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Effect of inbreeding on reproduction and juvenile performance in two marine gastropods with contrasting reproductive patterns

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ABSTRACT: Many species avoid inbreeding through dispersal of individuals away from their natal groups or sites. Benthic marine invertebrates with long free-living larval stages generally possess great dispersal potential and are therefore expected to have a small chance of inbreeding in the field. Species that lack such dispersive larval stages should have a higher likelihood of inbreeding. If such direct-developing species do often mate with close relatives, we expect them to exhibit less inbreeding depression than those with dispersive larvae, since frequent inbreeding can lead to the purging of deleterious alleles through natural selection. To test this, we compared the effects of inbreeding in 2 *Crepidula* species with contrasting reproductive patterns: *C. fornicata*, which has a long dispersive larval stage; and *C. convexa*, a direct developer without a free-living larval stage. In laboratory studies, we compared reproductive output and several fitness traits of juveniles from snails that were forced to mate with full siblings with those from snails forced to mate with unrelated individuals. In contrast to our expectations, *C. convexa* showed much stronger inbreeding depression than *C. fornicata*. For example, inbreeding decreased mean juvenile growth rate for *C. convexa* by 20 to 44 %, but did not affect mean growth rate for *C. fornicata.* It appears that larval dispersal potential alone can be a poor predictor of the amount of inbreeding that occurs in natural populations and of the effect of inbreeding on offspring fitness.

KEY WORDS: Inbreeding depression · Reproductive pattern · *Crepidula* · Direct development · Dispersal · Larvae

INTRODUCTION

The amount of inbreeding that occurs in a species is often inferred from how far individuals in that species can disperse in their lifetime (Knowlton & Jackson 1993, Smith 1993, Peters & Michiels 1996). In many bird and mammal species, dispersal of individuals away from their natal groups or sites, especially those of just 1 sex, separates close relatives and thus prevents inbreeding (Hoogland 1992, Bollinger et al. 1993, Pusey & Wolf 1996, Daniels & Walters 2000). On the other hand, species with low dispersal ability often inbreed routinely (Riechert & Roeloffs 1993, Clarke & Faulkes 1999, Bilde et al. 2005). Organisms that inbreed often experience reduced reproductive fitness and reduced offspring performance (a phenomenon termed inbreeding depression; Wright 1977, Charlesworth & Charlesworth 1987). According to the partial dominance hypothesis, inbreeding depression results from the phenotypic expression of deleterious recessive or partially recessive alleles that are at least partially masked in the heterozygous condition: inbreeding increases the proportion of individuals that are homozygous for such recessive alleles, allowing their full expression (Charlesworth & Charlesworth 1987, Crnokrak & Barrett 2002). Alternatively, the overdominance hypothesis assumes that inbreeding depression occurs because heterozygotes are superior to homozygotes in fitness, through beneficial interactions between alleles (Dudash & Carr 1998). Although

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some studies support the effect of overdominance (Mitton & Koehn 1975, Karkkainen et al. 1999, Crnokrak & Barrett 2002), alleles with partial dominance are considered to make the major contribution to inbreeding depression (e.g. Charlesworth & Charlesworth 1987, Dudash & Carr 1998, Frankham et al. 2002).

Species that have a long inbreeding history, likely caused by their low dispersal ability, tend to exhibit less inbreeding depression than those that predominantly outbreed (Cohen 1996, Husband & Schemske 1996, Ballou 1997). According to the partial dominance hypothesis, that is because a species' mutation load can be purged through frequent inbreeding: inbreeding exposes deleterious recessive alleles to selection and potentially removes them from populations over many generations (Charlesworth & Charlesworth 1987, Frankham 1996, Byers & Waller 1999, Crnokrak & Barrett 2002). In marine invertebrates, the relationship between dispersal potential and the effect of inbreeding has been little examined.

Benthic marine invertebrates can differ greatly in dispersal potential (Thorson 1950, Pechenik 1999). Many species have a free-living larval stage (Thorson 1950, Pechenik 1999) that spends weeks to months in the plankton, potentially dispersing individuals over great distances (Jackson 1986, Scheltema 1995, Pechenik 1999), while others (called direct developers, Thorson 1950) lack such a stage and emerge instead as crawling juveniles. Even though juveniles or adults of some direct-developing benthic marine invertebrates possess the ability to disperse through drifting or rafting (e.g. Martel & Chia 1991a,b), their dispersal potential is probably much smaller than that of long-lived larvae (Jackson 1986, Palumbi 1995, Scheltema 1995). Therefore, species with a long free-living dispersive larval stage should have a lower likelihood of encountering close relatives as adults — and mating with them — than species that lack such a free-living larval stage (Knowlton & Jackson 1993, Pechenik 1999). If direct-developing benthic marine invertebrates do often inbreed in the field, we would expect them to exhibit less inbreeding depression when forced to mate with close relatives than species that have a long free-living larval stage.

We tested this hypothesis using the 2 marine gastropods *Crepidula fornicata* and *C. convexa*. *C. convexa* is a direct developer while *C. fornicata* has a larval period that can last up to 9 wk in the laboratory at 18°C (Pechenik 1984). Molecular data for the east coast of the USA showed little population structure for *C. fornicata*, but strong genetic differentiation for populations of *C. convexa*, supporting the idea that larvae of *C. fornicata* achieve much wider dispersal than the juveniles or adults of *C. convexa* (Collin 2001). In addition, individuals of *C. convexa* either cannot or simply do not avoid mating with close relatives (authors' unpubl. data), so that there are no apparent behavioral barriers to inbreeding for this direct-developing species. Whereas freshwater gastropods have become a model system for studies of mating system variation (Jarne et al. 1991, Trouve et al. 2003, Glow et al. 2005), we know of no previous studies on this topic concerning marine gastropods.

Crepidula fornicata and *C. convexa* are ideal for our study for many reasons. First, they are closely related, with similar morphology and physiology, and they often co-occur in the same habitats. Therefore, differences seen in the magnitude of inbreeding depression between the 2 species likely reflect differences in reproductive patterns. Second, individuals in the genus *Crepidula* are protandrous hermaphrodites, so that every male has the potential of becoming a female (Coe 1936). This facilitates laboratory breeding studies, since 2 associated males will always eventually become a mating pair (Pechenik et al. 1996, Hilbish et al. 1999). Third, both species are suspension feeders, so it is easy to ensure that all juveniles have equal access to food (phytoplankton) at identical concentrations, enabling us to document with confidence any growth rate differences occurring among individuals in different treatments. Our study is the first we know of that compares the levels of inbreeding depression in 2 non-colonial marine invertebrates with contrasting reproductive patterns.

In this study, we conducted laboratory experiments to determine the consequences of inbreeding for *Crepidula fornicata* and *C. convexa* by comparing reproductive output and the values of several fitness traits of offspring from individuals that mated with their full siblings and those that mated with unrelated individuals. We define fitness trait as any particular trait of an organism upon which natural selection acts. The term inbred is sometimes used to mean different things by different authors, or used ambiguously. In the present study, parents that were set up to form inbreeding or outbreeding matings are referred to as either inbreeding or outbreeding individuals. Offspring resulting from those matings are referred to as either inbred or outbred individuals.

MATERIALS AND METHODS

Measuring inbreeding depression in *Crepidula convexa*. To obtain full sibling families, 10 males of *Crepidula convexa* were collected from Nahant, Massachusetts, USA, during low tide. In the laboratory, they were haphazardly arranged into 5 pairs. All snails were kept in 1 µm filtered seawater at room temperature (~23°C) and fed with a mixture of the unicellular

phytoplankton *Dunaliella tertiolecta* (clone DUN) and *Isochrysis galbana* (clone T-ISO). Over 2 to 3 mo, the larger male in each pair became a female and the smaller one remained a male to form a mating pair. Pairing snails when both individuals were still male ensured that the female in each pair could only mate with 1 male and produce full-sibling offspring. The offspring released from each pair were designated as the parental generation: some of them were raised to adulthood in the laboratory, and were eventually selected to be the parents of either inbred or outbred offspring. All snails from the same parents were considered a family. Therefore, we obtained 5 full-sibling families of *C. convexa*, named CC-A, CC-B, CC-C, CC-D, and CC-E.

For each family, we measured the shell lengths of 20 haphazardly chosen newly hatched juveniles at 50× magnification using a dissecting microscope to determine initial shell length. The shell lengths of both male and female parents were also measured. Ten of the juveniles were then each raised in 20 ml of phytoplankton suspension (T-ISO) at 18×10^4 cells ml^{-1} for 8 d. The juveniles were kept in an incubator set at 25°C with a photoperiod of 12:12 h light:dark; water and food were changed daily. Shell lengths of these juveniles were measured non-destructively at 50× magnification using a dissecting microscope on Days 0, 4, and 8. Juvenile growth rate was calculated from the slope of the regression relating juvenile shell length to age.

For each family, we created an inbreeding and an outbreeding group, each consisting of 10 pairs of snails. The snails in the inbreeding group were all haphazardly chosen from this full-sibling family, whereas in the outbreeding group, only females were from this family. The males in the outbreeding group were offspring obtained from females of *Crepidula convexa* collected at Nahant. Each male was from a different mother. Because the females in these 2 groups (but not the males) were siblings, our design minimized the differences in offspring performance that could be due to maternal effects. All snail pairs were maintained in the same aquarium at 20°C, but in separate plastic cages. The cages, with dimensions of 53 mm × 53 mm × 45 mm, had mesh sides to permit water flow for feeding and respiration. The snails were fed with a mixture of the phytoplankton clones DUN and T-ISO twice daily at approximately 2×10^6 cells ml^{-1} for about 6 mo, by which time 40 of the outbreeding and 36 of the inbreeding pairs had reproduced. The snail pairs were kept in the aquarium until their offspring were nearly ready to hatch out from the egg mass being brooded conspicuously under the female's shell; advanced development is indicated when the egg mass turns from a yellow color to grey. Each pair of snails was then kept individually in 1 µm filtered seawater until juveniles were released, so that we knew the parentage of all the juveniles.

We measured the cost of inbreeding in *Crepidula convexa* by measuring fitness traits. After some females in both groups produced juveniles forming the F_1 generation, we collected the first batch of juveniles (n = 11 to 523 juveniles per female) from each female (n = 76 females) and measured the following traits: mean time to offspring release, mean fecundity of the parental generation, mean initial size at hatching, mean juvenile growth rate, and mean juvenile survival (Table 1). Each trait was measured as described below.

To calculate the time from when the snails were paired together to the day that offspring were released, we recorded the date that each female released her first batch of offspring (F_1 generation). To determine the fecundity of the parental generation, we counted the total number of offspring released in the first batch produced by each female. Because female size affects fecundity in *Crepidula convexa* (Hendler & Franz 1971), we also measured the shell lengths of both parents after the offspring were released. As soon as we noticed that a batch of offspring had hatched out (always within 16 h after release), we determined their initial size by measuring the shell lengths of 20 haphazardly selected individuals from that batch using a dissecting microscope (magnification 50×).

To determine the effect of inbreeding on juvenile growth rate, we reared 5 haphazardly chosen juveniles (F_1 generation) from each female separately in 20 ml of phytoplankton (T-ISO) suspension at 18×10^4 cells ml^{-1} (excess food treatment) at each of 3 temperatures: 15,

Table 1. *Crepidula convexa*. Inbreeding depression coefficients (δ) for 5 families (CC-A to CC-E). na: not applicable; ns: not significant

Fitness trait	CC-A	CC-B	CC-C	CC-D	CC-E	Avg.
Time to offspring release	ns	ns	0.47[a]	ns	ns	na
Fecundity	0.65	0.43	0.40	0.89	0.35	0.54
Initial offspring size	ns	ns	ns	−0.18	ns	na
Juvenile survival	0.34	0.29	0.47	0.70	0.29	0.42
Juvenile growth rate (in presence of excess food)						
at 15°C	0.32	0.25	0.25	0.12	0.04	0.20
at 25°C	0.63	0.34	0.39	0.28	0.17	0.36
at 29°C	0.56	0.43	0.47	0.49	0.25	0.44
Juvenile growth rate (in presence of limited food)						
at 25°C	0.63	0.23	0.30	0.36	0.06	0.32

[a]For time to juvenile release, δ is calculated as 1 – (mean value of outbred individuals/mean value of inbred individuals)

25 (optimal temperature), and 29°C. Another 5 juveniles from each female were reared separately in 20 ml of phytoplankton (T-ISO) suspension at 1×10^4 cells ml^{-1} (food-limited treatment) at 25°C. For all treatments, water and food were changed daily and the photoperiod was kept at 12:12 h light:dark. Juvenile shell lengths were measured on Days 0 and 8, with Day 0 being the day they were released from the parents. Juvenile growth rate (µm d^{-1}) was calculated as follows:

$$GR = \frac{SL_8 - SL_0}{8} \quad (1)$$

where GR is juvenile growth rate, and SL_8 and SL_0 are juvenile shell length (µm) at Day 8 and Day 0, respectively. If there were not enough juveniles ($n < 20$) in the first batch of offspring for all treatments, juveniles from a second batch of offspring from the same female were added to the experiment.

All juveniles (F_1 generation) from each female other than the 20 used in the growth rate experiment were kept at 25°C in the presence of excess phytoplankton (T-ISO). Water and food were changed daily for 8 d. We recorded the number of juveniles that died in the first 8 d after hatching to calculate percent survival. A small proportion (usually <5%) of juveniles climbed out of the water and died of desiccation. These juveniles were not counted in the experiment, since in the field, with natural tide cycles, this climbing behavior might not have led to death.

Inbreeding depression coefficient δ was calculated as follows (Charlesworth & Charlesworth 1990):

$$\delta = 1 - \frac{W_I}{W_O} \quad (2)$$

where W_I is the mean value of a particular fitness trait for inbred offspring, and W_O is the mean value for the same fitness trait of outbred offspring.

Measuring inbreeding depression in *Crepidula fornicata*. Five full-sibling families of *Crepidula fornicata* were obtained using the same method as that used for *C. convexa*. These 5 families were named CF-A, CF-B, CF-C, CF-D, and CF-E.

To measure the juvenile growth rate of the parental generation, we haphazardly selected about 200 larvae for each family and reared them for 2 wk in the laboratory on a diet of clone T-ISO. When these larvae grew to an average size of 800 µm, they were induced to metamorphose using 20 mM excess K^+ in seawater (Pechenik & Heyman 1987). We then haphazardly selected 10 juveniles and reared them under the same conditions as for the parental generation juveniles of *Crepidula convexa*. For these *C. fornicata* juveniles, food was added 1 to 2 times daily to ensure adequate food supply. We non-destructively measured the shell lengths of these juveniles at a magnification of 32 to 50× using a dissecting microscope on Days 0 and 8. The juvenile growth rate of the parental generation (µm d^{-1}) was calculated using Eq. (1).

To create the inbreeding and outbreeding groups, we used essentially the same method as for *Crepidula convexa*, except that instead of setting up 10 pairs of snails in each group for each family, we set up 5 pairs of snails in each group. Because of an incubator malfunction 7 mo into the experiment, some of the snail pairs died before reproducing. As a result, we were only able to obtain data from both inbreeding and outbreeding groups for 3 families (CF-A, CF-B, CF-C). Partial results from the other 2 families were discarded.

To assess the cost of inbreeding in *Crepidula fornicata* we measured fitness traits on offspring. After some females in both inbreeding and outbreeding groups produced larvae forming the F_1 generation, we collected the first batch of larvae (n = 1282 to 10 176 larvae per female) from each female (n = 20 females) and measured the following fitness traits: mean brooding time, mean fecundity, mean initial size at hatching, and mean juvenile growth rate (Table 2). Each trait was measured as described below.

We recorded the date each female deposited an egg mass and the date she released her first batch of offspring to calculate brooding time for F_1 generation offspring (in days). To measure fecundity and initial offspring size for *Crepidula fornicata* we used the same methods as we did for *C. convexa*.

To determine the effect of inbreeding on juvenile growth rates, we haphazardly selected about 200 larvae from each hatching, and reared them for about 2 wk on a diet of clone T-ISO. When these larvae grew to an average size of 800 µm, they were induced to metamorphose using the same method as for the parental generation larvae. These newly metamorphosed juveniles were reared in the same 4 treatments of different temperature and food concentrations as used for examining the juvenile growth rates of *Crepidula convexa*, except that there were 10 juveniles in each treatment instead of 5. Juvenile growth rates of *C. fornicata* were determined using the same method as used for *C. convexa*.

Statistical analysis. All data were checked for normality and homogeneity before statistical analysis, and appropriate transformations were used when necessary to improve data distributions (Zar 1999). All percentage data were arcsine-transformed before statistical analysis. Data on mean time to offspring release, mean brooding time, mean initial size, mean juvenile survival, and mean juvenile growth rate at 25°C in the presence of limited food were each analyzed using a 2-way mixed-model ANOVA with

Table 2. *Crepidula convexa* and *C. fornicata*. Fitness traits examined in both species and whether these traits were negatively affected by inbreeding. na: not examined

Fitness trait	*C. convexa*	*C. fornicata*
Time to release of F_1 generation offspring	Yes, for 1 of the 5 families	na
Brooding time of F_1 generation offspring	na	No
Fecundity	Yes	No
Initial size of F_1 generation offspring	No	No
Juvenile survival	Yes	na
Juvenile growth rate (in presence of excess food)		
at 15°C	Yes	No
at 25°C	Yes	No
at 29°C	Yes	No
Juvenile growth rate (in presence of limited food)		
at 25°C	Yes	No
Comparison of:		
Fecundity of inbreeding females to that of their mothers	Yes, for 3 of the 4 families examined	na
Initial sizes of parental generation and F_1 generation individuals	No	na
Growth rates of parental generation and F_1 generation juveniles reared at 25°C in the presence of excess food	Yes, for 2 of the 5 families	Yes, for 1 of the 3 families

breeding type (inbreeding or outbreeding) as the fixed factor and family as the random factor. Data on mean juvenile growth rates at 15, 25, and 29°C in the presence of excess food were analyzed using 4-way partly nested ANOVA (split-plot design, Quinn & Keough 2002) with breeding type and temperature as fixed factors, and family and parent of F_1 generation juveniles as random factors. Breeding type, temperature, and family were all crossed with each other, and parent of the juveniles was nested within breeding type and family, but was crossed with temperature. Data on mean fecundity of the parental generation were analyzed using a mixed model ($y = XB + u + e$), where y is the square root of fecundity, X is the matrix of the covariate factor female size, B is the regression coefficient, u accounts for the variance of family random effects, and e accounts for error. When comparing data on mean fecundity of the inbreeding and outbreeding females to that of their mothers using 1-sample t-tests, we used size-adjusted female fecundity (fecundity/female shell length). Data on mean initial sizes and mean growth rates of the parental generation juveniles and those of the F_1 generation juveniles were compared using ANOVA and Bonferroni post hoc tests.

In any of the ANOVA tests examining the interaction between breeding type and other factors, such as family and temperature, if the interaction was significant, multiple comparisons (multiple t-tests) were performed for each family or temperature, with the significance level adjusted using the sequential Bonferroni procedure (p-values were ranked from largest to smallest, and the smallest p-value was tested at $0.05/n$, the next at $0.05/(n - 1)$, etc., where n is the number of tests performed, Quinn & Keough 2002).

Data were analyzed using Prism 3.0 (GraphPad), SAS 9.1, and Systat 10 (SPSS), with adjustments made as needed for 4-way partly nested ANOVA (Zar 1999). Power analysis was performed using Java applets for power and sample size (retrieved February 10, 2006, from www.stat.uiowa.edu/~rlenth/Power).

RESULTS

Crepidula convexa

The mean initial shell lengths of the parental generation snails obtained from the 5 full-sibling families used in this study ranged from 785.6 to 948.1 µm (Fig. 1). Parental generation juveniles grew at a constant rate at 25°C in the presence of excess food (linear regression: $r^2 = 0.95 \pm 0.10$ SD, n = 50). The mean growth rates of these parental generation juveniles obtained from the 5 full-sibling families ranged from 57.8 to 79.5 µm d^{-1} (Fig. 2B; see Fig. 2 for all 4 treatments). Juvenile growth rate was not significantly correlated with initial size (linear regression: $r^2 = 0.020$, $F_{1,48} = 0.991$, p = 0.324).

Overall, inbreeding did not affect mean time from the start of the experiment to release of F_1 generation offspring (Fig. 3, Table 3), although there was a significant interaction ($p < 0.05$) between breeding type and family (Table 3). When examining time to release of inbred and outbred offspring for each family separately and adjusting the significance level for multiple comparisons, we found that inbreeding marginally (but significantly) increased mean time to offspring release for family CC-C (unpaired t-test: $t = 3.341$, df = 7, p = 0.012 > 0.05/5, Table 1), but not for any of the other 4 families in the study (unpaired t-test, CC-A: $t = 0.183$, df = 12, p = 0.858; CC-B: $t = 0.142$, df = 12, p = 0.889; CC-D: $t = 1.661$, df = 14, p = 0.119; CC-E: $t = 0.113$, df = 13, p = 0.912).

Inbreeding significantly decreased female fecundity for *Crepidula convexa* ($F_{1,68} = 33.09$, $p < 0.0001$,

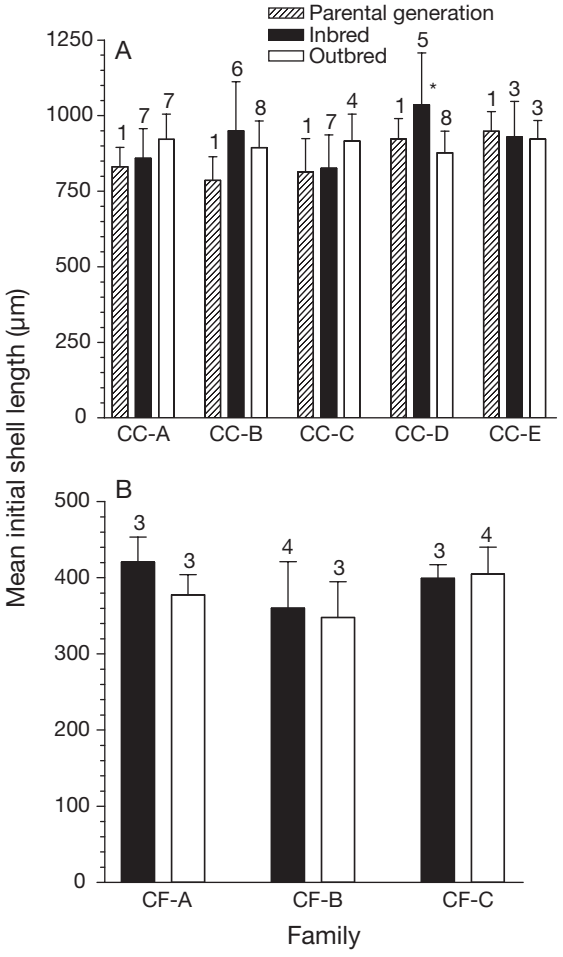

Fig. 1. *Crepidula convexa* and *C. fornicata*. Mean initial shell length of parental and F_1 generation individuals in (A) 5 families of *C. convexa* and (B) 3 families of *C. fornicata*, including shell lengths of 20 to 40 offspring from each female. Number above each bar: number of female parents for each group of snails. Error bars: +1 SD. *Significant difference between mean initial shell length of inbred and outbred offspring at p = 0.01 (significance level adjusted for multiple comparisons)

Table 3. *Crepidula convexa* and *C. fornicata*. Two-way mixed-model ANOVA results for the effect of inbreeding on 5 fitness characteristics of both species. 'Breeding type' indicates whether the F_1 generation offspring were inbred or outbred

Source	df	*F*	p
Time to release of F_1 generation offspring			
C. convexa			
Breeding type	1	2.065	0.224
Family	4	4.168	0.005
Breeding type × Family	4	3.399	0.014
Error	58		
Brooding time of F_1 generation offspring			
C. fornicata			
Breeding type	1	0.014	0.917
Family	2	1.592	0.238
Breeding type × Family	2	0.310	0.739
Error	14		
Initial size of F_1 generation offspring			
C. convexa			
Breeding type	1	0.045	0.843
Family	4	2.099	0.096
Breeding type × Family	4	5.066	0.002
Error	48		
C. fornicata			
Breeding type	1	2.345	0.265
Family	2	2.508	0.117
Breeding type × Family	2	0.782	0.477
Error	14		
Juvenile survival			
C. convexa			
Breeding type	1	21.313	0.010
Family	4	2.269	0.072
Breeding type × Family	4	4.690	0.002
Error	60		
Juvenile growth rate at 25°C in the presence of limited food			
C. convexa			
Breeding type	1	15.681	0.017
Family	4	0.899	0.470
Breeding type × Family	4	2.221	0.077
Error	62		
C. fornicata			
Breeding type	1	1.860	0.306
Family	2	2.149	0.153
Breeding type × Family	2	0.628	0.548
Error	14		

Fig. 4). The inbreeding depression coefficients (δ) for fecundity of all 5 families are listed in Table 1. For inbreeding females, there was a linear relationship between the number of juveniles released (y) and female shell length (x in mm) as follows: $y = 11.67x - 72.45$ (linear regression: $r^2 = 0.121$, $F_{1,35} = 4.837$, p = 0.034, Fig. 4). The relationship for outbreeding females was recast as $y = 42.89x - 395.5$ (linear regression: $r^2 = 0.282$, $F_{1,36} = 14.11$, p = 0.0006, Fig. 5). However, there was no significant linear relationship between female fecundity and shell length of the associated male (linear regression: $r^2 = 0.011$, $F_{1,62} = 0.682$, p = 0.412).

Overall, inbreeding did not affect mean initial shell lengths of F_1 generation snails (Fig. 1, Table 3). There was a significant interaction between breeding type and family. Inbreeding significantly increased the initial juvenile shell lengths for family CC-D (unpaired *t*-test: $t = 3.750$, df = 11, p = 0.003 < 0.05/5), but did not have any significant effects for the other 4 families (unpaired *t*-test, CC-A: $t = 2.272$, df = 12, p = 0.042 > 0.05/4; CC-B: $t = 1.077$, df = 12, p = 0.303; CC-C: $t = 1.992$, df = 9, p = 0.078; CC-E: $t = 0.131$, df = 4, p = 0.902).

Overall, inbred juveniles of *Crepidula convexa* had significantly lower mean survival than outbred juveniles for the first 8 d after hatching (Fig. 6,

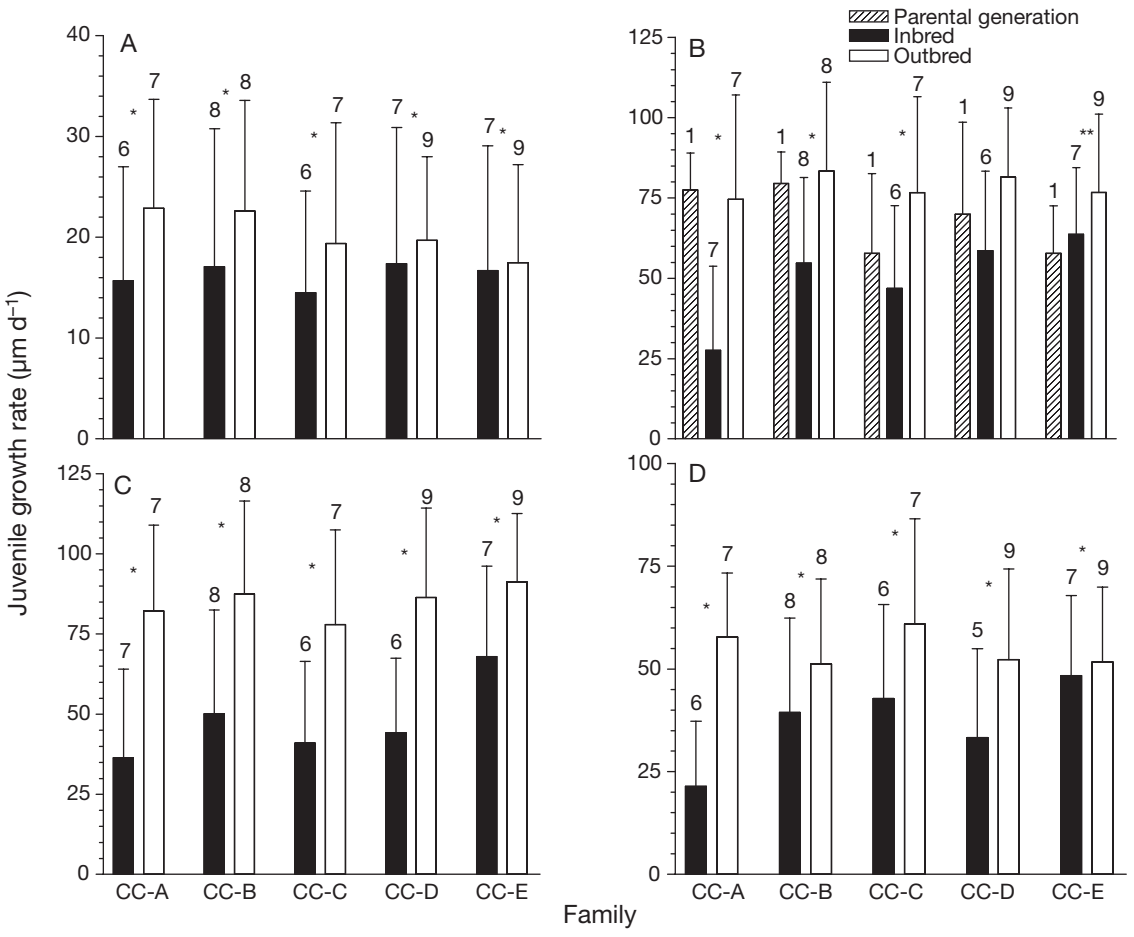

Fig. 2. *Crepidula convexa*. Effect of inbreeding on mean juvenile growth rate in 5 families. Ten parental generation juveniles and 5 F_1 generation juveniles from each female were reared in each treatment. (A) 15°C; (B) 25°C; and (C) 29°C in the presence of excess food; and (D) 25°C in the presence of limited food. Number above each bar: number of female parents for each group of juveniles. Error bars: +1 SD. *Significant difference between mean growth rates of inbred and outbred juveniles from the same family

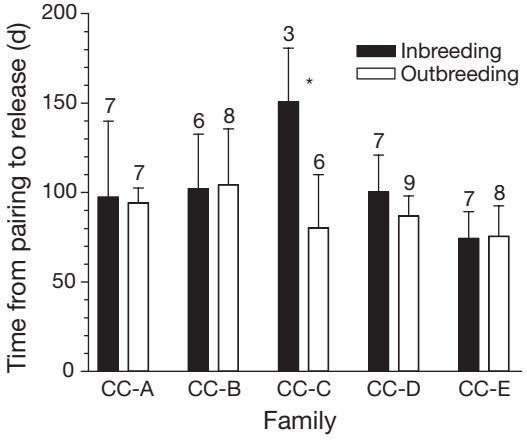

Fig. 3. *Crepidula convexa*. Effect of inbreeding on mean time from when full-siblings were paired together to releasing their first offspring. Number above each bar: number of female parents in each group. Error bars: +1 SD. *Marginally significant difference between means at p = 0.01 (significance level adjusted for multiple comparisons)

Tables 1 & 3). There was a significant interaction between breeding type and family, even though inbreeding significantly decreased mean survival of inbred juveniles for all 5 families of *C. convexa* when each family was examined separately (unpaired *t*-test, CC-D: $t = 6.938$, df = 13, $p < 0.0005 < 0.05/5$; CC-E: $t = 5.275$, df = 13, $p < 0.0005 < 0.05/4$; CC-C: $t = 3.886$, df = 11, $p = 0.003 < 0.05/3$; CC-A: $t = 3.446$, df = 10, $p = 0.006 < 0.05/2$; CC-B: $t = 2.963$, df = 13, $p = 0.011 < 0.05/1$).

Overall, inbred juveniles reared in the presence of excess food had significantly lower mean growth rates than outbred juveniles, regardless of rearing temperature (Fig. 2A–C, Tables 1 & 4). There was a significant interaction between breeding type and temperature, even though inbreeding significantly decreased mean growth rates of inbred juveniles at all 3 temperatures when each temperature was examined separately

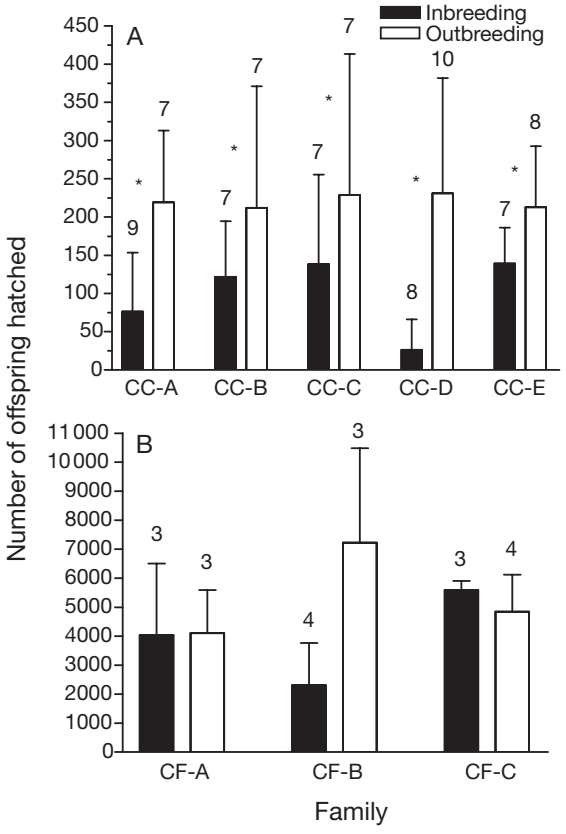

Fig. 4. *Crepidula convexa* and *C. fornicata*. Effect of inbreeding on mean fecundity of the parental generation females for (A) 5 families of *C. convexa* and (B) 3 families of *C. fornicata*. Number above each bar: number of female parents in each group. Error bars: +1 SD. *Significant difference between the mean fecundity of inbreeding and outbreeding females

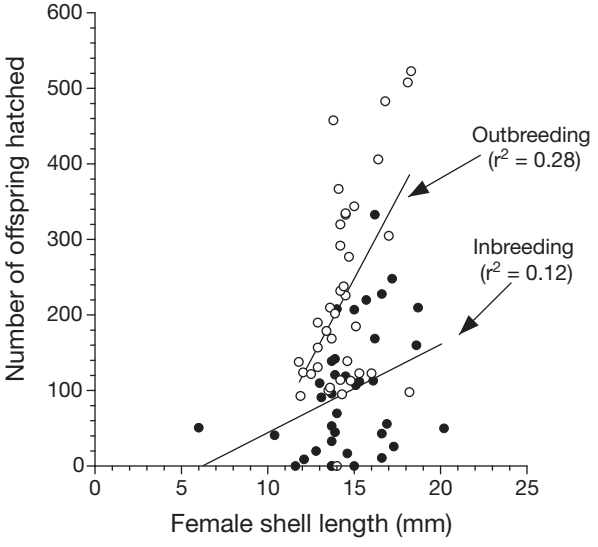

Fig. 5. *Crepidula convexa*. Effect of inbreeding on the relationship between number of offspring hatched and female shell length. Results from 36 inbreeding (●) and 37 outbreeding (○) females

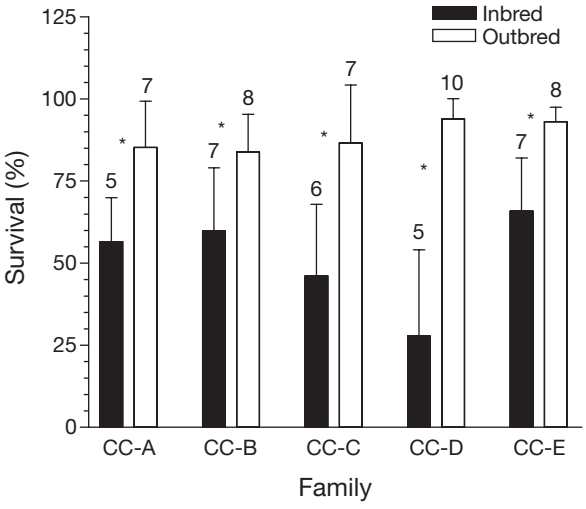

Fig. 6. *Crepidula convexa*. Effect of inbreeding on mean survival of F_1 generation juveniles for 5 families. Number above each bar: number of female parents for each group of juveniles. Error bars: +1 SD. *Significant difference between means after significance level was adjusted for multiple comparisons

(unpaired *t*-test, 29°C: $t = 8.012$, df = 72, $p < 0.0005 < 0.05/3$; 25°C: $t = 6.198$, df = 72, $p < 0.0005 < 0.05/2$; 15°C: $t = 2.176$, df = 72, $p = 0.033 < 0.05/1$). Inbreeding also significantly decreased mean juvenile growth rates at 25°C in the presence of limited food (Fig. 2D, Tables 1 & 3).

Mean size-adjusted fecundity of the inbreeding females was significantly lower than that of their respective mothers for 3 of the 4 families in which fecundity was examined (extent of reduction in fecundity: CC-A, 54.6%, CC-B, 63.0%, CC-E, 60.1%), but mean size-adjusted fecundity of the outbreeding females was only significantly lower than that of their mother for 1 of the 4 families (extent of reduction: CC-E, 36.0%, Table 5).

The mean initial sizes of inbred snails F_1 generation at hatching were significantly larger than those of the parental generation juveniles for 2 of the 5 families examined (extent of increase in initial size: CC-B, 20.8%, CC-D, 12.3%, Fig. 1), and not significantly different from those of the parental generation juveniles for the other 3 families (CC-A, CC-C, and CC-E, Table 6). In 2 of the 5 families, inbred juveniles had significantly lower mean growth rates than those of the parental generation when reared at 25°C with excess food (extent of reduction in growth rate: CC-A, 64.4%, CC-B, 31.2%, Table 7). In 2 other families, the differences were in the same direction but were not significant (Table 7).

Crepidula fornicata

Inbreeding did not affect the average time that females of *Crepidula fornicata* spent brooding their offspring (Fig. 7, Table 3), the fecundity of the parental generation ($F_{1,15} = 3.63$, p = 0.076, Fig. 4), the mean initial sizes of F_1 generation offspring (Fig. 1, Table 3), or the mean growth rates of F_1 generation juveniles at all

Table 4. *Crepidula convexa* and *C. fornicata*. Partly nested 4-way ANOVA results for the effect of inbreeding on mean growth rates of F_1 generation juveniles of both species, when juveniles were reared in the presence of excess food. 'Breeding type' indicates whether the F_1 generation juveniles were inbred or outbred

Source	SS	df	MS	*F*	p
C. convexa					
Between plots					
Breeding type	33421.011	1	33421.011	51.986	<0.0025
Family	3518.317	4	879.579	1.367	0.255
Breeding type × Family	2571.551	4	642.888	0.999	0.415
Parent (Breeding type, Family)	40532.059	63	643.366		
Within plots					
Temp.	102254.302	2	51127.151	129.791	<0.0005
Breeding type × Temp.	12393.992	2	8.529	48.456	<0.025
Family × Temp.	3151.341	8	393.918	3.080	0.003
Breeding type × Family × Temp.	1453.187	8	181.648	1.420	0.194
Parent (Breeding type, Family) × Temp.	16114.026	126	127.889		
C. fornicata					
Between plots					
Breeding type	5017.395	1	5017.395	2.149	>0.25
Family	36286.130	2	18143.065	10.077	0.002
Breeding type × Family	4669.401	2	2334.700	1.297	0.304
Parent (Breeding type, Family)	25204.957	14	1800.354		
Within plots					
Temp.	180448.167	2	90224.084	23.177	<0.01
Breeding type × Temp.	2911.158	2	1455.579	1.172	>0.25
Family × Temp.	15571.210	4	3892.803	6.879	0.001
Breeding type × Family × Temp.	4967.069	4	1241.767	2.194	0.095
Parent (Breeding type, Family) × Temp.	15844.349	28	565.870		

Table 5. *Crepidula convexa*. Results of 1-sample *t*-tests comparing size-adjusted female fecundity of the inbreeding and outbreeding females to that of their respective mothers. I: inbreeding female; O: outbreeding female; M: mother; < : significantly lower; = : not significantly different

Family	Inbreeding group				Outbreeding group			
	t	df	p	Results	*t*	df	p	Results
CC-A	3.78	7	0.0069	I < M	1.30	6	0.2421	O = M
CC-B	8.42	6	0.0002	I < M	1.78	5	0.1345	O = M
CC-C	0.80	6	0.4545	I = M	0.86	6	0.4219	O = M
CC-E	13.07	6	<0.0001	I < M	4.72	7	0.0022	O < M

Table 6. *Crepidula convexa*. Results of ANOVA and Bonferroni post hoc tests comparing mean initial offspring sizes of the parental generation to those of the F_1 generation. I: inbred offspring; O: outbred offspring; Pa: parental generation individuals; < : significantly smaller; = : not significantly different; > : significantly larger

Family	*F*	p	Inbreeding group			Outbreeding group		
			t	p	Results	*t*	p	Results
CC-A	$F_{2,288} = 20.95$	<0.0001	1.30	>0.05	I = Pa	4.24	<0.001	O > Pa
CC-B	$F_{2,297} = 17.46$	<0.0001	5.49	<0.001	I > Pa	3.73	<0.001	O > Pa
CC-C	$F_{2,232} = 20.93$	<0.0001	0.46	>0.05	I = Pa	3.94	<0.001	O > Pa
CC-D	$F_{2,278} = 56.96$	<0.0001	3.94	<0.001	I > Pa	1.64	>0.05	O = Pa
CC-E	$F_{2,137} = 0.602$	0.549	0.80	>0.05	I = Pa	1.10	>0.05	O = Pa

Table 7. *Crepidula convexa* and *C. fornicata*. Results of ANOVA and Bonferroni post hoc tests comparing mean growth rates of the parental generation juveniles of both species at 25°C in the presence of excess food to those of F_1 generation juveniles reared in the same conditions. Critical value is 0.05. I: inbred juveniles; O: outbred juveniles; Pa: parental generation juveniles; < : significantly lower; = : not significantly different; > : significantly higher; na: no significant difference overall, therefore Bonferroni post hoc test was not performed

Family	*F*	p	Inbreeding group			Outbreeding group		
			t	p	Results	*t*	p	Results
C. convexa								
CC-A	$F_{2,64} = 20.65$	<0.0001	4.60	<0.001	I < Pa	0.30	>0.05	O = Pa
CC-B	$F_{2,83} = 12.43$	<0.0001	2.70	<0.05	I < Pa	0.42	>0.05	O = Pa
CC-C	$F_{2,59} = 7.39$	0.0014	1.02	>0.05	I = Pa	1.85	>0.05	O = Pa
CC-D	$F_{2,72} = 6.82$	0.0019	1.25	>0.05	I = Pa	1.42	>0.05	O = Pa
CC-E	$F_{2,78} = 4.53$	0.0137	0.73	>0.05	I = Pa	2.41	<0.05	O > Pa
C. fornicata								
CF-A	$F_{2,82} = 3.881$	0.0245	2.32	<0.05	I < Pa	0.86	>0.05	O = Pa
CF-B	$F_{2,67} = 5.567$	0.0058	3.28	<0.05	I > Pa	2.15	>0.05	O = Pa
CF-C	$F_{2,54} = 0.513$	0.6018	na	na	I = Pa	na	na	O = Pa

3 temperatures and both food levels examined (Fig. 8, Tables 3 & 4). Fecundity of both inbreeding and outbreeding females (y) increased linearly with female shell length (x in mm): $y = 558.4x - 10\,380$ (linear regression: $r^2 = 0.314$, $F_{1,21} = 9.622$, p = 0.005), but was not related to male shell length (linear regression: $r^2 = 0.061$, $F_{1,21} = 1.363$, p = 0.256).

When comparing juvenile growth rates of the parental generation and F_1 generation for juveniles of *Crepidula fornicata* that were reared at 25°C in the presence of excess food (Fig. 8B), we found that for all 3 families, mean growth rates of outbred juveniles were not significantly different from the growth rates of parental generation juveniles (Table 7). In contrast, the relationship between mean growth rates of inbred juveniles and mean growth rates of parental generation juveniles varied among families (Table 7).

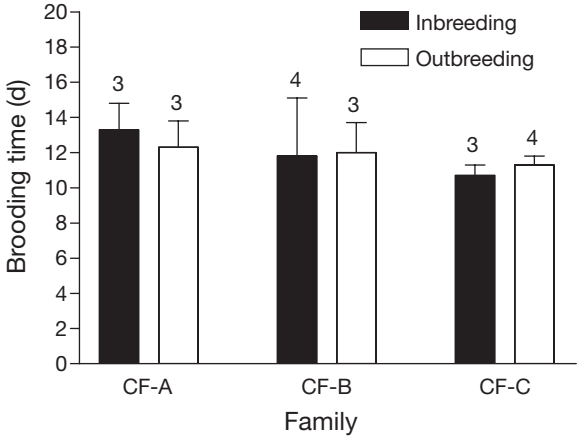

Fig. 7. *Crepidula fornicata*. Effect of inbreeding on mean brooding time of F_1 generation embryos for 3 families. Number above each bar: number of female parents in each group. Error bars: +1 SD

DISCUSSION

The data reported here on reproductive parameters for *Crepidula fornicata* agree well with those published in earlier studies. For example, Collin (1995) found that, in *C. fornicata*, female fecundity (measured as egg mass dry weight) varied with shell length ($p < 0.0001$), as also seen in our study (measured as number of offspring, p = 0.005). In addition, the mean juvenile growth rates of *C. fornicata* at 25°C in the presence of excess food reported in our study (126.7 to 246 µm d^{-1}) are very similar to those reported earlier (121 to 233 µm d^{-1}, Eyster & Pechenik 1988).

The present study seems to provide the first data on several reproductive characteristics for *Crepidula convexa*: juvenile growth rates at a wide range of temperatures (15 to 29°C) and 2 levels of food supply (excess and limited), and juvenile mortality. Our other data for this species agree well with those reported for a population of *C. convexa* at Delaware Bay, New Jersey, USA (Hendler & Franz 1971). For example, Hendler & Franz (1971) found that most females of *C. convexa* at Delaware Bay did not reproduce until their shell lengths reached 10 to 11 mm, which is also what we found for snails from Nahant: in our study, 74 of the 75 females had shell lengths >10 mm when they released their first batch of offspring. In addition, juveniles of *C. convexa* at Delaware Bay had a mean hatching shell length of 0.95 ± 0.09 mm (Hendler & Franz 1971), which is only slightly larger than what we report here (0.90 ± 0.06 mm for outbred juveniles, mean ± 1 SD). The relationship between female shell length and fecundity is also very similar for individuals of *C. convexa* from the 2 populations (compare our Fig. 5 with Fig. 4 in Hendler & Franz 1971).

It is very clear from our results that individuals of *Crepidula convexa* exhibited much stronger inbreed-

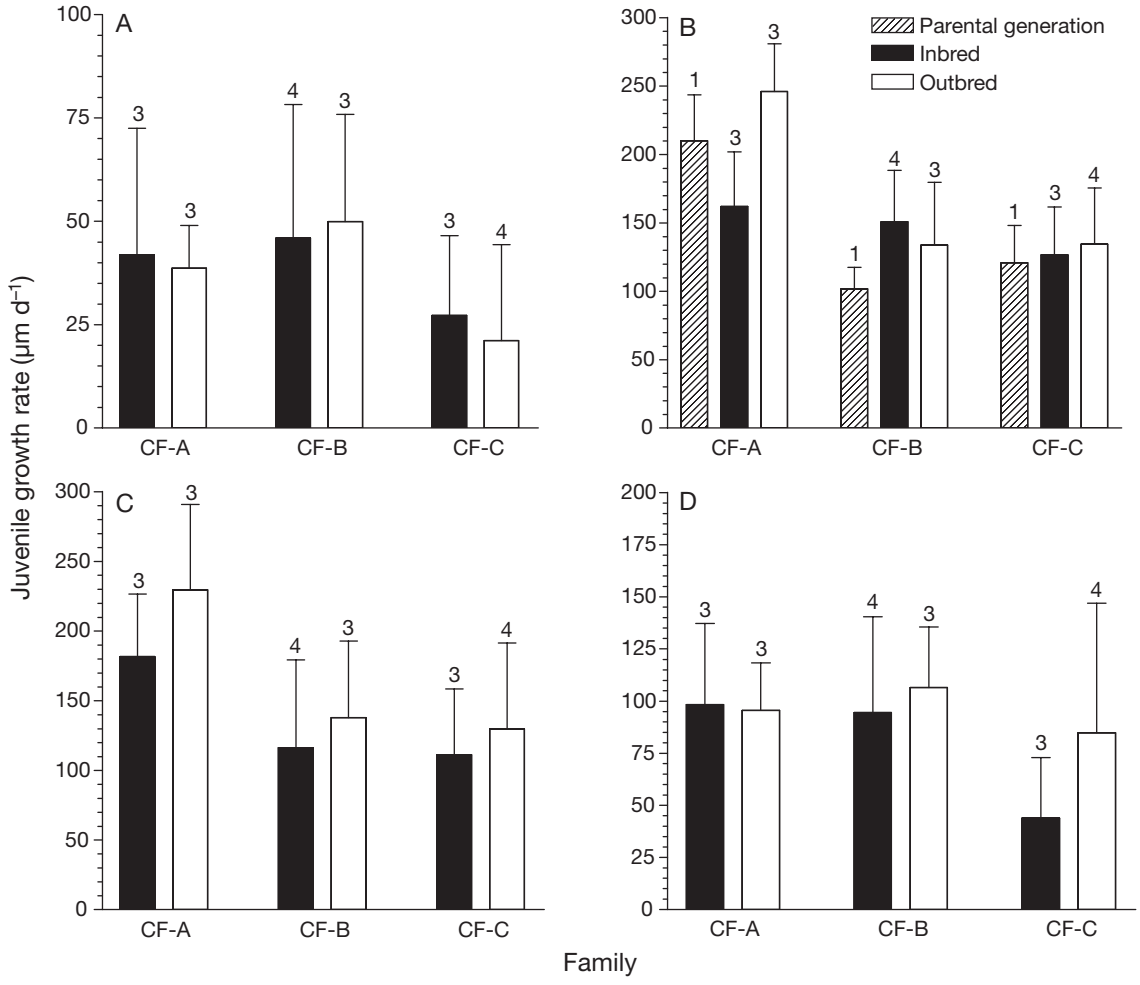

Fig. 8. *Crepidula fornicata*. Effect of inbreeding on mean juvenile growth rate for 3 families. Ten juveniles from each female were reared in each treatment. (A) 15°C; (B) 25°C; and (C) 29°C in the presence of excess food; and (D) 25°C in the presence of limited food. Number above each bar: number of female parents for each group of juveniles. Error bars: +1 SD

ing depression than did individuals of *C. fornicata* (Table 2). For example, in *C. convexa*, inbreeding substantially decreased mean female fecundity (>11% reduction, Fowler & Whitlock 2002), an effect not seen in *C. fornicata*. Inbreeding also largely decreased mean growth rates for juveniles of *C. convexa* reared at all 3 temperatures and both food levels tested, but had no effect on juvenile growth rates for *C. fornicata* reared under the same conditions. Similarly, inbreeding significantly increased time to juvenile release in 1 family of *C. convexa*, but did not affect brooding time of F_1 generation offspring in *C. fornicata*. Inbreeding also strongly decreased mean offspring survival in all 5 families of *C. convexa*. Overall, in none of the traits examined did inbreeding have a stronger detrimental effect in *C. fornicata* than in *C. convexa*. These results therefore contradict our expectation that *C. convexa* would show less inbreeding depression than *C. fornicata* because of *C. convexa*'s lower dispersal potential and higher likelihood of inbreeding in the field.

One possible reason for these surprising but clear results is that individuals of *Crepidula convexa* at our study site in Nahant may disperse farther than expected. At this site, most individuals of *C. convexa* live on periwinkle shells inhabited by hermit crabs *Pagurus longicarpus* (Li & Pechenik 2004). Indeed, the hermit crabs preferred shells with *C. convexa* on them to those that had been drilled by naticid snails, even though the drilled shells are more abundant in the field (Li & Pechenik 2004). This association between the snails and the highly motile hermit crabs might substantially increase the dispersal of the snails, thus potentially reducing the likelihood of inbreeding in this population. Individuals of *C. convexa* might also disperse by drifting or rafting, as has been documented for the juveniles and small adults of some other benthic marine inverte-

brate species (e.g. Martel & Chia 1991a,b). However, we took plankton tows for about half an hour during low tide at our study site in Nahant on August 12 and 16 and September 1, 2002, and found no individuals of *C. convexa* in any of the samples (authors' unpubl. data). There is thus no evidence that individuals of *C. convexa* disperse by drifting or rafting.

Inbreeding might also be unlikely to occur in natural populations of *Crepidula convexa* if juvenile mortality in the natural environment is so high that very few siblings or other close relatives survive to maturity. At least some benthic marine invertebrates experience very high juvenile mortality by both biotic and abiotic factors such as predation, competition, desiccation, and solar radiation (Gosselin & Qian 1997, Hunt & Scheibling 1997); in one study on juvenile barnacles, for example, up to 40% of individuals died within 24 h of metamorphosis, and a further 5 to 20% died during the next 24 h (Gosselin & Qian 1996). As far as we know, juvenile mortality rates in the field have not been reported for *C. convexa*.

Even if individuals of *Crepidula convexa* inbreed routinely in the field, they may still harbor a heavy mutation load, and therefore exhibit strong inbreeding depression. The effectiveness of purging can be influenced by many factors, such as the severity of the deleterious alleles' effects on fitness and the degree of dominance of these deleterious alleles (Charlesworth & Charles-worth 1987). Purging is highly effective for alleles of large effect (e.g. lethals), but much less so for alleles of small effect (Frankham et al. 2002). In addition, if deleterious alleles have even slight dominance (4 to 5%), purging of these alleles in inbred lines will be much less likely to occur (Morton et al. 1956, Charlesworth & Charlesworth 1987). Kokko & Ots (2006) predicted that species that are capable of breeding many times in their lifetime and whose reproductive success is limited by low mate encounter rates should show high inbreeding tolerance even when the level of inbreeding depression is high. This helps to explain the apparent lack of inbreeding avoidance towards full-siblings in *C. convexa* (authors' unpubl. data) despite the high levels of inbreeding depression that we found in this species.

On the other hand, the fact that inbreeding did not affect many fitness characteristics for *Crepidula fornicata* suggests that individuals of this species at our study site may harbor relatively few deleterious alleles that contribute to inbreeding depression. Genetic studies on field populations will be necessary to compare the composition and function of the mutation load in these 2 *Crepidula* species. Populations of *C. fornicata* at Nahant might harbor a lighter mutation load than expected if individuals in this population disperse less, and consequently inbreed more often than expected. Despite a long-lived larval stage and substantial dispersal of offspring over large distances (Collin 2001), it is possible that some fraction of the larvae of *C. fornicata* from our study population are in fact retained in the local area and recruit to the parental population, thus increasing opportunities for substantial inbreeding. Local recruitment has been suggested by genetic studies for 2 populations of *C. fornicata* in France: significant heterozygote deficiency was observed in both populations, suggesting that substantial inbreeding occurs within these populations (Dupont 2004). A number of recent studies on other marine species have also suggested at least some degree of larval retention within parental populations despite long larval lives (Petersen & Svane 1995, Kyle & Boulding 2000, Lambert et al. 2000). Populations of *C. fornicata* in which a higher proportion of larvae disperse might be more vulnerable to the effects of inbreeding than the population that we studied. This issue can be explored by estimating the extent of inbreeding in several different natural populations of *C. fornicata*.

It is also possible that inbred individuals of *Crepidula fornicata* do suffer strong inbreeding depression, but that the effects appear in life stages other than those we have examined here. Life-stage-dependent inbreeding depression has been found in many plant species. For example, in compiling results from 40 predominantly outcrossing plant species, Husband & Schemske (1996) found that outcrossing species expressed much of their inbreeding depression at early stages (seed production) and late stages (growth and reproduction), but much less at the germination stage. They suggested that the expression of some deleterious alleles might be stage-specific, or that life stages might differ in the number of genes expressed and/or in the frequency of deleterious mutations for those genes (Husband & Schemske 1996). For *C. fornicata*, more deleterious alleles may be expressed in the larval stage than in the juvenile stage, or the frequency of deleterious mutations for genes that are specifically expressed in the larval stage may be higher than that of genes in the juvenile stage. Some individuals of *C. fornicata* have expressed strong inbreeding depression as larvae (authors' unpubl. data). Inbreeding depression in the larval stage was also reported in other marine invertebrates, such as the Pacific oyster *Crassostrea gigas* (Hedgecock et al. 1995), the Mediterranean mussel *Mytilus galloprovincialis* (Beaumont & Abdul-Matin 1994), and the scallop *Pecten maximus* (Beaumont & Budd 1983).

Finally, in the present study, we may not have detected significant effects of inbreeding in *Crepidula fornicata* because of inadequate statistical power. The power of detecting a significant effect of inbreeding at an alpha level of 0.05 from our data for *C. fornicata* was

estimated at 0.1. However, the power of detecting significant inbreeding effects from our data for *C. convexa* was only 0.2, and yet strong inbreeding depression was indeed detected for this species. Even though statistical power can be increased by examining a larger number of families (Quinn & Keough 2002), substantial inbreeding depression has been found in some other marine invertebrates even when only 1 to 4 families were examined (Beaumont & Budd 1983, Beaumont & Abdul-Matin 1994, Keys et al. 2004).

The levels of inbreeding depression we report here for several fitness traits are very high compared to what has been reported for many other organisms. For example, inbreeding reduced mean female fecundity in *Crepidula convexa* by 35 to 89% (δ = 0.35 to 0.89). In studies on other marine invertebrates, Brown (1991) reported only a 33% reduction in fecundity for the copepod *Tigriopus californicus* for sibling matings, and Gee & Williams (1965) reported no effect of self-fertilization on fecundity in the 2 polychaetes *Spirorbis borealis* and *S. pagenstecheri*. The effect of inbreeding on fecundity seen for *C. convexa* is also very high compared to findings for non-marine organisms. For example, self-fertilization (the most extreme form of inbreeding) on average caused only about a 30% reduction in seed production in 40 outbreeding plant species (Husband & Schemske 1996). In terrestrial animals, full-sibling matings generally cause <20% reduction in fecundity (e.g. Barnard & Fitzsimons 1989, Hoogland 1992, Kruuk et al. 2002, Bilde et al. 2005), which is again much lower than what we have documented for *C. convexa*.

The levels of inbreeding depression we report here for juvenile survival of *Crepidula convexa* (29 to 70% reduction) are also very high compared to those found in the few previous studies that had examined this trait in other marine invertebrates. For example, in the Japanese pearl oyster *Pinctada fucata martensii*, survival of inbred juveniles was only 5% less than that of outbred juveniles (Wada & Komaru 1994), and in the ascidian *Corella willmeriana*, inbreeding had no effect on juvenile survival at all (Cohen 1996). In 40 captive mammalian populations belonging to 38 species, offspring from sibling matings had on average 33% lower survival than those from unrelated parents (Ralls et al. 1988), which is still lower than the average decrease we found for *Crepidula convexa* (41.8%). The reported levels of inbreeding depression (δ) on survival for other animals, such as the mouse *Mus musculus* (Barnard & Fitzsimons 1989), the golden lion tamarin *Leontopithecus rosalia* (Dietz & Baker 1993), the collared flycatcher *Ficedula albicollis* (Kruuk et al. 2002), and the spider *Stegodyphus lineatus* (Bilde et al. 2005), range substantially from 0% (total survival) to 100% (total death of inbred offspring), with most reported values being below 30%, which is again lower than what we found for *C. convexa*.

Inbreeding also had comparatively large depressive effects on juvenile growth rates for *Crepidula convexa*, at all 3 temperatures and both food levels examined. In sharp contrast, inbreeding did not depress mean juvenile growth rates for other marine invertebrates that have been studied, such as the scallop *Argopecten purpuratus* (Winkler & Estévez 2003), the Pacific oyster *Crassostrea gigas* (Hedgecock et al. 1995), the Japanese pearl oyster *Pinctada fucata martensii* (Wada & Komaru 1994), and *C. fornicata* (the present study). In compiling results from 40 outbreeding plant species, Husband & Schemske (1996) found that on average self-fertilization decreased plant growth and reproduction (flower number, seed number/flower, etc.) by about 25%. If inbreeding depression increases linearly with inbreeding coefficient (F), as genetic models and available data suggest (Lynch & Walsh 1998, Frankham et al. 2002), then the effect of inbreeding on growth in normally outbreeding plants at the level of sibling mating (F = 0.25, while F = 0.5 for self-fertilization) should be about 12.5%, which is much lower than the average inbreeding depression that we found for *C. convexa* at any of the temperature and food levels examined (20 to 44%, Table 1).

In 4 of the 5 families of *Crepidula convexa* examined, the magnitude of inbreeding depression on mean juvenile growth rate increased with increasing rearing temperature over the range of 15 to 29°C. If temperature harshness can be judged by relative juvenile growth rate at that temperature (juveniles grew most slowly at 15°C), this result is the opposite of what we expected to see: inbreeding depression is commonly found to be greater in harsher environments, probably because the burden of deleterious alleles is stronger when inbred organisms live under physiologically stressful conditions (Roff 1997, Lynch & Walsh 1998, Keller et al. 2002). Stronger inbreeding depression under harsher conditions was also not found in our study when food was the limiting factor; for 3 of the 5 families of *C. convexa* examined, inbreeding depression was in fact less severe when juveniles were given a limited food supply. For only 1 of the 5 families was the level of inbreeding depression greater when food was limited. A smaller inbreeding effect under suboptimal conditions has been reported for the plant *Schiedea menziesii*: the unfavorable conditions might have suppressed the expression of inbreeding depression by limiting the growth of all individuals, overwhelming the effect of inbreeding on growth rate (Rankin et al. 2002).

It is clear for *Crepidula convexa* that the effect of inbreeding varied among families. For example, family CC-E always showed the lowest effect of inbreeding

for all of the fitness traits that exhibited inbreeding depression. At the other extreme, family CC-A showed the greatest effect of inbreeding on juvenile growth rates at all 3 temperatures and both food levels examined. Different families can harbor different proportions of deleterious alleles (mutation load) simply through random genetic drift (Frankham et al. 2002). It is possible that family CC-E carried a lighter mutation load than other families and therefore suffered the least from inbreeding. Compared with other families, family CC-A may carry the strongest deleterious allele (or alleles) that affect juvenile growth. Different effects of inbreeding for different families has also been found in several plant species (Ågren & Schemske 1993, Holtsford 1996, Fishman 2001) and in the warbler *Acrocephalus sechellensis* (Richardson et al. 2004).

Inbreeding did not decrease the mean initial sizes of inbred offspring in either of the 2 *Crepidula* species. To the contrary, inbred offspring were 18% larger than outbred offspring for 1 family of *C. convexa*. A similar lack of detrimental effect of inbreeding on hatching size has been reported for terrestrial animals such as the mouse *Mus musculus* (Barnard & Fitzsimons 1989), the prairie dog *Cynomys ludovicianus* (Hoogland 1992), and the butterfly *Bicyclus anynana* (Saccheri et al. 1996). However, our study seems to be the first to report a larger size for inbred hatchlings than for outbred hatchlings. It is possible that inbreeding in *C. convexa* caused a higher failure in egg development (a sign of inbreeding depression), which then provided extra embryonic nutrients for the surviving embryos (Hendler & Franz 1971, Hoagland 1979), resulting in both bigger inbred offspring and lower fecundity.

Even though inbreeding effects have most often been studied by comparing the fitness traits of inbred individuals to those of outbred individuals from the same generation (Charlesworth & Charlesworth 1987, Husband & Schemske 1996, Frankham et al. 2002), evidence for inbreeding depression can also be found by comparing the performance of inbred individuals to that of individuals from their parental generation. For example, the only evidence of inbreeding depression in *Crepidula fornicata* was found in 1 of the 3 families when comparing growth rates of the parental generation with those of F_1 generation juveniles reared under the same condition. For the other species, *C. convexa*, size-adjusted fecundity of inbreeding females was significantly lower than that of the parental generation for 3 of the 4 families examined and was not significantly different in only 1 family, suggesting inbreeding depression. At the same time, however, the mean size-adjusted fecundity of outbreeding females was not significantly different from that of the parental generation for 3 families of *C. convexa* and was significantly lower in only 1 family.

From our results with the 2 closely related marine gastropods *Crepidula fornicata* and *C. convexa*, we found that dispersal potential alone was a surprisingly poor predictor of the amount of inbreeding that occurs in natural populations, or the effect of inbreeding on certain offspring fitness traits. The effect of inbreeding in a given species or population may be influenced by many factors, such as the composition of its mutation load, the effectiveness of purging, the harshness of the environmental conditions, the effect of juvenile mortality, and even the animal's relationship with individuals of other species (such as the symbiotic relationship between *C. convexa* and the hermit crab *Pagurus longicarpus*). The effect of the association between *C. convexa* and *P. longicarpus* on realized dispersal of *C. convexa* merits additional study.

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