presents the results of two experiments designed to determine whether the Allee effect due to mate limitation prevents *H. shoshone* populations from recovering following the removal of fish from alpine lakes. The primary experiment is a whole-lake reintroduction experiment in six Sierra Nevada lakes to test for a critical density for population recovery. Stocked copepod densities bracketed a hypothesized critical density ($0.5\text{-}5/\text{m}^3$) based on a mechanistic encounter model. The results are consistent with an Allee effect, with recovery in only 1 lake stocked at high density and population decline in other lakes. Data on mating probability across experimental and natural *H. shoshone* densities ($n=18$) shows significant positive correlation with population density, supporting mate limitation as the mechanism leading to population recovery or decline. This is one of only a handful of experiments to detect a critical density and establish a mechanism for the Allee effect and is the only experiment we are aware of that does this at the scale of natural populations. A second mesocosm experiment conducted in Michigan with local copepods provided higher replication ($n=38$) and showed increased growth rate with increasing density, i.e., inverse density dependence. This mesocosm

experiment provides further support for the Allee effect and extends it from single species to a phenomenon common to calanoid copepods.

Chapter 2 explores the consequences that a critical density has for the population genetics of calanoid copepods and other species subject to the Allee effect in a similar way. By setting a lower limit on population size, Allee effects constrain the loss of genetic variability due to genetic drift. In fact, the minimum population size required by Allee effects can be much larger than the extremely small population sizes generally assumed possible in the literature on bottlenecks. As a result, much of the original genetic variation may be retained in recovered or newly-founded populations. Combining our findings regarding *H. shoshone* critical density with reproductive characteristics and habitat sizes we estimated minimum effective population sizes for *H. shoshone*. The distribution of habitat sizes for this species in the Sierra Nevada suggests that >90% of populations are resistant to significant changes in heterozygosity or genetic distance and 70-75% of populations will lose <10% of allelic richness during bottlenecks or founder events. To our knowledge, this is the first consideration of the role of Allee effects in the loss of genetic variability during population bottlenecks. The results reinforce the fact that the existence of a critical density can lead to significantly different genetic outcomes than those predicted when ignoring limits on minimum population size, and suggest Allee effects could potentially decrease the influence of genetic drift in other taxa.

Chapter 3 presents data from laboratory experiments conducted to improve the understanding of mate encounter, and therefore the critical density determined by mate limitation, in *H. shoshone* and freshwater calanoid copepods. Advanced imaging techniques allow characterization and quantification of individual mating encounters.

Earlier work with our collaborators confirmed the ability of male *H. shoshone* to increase mate encounter rate by following pheromone trails produced by females, and we assess the effect of trail following and swimming speed on critical density. At the same time we explore the impact of environmental variation, specifically temperature, on critical density. Because temperature should impact several parameters of the encounter, such as swimming speed and pheromone production, trials were conducted over a range of temperatures applicable to natural environmental variation. These results indicate a large effect of temperature on swimming speed, and that critical density is more sensitive to this change in encounter rate than to proportionally larger changes in reproductive rate. The relationship between temperature, swimming speed and encounter rate suggests an unconsidered pathway through which temperature changes could affect reproductive rate in copepods, at least in species that occur at low densities.

CHAPTER 1
ALLEE EFFECT LIMITS COLONIZATION SUCCESS OF SEXUALLY
REPRODUCING ZOOPLANKTON

ABSTRACT

Understanding the dynamics of populations at low density and Allee effects is a priority largely 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 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 mating 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 initial densities that bracketed a hypothesized critical density ($0.5\text{-}5\text{m}^{-3}$). Successful recovery by the copepod was observed in only the lake that was stocked at the highest density. Copepods stocked into small cages at high density 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. Further evidence for Allee effects was obtained from a mesocosm experiment with a related 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. This appears to be the first experimental demonstration of the Allee effect in aquatic animals in nature.

INTRODUCTION

Understanding the dynamics of populations at low density has become a priority, largely due to concern about rare and endangered species, but also because of 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 being increasingly recognized as an important phenomenon in natural systems (Stephens and Sutherland 1999, Courchamp et al. 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 very 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). Observational studies have offered evidence for both decreased growth rate and the existence of critical densities in natural populations (Veit and Lewis 1996, Kuussaari 1998, Morris 2002, Serrano et al. 2005, Stoner and Culp 2005), and theoretical models have explored 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 1999, Drake and Lodge 2006, Taylor and Hastings 2005), and predator-prey dynamics (Kent et al. 2003).

Despite long-standing interest in Allee effects, manipulative experiments in natural systems are rare. A handful of experiments 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 examine Allee effects experimentally in a natural system arose in the context of fish introduction and removal in alpine lakes of the Sierra Nevada. Introduction of exotic fish to alpine lakes has led to local extinction of large-bodied zooplankton, and in some cases cessation of fish stocking or active fish removal has returned lakes to a fishless state (Knapp et al. 2001b). Following the removal of fish, the diaptomid copepod *Hesperodiaptomus shoshone* sometimes fails to recover despite the recovery of another extirpated zooplankton species, *Daphnia melanica* (Sarnelle and Knapp 2004). Both *Daphnia* and copepods have long-lived eggbanks (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 markedly in their mode of reproduction. Unlike asexually-reproducing *Daphnia*, diaptomid copepods are obligately sexual and do not store sperm (Watrás 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 too low, the probability of male-female encounter may be too low to sustain the population, in other words they would be subject to the Allee effect via mate limitation. As a result, *H. shoshone* would need a much larger

number of colonists in a given year in order to successfully re-establish after extirpation from the water column, relative to *Daphnia* (Sarnelle and Knapp 2004).

Because some populations have failed to recover, alpine lakes in the Sierra Nevada can be used to test the mate limitation hypothesis at the ecosystem scale, via a whole-lake reintroduction experiment. This study uses a four-tiered approach to examine the Allee effect in diaptomid copepods. First, we designed a whole lake reintroduction experiment using multiple lakes from which *H. shoshone* had been extirpated, allowing us to test the effect of initial population density on population recovery across a gradient of densities. Second, we assessed the evidence for mate limitation and its relationship to density across our experimental lakes and a set of un-manipulated lakes with established *H. shoshone* populations. Third, we used an *in situ* caging experiment to determine whether the experimental lakes are suitable habitat for *H. shoshone* reproduction when mate limitation is alleviated. Fourth, we conducted a mesocosm manipulation of initial density using a related species of copepod, to allow for more replication and 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 these animals.

METHODS

Hesperodiaptomus shoshone, is a large (>2mm) deeply-pigmented diaptomid copepod that occurs in alpine lakes in the Sierra Nevada and the Rocky Mountains from Colorado to British Columbia. As such, *H. shoshone* is highly sensitive to local extinction after fish introduction, and is largely restricted to fishless lakes (Knapp et al. 2001). Using paleolimnological techniques (Knapp et al. 2001a) we identified six lakes

in two basins (Fig. 1) from which *H. shoshone* had been extirpated by fish (Knapp et al. 2001a, Knapp unpublished data). The lakes had been fishless for a variable amount of time, ranging from 1 to 25 years (Table 1). Intensive sampling using vertical tows of a 1 m diameter zooplankton net in the weeks prior to the reintroduction, as well as the previous summer, detected no *H. shoshone* in any of the lakes (detection limit = 0.009-0.015 m⁻³). Sediment samples detected the presence of viable *H. shoshone* eggs within 5 cm of the surface in only 1 of the 6 lakes, No Good Lake (Fig. 1, A) (Sarnelle and Knapp 2004, Knapp unpublished data). The lakes upstream of Cony and No Good Lakes (Fig. 1, A) both contain established populations of adult *H. shoshone*.

Whole-lake experiment

We stocked lakes with *H. shoshone* across a density gradient (Table 2) that bracketed our estimate of the critical density for population establishment (0.5-5 m⁻³) (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 roughly consistent with the range of natural densities of established populations in Sierra Nevada lakes (6 - 22,000 m⁻³, Sarnelle and Knapp 2004). One would not expect to encounter populations below the critical density, which we have not. Given the logistic hurdles associated with stocking whole lakes with hundreds of thousands of copepods, imprecision in the actual number stocked was expected, so we chose widely spaced target densities, with replicate treatments at the intermediate densities closest to estimated critical densities (Table 2).

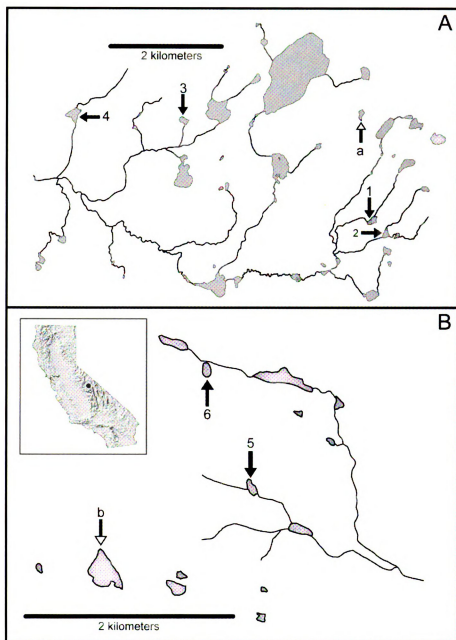


Figure. 1. Map of experimental lakes. A) Humphreys Basin is on the west side of the Sierra Crest in the Sierra National Forest, a) Dissertation Lake, source of *H. shoshone* used in reintroduction, 1) No Good Lake, 2) Cony Lake, 3) Square Lake, 4) Knob Lake. B) Morgan Creek Basin is located on the east side of the crest in the Inyo National Forest is 12 km north of Humphreys Basin, b) Spire Lake, source of *H. shoshone* used in reintroduction, 5) Finch Lake, 6) Middle Morgan Lake.

Table 1. Lake morphometry and fish history for the 6 experimental and 2 source lakes.

Lake	Surface Area			Mean depth		Max depth	Fishless since
	Altitude (m)	(ha)	Volume (m ³)	(m)	(m)		
Square	3443	1.71	30282	1.8	3.5		1997
No Good	3516	1.67	30939	1.9	5		2002
Knob	3358	3.39	65336	1.9	5.5		2001
Cony	3492	1.43	17349	1.2	3.3		2002
Finch	3254	1.14	29543	2.6	7.1		1972
M. Morgan	3321	0.57	17656	3.1	5.5		1976
Dissertation	3602	1.08	ND	ND	7		Never fish
Spire	3523	6.69	ND	ND	30		Fish present

Table 2. Experimental design, detailing the target stocking density for each experimental lake, the number of copepods needed to attain that density, the source of the stocked animals, and the logistical details of the stocking event.

Lake	Target density		Source of stocked animals		Transport duration		Date stocked
	(m ⁻³)	# of copepods			(hours)		
Square	10	300,000	Dissertation		6		7/14/03
No Good	1	31,000	Dissertation		2		7/17/03
Knob	1	66,000	Dissertation		5		7/14/03
Cony	0.1	1800	Dissertation		4.5		7/14/03
Finch	0.1	3000	Spire		2.5		7/24/03
M. Morgan	0.01	180	Spire		3		7/24/03

Late stage *H. shoshone* copepodites (pre-adults) to be reintroduced were collected from two lakes close to the two groups of experimental lakes (Figure 1, Table 1).

“Dissertation” Lake, formally Lake #52121, is fishless, while in Spire Lake, *H. shoshone* co-exist with fish via a deep-water refuge. *H. shoshone* are univoltine and emerge from the egg bank synchronously so we were able to collect large numbers of animals at a similar life-history stage (i. e, pre-adults) in a short period of time. Animals were collected using vertical tows of a 1 m diameter zooplankton net with 350 μm mesh. 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^{-1} . 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 2-6 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 a float tube. Animals were observed swimming in the containers prior to release and in the water following release.

The low densities associated with tests of the Allee effect necessitated a special sampling regime with a very low detection limit. Reintroduction lakes were intensively sampled using vertical tows with a 1m diameter, 350 μm mesh zooplankton net. Detection limits, calculated from the total volume towed during 2-6 visits per lake each summer were always $\leq 0.005 \text{ m}^{-3}$, and often much lower (Table 3). Effort was generally focused on the deepest area of the lake in order to maximize volume sampled, minimize

Table 3. The volume sampled in each lake each year. The total number of copepods collected in that volume is in parentheses.

Lake	Volume sampled each year in m ³			
	(# H. shoshone collected)			
	2003	2004	2005	2006
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	198 (0)	214 (0)	211 (0)

collection of sediment and enable detection of *H. shoshone* if they are undergoing vertical migration. On at least one occasion in each lake horizontal tows and tows in shallow areas were conducted to make sure animals were not residing in under-sampled areas. *H. shoshone* were never observed in these samples. Density was estimated as the total number of individuals collected in a lake in a given year divided by the total volume sampled in that year because individual samples often contained only a handful of animals.

Assessment of mate limitation

H. shoshone collected from the experimental lakes, *in situ* cages (see below) within the experimental lakes, and eight un-manipulated lakes were examined in order to assess the relationship between the relative probability of mating and population density. These samples were collected between 2003 and 2005. Because *H. shoshone* are large and pigmented, the female reproductive state is easily determined. When female diaptomid copepods are ready to mate (“gravid”), their ripened ovaries become dark and clearly visible. After mating the fertilized eggs are extruded into an egg sac that is carried by the female for several days. Females may return to the gravid state before dropping their egg sac. Comparing the number of egg-carrying females to the number of gravid females provides a relative index of mate limitation among populations (Williamson and Butler 1987).

To maximize confidence in our estimates of mate limitation, each sample was examined live with the naked eye at the time of collection, preserved, and then re-examined under the microscope. In both examinations, individuals were scored as copepodite, male, non-gravid female, gravid female, non-gravid female with eggs, or

gravid female with eggs. Live examination was necessary because a portion of females drop their eggs during preservation and transport, making it impossible to determine whether unattached eggs observed in the preserved sample were carried by a gravid or non-gravid individual. Examination under the microscope allowed maturity of individuals to be confirmed, making certain that animals identified as male, non-gravid female, and gravid female were correctly characterized. Live and preserved counts were compared, with close correspondence between the two (less than 2.5% of individuals mischaracterized).

Reproductive females were scored as mated if egg-bearing, or unmated if gravid and not bearing eggs. The probability of mating success of a reproductive female was modeled as a logistic function of population density. Because conditions such as food and temperature may influence both proportion of females ready to mate (Williamson and Butler 1987) and the probability of mating successfully (Chapter 3), and these conditions will vary among lakes and between years, a random effect of lake by year was included in the model. Each data point represents one lake-year. For lakes sampled multiple times in a single year (i. e., experimental lakes), all data were pooled to give a single estimate of the proportion of females mated across the summer. Density was defined as adult density since encounters involving immature copepods cannot result in mating events.

Caging experiment

In 2004, immature copepods were collected from Dissertation Lake and placed in 4, 33 L mesh cages (400 μ m mesh cylinder 25 cm in diameter, 50 cm long, with 40 cm conical collector), suspended in each of Dissertation Lake, Knob Lake, and Cony Lake,

the latter two being lakes in which copepods showed no obvious signs of recovery after the reintroduction in 2003. Animals were subjected to collection and transport conditions closely matched to the whole-lake experiment, (e. g., confinement at $\sim 2000 \text{ L}^{-1}$ for 4 hours, etc.). After animals were added, cages were submerged at 1.5 m below the surface. Initial density in each cage was $3,333 \text{ m}^{-3}$. After two weeks, cages were retrieved and all animals and eggs enumerated live. Animals were then returned to the cages for two additional weeks, after which the contents of each cage was preserved for microscopic analysis.

Mesocosm experiment

A grid of 38, 1100 L cattle tanks was established at Michigan State University's Kellogg Biological Station, Hickory Corners, Michigan in July, 2006. The tanks were scrubbed with hydrochloric acid solution and rinsed before use. A thin layer of sand was placed in the bottom of each tank, and tanks were filled with well water to a volume of $\sim 1000 \text{ L}$. Tanks were allowed to equilibrate for 4 days, after which nutrients were added to establish an initially eutrophic environment with a phosphorus concentration of $100 \mu\text{g L}^{-1}$ and an N:P ratio of 30:1 (by atoms) (Hall et al. 2004). On the same day, an inoculum of filtered 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 initially isolated from the whole sample using a pipette and then separated by sex

into two aquaria. Animals were kept in the aquaria overnight. They were then transferred into a bucket and concentrated into a flask. Animals were examined independently by two observers at 10X magnification to confirm sex and eliminate females that were carrying eggs. Animals were then introduced into the experimental tanks, with males and females released on opposite sides of the tank. Tanks were covered with screening to reduce transfer of material between tanks.

Tanks were stocked at the following densities: 2 (n=10), 4 (n=10), 8 (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 4 weeks, long enough for at least one additional generation to mature and reproduce (Geiling and Campbell 1971), each tank was sampled by pulling a 30 cm zooplankton net vertically through the tank 3 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:

$$\frac{\ln Density_{final} - \ln Density_{initial}}{days}.$$

After four weeks we failed to detect *S. pallidus* in 4 tanks: three stocked with 4 individuals, and one stocked with 8. Three of the tanks were immediate neighbors, so it seems possible that failure to establish could have been a function of factors unrelated to

stocked density (all populations established successfully at lower and higher densities). Statistically we handled these non-detections by adding a constant to the observed density of zero. The constant added was $1/6 * \text{the detection limit of } 3/\text{m}^3$, as recommend by Mosteller and Tukey (1977). We then calculated a regression analysis of daily growth rate vs. initial (stocked) density. The results are relatively insensitive to the added constant, in fact the common practice of adding 1 to calculate the natural logarithms would have improved the fit in this case.

RESULTS

We successfully introduced live *H. shoshone* into the experimental lakes (Figure 2). In the two weeks following stocking in 2003, live animals were detected in all but Middle Morgan Lake, the lake that was stocked at the lowest density (Table 2). Because of the amount of time and effort necessary to intensively sample wilderness lakes, we focused our sampling effort in 2003 on the 5 lakes where we expected *H. shoshone* to be detectable given the limitations on sampling effort (Table 3). Observed density was significantly less than density expected based on lake volume and estimated inoculum size (Figure 2). This discrepancy can be attributed to three main causes. Firstly, there was likely some mortality due to the stress of handling, transport and adjustment to the new lake (see caging experiment results below). Secondly, natural mortality reduces population size over the course of the summer even in unmanipulated lakes since all reproduction results in resting eggs that do not hatch until later years (Appendix A). Finally, our sampling methods may underestimate density due to net efficiency and perhaps clearing of the water column during the net lowering phase. However, our sampling likely reduced population size by no more than 1% on each visit.

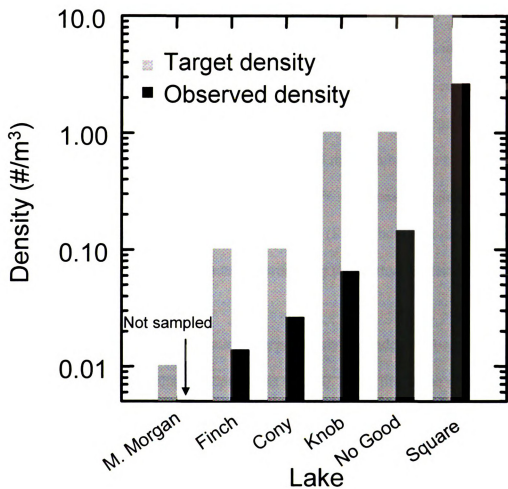


Figure. 2. Target stocking density and observed density in the weeks following (2003) in the experimental lakes (log₁₀ scale).

Population recovery occurred only in Square Lake, the lake with the highest stocking density (Fig 3). This population remained steady for two years before increasing from 2005- 2006 by a factor of 2.5 to 6.4 m^{-3} , which is within the range observed for unmanipulated lakes (Sarnelle and Knapp 2004). The remainder of the populations declined after stocking (Fig. 3). The No Good Lake population, stocked at 1 m^{-3} but with an observed first-year density of 0.14 m^{-3} , exhibited an exponential decline from the initial stocked population size (Fig 3). *H. shoshone* were detected in 2005 and 2006 in Cony Lake, which was stocked at 0.1 m^{-3} (Fig 3), but these rare individuals (Table 3) probably do not constitute a reproducing population, as discussed further below.

The degree of mate limitation was positively dependent on population density across stocked and established populations (Fig. 4). Mate limitation was also highly variable in established populations such that the lowest values of mating success in established populations were similar to the highest values in the stocked populations, despite the fact that population densities in stocked lakes were more than an order of magnitude lower.

The results of the caging experiment (Table 4) showed that *H. shoshone* were able to reproduce successfully when caged at high density (3333 m^{-3}) in Knob lake and Cony Lake, at the same time that stocked populations in the lakes declined (Fig. 3, Table 4). Clutch size and clutches per female for the caged copepods in Knob Lake and No Good Lake were similar or higher than those observed in caged and uncaged copepods in the source lake (Dissertation Lake) and in Square Lake (Table 4). Mortality was also similar

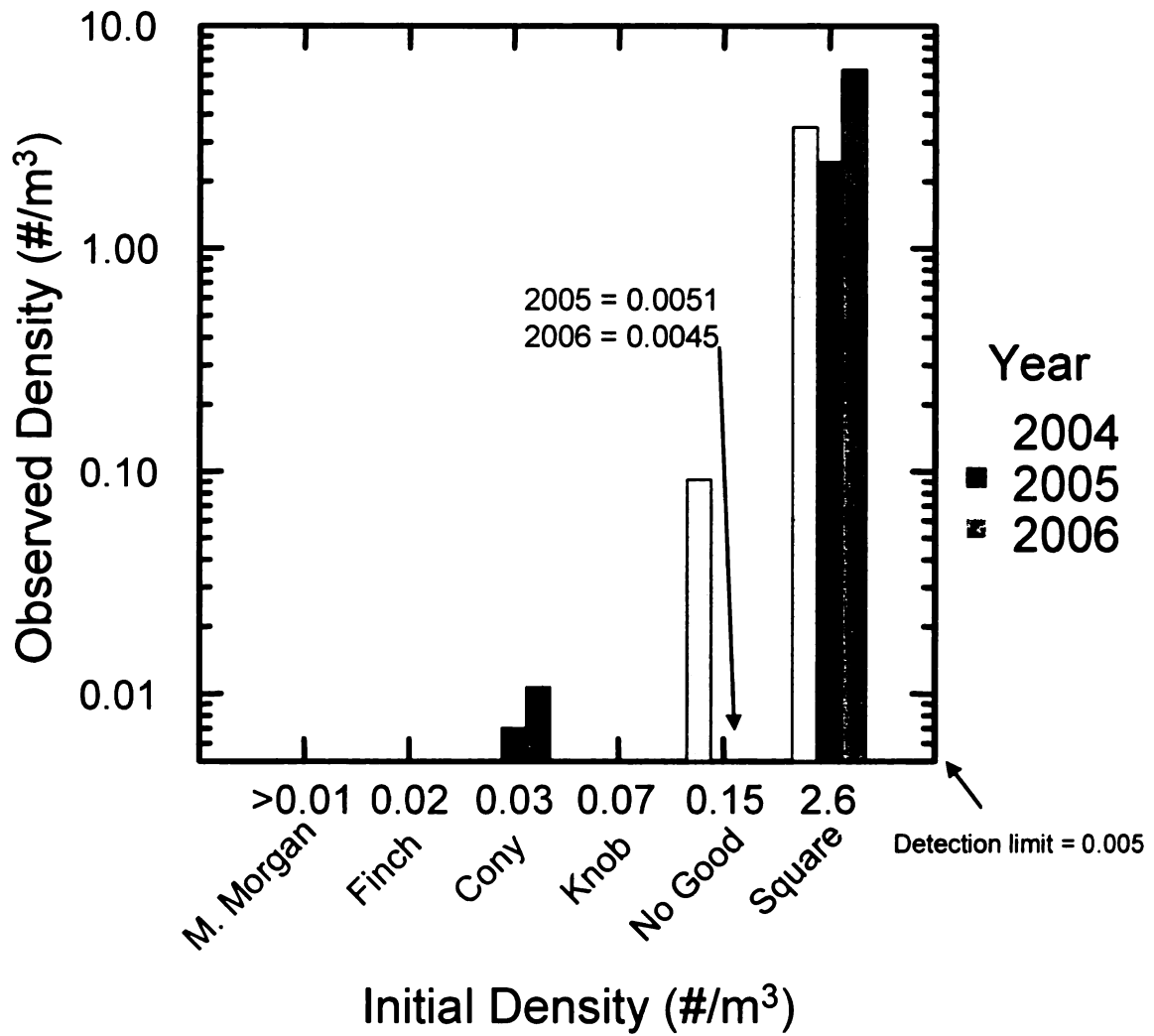


Figure. 3. Observed densities in the experimental lakes ($\log_{10}$ scale) immediately following reintroduction (2003) and in the following years. Lakes are identified by target density and are in the same order as Fig. 2. Detection limit $< 0.005 \text{ m}^{-3}$ unless noted otherwise.

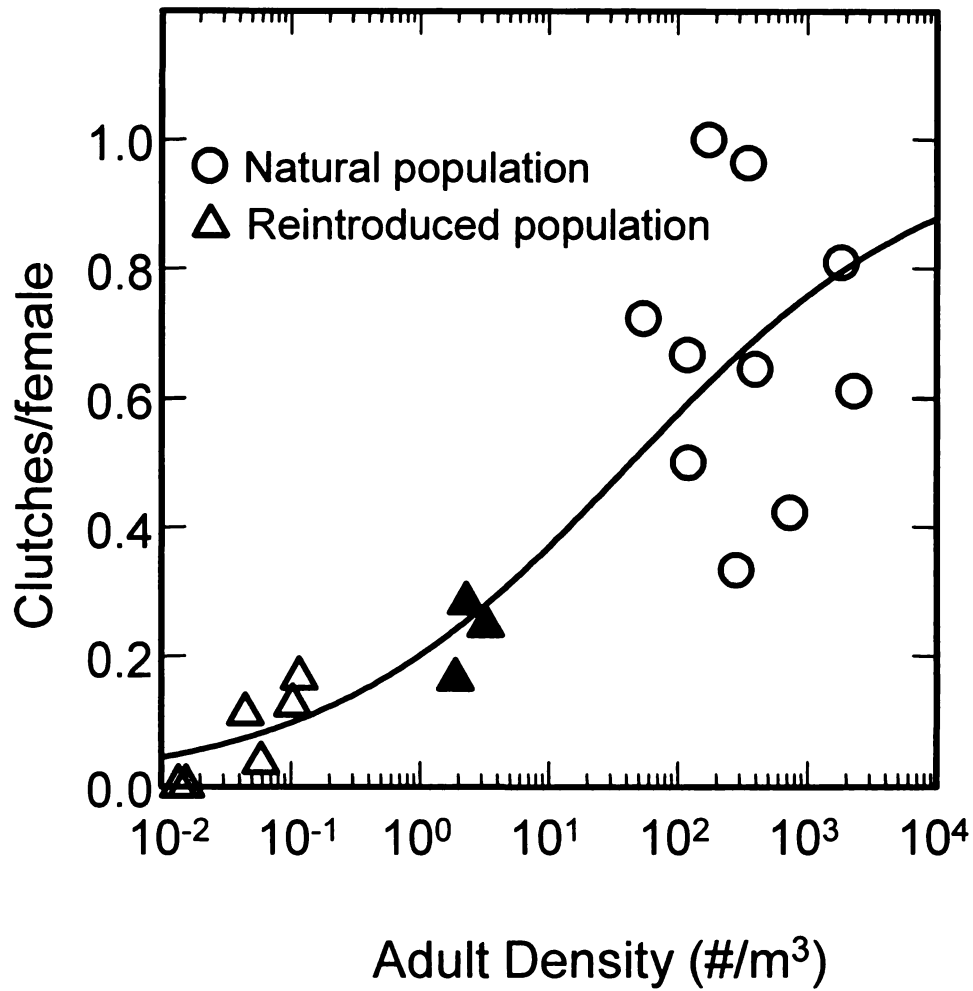


Figure. 4. Proportion of reproductive females bearing eggs vs. density of adult copepods in reintroduced populations (Δ) and natural populations ($\circ$). Filled triangles ($\blacktriangle$) represent Square Lake, the reintroduction lake where the population recovered. The line is the logistic regression function: $\text{WithEggs} = (e^{-1.184 + 0.74 * \text{AdultDensity}}) / (1 + (e^{-1.184 + 0.74 * \text{AdultDensity}}))$ ($p < 0.0005$).

Table 4. Reproductive and survival data from the caging experiment, the source of the animals used in the caging experiment (Dissertation Lake), and the successfully reintroduced population (Square Lake). Clutch size is average and standard deviation of eggs per egg bundle over the course of the experiment (cages) or the summer of 2004 (Dissertation and Square Lake). # clutches/female is the number of clutches per reproductive (gravid and/or egg-bearing) female for 2 cages per lake after 2 weeks in the cages (cages) or for the single closest sampling date (lakes). All sampling took place between 7/19/2004-7/21/2004. Mortality is the average daily mortality calculated from the regression of $\ln(\text{density})$ vs. time. ^A-Mortality is mean of 4 cages sampled on 3 dates from 7/11/2004-8/8/2004. ^B- Dissertation Lake mortality is from 3 sampling dates from 7/29/2004 – 8/22/2004, equivalent data is not available for same time period as the cages. ^C – Square Lake mortality data is from 4 sample dates.

Location	Mean clutch size (s.d)	#clutches/female (s.d.)	Daily mortality (s.d)
Knob (cages)	9.86 (0.75)	0.39 (0.22)	0.040 (0.004) ^A
Cony (cages)	15.25 (0.74)	0.20 (0.03)	0.033 (0.007) ^A
Dissertation (cages)	13.50 (0.51)	0.44 (0.08)	0.013 (0.004) ^A
Dissertation (lake)	12.38 (0.06)	0.27 (NA)	0.074 ^B
Square (lake)	19.49 (1.29)	0.22 (NA)	0.112 ^C

to the source lake and substantially lower than the mortality observed following the 2003 reintroduction in Square Lake (Table 4).

Results from the mesocosm experiment provide clear evidence of inverse density dependence (i.e., the Allee effect) in the Midwestern diaptomid, *S. pallidus* (Fig. 5). In contrast to the whole-lake experiment, *S. pallidus* successfully colonized most of the mesocosms, including all at the lowest stocking density of 2 m^{-3} , but population growth rate increased significantly with stocking density ($p=0.025$, $R^2=0.13$). Mean growth rate at the highest stocking density was 0.214, which is below the reported range of 0.25-0.4 for maximum copepod growth rates (Allan 1976). The observed differences in growth rate resulted in two orders of magnitude difference in mean population size over the 1 month-long experiment, with mean population size = $114 / \text{m}^3$ (± 49) when 2 individuals were stocked and mean population size = $12200 / \text{m}^3$ (± 6600) when 32 individuals were stocked.

DISCUSSION

This paper presents four lines of evidence that the Allee effect operates to reduce re-establishment success after local extinction in diaptomid copepods. In the whole-lake experiment, we found recovery only in Square Lake, the lake with the highest density of reintroduced animals (Fig 3). Copepods caged at high density in two of the lakes with failed recovery survived and reproduced at rates comparable to or higher than in natural populations (Table 4). This suggests that recovery failure was not due to the experimental lakes being unsuitable habitat for *H. shoshone*, the most likely alternative hypothesis

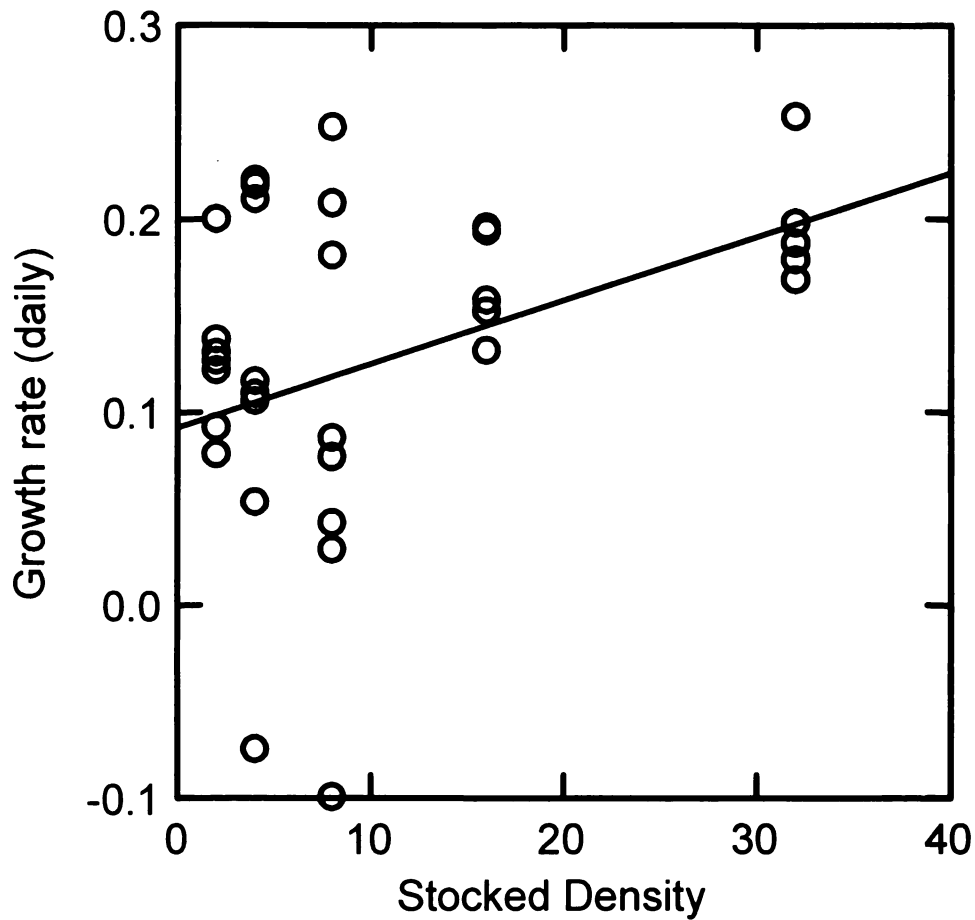


Figure. 5. Daily population growth rate vs. introduced density of *S. pallidus* in 1000 L mesocosms. The line is the linear regression function: $y = 0.003 \cdot x + 0.092$, $p = 0.025$, $R^2 = 0.13$, $n = 38$. Final density was undetectable in four cases resulting in negative growth rate (see Methods). Experiment lasted for 4 weeks.

s for recovery failure. We expected all the lakes to be suitable habitat for *H. shoshone* because the species was present in the past (Knapp et al. 2001a, Knapp unpublished data), but it is possible that the lakes could have become unsuitable over the last several decades as a result of climate change or some other factor. Our caging results, and the success of *H. shoshone* in Square Lake, provide evidence against this hypothesis. Further, pelagic predators such as cyclopoid copepods and *Chaoborus* (Parker et al. 2001) are absent from these lakes (Sarnelle and Knapp 2004), so caged animals would not be expected to have higher survival relative to free-swimming animals. In addition, the degree of mate limitation was greatly intensified at the low population densities of the experimental lakes (Fig 4). Female mating success was comparable to levels found in natural populations only in Square Lake, the only experimental lake showing evidence of successful recovery. Taken together, these three lines of evidence strongly suggest that the primary reason for recovery failure of *H. shoshone* in 5 of the 6 experimental lakes was reduction in population growth rate below zero by insufficient mating success. Finally, the results of the mesocosm experiment with *S. pallidus* were congruent with the alpine lake data in that population growth rate of a diaptomid copepod was found to be a positive function of initial density (Fig. 5).

Our detection of isolated, rare *H. shoshone* individuals in Cony and No Good Lakes (Fig. 3, Table 3) does not seem to represent ongoing recovery of these two populations. No mated females have ever been detected in Cony Lake, and mated females were not detected in 2005 or 2006 in No Good Lake (Fig. 4). The individuals detected by our sampling could instead arise from viable resting eggs that were deposited prior to fish presence or from downstream transport of eggs or live copepods. No Good

Lake has viable eggs in the top 5 cm of sediment (700 m^{-2} , Sarnelle and Knapp 2004), and while eggs were not detected in the top 5 cm of Cony Lake sediment, our intensive sampling disturbs sediment across a wide swath of the deepest part of the lake, and the disturbance could extend more than 5 cm into soft sediment at the bottom of these lakes (Kramer personal observation). Additionally, the upstream lakes contain *H. shoshone* (see Methods), and while downstream transport of adults is unlikely (Kramer, unpublished data), egg transport may be more common. The fact that *H. shoshone* have not recovered in No Good Lake in the 5 years since fish removal (Fig. 3, Sarnelle and Knapp 2004) despite these possible inputs suggests that these isolated detections are not a prelude to population recovery.

The results of the whole-lake experiment are congruent with studies on *H. arcticus*, an ecologically similar and closely-related species in the Canadian Rockies. *H. arcticus* is also driven locally extinct by the stocking of non-native fish (Parker 1996), followed in some cases by recovery failure where the egg bank is 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 m^{-3} (McNaught et al. 1999), which is within the range of *H. shoshone* critical densities suggested by our experiment (see below). 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 the evidence supporting operation of the Allee effect via mate limitation in diaptomid copepods is strong (Figs 3 - 5), estimating the critical density below which populations will fail to establish is less certain. If we assume that the density we

observed over the weeks immediately following stocking (not actual numbers stocked) is the more relevant measure of initial density, the results of the whole-lake experiment suggest a critical density between 0.2 m^{-3} (No Good Lake) and 3 m^{-3} (Square Lake) for *H. shoshone* (Fig 3), which is slightly lower than we originally estimated based on laboratory data alone ($0.5 - 5 \text{ m}^{-3}$). Calculating lake densities based on the entire lake volume as we have done, rather than for the pelagic zone only, seems justified because we regularly observed *H. shoshone* in the littoral zone in established populations. For *S. pallidus*, the tanks were apparently too small to permit an accurate estimate of critical density, since copepods established in all tanks stocked at 2 m^{-3} (Fig. 5). Based on encounter models (Gerritsen 1980, Kiorboe and Bagioen 2005), *S. pallidus* should have a higher critical density due to its smaller size, which reduces swimming speed and detection radius. However, decreased encounter rate could have been offset by increased population growth rate in the warmer and more productive mesocosms. In addition, *S. pallidus* produces eggs that hatch immediately, in contrast to the exclusive production of resting eggs by *H. shoshone*, which also contributes to higher population growth, and therefore lower critical density. In cases where an egg bank is absent, such as Square Lake, resting eggs will become buried by 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. 3), despite our estimate that the Square Lake population was able to produce $5.6 \text{ eggs individual}^{-1}$ 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 which switch between the two egg types (Ellner et al. 1999).

The results in this paper support the hypothesized explanation for the pattern of *H. shoshone* recovery 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, a rate much lower than that of *Daphnia melanica*, and failure was correlated with fish residence time (Knapp and Sarnelle in prep). During fish presence the egg bank becomes depleted over time due to hatching and egg mortality, so longer fish residence time leads to a lower density when fish disappear. If that initial density is much below $\sim 1 \text{ m}^{-3}$, our whole-lake results suggest that recovery is unlikely.

If *H. shoshone* are unlikely to recover from depleted, yet viable, egg banks or 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 $\sim 60\%$ of never-stocked and stocked-now-fishless lakes (Knapp et al. 2001b). Our results suggest that these lakes required many thousands of individuals to be present simultaneously for recovery to be probable, and that larger lakes would require many more. Resting eggs are likely the key to resolving this paradox. One possible explanation is that large numbers of eggs can be transferred by events that are either rare, or the result of past transport mechanisms that are no longer relevant. Another possibility, that we plan to explore further, is whether the egg bank provides a way to accumulate the reproductive effort of repeated, individual colonization events even if no single event exceeds the critical density. This explanation would require that a substantial fraction of resting eggs not hatch in the first few years after deposition (to allow a build-up of individuals) and

would predict that establishment would take many years or even decades. Alternatively, it is possible that copepods spread through surface water at a time when the lakes were more connected, as argued by Stemberger (1995) for lowland lakes. However, this explanation seems less likely for high-altitude lakes (Stemberger 1995). Finally, perhaps the widespread distribution of *H. shoshone*, is the result of a highly stochastic process with eventual colonization success following one of many repeated dispersal events, with each individual event having a very low probability of success because of mate limitation.

This study highlights the difficulty of testing the Allee effect, and estimating critical density in particular. At spatial scales that are substantially smaller than whole lakes, the critical density would be difficult or impossible to assess. This is illustrated by the results of our mesocosm experiment where the tanks were too small to measure critical density in a small copepod.

Difficulty of detection does not mean the Allee effect is unimportant. This study shows that Allee effects are an important force in a range of dynamics, even in species known for large abundances. Natural populations of diaptomid copepods are in the millions even in small habitats, yet our study suggests Allee effects can play an important role in the establishment and persistence of each of those large populations. We believe this to be the first experimental evidence for the Allee effect in an aquatic animal and it provides experimental confirmation that theoretical models of the Allee effect have relevance to natural populations. These results are relevant to other taxa expected to be subject to mate limitation in particular, including conch (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. These experimental results reinforce the importance of considering and testing Allee effects in the context of the recovery of populations following anthropogenic disturbance, natural colonization processes and species invasions.

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CHAPTER 2

LIMITS TO GENETIC BOTTLENECKS AND FOUNDER EVENTS IMPOSED BY THE ALLEE EFFECT

ABSTRACT

Allee effects create a minimum (critical) density for population persistence when population growth rate becomes negative at low population density. Critical density and habitat size combine to define a minimum population size during population bottlenecks or founder events. By setting a lower limit on population size, Allee effects constrain the loss of genetic variability due to genetic drift. As a result, most of the original genetic variation may be retained in recovered or newly-founded populations. In the alpine copepod, *Hesperodiaptomus shoshone*, models, surveys and experimental data suggest populations at densities less than 0.5 - 5 individuals/m³ are unable to persist due to mate limitation. Combining this range of critical density with reproductive characteristics and the distribution of habitat sizes in nature, we estimated minimum effective population sizes for *H. shoshone*. Our calculations suggest that >90% of *H. shoshone* populations in the Sierra Nevada are resistant to significant changes in heterozygosity or genetic distance, and 70-75% of populations will lose <10% of allelic richness, during bottlenecks or founder events. This appears to be the first attempt to account for Allee effects as a factor limiting the loss of genetic variability during population bottlenecks.

INTRODUCTION

The Allee effect, viewed broadly, is a decrease in population growth rate as population density declines (inverse density-dependence), resulting from mechanisms such as mate limitation, obligate cooperation, and group-based antipredator strategies (Allee et al. 1949, Odum 1959, Courchamp et al. 1999). A specific consequence of strong inverse density dependence is the existence of a critical population density, the density below which population growth rate is negative (Courchamp et al. 1999). In effect, critical density represents a minimum population density, and depending on habitat size, a minimum population size, because at lower densities both the persistence of established populations and the establishment of new populations will be unlikely. To date, most discussions of the Allee effect have focused on issues relating to the establishment of invasive species (Lewis and Kareiva 1993, Veit and Lewis 1993, Leung et al. 2004), or to population decline and recovery of endangered species (Kuussaari et al. 1998, Lamont et al. 1993, Forsyth 2003) or collapsed populations of harvested taxa (Myers et al. 1995, Liermann and Hilborn 1997, Gascoigne and Lipcius 2004). Here, we draw attention to an unappreciated connection between critical density, a phenomenon drawn from population dynamics, and loss of genetic diversity in small populations, a phenomenon drawn from population genetics.

By constraining the minimum effective population size, the Allee effect will also constrain the genetic outcome of bottleneck events. Here we define a bottleneck event as a severe decrease in population size or the founding of a new population by a small number of colonists. Theory predicts that for sufficiently severe bottlenecks, the change in gene frequency depends only on the effective population size during the bottleneck and

the duration of the bottleneck, because genetic drift overwhelms selection in small populations (Nei et al. 1975, Maruyama and Fuerst 1985). Because many populations rebound rapidly from small size, with threatened and endangered species a notable exception, the minimum effective population size sustained during a bottleneck event largely determines the genetic makeup of the post-bottleneck population, affecting both the absolute genetic variation remaining and the degree of genetic divergence from the pre-bottleneck population's genetic state (Nei et al. 1975, Wade and McCauley 1988).

The theoretical and empirical literature tends to focus on extreme bottlenecks with very small effective population sizes. The minimum effective population size in theoretical analyses is often ten or fewer individuals (Nei et al. 1975, Pannell and Charlesworth 1999, Whitlock and McCauley 1990, Leberg 2002). Empirical work, especially on species of conservation concern, has confirmed both the occurrence and the genetic impact of such extreme bottlenecks (Le Page et al. 2000, Robichaux et al. 1997, Glenn et al. 1999). As a result, it is typical for observations of very low genetic variability in natural populations or high genetic divergence between populations to be automatically ascribed to genetic drift associated with severe population bottlenecks. In effect, most analyses of genetic variability and genetic differentiation via drift contain an implicit assumption that the population could have established or recovered from an arbitrarily small size; 1 or 2 individuals in the case of asexual or sexual species, respectively. This assumption affects not only direct assessments of population bottlenecks, but also analyses which use genetic differentiation to infer other processes such as migration (Boileau et al. 1992, Ramstad et al. 2004). If the minimum effective population size determined by the Allee effect is relatively large, as suggested for some

species such as zooplankton (Gerritsen 1980), conch (Stoner and Ray-Culp 200) and several insects (Hopper and Roush 1993, Berggren 2001, Liebhold and Bascombe 2003), bottlenecks or founder events may predominantly result in little loss of genetic variability via drift.

Because the Allee effect is a function of population density, habitat size is the key to predicting minimum possible population size, and therefore the change in genetic variability and divergence, for species subject to an Allee effect. We present a quantitative analysis of how habitat size constrains the loss of genetic diversity and the increase in genetic divergence in a diaptomid copepod, a group of organisms potentially subject to strong mate limitation. These small planktonic crustaceans reproduce sexually (unlike cladocerans such as *Daphnia*) and have limited mobility in a potentially gigantic habitat of a lake or ocean. These constraints should create a minimum critical density below which the probability of encountering a mate is too low for a population to persist (Gerritsen 1980). Data confirms the existence of mate limitation in diaptomid copepods (Chapter 1), and there is observational and experimental support for the existence of a critical density (Sarnelle and Knapp 2004, Parker et al. 1996, McNaught et al. 1999, Chapter 1). Copepod critical density can be estimated from information on body size, swimming speed and reproductive characteristics (Gerritsen 1980, Sarnelle and Knapp 2004). Because copepods live in lakes and ponds, as well as the ocean, we can also define and quantify habitat sizes encountered in nature. We use this information to assess the relationship between habitat size and the genetic impact of bottleneck events and determine if Allee effects should be considered when analyzing population genetic data of these copepods and other species with analogous characteristics.

METHODS

Our analysis focuses on *Hesperodiaptomus shoshone*, a large (>2mm) diaptomid copepod that occurs in alpine lakes in the Sierra Nevada and the Rocky Mountains from Colorado to British Columbia. *H. shoshone* is univoltine and susceptible to eradication by fish introduction (Knapp et al. 2001). The critical density of *H. shoshone* has been estimated by Sarnelle and Knapp (2004). Using a range of values for population growth rate and length of reproductive season and Gerritsen's (1980) encounter model, they predicted a critical density of 0.5-5/m³ (Sarnelle and Knapp 2004). This estimated range is supported by observations on natural populations (Sarnelle and Knapp 2004) as well as data from experimental reintroductions (Chapter 1). Critical density multiplied by habitat size (in this case lake volume) gives the minimum population size. For example, an average size *H. shoshone* lake in the Sierra Nevada has a minimum population size of 64,000-640,000 individuals.

The range of relevant habitat sizes was determined using the surface area and maximum depth of 169 *H. shoshone*-containing lakes which constituted a representative sample of *H. shoshone* habitat in the Sierra Nevada (Knapp, unpublished data). We estimated mean depth from maximum depth with the regression equation: mean depth = $0.782 + 0.315 * \text{max depth}$ ($R^2=0.77$), which is based on 2345 northern U.S. lakes spanning a range of maximum depths similar to the Sierra Nevada lakes (Soranno, personal communication). Volume was then estimated as surface area * mean depth.

The key link in examining the relationship between minimum population size and population genetics is the effective population size. Changes in gene frequency due to drift are determined by effective population size rather than census population size

(Wright 1931), and fluctuations in population size, skewed sex ratios and variance in reproductive success act to reduce the effective population size (Wright 1938). We used life history data and made several assumptions to develop a simple, deterministic estimate of the effective population size of *H. shoshone* populations at the critical density. First we assume population size during the bottleneck remains at the minimum and that the sex ratio of *H. shoshone* is 1:1 (Appendix A). Therefore, any reduction in effective population size is due to variance in reproductive output.

We expect high variance in reproductive success because the low encounter rate at critical density will lead to zero reproductive output in the majority of individuals. To estimate the number of individuals that do contribute offspring to the next generation, we use the clutch size (mean = 16.02, sd = 4.18) measured for *H. shoshone* stocked into mesh enclosures in 4 lakes (Appendix A), and the conservative assumption that successful individuals (male and female) are able to mate twice and each of these females produce two clutches during the reproductive season. Our aim is to err towards overestimating variance in reproductive success by assuming all reproduction is contributed by a somewhat unrealistically limited pool of individuals, but this may be somewhat offset by an assumption of zero variance in reproduction among successful pairs. Based on the assumptions above, we can estimate the minimum number of reproducing individuals (N_e) necessary to maintain population size at the critical density as follows:

$$N_e = \frac{\text{habitat size} \times \text{critical density}}{\text{clutch size} \times \# \text{clutches/pair}} = \frac{\text{habitat size} \times \text{critical density}}{16 \times 2}$$

We also conservatively assume 100% hatching success and zero mortality before reproduction.

We consider bottlenecks that last for 1 to 10 generations. Longer bottlenecks are unlikely for *H. shoshone* and diaptomid copepods in general (Sarnelle and Knapp 2004, McNaught et al. 1999). McNaught et al. (1999) found population growth to be exponential, with natural population density nearly attained in only 4 generations [following reintroduction at a density of 1.5 individuals / m³]. Short bottleneck duration is also a likely scenario for any species with high reproductive rates not subject to habitat loss, harvest or other external forces.

The ranges of bottleneck duration and minimum effective population size determined above are used to examine how two measures of genetic variability and two measures of genetic divergence are affected by bottlenecks in populations subject to Allee effects. Change in genetic variability is estimated by heterozygosity; perhaps the most widely used measure for the maintenance of genetic variation, and allelic richness, which is much more sensitive to changes in population size (Nei 1975, Nei 1987). For reasons of simplicity and consistency with existing analyses, we estimate change in heterozygosity and allelic richness as a proportion of the variation present before the bottleneck, as in Nei et al. (1975). We calculate both measures in the same way as Nei et al. (1975), using their analytical solution for heterozygosity and their simulation method for allelic richness (see Nei et al. 1975).

To represent genetic divergence we consider Nei's genetic distance (D) because it allows us to consider two currently or historically linked populations (Chakraborty and Nei 1977). We estimate D between two populations, where one is founded by a random sample from a second population which is at mutation-drift equilibrium, using the infinite alleles model as in equation (21) from Chakraborty and Nei (1977). We also consider

F_{ST} , which measures the overall genetic similarity of a group of populations, following the assumptions for the migrant pool model and a small number of colonists as in equation (1) from Wade and McCauley (1988). Under this model F_{ST} measures the genetic differentiation among a group of populations founded from a single source population (Wade and McCauley 1988). This model was selected because it is the only formulation which does not require parameters, such as migration, which are not present in our other analyses.

RESULTS

The sample of lakes inhabited by *H. shoshone* in the Sierra Nevada had volumes ranging from 77 m^3 to $7.3 * 10^6 \text{ m}^3$, with a median volume of $1.3 * 10^5 \text{ m}^3$ (Fig. 1). The corresponding range of minimum effective population size for a minimum population density of $0.5/\text{m}^3$ is $2.4 - 2.3 * 10^5$ individuals, or $1.2 - 1.15 * 10^5$ pairs, with a median effective population size of $4.0 * 10^3$ (Fig. 1). With a critical density of $5/\text{m}^3$ the lower limit on effective population size for similarly-sized habitat is ten times higher.

The proportion of the original heterozygosity retained in a bottlenecked population rises rapidly as habitat size increases from the minimum habitat size (Fig. 2). It asymptotes at a small habitat size; over 90% of the original variability is retained in habitats larger than 1500 m^3 , even for the lowest critical density and longest bottleneck duration considered (Fig. 2). This size is exceeded by 97.5% of the *H. shoshone* habitat in the Sierra Nevada (Fig. 1). At the median habitat size, >99% of heterozygosity is retained for all combinations of critical density and bottleneck duration. Not surprisingly, similar retention percentages for allelic richness require much larger habitat size (Fig. 3). What is important to note is that even our most extreme scenario, consisting of a ten

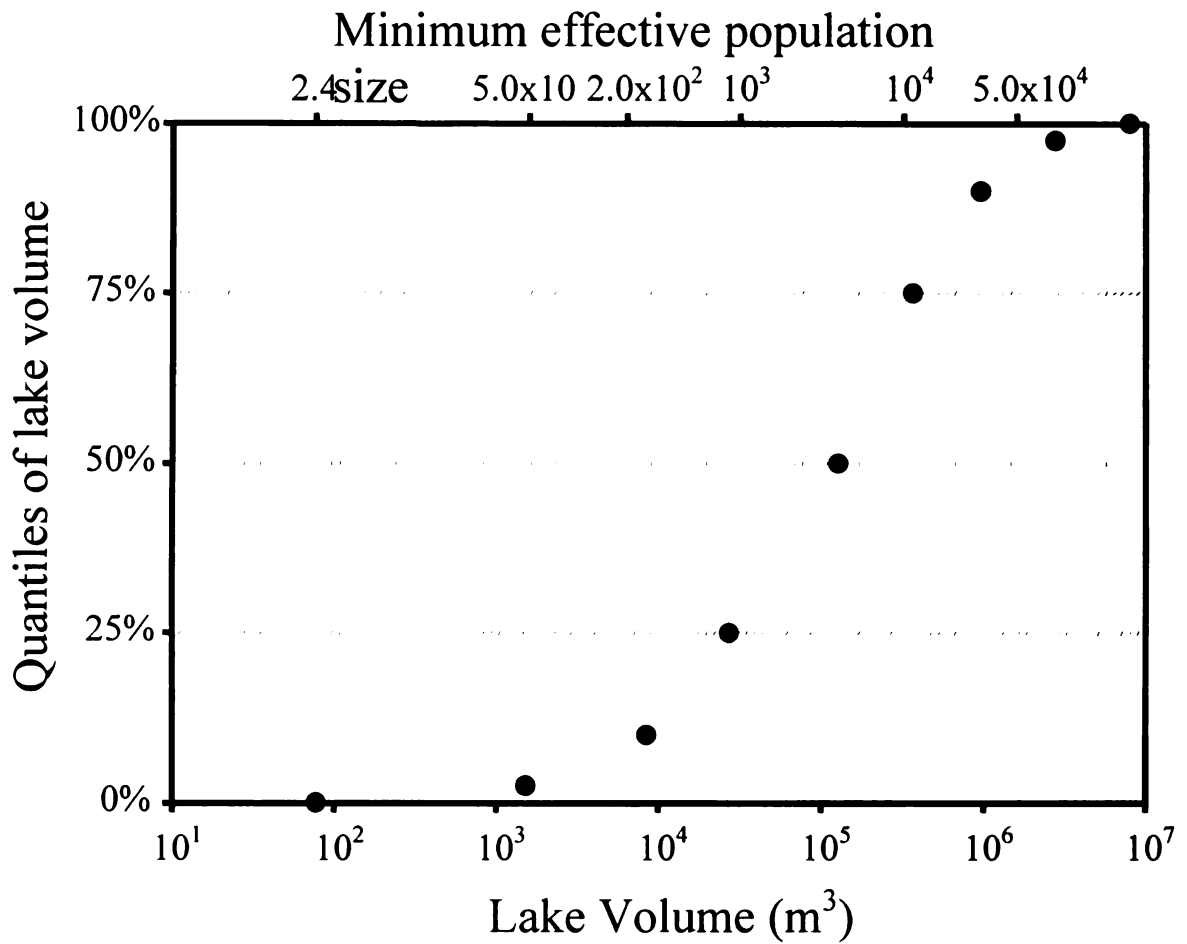


Figure 1. The quantile distribution of lake volume for a representative sample of *H. shoshone* populations in the Sierra Nevada (n=169), and the corresponding minimum effective population size for a critical density of $0.5/m^3$.

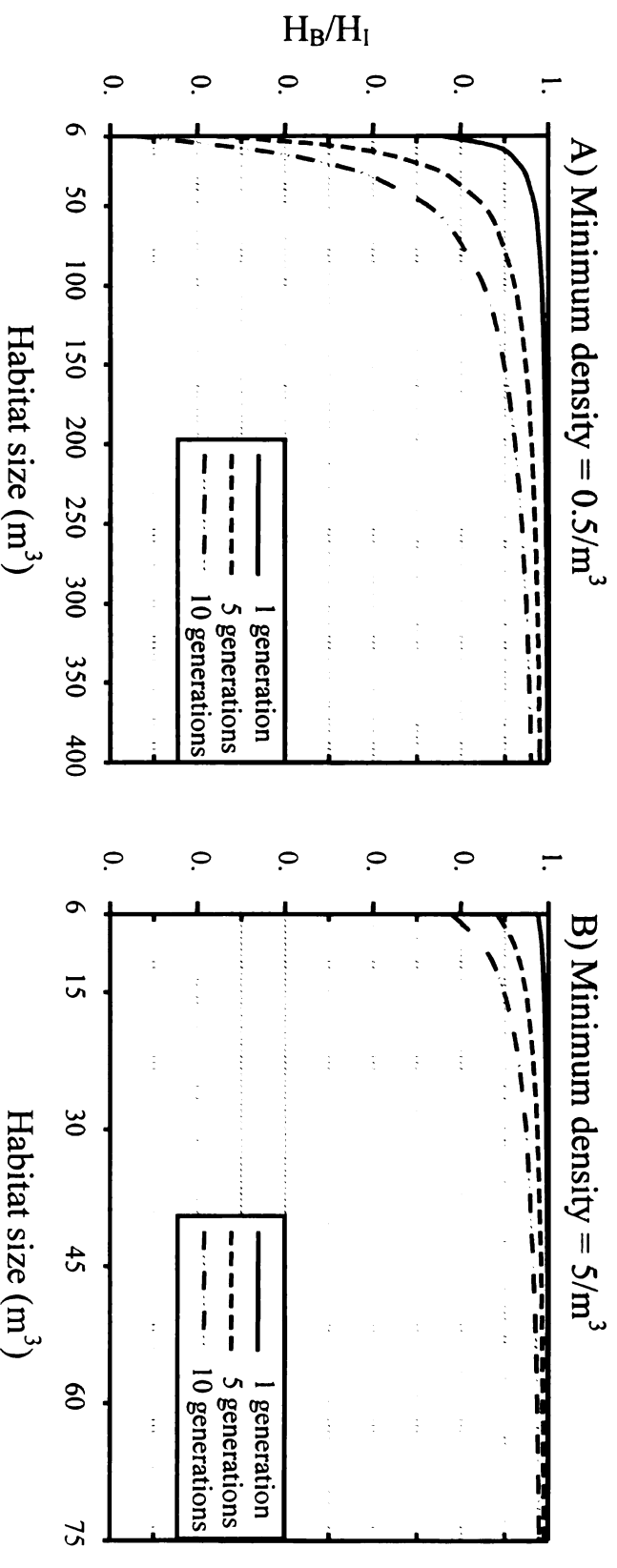


Figure 2. The expected ratio of post-bottleneck heterozygosity (H_B) to initial heterozygosity (H_I) as impacted by habitat size and duration of bottleneck for (A) minimum density of 0.5 individuals/ m^3 ; (B) minimum density of 5 individuals/ m^3 . Bottleneck duration is the number of generations population is at minimum effective population size. A habitat of volume $64\ m^3$ is the largest habitat in which effective population size can decline to one male and one female when critical density = $0.5/m^3$.

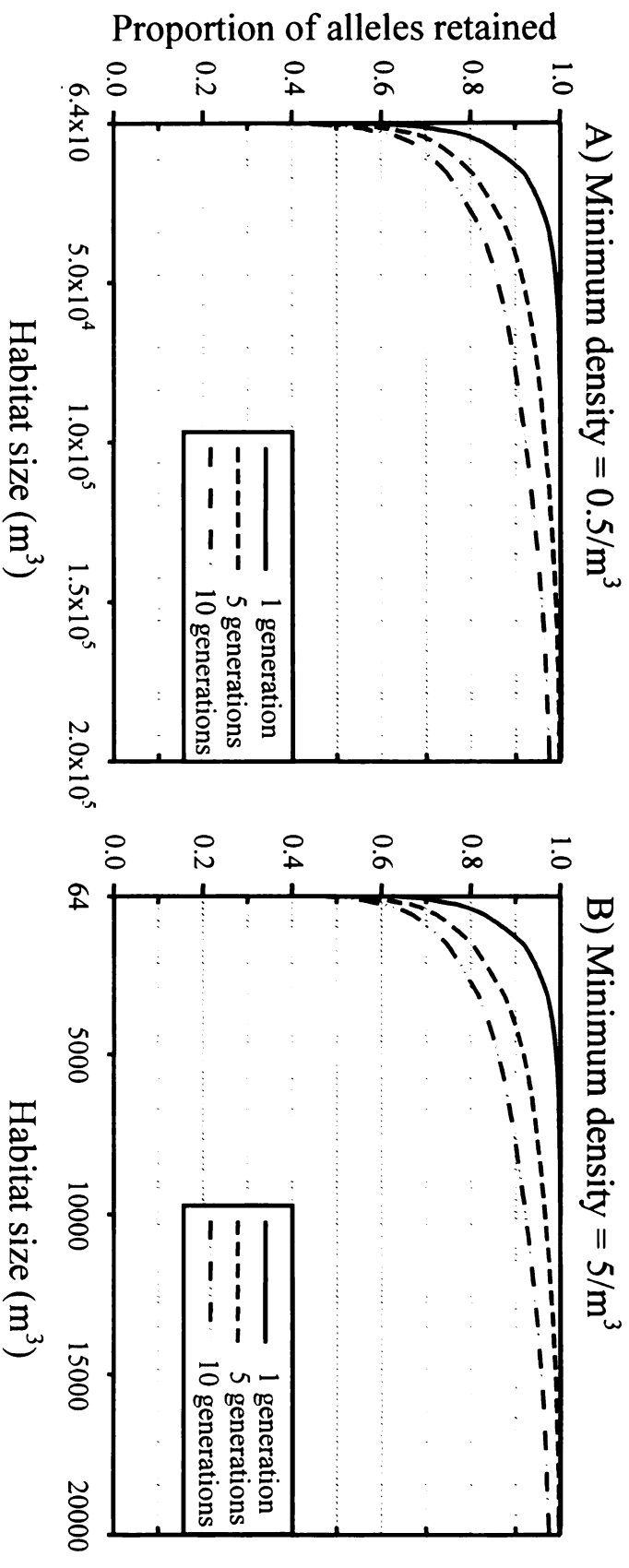


Figure 3. The proportion of original allelic richness retained after population bottleneck as determined by habitat size and bottleneck duration for (A) minimum density of 0.5 individuals/ m^3 ; (B) minimum density of 5 individuals/ m^3 . Bottleneck duration is the number of generations population is at minimum effective population size. Data was produced using the simulation technique of Nei et. al. (1975). Initial effective population size = 4×10^6 , mutation rate = 10^{-8} , number of iterations = 5000.

generation bottleneck at the critical density of $0.5/\text{m}^3$, results in over 90% retention of the original alleles in roughly 65% of the sample of *H. shoshone* lakes (Fig. 3). Higher critical densities rapidly increase retention, leading to the expectation of 90% retention for 90% of populations when the critical density is $5/\text{m}^3$ and the bottleneck last for 10 generations (Figs. 1 and 3). 93 – 99 % of allelic richness is retained at the median habitat size for the combination of parameters considered.

The pattern for genetic distance is similar to the pattern seen in heterozygosity (Fig. 4). Nei's D cannot be strictly interpreted as a percentage difference between two populations, but in this case a value of 0.1 is roughly equal to 90% identity of alleles between the two populations (Nei 1987). For our most extreme scenario, habitats larger than 4500 m^3 result in $D < 0.1$. This includes over 90% of Sierra Nevada *H. shoshone* habitat. Genetic distance of a median size habitat from its founding population is <0.01 .

The relationship between habitat size and F_{ST} shows populations in habitats $>500 \text{ m}^3$ have very low F_{ST} (Fig. 5). We include F_{ST} because of its popularity as a measure of genetic differentiation, especially for researchers estimating migration from genetic data. At the same time, it is less than ideal for our purposes due to its more restrictive, and less applicable, assumptions of the relationship between populations. The F_{ST} shown (Fig. 5) is that expected among populations when those populations are founded by the same number of colonists from a single mixture of potential colonists. In addition these populations grow to a large size in a single generation. The most we can safely draw from this is that all the populations must be very small in order for very short bottlenecks to substantially increase the amount of genetic variance distributed among populations. F_{ST} is further discussed below.

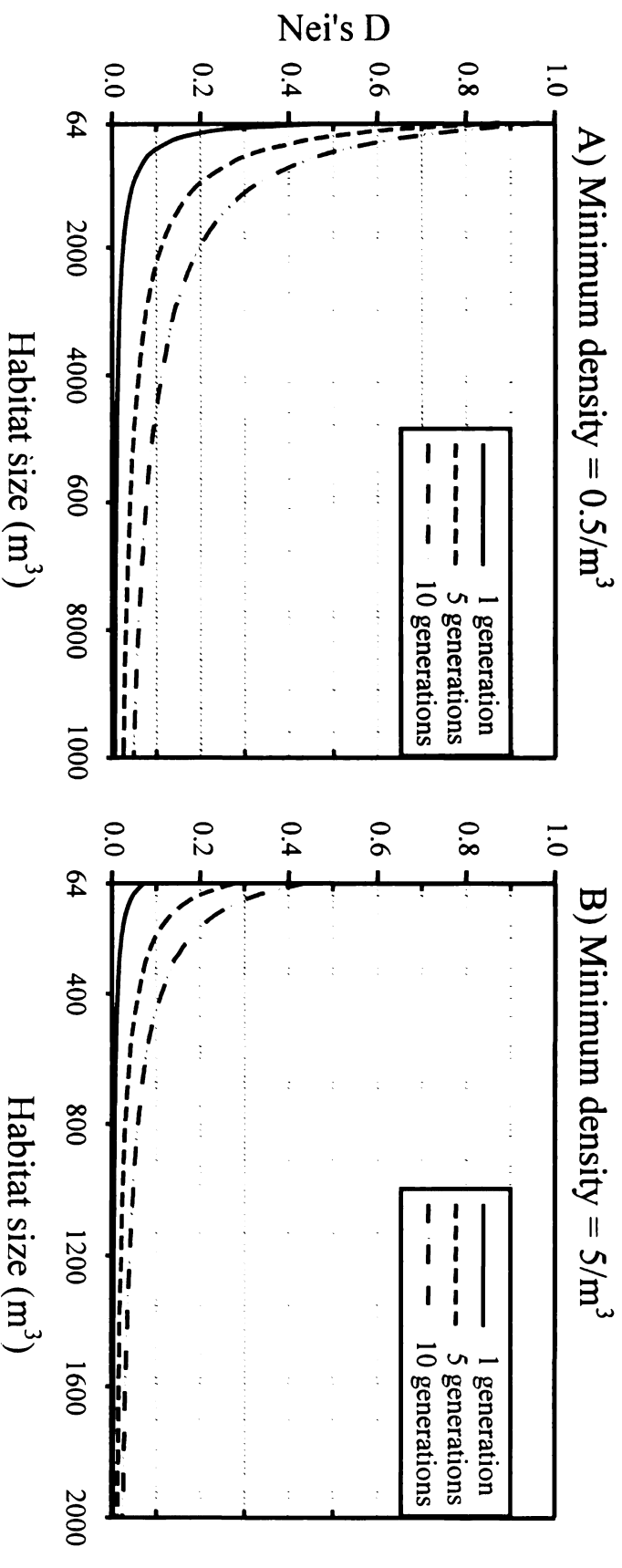


Figure 4. Expected Nei's D for a population founded at the critical population density from a founding population at equilibrium ($4n_v = 0.16$) as determined by habitat size and duration of the bottleneck (A) minimum density = $0.5/\text{m}^3$; (B) minimum density = $5/\text{m}^3$. Bottleneck duration is the number of generations population is at minimum effective population size.

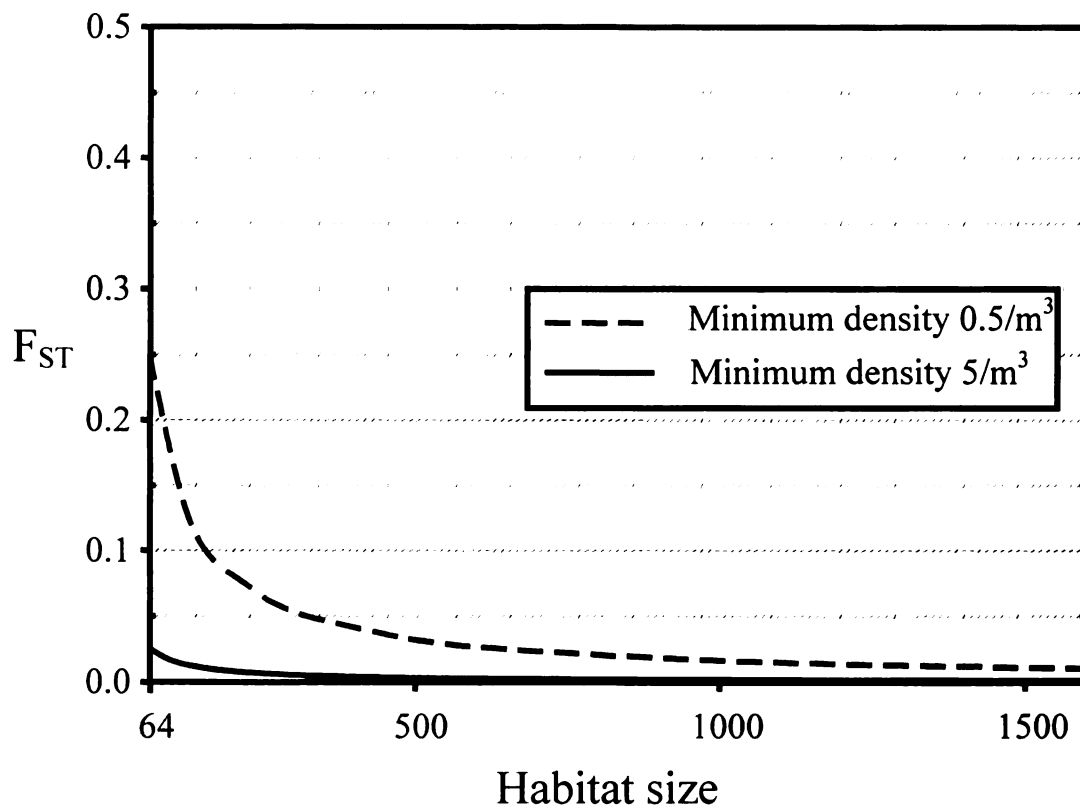


Figure 5. Expected F_{ST} for a group of identical populations founded at the critical density from a single source population as determined by habitat size for minimum density = 0.5/m³ and 5/m³. See Wade and McCauley (1988).

DISCUSSION

Our analyses show that habitat size has a very large influence on the potential for loss of genetic variation and increase of genetic divergence due to population bottlenecks. A likely conservative scenario is to assume actual minimum densities are closer to $0.5/\text{m}^3$ and that bottleneck duration is close to the 3-4 generations suggested by past observation (Boileau et al. 1992, McNaught et al. 1999). Under this scenario colonization/recovery events in 70-75% of *H. shoshone* habitat result in <10% loss of allelic richness (Fig. 3). Furthermore, over 90% of *H. shoshone* populations are resistant to significant changes in heterozygosity or genetic distance (Fig. 2, 4). This is a hugely different outcome than expected under the common assumption that there is no lower limit to population size.

If *H. shoshone* populations can often recover from lower densities, the genetic impact of population bottlenecks will obviously be increased. Therefore, it is important to examine the potential for bias due to our assumptions about copepod population dynamics and genetic structure. Our estimate of critical population density is intentionally broad, but the actual critical density could be less than $0.5/\text{m}^3$, because of the potential for chemical detection in diaptomid copepods (Katona 1973, Watras 1983, Doall et al. 1998, Sehn et al. in prep., Chapter 3) or behaviors that increase local density of copepods (Buskey et al. 1996, Tsuda and Miller 1998). However, multiple years of data on recovery of stocked populations in natural habitats are consistent with a critical density within the range $0.5\text{-}5/\text{m}^3$ (Chapter 1). At the same time, several of our conservative assumptions about reproduction likely offset possible over-estimation of critical density and have the potential to make populations resistant to the genetic effects of bottlenecks in all but the smallest habitats. Namely we have assumed all eggs

produced are viable, hatch the next year and suffer zero mortality before reproduction. The assumption of 100% hatching success is the most unrealistic assumption since large numbers of eggs are known to remain dormant in the sediments. We were also extremely conservative in presuming our reproductive males and females are able to reproduce twice in a season. Multiple clutches are definitely common in good years (Appendix A), but a single pair producing two clutches in a low density population should be rare because *H. shoshone* must mate before each viable clutch (Watras and Haney 1980) and the probability of mating at all is very low.

Finally, our estimates of D and loss of allelic richness are dependent on the initial levels of variability present in the pre-bottleneck population. Increased variability results in larger D and increased loss of allelic richness when other parameters are held constant. For our analysis we used the same initial parameters as Nei et al. (1975) and Chakraborty and Nei (1977) for their hypothetical fruit fly population. We thought this suitable since we are also considering invertebrate populations with high carrying capacities and rapid population growth. This similarity makes our results directly comparable with analyses from Nei, which were the first to quantify the major impact that severe population bottlenecks have on the genetics of a population. Additionally, we found no reported values for average effective population size or mutation rate for nuclear genes in freshwater copepods which would be more directly applicable to our calculations.

Given the limits the Allee effect should impose on the genetic outcome of population bottlenecks and colonization events, it seems crucial to consider habitat size in making inferences from genetic data. For example, genetic information has been used to infer population structure and phylogeography in arctic copepods (Boileau and Hebert

1988, Boileau et al. 1992). These studies found significant genetic differentiation among nearby ponds, suggesting very low migration, which seemed inconsistent with the ponds proximity (Boileau and Hebert 1988). This paper and subsequent analyses (Boileau and Hebert 1991, Boileau et al. 1992) led the authors to suggest a model in which very rapid initial growth rates--carrying capacity is reached in 5 generations or less--retain genetic differences resulting from genetic drift due to founder events, despite migration between populations.

Specifically, measured F_{ST} values suggested 5 or fewer individuals founded most of the populations (Boileau and Hebert 1992). The habitats surveyed ranged from large tundra ponds (volume = $20166 \pm 9882 \text{ m}^3$) to small rock pools (volume $< 50 \text{ m}^3$) (Boileau and Hebert 1988). The Allee effect seems to make it highly improbable that 5 individuals could successfully colonize the large ponds. In fact, measured gene frequency divergence for the large tundra ponds were consistently lower than for populations in much smaller rock pools in the same species (Boileau and Hebert 1988), consistent with less severe bottlenecks in the larger habitats. Our results highlight the fact that inclusion of very small habitats prone to severe bottlenecks could bias estimates of metapopulation wide estimates such as F_{ST} . We suggest that the use of genetic statistics, and F_{ST} in particular, to estimate population size during colonization events in species subject to the Allee effect should acknowledge the ecological limitations on the populations.

Boileau and Hebert (1991) also bring up an important additional force, colonization pattern, which interacts with the number of colonists to create the observed population genetics. They propose that Arctic habitats were serially colonized and genetic distance built up as a small number of colonists from a previously colonized habitat

successively filled each empty habitat (Boileau and Hebert 1991). The successive colonization events proposed by the authors would act very strongly to increase differentiation (Wade and McCauley 1988), especially in areas dominated by small pools. In the terms of our analysis, bottleneck duration is effectively extended by each subsequent colonization event, which could significantly increase genetic differentiation even when habitat is dominated by large lakes.

The potential of the Allee effect to maintain genetic variation and minimize differentiation has implications for both ecology and evolutionary biology. As in the arctic copepod example above, population genetics are being increasingly used to try to infer ecological processes such as migration and historical population size. This example underscores the need for studies to directly consider minimum population size when analyzing genetic variation in species, such as copepods, thought to experience the Allee effect. Because the minimum effective population size can be dependent to a great degree on habitat size, bias will result if the distribution of habitat sizes sampled does not match the overall distribution of habitat size. The Allee effect creates differences in population genetic expectations that are dependent on ecology, and recognizing the potential impact of the Allee effect should allow ecologists to better understand their results and the ways in which model assumptions are violated in their analyses. Hopefully this will improve the accuracy of the inferences made using population genetics.

For evolutionary biologists, a relatively large lower constraint on population size in a species could reduce the likelihood of speciation according to some of the models that depend on genetic reorganization due to small population size and genetic drift

(Wright 1931, Mayr 1963, Templeton 1980). A higher mean population size will decrease the influence of drift on the population's genetic makeup, presumably increasing the influence of natural selection. Fewer bottlenecks will also preserve more genetic variability on which natural selection can act. Additionally, in several cases very low heterozygosity in wild populations is attributed to severe historic bottlenecks (Nei et al. 1975, O'Brien 1994). Evidence for the Allee effect, and therefore larger critical population size, in such species with very low genetic variability suggests other causes may need to be considered. However, it is also possible that the observed populations of species subject to the Allee effect, such as the arctic copepods discussed above, could be the result of successful colonization by an unexpectedly small population, following the stochastic success of one of many repeated dispersal events with very low individual probabilities of success.

Our analysis suggests habitat size places a crucial role in population genetics of *H. shoshone* due to the Allee effect. Similar effects are possible in other species with minimum population size determined by the Allee effect, such as conch (Stoner and Ray-Culp 2000), and several insects (Hopper and Roush 1993, Berggren 2001, Liebhold and Bascompte 2003). We are not suggesting a species or population becomes immune to genetic drift or loss of genetic variability. Species with very large population size can harbor enormous genetic variability, and a reduction of population size, even if to a large absolute size, will cause some of that variability to be lost and increase the influence of genetic drift (Bucklin and Wiebe 1998). Rather, this paper proposes that Allee effects, caused by mechanisms such as mate limitation, which impose limits on minimum population size, will have measurable and important results for the genetic dynamics of

the species subject to them and that this possibility, to our knowledge, is effectively ignored in the current literature. Our results reinforce the fact the even moderate limits on minimum population size lead to significantly different genetic outcomes than those seen when limits on bottleneck size are ignored.

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CHAPTER 3

THE EFFECT OF MATING BEHAVIOR AND TEMPERATURE VARIATION ON THE CRITICAL POPULATION DENSITY OF A FRESHWATER COPEPOD

ABSTRACT

Population growth rates of dieocious zooplankton depend on the encounter rate of potential mates, resulting in a predicted critical density for population establishment and persistence. Existing evidence confirms a critical density for the calanoid copepod, *Hesperodiatomus shoshone*, but estimates of the critical density span an order of magnitude. We improve the precision and accuracy of previous estimates of *H. shoshone* critical density by combining mating behavior from 3-dimensional video analysis with life history data from natural populations. We also investigate the impact of temperature variation on mating behavior and sensitivity of critical density to changes in mating behavior vs. changes in fecundity and mortality. We estimate *H. shoshone* critical density to be $0.56 - 1.3 \text{ m}^{-3}$ and to be highly dependent on body size, primarily due to changes in swimming speed. *H. shoshone* swimming speed increased >25% as temperature increased from 5°C to 16°C, a temperature range relevant to their alpine lake habitat. The corresponding decrease in critical density was roughly equivalent to the decrease resulting from the six-fold variation in net reproductive rate observed for large-bodied alpine copepod species. The average swimming speeds measured for *H. shoshone* (1.7 - 2.4 cm/s) are dramatically faster than those previously reported for similar sized calanoid copepods. Rapid swimming and the ability to follow pheromone trails greatly improve the ability of *H. shoshone* to find mates, and the relationship between temperature and critical density suggests that recovery or colonization events may be more likely to succeed in warmer lakes and/or warmer years.

INTRODUCTION

The stocking of non-native trout species in the alpine lakes of North America has had dramatic effects on the biotic community of these previously fishless ecosystems (Bradford 1998, Parker 2001, Knapp et al. 2001). In the Sierra Nevada, for example, trout stocking typically eliminates several benthic invertebrates, and the two dominant zooplankton species, the cladoceran *Daphnia melanica* and the copepod *Hesperodiaptomus shoshone* (Knapp et al. 2001). If stocking is halted and the fish die-out or are removed, *H. shoshone* often fails to recover despite the recovery of benthic invertebrates and of *D. melanica* (Sarnelle and Knapp 2004). Inability to recover was also observed with a related species under a similar scenario in the Rocky Mountains (Parker et al. 1996). Lack of recovery is surprising given the widespread distribution of these species (Knapp et al. 2001) and the persistence of long-lived diapausing eggs in lake sediments (Hairston 1996, Parker 1996).

One possible explanation for the reduced resiliency of large copepods relative to other alpine lake taxa is the difficulty of finding a mate. In a 3-dimensional habitat that is large relative to copepod body size, the rate of male-female encounter can have an important impact on population growth and persistence (Gerritsen 1980, Buskey 1998, Strickler 1998). Mechanistic models based on population growth rate and encounter rate predict a critical density for copepod population viability (Gerritsen 1980, Kiørboe 2006). Below this threshold, mate limitation results in a negative population growth rate. This critical density has been hypothesized to explain observed population densities (Kiørboe 2006) and the failure of *H. shoshone* populations to recover following extirpation by non-native trout (Sarnelle and Knapp 2004).

Using Gerritsen's (1980) diffusion model of encounter rate and very limited information about swimming speed, detection distance, and life history parameters, the critical density for *H. shoshone* recovery was estimated to be $0.5\text{-}5\text{m}^{-3}$ (Sarnelle and Knapp 2004). Results of a recent multi-lake reintroduction experiment suggested that the critical density was somewhat lower, between 0.2 and 3m^{-3} (Chapter 1). Here our aim is two-fold, to use data on *H. shoshone* mating behavior to obtain a more precise and accurate estimate of critical density, and to assess how environmental variation affects the critical density of *H. shoshone*.

Improving our estimate of critical density depends on obtaining a quantitative understanding of *H. shoshone* mating behavior. Various adaptations to increase encounter rate have been identified in copepods, including swarming (Ambler et al. 1996), pheromone clouds (Nihongi et al. 2004, Kiørboe et al. 2005) and pheromone trails (Doall et al. 1998, Tsuda and Miller 1998, Yen et al. 1998, Bagøien and Kiørboe 2005). Earlier studies on freshwater copepods have not found evidence of pheromone trails (van Leeuwen and Maly 1991, Nihongi et al. 2004), but recent observations by our group indicate that *H. shoshone* males use pheromone trails to locate females (Sehn et al. in prep). Theory suggests that trail-following behavior should be more common in larger species, such as *H. shoshone*, and results in higher encounter rates than hydromechanical or pheromone cloud methods of detection (Kiørboe and Bagøien 2005). Here we analyze a large number of *H. shoshone* mating interactions and use the results to estimate critical density with a model incorporating pheromone trails (Kiørboe and Bagøien 2005).

A second objective was to examine whether environmental variation affects encounter rate and critical density of *H. shoshone*. We focus specifically on the role of

temperature in this paper. Temperature has been shown to affect many aspects of copepod biology, including reproductive rates (Watras 1983, Williamson and Butler 1987, Chow-Fraser and Maly 1991), developmental rates (Roberston et al. 1974, Hart and McLaren 1978), and life span (Jersabek and Schabetsberger 1995, Hirst and Kiørboe 2002). Temperature may affect encounter rate both via physiological mechanisms, such as changes in activity or pheromone production, and physical mechanisms, including changes in viscosity and diffusion rates. For example, Podolsky and Emlet (1993) found that 40% of the decrease in swimming speed of sand dollar larvae (*Dendraster excentricus*) over a declining temperature gradient was due to increases in viscosity. Therefore, it is important to consider how temperature variation is likely to alter the critical density of copepod populations, and whether significant differences can arise solely from changes in encounter rate.

METHODS

Animal collection and maintenance

Adult *H. shoshone* were collected from an alpine lake (Name Lake) in the Sawtooth range, Utah, USA on August 25, 2006. Animals were shipped overnight in bottles on ice, to the Georgia Institute of Technology in Atlanta, GA. Animals were maintained as a mixture of males and females in bottled spring water at 12°C and fed rotifers. Mortality was $<5\% \text{ d}^{-1}$ and surviving animals remained healthy and active for >3 weeks. Mating activity was observed throughout this time period. Two days prior to experiments, randomly-selected males and gravid females were separated and acclimated to experimental temperatures.

Temperature experiments

Observations of mating behavior were conducted at 5, 12, and 16°C. Experiments took place in a 3.0 L vessel containing spring water, maintained at the treatment temperature by circulating distilled water in a large water jacket through a refrigeration unit. Mating behavior was recorded using three-dimensional Schlieren laser videography as developed by Strickler (Strickler & Hwang 1998) and further described by Doall (Doall *et al.* 1998).

Experiments were initiated by adding males and females to the vessel at a nearly 1:1 ratio (Table 1). We observed mating in mixed groups of individuals in a relatively large vessel because this method better mimics nature compared to interactions between a single male and female in a more restricted container. Mating interactions at each temperature were recorded for 4 hour periods on two consecutive days, August 5th and 6th (8 hours total). Males and females were separated between trials, maintained at their acclimation temperature and fed rotifers. To keep sex ratio and density as constant as possible, dead animals were replaced with randomly selected replacement individuals of the appropriate sex that had been acclimated to the treatment temperature for > 24 hours (Table 1).

Video recordings were processed to identify *H. shoshone* mating interactions. We defined mating events as behavior culminating in one individual being clasped by a pursuing individual, followed by the pair entering a characteristic tumbling behavior. The sex of animals was not obvious from the recording, so the pursuer was assumed to be a male and the pursued a female. Previous observations indicated that males preferentially pursue females (Yen, unpublished data). We digitized several seconds of swimming at 1/30 sec intervals prior to each attempted mating for which both animals

Table 1: The conditions and results of mating trials. Density and sex ratio varied based upon mortality. The same individuals were used in trial 1 and 2, except for replacements due to mortality between trials. Observed mating attempts are the total seen in the recording of the 4 hour trial and analyzable encounters are the subset from which complete 3D positions were obtainable.

Temperature		Density (# L ⁻¹)	Males : Females	Observed mating attempts	Analyzable encounters
5	Trial 1	8	11 : 12	44	14
	Trial 2	7	10 : 11	21	11
12	Trial 1	7	10 : 11	27	10
	Trial 2	7	10 : 11	31	13
16	Trial 1	7	9 : 8	77	22
	Trial 2	7	10 : 10	106	35

were visible in both the x-z and y-z views. This resulted in 105 analyzable events (Table 1).

We quantified the swimming speeds of the animals prior to detection and the length of the trail followed by the male (see Doall et al. 1998 and Sehn et al. in prep for calculations) from the digitized video. Initiation of trail following was recognized by an abrupt change in direction and noticeable acceleration, and confirmed by visualization of the digitized male and female paths. Trail length was estimated as the length of the female's swimming path between her and the male at the point pursuit began. This was defined as the point along the female path closest to the male when he reacted to the presence of the trail. Maximum trail length is the most relevant measure for an encounter model, because we are interested in how far away males are able to detect females, whereas average trail length is determined by where the males encounter the trail, even if they would have been able to successfully follow a longer trail. We wanted a quantitative estimate of maximum trail length rather than the absolute limit, which may only occur under ideal conditions or for extraordinary individuals, so we arbitrarily took the average of the 90th percentile across all treatments and used this as our estimate of L in the encounter model (Equation 1 below).

ANOVA was used to evaluate the effect of temperature on swimming speed, average trail length and average trail age. There were no significant differences in these parameters between experiments performed on different days ($P > 0.3$), so we pooled observations across days. Post—hoc pairwise comparisons were done using Tukey-Kramer HSD (SYSTAT version 9).

Encounter model and estimate of critical density

We estimated the search volume rate (β , $\text{m}^3 \text{ day}^{-1}$) for *H. shoshone* using an equation for a cruising male and a female pheromone trail (Bagøien and Kjørboe 2005):

$$\beta = 2Lu_{2D} \left(\sqrt{\frac{D_p L}{v}} + S \right) \quad (1)$$

where S is the sensory reach of the copepod (the length of one antennule, here roughly equivalent to the body length), L is the length of the pheromone trail, D_p is the diffusion coefficient of the pheromone, v is the swimming speed of the female prior to detection, and u_{2D} is the two-dimensional component of male swimming speed prior to pursuit. D_p was assumed to be 10^{-5} , typical of small biological molecules (Yen et al. 1998), and two-dimensional component of velocity was estimated based on random 3-dimensional swimming (Kjørboe and Bagøien 2005).

The search volume rate was then used to estimate critical density (N_C) following Gerritsen (1980). We chose this formulation because we were able to estimate R_0 with existing data, and could not accurately estimate the stage-specific mortalities required by the alternate formulation (Kjørboe 2006). The critical sexual encounter rate (Z) necessary to maintain a population without growth is (Gerritsen 1980):

$$Z = \frac{-1}{t} \ln \left(\frac{R_0 - 1}{R_0} \right), \quad (2)$$

where t is the length of the breeding season and R_0 is the net reproductive rate.

We replaced the spherical, diffusion model of encounter (Gerritsen 1980) with the estimate based on the detection of pheromone trails. Assuming a 1:1 sex ratio, we obtain:

$$N_C = \frac{-2 \ln \left(\frac{R_0 - 1}{R_0} \right)}{t\beta}. \quad (3)$$

Values for annual rate of increase from small population size were estimated from the first 4 years of successful reintroductions of *H. shoshone* ($r = 0.236$, from Chapter 1) and *Hesperodiaptomus arcticus* ($r = 1.57$, McNaught et al. 1999). These reintroductions offer a unique opportunity to directly assess net population growth rate since abundances were below equilibrium. *H. shoshone* are univoltine and encounter rate is per day, so $R_0 = e^{r/365}$. Maximum length of breeding season is assumed to be 60 days based on extensive sampling of Sierra Nevada *H. shoshone* populations from 2002-2006 (Kramer unpublished data) and estimated adult mortality rates (Chapter 1).

RESULTS

Temperature had a significant effect on the swimming speed prior to pursuit of both males and females (ANOVA, $p < 0.001$, Figure 1). Male swimming speeds were higher at 16°C ($p < 0.001$), and not different between 5°C and 12°C. (Figure 1). Female swimming speeds were also indistinguishable between 5°C and 12°C, and at 16°C were significantly higher than 12°C ($p < 0.001$) and nearly significantly different than 5°C ($p = 0.06$). Average trail lengths varied from 1.5 cm at 5 to 1.2 cm at 16°C, but there was no significant effect of temperature ($P > 0.2$). We estimated maximum trail length to be $L = 3.14$ cm.

Given our estimates of trail length and swimming speed (Fig. 1) and our range of estimates of growth rate (see Methods), we estimated a range of critical densities, 0.56 – 0.98 m^{-3} , for a population of 3mm long *H. shoshone* (Fig. 2). Average body length in many Sierra Nevada populations is 2.5mm (Appendix A), causing decreases in swimming speed and antennule length; using swimming speeds at 12°C from a small

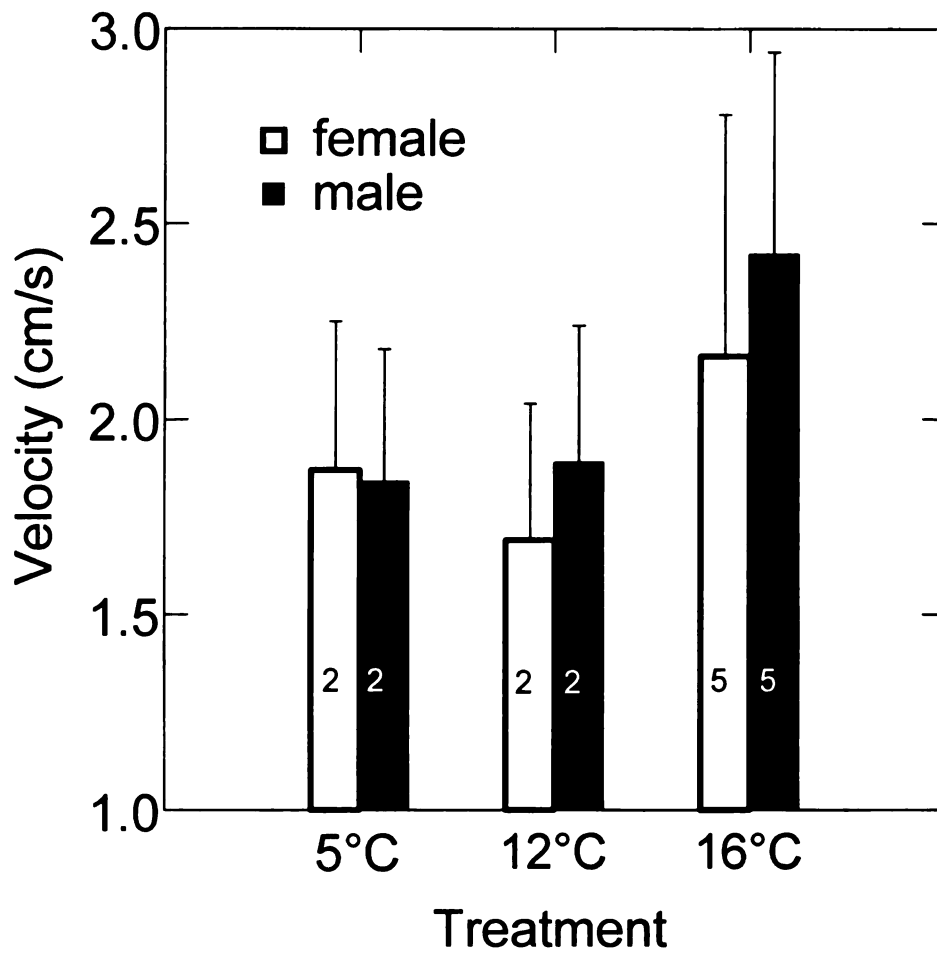


Figure 1. Average pre-pursuit swimming velocity (cm/s) for female and male *H. shoshone*. Sample size is indicated inside each bar and error bars represent the standard deviation. According to ANOVA results, treatment temperature had a significant effect on both female ($p < 0.001$) and male ($p < 0.001$) velocity.

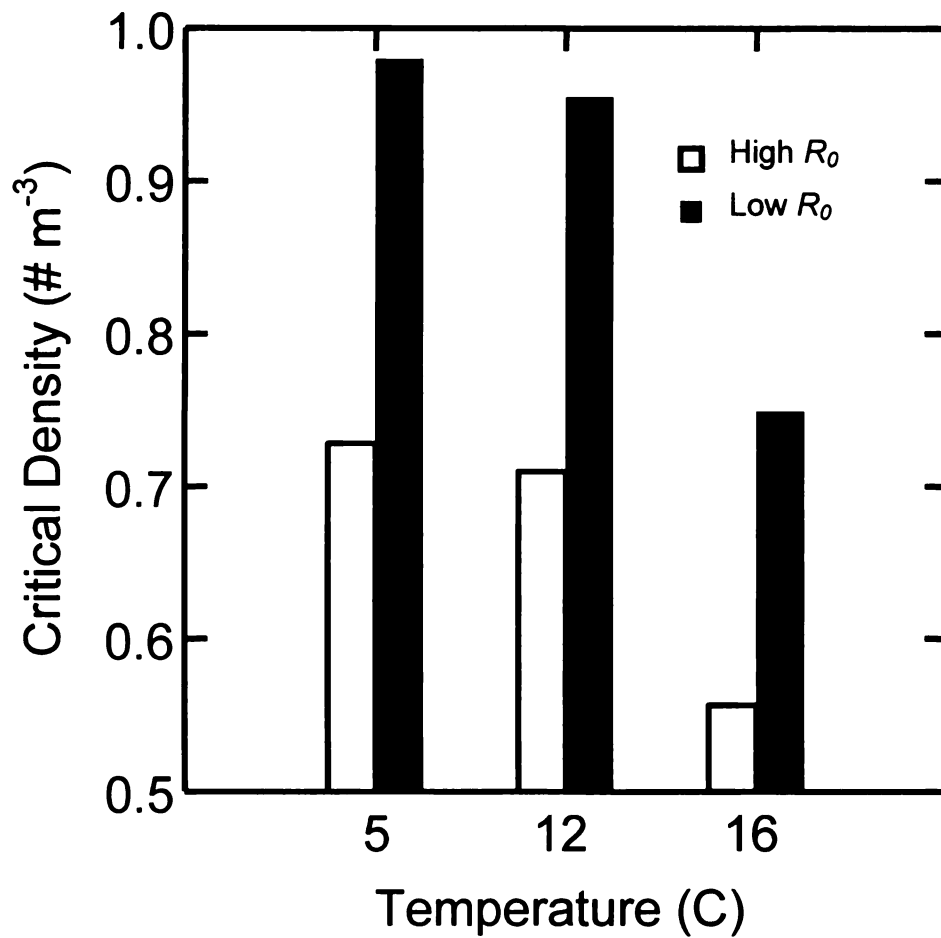


Figure 2. Critical density calculated using male and female velocity from each temperature treatment and low (1.00065 day^{-1}) or high (1.0043 day^{-1}) net reproductive rate. Breeding season length is 60 d.

sample of Sierra Nevada animals (Sehn et al. in prep) the resulting estimate of critical density is $0.86 - 1.30 \text{ m}^{-3}$.

An increase in swimming speed associated with an increase in temperature from 5 to 16°C decreases critical density nearly as much as the difference across the range of observed annual rates of increase (Fig. 2). In order to further assess the relative importance of variation in parameters influencing encounter rate vs. variation in reproductive rate we looked at the sensitivity of critical density to swimming speed, trail length and population growth rate. Proportionally equivalent changes in swimming speed and trail length had very similar impacts on critical density (Fig. 3b, c). Conversely, changes in population growth rate had little influence on critical density (Fig. 3a). It is also apparent that proportional decreases have a larger absolute effect than increasing the parameters by the same proportion (Fig. 3).

DISCUSSION

Our behavioral data enabled estimation of a range of critical density for *H. shoshone* in the Sierra Nevada ($0.86 - 1.3 \text{ m}^{-3}$) that was more precise than in a previous paper ($0.5 - 5 \text{ m}^{-3}$, Sarnelle and Knapp 2004), and congruent with the results of a multi-lake reintroduction experiment ($0.2 - 3 \text{ m}^{-3}$, Chapter 1). We also found that increased swimming speeds at higher temperatures (Fig. 1) have a relatively large effect on estimates of critical density (Fig 2). Model calculations suggest that critical density is more sensitive to temperature-induced changes in encounter rate than to changes in reproductive rate (Fig. 3) over the range of temperatures found in *H. shoshone* habitat. Because both encounter rate and life history parameters are expected to vary with

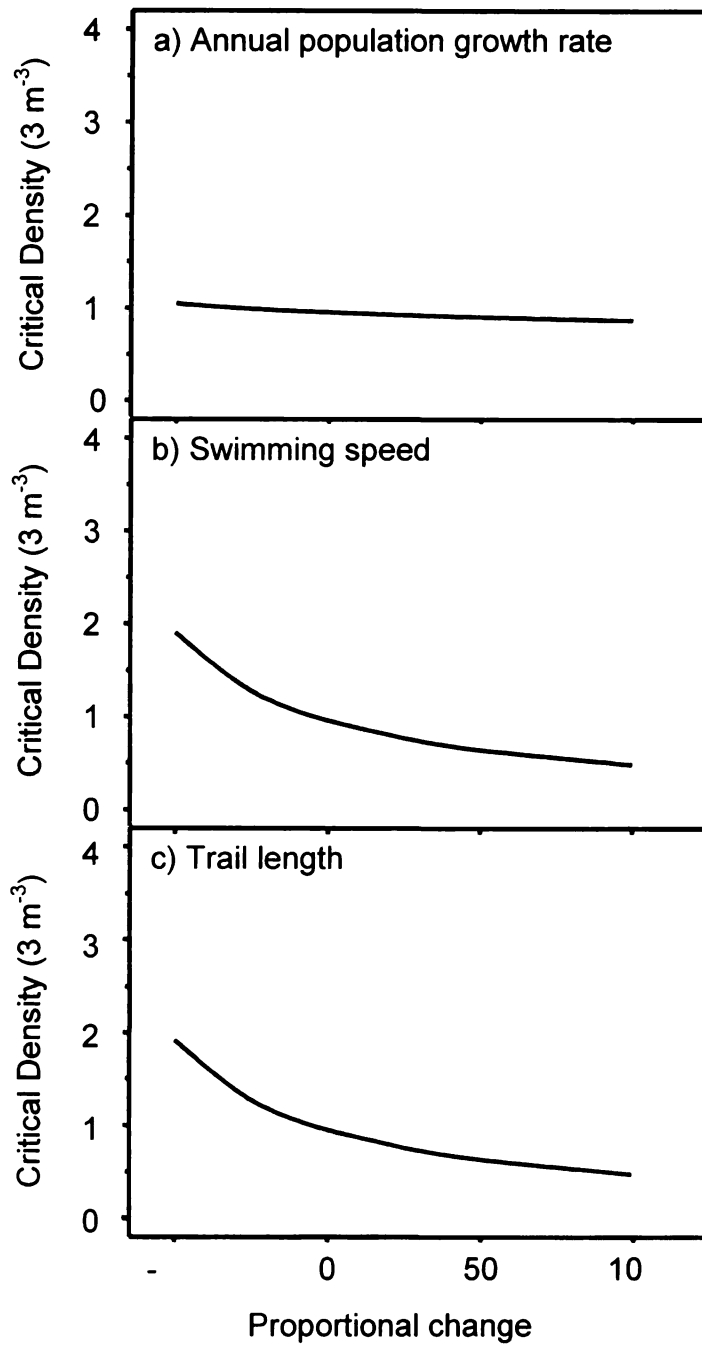


Figure 3. Sensitivity of estimated critical density to proportional changes in: a) annual population growth rate, b) swimming velocity, and c) trail length. Default value is velocity and trail length for males and females at 12°C (see text), 60 day breeding season, and net reproductive rate = 1.00065 d⁻¹.

temperature, critical density in this species likely varies substantially among lakes and between years.

Much of the increase in the precision of the critical density estimate is due to our use of field-measured, *H. shoshone*-specific life history parameters, however we introduce variation due to swimming speed that has not been considered in previous estimates of critical density. We have also increased the accuracy of the critical density estimate by using a realistic model of mating behavior in combination with experimentally measured parameters.

In addition to expected improvements in our estimates of critical density, this experiment provided evidence that temperature strongly influences the critical density of copepod populations by affecting swimming speed (Fig 2). Notably, male swimming speed tended to increase more than female speed across our temperature gradient (Fig. 1), and increases in male speed have a much larger effect on critical density than equivalent increases in female speed (Eq. 1). Whether temperature affects pheromone trails is unclear from these results. To accurately test L , the trail length relevant to encounter rate, a sample size large enough to ensure sufficient sampling of the longest trails is necessary, something we didn't achieve at 5 or 12°C. It is interesting to note that we observed a significantly higher number of mating events (ANOVA, $p=0.043$) in trials at 16°C (Table 1) and this increase exceeds that predicted by differences in swimming speed alone, implying that perhaps some other factors related to temperature were affecting either encounter rate or another aspect related to the initiation of mating behavior. Alternatively, animals may have been distributed closer to the bottom or sides of the chamber at lower temperature, reducing our ability to observe their activity.

Increased water temperature reduces critical density by increasing encounter rates, but can also affect at least one other component of critical density, namely reproductive rate. In general, warmer water results in increased egg production rate by copepods (Hirst and Kiørboe 2002). In one alpine copepod species, egg production rate was maximized at 20°C, while another species experienced highest fecundity at an intermediate (10°C) temperature (Jersabek and Schabetsberger 1995). However, reproductive rates appear to have little impact on critical density relative to other factors.

Temperature could also have an effect on breeding season length and proportional increases in breeding season length have effects similar to increases in swimming speed and trail length for the relevant range of parameter values (Equation 3). In warm years alpine lakes will experience longer ice-free periods, presumably allowing adults to appear earlier in the year and likely improving food quality and/or quantity, but increased temperature also results in increased mortality rates (Hirst and Kiørboe 2002). Because of these opposing influences, we used a single estimate of breeding adult presence in the lake, 60 days, which is equal to or greater than the maximum observed in 5 years of sampling Sierra Nevada *H. shoshone* populations, and is conservative with respect to the lower bound of the critical density.

Combining the effect of temperature on mating behavior and other components of critical density in *H. shoshone*, it seems likely that warmer temperatures result in lower critical density for these alpine copepods. Mean temperatures for the ice-free period of seven Sierra Nevada lakes (3358 – 3583 m elevation) measured from 1996-2002 ranged from 6.4 – 16.5°C (Knapp and Sarnelle unpublished data). This range is nearly identical to that examined in this experiment, and our results suggest ~25% reduction in critical

density at higher temperatures, due to increased swimming speed. A 25% decrease in critical density is a large change when the absolute number of individuals involved is considered. The median volume of lakes with established populations of *H. shoshone* in the Sierra Nevada is $1.3 \times 10^5 \text{ m}^3$ (Chapter 2). A decrease in critical density from 1 m^{-3} to 0.75 m^{-3} is a decrease of 33,000 animals that must initially colonize in order for the population to become established. It follows that recovery or colonization events taking place in warmer lakes or in warmer years may be more likely to succeed in establishing a population. The results also highlight the importance of body size. Populations of *H. shoshone* with body size closer to 2.5 mm than to the 3 mm animals used here are likely to have significantly lower swimming speeds (see Sehn et al. in prep) and, therefore, a higher critical density.

More generally, these results suggest *H. shoshone* deals with the potential challenges of finding mates through two important adaptations, the ability to produce and follow pheromone trails (Sehn et al in prep) and rapid swimming. The swimming velocity of *H. shoshone* is much higher than published swimming speeds for other calanoid copepods (Fig. 4), and several times higher than the similarly sized marine copepod *Calanus marshallae* (Tsuda and Miller 1998). A potential explanation for this difference is that *H. shoshone* evolved in the absence of fish, meaning adults have no pelagic predators, and many of the lakes also lack potential predators of juveniles, such as cyclopoid copepods and *Chaoborus* (Sarnelle and Knapp 2004). Increases in swimming speed increase the encounter rate with predators in much the same way as they increase the encounter rate with mates (Gerritsen 1980) and the lack of predators may have removed selection against rapid swimming in these large animals, or at least reduced the

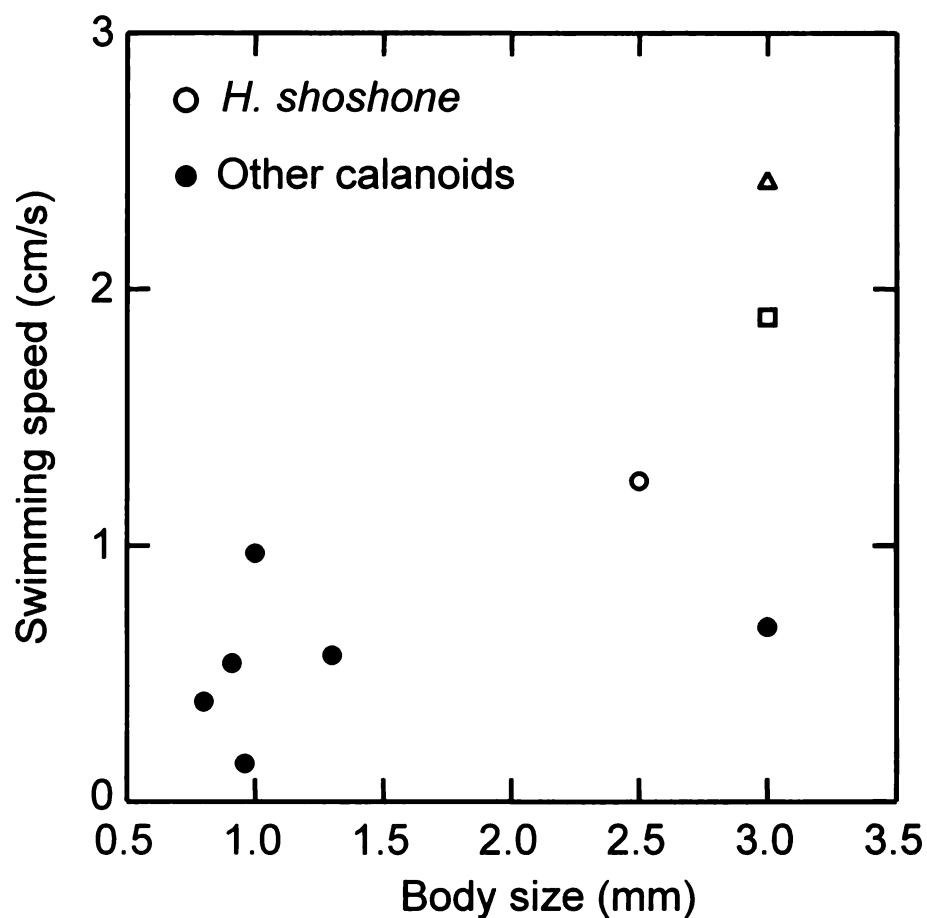


Figure 4. Swimming speed as a function of body size for *H. shoshone* (open shapes) and six other calanoid copepod species (filled circles). Measured swimming speeds are shown for Sierra Nevada *H. shoshone* at 12°C (○), and Utah *H. shoshone* at 12 (□) and 16°C (Δ). Data from Tsuda and Miller (2004), Doall et al. (1998), Nihongi et al. (2004), Kiørboe and Bagøien (2005), and Sehn et al. (in prep).

cost of large size, allowing rapid swimming. Large body size is expected to favor pheromone production, due to metabolic constraints, and existing data on copepod mate search capability suggest larger species are more likely to produce pheromones (Kjørboe and Bagøien 2005). Adaptations favoring low critical density are important to an animal that must colonize and persist in isolated habitats, especially because calaniod copepods have limited dispersal capabilities (Jenkins and Buikema 1998, Cáceres and Soluk 2002).

This study suggests that when densities are low, behavioral components of mating are equal to or more important in *H. shoshone* population growth than factors influencing egg production and mortality. It seems likely that other large freshwater copepods, such as *H. arcticus*, will have comparable mate search capabilities due to similarities in habitats and body size. Additionally, the relationship between temperature, swimming speed and encounter rate suggests a pathway through which temperature changes could affect reproductive rate in copepods at densities near the critical density.

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APPENDIX A

Hesperodiaptomus shoshone is a large (>2mm) deeply-pigmented copepod that occurs in alpine lakes in the Sierra Nevada and the Rocky Mountains from Colorado to British Columbia (Pennak 1978). As part of a multi-year project on the recovery ability of *H. shoshone* populations, I collected samples from multiple populations in the Sierra Nevada. Some populations were sampled a single time, but others were sampled in multiple years and multiple times per year. I also conducted a month long translocation experiment in which *H. shoshone* were placed in cages in 4 lakes. Some data from these samples is summarized here in order to improve the understanding of the life-history and population dynamics of this species.

SAMPLE AREA

Animals were collected between 2001 and 2006 from 19 persistent populations, 5 experimentally reintroduced populations and 1 naturally recovering population. All lakes were above 3290 meters in elevation and located in the Sierra National Forest, Inyo National Forest and Kings Canyon National Park (Table 1). Persistent populations can be categorized based on trout stocking history as: fish present, never stocked, or stocked but now fishless (Knapp et al. 2001). Populations in the final category are expected to have been extirpated by fish predation and to have recovered from the egg bank following fish disappearance (Knapp et al. 2001, Knapp and Sarnelle in prep).

Table 1: Lakes sampled, with lake ID (when known), name (when in quotes the name is not found of topographical maps), morphological data, stocking history(S-F=stocked, fish present, NS=never stocked, S-FL=stocked previously, now fishless, S-FL, Exp=Now fishless, experimentally reintroduced), the number and years each lake was sampled, additional samples which provided no information to this analysis are not included. (sampling differs for experimentally populations, see Methods).

Lake				Max		# of	Years
ID	Lake Name	Elevation	Area	Depth	Status	samples	sampled
40222	Ramona	3290	12.5	11.25	S-F	1	2003
41203	"Pavilion, Lower"	3323	8.2	20	NS	1	2003
50153	Goethe, Lower	3513	4.5	19.5	S-F	6	2001-2003
50154	Goethe, Upper	3514	23.1	30	S-F	4	2001-2003
50176	Lobe, Upper	3291	2.2	8.5	S-F	1	2003
50193	Petite, Lower	3504	1.0	7	S-FL	3	2001-2003
50194	Petite, Upper	3504	0.6	6.5	NS	3	2001-2003
50207	Puppet	3422	20.7	5.3	S-F	1	2003
50224	Wahoo 2	3443	3.5	13	S-FL	5	2001-2003
50423	Spire	3523	6.7	30	S-F	4	2001-2003
50424	Split	3413	1.6	20.5	S-F	5	2001-2003
52103	"Frog"	3632	0.9	5	NS	2	2003-2004
52121	"Dissertation"	3602	1.1	7	NS	8	2002-2005
52127	"Freedom"	3547	1.8	5.5	NS	1	2003
10222	"Barrett, Upper"	3554	1.1	5.2	NS	1	2004
10223	"Barrett, Middle"	3495	4.0	14.8	S-FL	1	2004
10230	"Glacier	3533	14.3	10.3	NS	1	2004
10257	"Southfork Pass"	3587	17.8	19.5	NS	1	2004

Table 16 (cont'd)

Lake				Max		# of	Years
ID	Lake Name	Elevation	Area	Depth	Status	samples	sampled
10263	"Palisade, Upper"	3450	4.8	13.2	S-FL	1	2004
50183	Marmot	3583	3.03	8	S-FL	2	2004-2005
50219	Square	3443	1.71	3.5	S-FL, Exp	10	2003-2005
	"No Good"	3516	1.67	5	S-FL, Exp	6	2003-2005
	Knob	3358	3.39	5.5	S-FL, Exp	2	2003
50133	Cony	3492	1.43	3.3	S-FL, Exp	1	2003

METHODS

Animals were sampled using 2 or more vertical tows of a 30 cm diameter plankton net with 64µm mesh. Tows were conducted in the deepest area of the lake from a float tube. In the case of the experimentally reintroduced populations, samples were collected with a 1 m diameter, 350 µm mesh net. This mesh is big enough to capture egg bundles and copepodites (Chapter 1). Samples were preserved in 90% ethanol. Three or more replicate, 1ml subsamples were examined at 40X with a Sedgewick-Rafter chamber, and the *H. shoshone* were assigned to the following categories, adult male, copepodite male, adult female, copepodite female, sex-indeterminate copepodites, and nauplii. Adult females were additionally assigned a reproductive condition of gravid, gravid w/eggs, non-gravid w/eggs, or non-gravid. Observed egg bundles were measured and the number of eggs was counted. The length of the first 50 individuals of each category was measured. Co-occurring copepods and cladocerans were also measured and enumerated, but that data will not be presented here.

LIFE HISTORY

Hatching

The data strongly suggest that a single generation of *H. shoshone* hatches synchronously from the egg bank prior to ice-out. Observing nauplii was very rare, even when lakes were sampled while partially ice-covered. Only one sample from a persistent population contained adults and more than two nauplii, and only six of 47 samples containing adults also contain 2 or 1 nauplii. Seventeen of the samples from persistent population contain only adults. Only two samples taken after July contained nauplii.

Nauplii were never observed in the experimentally reintroduced populations or the Marmot Lake population. Eggs much hatch sufficiently early for substantial development to take place before the ice melts.

Nauplii were not observed in the caged populations, even though a large number of eggs were produced. This suggests all the eggs produced were resting eggs.

Development

Copepodites were not identified to stage, so development times for various stages can not be estimated. We can make some rough estimates at the population level. In six cases sampling provided a time series taking the population from >90% copepodites to 80-100% adults. These samples were 30 days apart for Spire and Split Lakes in 2001, and Dissertation Lake in 2003, and only 10 days (Square Lake 2003), 13 days (Marmot Lake 2005), and 16 days apart in the other 3 cases (No Good Lake 2004). While an estimate of development time is difficult to make from these data, it is clear that time to maturity can be prolonged and that development is usually highly synchronous.

Adults were still present in all lakes which were sampled a month or more after adults were first observed to be predominate (n=8). In a similar time period of four weeks, females in the caged populations produced multiple clutches, proving females can mate multiple times after maturity and allowing for an estimate of interclutch duration in these animals. In these four lakes (see Chapter 1) interclutch duration varied from 13 -20 days. We can assume females have enough time to produce 2 or more clutches each summer.

Body size

Average body size varied among lakes and years. Average body size among all lakes sampled in 2003 was similar to, but slightly smaller and less variable than, all lake*year combinations for which data was available (Fig. 1). Males tended to be smaller than females on average (Fig. 1) and in each lake*year combination a males were $83\ \mu\text{m}$ (± 121) smaller than non-gravid females (paired t-test, $p < 0.0001$, $n = 36$) and $152\ \mu\text{m}$ (± 156) than gravid females ($p < 0.0001$, $n = 38$). Non-gravid females were $71\ \mu\text{m}$ (± 133) smaller than gravid females ($p < 0.003$, $n = 36$). Males and females differed less in size within a given lake*year than sexes did between lakes. There was a large difference between the maximum average size observed, $3200\ \mu\text{m}$ for gravid females in No Good Lake (2004), and the minimum average size, $1820\ \mu\text{m}$ for males in Spire Lake (2001).

Sex ratio

Sex ratio was varied widely among lakes, but also varied within lakes among samples. Average sex ratio was 0.99 (± 0.61) among 40 lake*year combinations with a max of 2.5 males:female and minimum of 0 (two cases, fewer than 5 adults sampled). When multiple samples were taken from a single lake in a single year, all data were pooled to calculate the sex ratio for that lake. Average and standard deviation were similar among lakes with multiple samples vs. lakes sampled a single time. Within a lake sex ratio varied as much a 6-fold among samples within the same year. Further analysis should determine if sex ratio varies temporally or is it is merely highly stochastic with regard to a given sample, perhaps suggesting spatial variation in sex ratio.

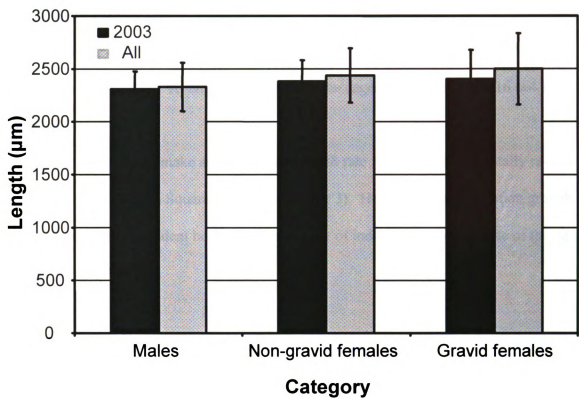


Figure 1. Average and standard deviation of body length (μm) for adult *H. shoshone* in 2003 (n=14 lakes) and all lake*year combinations (n=40).

Reproductive rate

Average clutch size was 12.3 eggs (± 5.1 , max = 23, min = 6) among 29 lake*year combinations. Average clutch size for the caged populations was 16 (± 4.1) (Chapter 3).

We were able to make an estimate of growth rate from the experimentally re-introduced population in Square Lake (see Chapter 3). However, this population growth rate is likely to be dependent both upon the density of individuals and the state of the egg bank (Chapters 1 and 3).

Mortality

Mortality was estimated for the caged populations and the two most thoroughly sampled populations (see Chapter 1).

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