

Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods

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ABSTRACT: In marine molluscs, sex change is often labile and is thought to be largely influenced by interactions with conspecifics. Previous studies of calyptraeid gastropods concluded that their social environment influences the timing of protandrous sex change. We conducted field surveys and laboratory experiments to examine the effects of conspecifics on size at sex change in 3 Panamanian calyptraeids. *Crepidula* cf. *onyx*, *C. incurva* Broderip, 1834 and *Bostrycapulus calyptraeformis* (Deshayes, 1830) vary in densities, sex ratio and mode of development, which suggests that they might respond to associations with conspecifics in different ways. However, our laboratory experiments showed that the response to interactions with conspecifics is generally similar. In all 3 species, individuals raised in isolation pass through a male phase and males raised alone change sex at the same size as males raised with another male. Both *C. cf. onyx* and *C. incurva* change sex at a larger size when kept with a female than when kept alone or with another male. The differences in size at sex change between the treatments is small and the treatment effect explains more of the variation in size at sex change in *C. cf. onyx*, the more solitary species, than in *C. incurva*, a species that is usually found in pairs. In all species, individuals with high initial growth rates change sex sooner and at a smaller size than those with slower initial growth rates. Growth rates increase significantly during sex change in *C. cf. onyx* and *C. incurva* but not in *B. calyptraeformis*.

KEY WORDS: Protandry · *Crepidula* spp. · Size advantage · *Bostrycapulus calyptraeformis*

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INTRODUCTION

Sex change is widespread among vertebrates and invertebrates. Most species show environmentally mediated lability in size or age at sex change. However, the number of factors influencing sex change make it difficult to make precise predictions of the size at which an individual changes sex. Interactions with conspecifics have been shown to influence the initiation or delay of sex change in sex-changing fishes (e.g. Robertson 1972, Alonzo & Warner 2000a,b, Mackie 2003, Perry & Grober 2003), shrimp (see e.g. Charnov 1982, Koeller et al. 2000, Chiba et al. 2003), and mollusks (e.g. Coe 1953, Hoagland 1978, Charnov 1982, Soong & Chen 1991, 2003). Studies of protogynous (females first) fish species have revealed a surprising diversity of strategies to increase reproductive output,

including alternate mating strategies for males, accelerated and delayed sex change, and reversed sex change (e.g. Warner & Swearer 1991, St. Mary 1993, Lutnesky, 1994, Munday 2002). Some fishes can make very fine adjustments in sex allocation in response to local social interactions (e.g. Ross et al. 1983, Petersen et al. 2001, Alonzo & Warner 2000b).

In contrast to the wide range of behavioral strategies displayed by sex-changing fishes, most sex-changing mollusks seem to employ simple strategies for sex change (Warner et al. 1996). This simplicity may be due to differences between the evolution of reproductive strategies in protandrous (males first) and protogynous species, differences in the complexity of vertebrate versus invertebrate behaviors, or may reflect the relatively large research effort focused on fishes compared to invertebrates. Strategies of sex change in

protandrous shrimp, which have been more intensively studied than mollusks, appear to be more variable, with mixed strategies as well as pure protandry occurring in several groups (e.g. Bauer & VanHoy 1996, Baldwin & Bauer 2003).

It is difficult to assess the variability of sex change strategies among mollusks because few groups have been examined in detail and the conclusions of studies on the same species often differ. Calyptraeids, protandrous, filter-feeding limpets, are the most well-studied sex-changing mollusks (see e.g. Gould 1917, 1919, 1952, Coe 1936, 1938, 1953, Hoagland 1978, Collin 1995, Warner et al. 1996, Chen & Soong 2000). The conclusions of these studies are often contradictory, and interpretation of many older studies is difficult because they may have employed experimental conditions that starved the limpets (e.g. Coe's [1948] criticisms of Gould [1947], but see Gould & Hsiao's rebuttal [1948]), the sample sizes were often low or not reported (e.g. only a single replicate of each density treatment in Hoagland 1978), the experimental design was not described (e.g. Coe 1938), or/and the size of the limpets in the different treatments were not reported (e.g. Coe 1953). Differing results from studies using the same species are common. For example, Coe (1936) stated that there are some 'true' males that never change, but Hoagland (1978), studying the same species, found no evidence to support this in *C. convexa* and thought it unlikely in *Crepidula fornicata*. Although Gould (1919) reports that small *C. plana* must be associated with a female to develop into a fully functional male, Coe (1936, 1938) stated that this association is not necessary for male development in either *C. plana* or 6 other species of *Crepidula*. Some of these conflicting conclusions could be due to differences or vagaries in interpretation of similar results. For example, several of these studies report a 'delayed' sex change, which could refer to a delay with respect to size or to a temporal delay (a distinction that is often not made clear).

Sex change strategies may vary in concert with mode of development, social behavior, and body size. Coe (1938) suggested that larger species with planktonic development (*Crepidula fornicata*, *C. onyx* and *C.*

plana) are gregarious and show large differences in size at sex change between solitary and 'mated' individuals while smaller species with direct development (*C. convexa* and *C. adunca*) are less gregarious and do not show a difference in size at sex change between solitary and mated individuals. The results of Hoagland (1978) for *C. fornicata*, *C. plana* and *C. convexa*, a subset of the species examined by Coe (1938), support the latter's conclusions. However, *C. nivea*, *Crucibulum spinosum*, and *Crepidula norissiarum* do not fit the pattern (Coe 1938, Warner et al. 1996) and there is no theoretical expectation that body size or mode of development should be associated with strategies of sex change.

We examined the lability of sex change in calyptraeid gastropods with the aim of understanding variation in sex allocation strategies among a wider range of species and to obtain parameters for subsequent use in quantitative models of sex change. We address the following questions for 3 species (*Crepidula cf. onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*) that display different life histories (Table 1) and gregariousness in the field: (1) Do individuals raised alone bypass the male stage and develop directly into females? (2) Do individuals kept alone, or in association with males or, in association with females change sex at different sizes? (3) Does growth rate increase during sex change?

MATERIALS AND METHODS

Study species and field survey. The 3 calyptraeid species *Crepidula cf. onyx*, *C. incurva* Broderip, 1834, and *Bostrycapulus calyptraeformis* (Deshayes, 1830) examined in this study co-occur commonly in muddy-rocky habitats in the intertidal along the Pacific coast of Panama. We used field-collected samples to survey the natural size frequencies of males and females for each species from Playa Venado and Playa Chumical (8° 30' N, 79° 40' W). The method of sampling differed for each species, based on their natural history.

Crepidula cf. onyx occurs at Playa Venado in densities of 1 or 2 individuals m⁻² in the low intertidal (–0.5

Table 1. *Crepidula cf. onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*. Life history differences between the 3 panamanian calyptraeids. Sex ratio: breeding ratio males:males + females

Species	Development	Stacking	Max. size (mm)	Abundance	Sex ratio
<i>C. cf. onyx</i>	Direct	Solitary	34	Uncommon	0.21
<i>C. incurva</i>	Planktotrophic	53% in pairs	18	Patchy	0.40
<i>B. calyptraeformis</i>	Planktotrophic	Solitary or paired	25	Very abundant (up to 1000 m ⁻²)	0.55

to -1.0 m). We collected them by carefully examining the rocks within an area of about 200 m^2 and collecting all the *C. cf. onyx* during low tide on February 2, 2003.

Crepidula incurva occurs patchily in the intertidal between -0.5 and -1.0 m. They are usually found attached to the shells of other snails, especially *Cerithium* sp., which congregate in large numbers in shallow tide pools and below large rocks. We collected *C. incurva* from tide pools within an area of 100 m^2 by examining every snail in the pool and collecting all that hosted a *Crepidula incurva*. All individuals were collected from January 19 to 23, 2003.

Because *Bostrycapulus calyptraeformis* can reach densities of more than 1000 m^{-2} in the low intertidal, we surveyed this species using 0.25 m quadrats: 3 quadrats were placed haphazardly within 200 m of each other in the intertidal where *B. calyptraeformis* are known to occur, and all individuals were collected from each quadrat from September 7 to 9, 2002. Quadrat sampling ensured that we obtained a representative sample including all the small individuals, which are cryptic.

Determination of sex. The shell lengths of all gastropods were measured with calipers to the nearest 0.1 mm and their sex was determined by presence or absence of the penis and the female genital papilla (fgp). For both *Crepidula cf. onyx* and *C. incurva* this was performed on living individuals which had been removed from the substrate. Individuals with both a penis and an fgp or with neither were classified as transitional if they had previously been observed as males, and as juveniles if they were small and had not been observed with male morphology.

Because female *Bostrycapulus calyptraeformis* do not possess an fgp, the sex of field samples was determined on the basis of the presence or absence of a penis and the presence or absence of a capsule gland (part of the female reproductive tract which can be seen when the gastropod is removed from the shell). In 544 field-collected individuals there were 5 individuals, presumably transitionals, for which the presence of a penis was not a perfect predictor of the absence of the capsule gland. Therefore, we used the presence or absence of the penis as an adequate indicator of sex for experimental individuals.

Isolation experiments. Previous literature on sex change in *Crepidula* species has suggested that juveniles raised in isolation bypass the male stage (Gould 1919). In order to test this hypothesis, juveniles of each species were raised in isolation. Larvae of *C. incurva* and *Bostrycapulus calyptraeformis* were raised following the methods of Collin (2000). After settlement they were raised in groups until they reached 2.0 to 2.5 mm and could be easily handled. Juvenile *C. cf. onyx* were held in groups for several days after hatching, until

they also reached a length of 2.0 to 2.5 mm . Juveniles of each species were transferred to individual 355 ml cups which were maintained in the laboratory at room temperature. They were fed 10 ml of a mix of algal cultures (*Nannochloropsis* sp., *Rhodomonas* sp., *Dunaliella tertiolecta*, *Isochrysis galbana* and *Chaetocerus gracilis*) daily; the proportion of each alga in the mix varied from day to day, but all gastropods were raised simultaneously and all were fed from the same mix on each day. The water was replaced with fresh unfiltered seawater every 2 d. Initially this diet was satiating; after several months, however, the gastropods were large enough to clear the containers between feedings and therefore food probably became limiting. The size and sex of each individual was recorded every 3 wk until female characteristics were observed. During the first few weeks there was some (10 to 15%) mortality when the tiny juveniles crawled out of the water and dried out. However, after reaching a size of 5 mm , mortality was negligible.

Sex change experiments. To examine the effects of association with conspecifics on size at sex change we raised individuals of each species in 3 different social environments. For both *Crepidula incurva* and *Bostrycapulus calyptraeformis* we collected individuals from the same locations as the field surveys. Males of sizes below the size of sex change (*C. cf. onyx* $<2.5\text{ mm}$; *C. incurva* $<8.5\text{ mm}$; *B. calyptraeformis* $<10.7\text{ mm}$) were randomly assigned to a 355 ml cup with one of the following treatments (1) alone, (2) 2 males together, or (3) male with a female. Because male *C. cf. onyx* were rare in the field, we arranged the same 3 treatments starting with laboratory-hatched juveniles and field-collected females. The average initial difference in size between the 2 males in the paired-male treatment were 0.38 , 1.4 , and 2.47 mm for *C. cf. onyx*, *C. incurva* and *B. calyptraeformis* respectively. There were initially 43 to 50 replicates of each treatment for each species. All treatments were fed 10 ml of a mix of algal cultures daily, which was satiating for small individuals. After the gastropods were large enough to clear the containers between feedings, we increased the ration to 20 ml algae d^{-1} for solitary individuals and 40 ml algae d^{-1} for paired individuals. We recorded the size and sex of each individual every 3 wk for the first 4 mo of the experiment and every 4 wk thereafter. The experiment was continued for more than 1 yr. After reaching a size of 4 to 5 mm , mortality was negligible, the gastropods grew considerably, and most female-phase individuals that had the chance to mate produced eggs.

Analyses. Size at sex change in the field was calculated using logistic regression (standard method described by Sokal & Rohlf 1995) to predict the size at which 50% of the individuals were male. We follow the

convention of Allsop & West (2003) in using 'L₅₀' to represent to the size at which 50% of the individuals were male.

The effect of each treatment on size at sex change was analyzed using 1-way ANOVA with 3 treatments and Fisher's post-hoc least-significant difference (PLSD) tests, in which the treatments were (1) solitary individuals, (2) first male to change sex in a pair of males, (3) male paired with a female. The second male to change sex in the paired male treatment was not included, since as soon as the first male changed sex, the treatment changed from being a male paired with a male to being a male paired with a female. This means that only the first male to change sex experienced the consistent treatment of being paired with a male. These analyses were conducted using the largest size that each individual was observed to be male and repeated using the smallest size it was observed to be female. Analyses were not conducted on the size of transitionals because many individuals that changed sex were not observed in the transitional phase and the transitional phase could not be identified in *Bostrycapulus calyptraeformis* without sacrificing them.

The time until sex change in each treatment was examined using survival analysis. We used Kaplan-Meier survival statistics with sex change recorded as the event; those individuals that died or had not changed sex by the end of the experiment were treated as censored.

Quantitative predictions of sex change and size at sex change often depend on the assumption that growth rates are the same for males, females and transitionals. Studies of *Crepidula* spp. often state that growth increases during sex change, although it is seldom measured explicitly. To test the hypothesis that growth rates increase during sex change we quantified growth rates for the solitary individuals. Individuals in

the other treatments were not used, as food availability may have been affected by competition with the other individual in the same container. The growth rates were binned into each time period between measurements and significant differences between the growth rates before, during and after the change were examined using paired *t*-tests, and ANCOVA using shell length as a covariate.

To determine if initial growth rates affect the timing of sex change, we divided the gastropods into those that changed sex before or after the census date by which about half of the individuals in the treatment had changed. Logistic regression was used to test for association between growth rates and early sex change, with initial size as a covariate.

RESULTS

Despite pronounced differences in field sex ratio, size distribution and population densities, the 3 species responded to the experimental conditions in similar ways. Juveniles raised in isolation passed through a male phase and males raised in isolation changed sex at the same size as a male raised with another male. Both *Crepidula* cf. *onyx* and *C. incurva* showed sex change at a larger size when males were paired with a female. Individuals with high initial growth rates changed sex sooner than those with slower initial growth rates.

Crepidula cf. *onyx*

Crepidula cf. *onyx* from the field survey had a pronounced female-biased sex ratio with 66% female, 16% transitionals and only 18% males (Table 2, Fig. 1).

Transitional individuals of this species show both a penis and an fgp. Transitionals ranged in size from 11.7 to 25.4 mm, but size overlap between males and females suggests that a sex change usually occurred around 13 to 14 mm. The size at sex change estimated by the logistic regression (L₅₀) was 13.8 mm.

Juveniles raised in isolation usually developed into males; 20 d after initiation of the experiment, 29 individuals had developed a penis and 6 had a penis rudiment; 20 d later, most individuals were already transitional. Despite the fact that most (82%) of these were observed with a penis, those were noticeably shorter and

Table 2. *Crepidula* cf. *onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*. Data from field surveys and sex change experiments showing size (mm) at sex change and size overlap. L₅₀: size at which 50% of individuals were male. -: not applicable

Treatment	Size overlap (min. female – max. male)	Size of transitional individuals	L ₅₀	Max. individual size
<i>C. cf. onyx</i>				
Field	13.0–13.9	11.7–19.0 ^a	13.8	29.6
Expt	10.5–14.9	11.1–15.7	–	26.1
<i>C. incurva</i>				
Field	6.3–9.8	6.8–12.2	8.05	18.1
Expt	10.4–13.7	8.1–12.7	–	20.5
<i>B. calyptraeformis</i>				
Field	12.7–19.7	9.3–14.6	14.6	23.1
Expt	12.2–17.2	–	–	19.2

^aOutlier at 25.4 mm

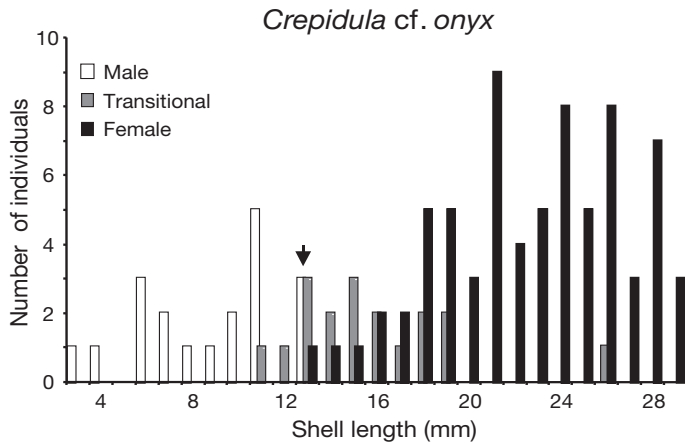


Fig. 1. *Crepidula cf. onyx*. Size–sex distribution for field-collected samples. Arrow indicates logistic regression estimate of size at which 50% were male (L_{50}). $n = 110$

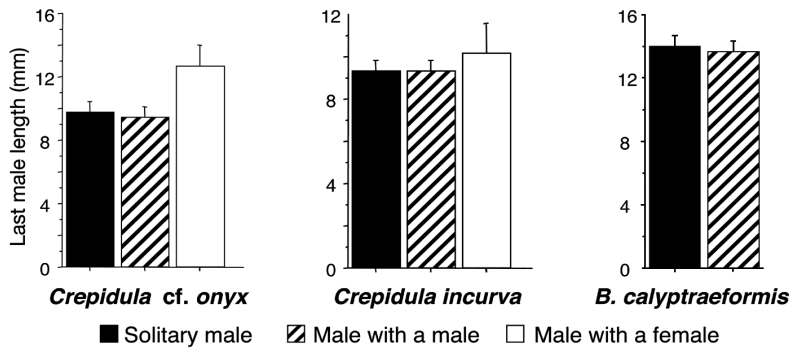


Fig. 2. *Crepidula cf. onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*. Differences in last recorded male size in 3 experimental treatments; error bars = 95% CI. Average sizes at sex change were *C. cf. onyx*: solitary males 9.8 mm, male with male 9.4 mm, male with female 12.7 mm; *C. incurva*: solitary males 9.2 mm, male with male 9.2 mm, male with female 10.6 mm; *B. calyptraeformis*: solitary males 14.0 mm, male with male 13.6 mm

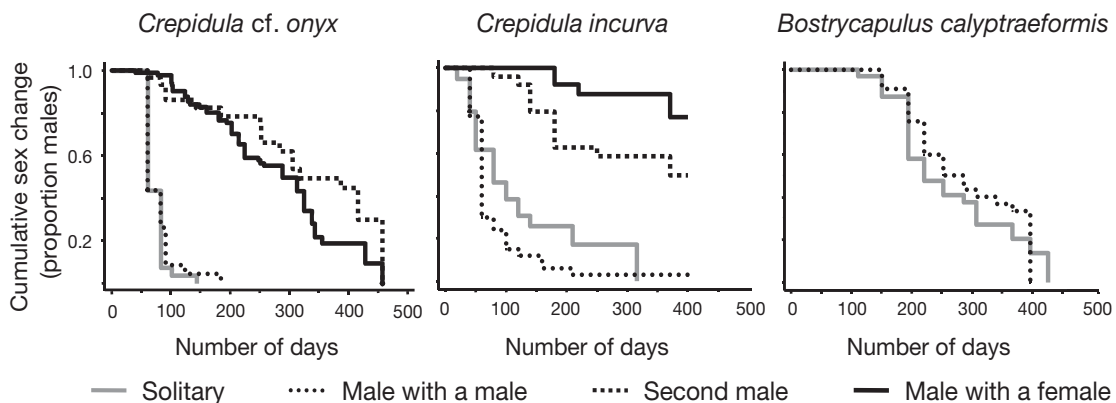


Fig. 3. *Crepidula cf. onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*. Results of Kaplan-Meier survival analysis of sex change for each species. Estimated mean (\pm SE) number of days until 50% had changed sex were *C. cf. onyx*: solitary males 61 ± 8 d, male with male 61 ± 11 d, second male 318 ± 91 d, male with female 289 ± 28 d. *C. incurva*: solitary males 80 ± 17 d, male with male 60 ± 5.9 d, second male in a pair 370 ± 137 d, male with female >400 d. *B. calyptraeformis*: solitary males 221 ± 31 d, male with male 286 ± 48 d

thinner than the penes developed by the small males kept in association with conspecifics. Those individuals not observed with a penis may have had one for so short a time that it was not detected by our infrequent sampling.

There was a significant effect of treatment on size at sex change (ANOVA $p < 0.0001$, Fig. 2, Table 3). Solitary males and the first of the paired males changed sex change at a significantly smaller size than did males placed with females (9.6 vs. 12.6 mm; Fisher's PLSD test, $p < 0.05$) Among-group variance accounted for 34% of the total variance. The second of the paired males to change showed the same size at sex change as males paired with females. The results were qualitatively the same when the analyses were repeated using the smallest recorded female size for each individual. Using the relationship between shell length and dry weight ($\ln[\text{wt}] = -6.883 + 0.127 \cdot \text{length}$; $r^2 = 0.89$; $p < 0.001$) showed that solitary and first males in a pair changed sex at 70% of the dry weight (0.0035 g) at which males paired with females changed sex (0.0051 g).

Sex change could be very rapid and took less than 3 wk. Many of the males that changed sex were not observed in a transitional phase; 50% of males in the solitary treatment and the first male in the paired males treatment had changed sex by 60 d (Fig. 3), while males paired with females and the second in a pair of males had changed by about 300 d. In the solitary and first male treatments, all individuals became female.

Table 3. *Crepidula* cf. *onyx* and *C. incurva*. Results of ANOVA testing effect of treatment on size at sex change in snails exposed to 3 experimental social treatments

	df	SS	MS	F	p
<i>C. cf. onyx</i>					
Treatment	2	184.9	92.4	15.86	<0.0001
Residual	82	477.8	5.8		
<i>C. incurva</i>					
Treatment	2	14.2	7.1	3.53	0.035
Residual	66	133.2	2.0		

Daily growth rates during the transitional period were significantly higher than prior to the sex change (paired *t*-test; $df = 30$; $p < 0.05$) or after the change ($df = 22$; $p < 0.0001$) (Fig. 4; $mean_{male} = 0.14 \text{ mm d}^{-1}$; $mean_{transitional} = 0.19 \text{ mm d}^{-1}$; $mean_{female} = 0.12 \text{ mm d}^{-1}$). An ANCOVA of growth rates using average size over the observation period as a covariate found a significant effect of sex ($p < 0.05$) but not of size ($p > 0.05$).

Solitary males were placed into 1 of 2 categories: those that had changed sex before and those that had changed sex after Day 61 of the experiment (the date by which half had changed). Those that changed before day 61 were larger on that day than those that had not yet changed (13.3 vs. 9.6 mm; *t*-test $p < 0.0001$). There was a significant effect of growth rate on the probability of changing sex ($p < 0.01$, $r^2 = 0.62$), but there was no significant effect of initial size ($p > 0.1$). Despite having faster growth rates, those individuals that changed sex before Day 61 changed at a smaller size (8.46 mm) than those that changed sex later (10.59 mm) (*t*-test; $p < 0.005$).

Among 23 paired males, the larger individuals always changed sex first.

Crepidula incurva

Field samples of *Crepidula incurva* had a slightly female-biased sex ratio, with 56% females (Fig. 5). Very few (3%) transitional individuals were collected and sex change occurred at 6 to 12 mm (Table 2). The L_{50} size at sex change was estimated as 8.05 mm (Table 2); 50% of these snails occurred in stacks, and those that were stacked had a larger L_{50} (8.47 mm) than solitary individuals ($L_{50} = 7.14 \text{ mm}$).

Of 38 individuals raised alone, 37 passed through a male phase with a distinct, well-developed penis; 1 of the individuals grew quickly, and was not observed in the normal male size range. In most cases, the penis was well-developed by the first census after 3 wk into the experiment and was observed for 1 or 2 subsequent intervals before sex change.

There was a significant effect of social treatment on size at sex change (ANOVA $p < 0.05$, Fig. 2, Table 3). Solitary males and males paired with males changed at a significantly smaller size (9.2 mm) than males paired with females (10.6 mm) (Fisher's PLSD test, $p < 0.05$). The among-group variance accounted for 11% of the total variance and the difference between treatments (1 mm) was surprisingly small. Shell lengths of 9.2 and 10.6 mm represent dry weights of 0.0038 and 0.0052 g respectively ($\ln[wt] = -10.479 + 2.209 \cdot \ln[\text{length}]$). The second male of paired males changed sex at the same size as males paired with females. Sex change occurred at slightly larger sizes in laboratory-reared individuals than in field-collected samples (Table 2). This is probably because *Crepidula incurva* lives attached to other small snails and therefore growth was likely to be substrate-limited in the field but not in our containers.

After 60 to 80 d, 50% of solitary males and the first male of a pair to change had changed sex (Fig. 3),

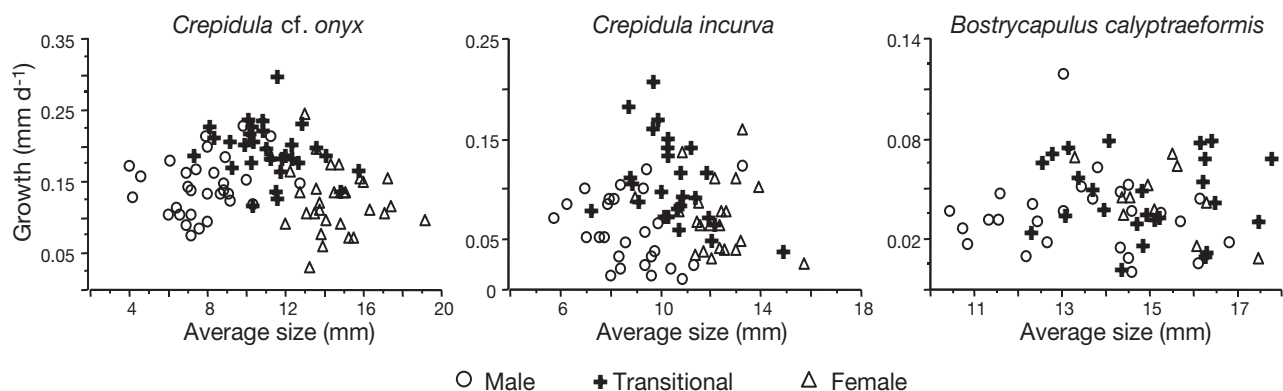


Fig. 4. *Crepidula* cf. *onyx*, *C. incurva* and *Bostrycapulus calyptraeformis*. Relationship between daily growth rate and average size during observation period for individuals during male, transitional and female phases. Average growth rates for all 3 categories were significantly different for *C. cf. onyx*, growth rates for transitionals was higher than for both male and females in *C. incurva*, and there was no difference between the categories in *B. calyptraeformis*

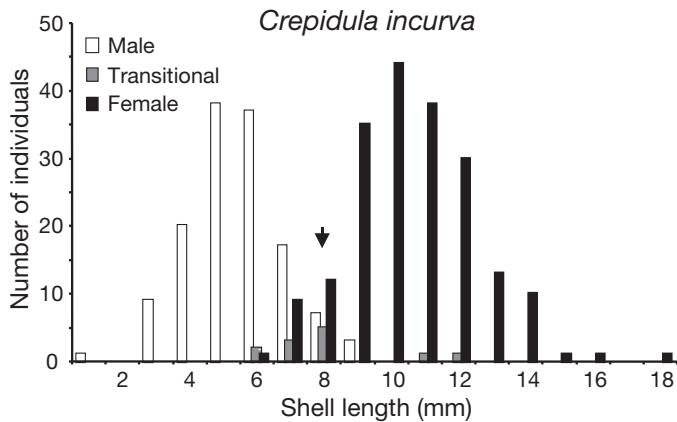


Fig. 5. *Crepidula incurva*. Size-sex distribution for field-collected individuals. Arrow indicates logistic regression estimate of size at which 50% were male (L_{50}). $n = 347$

while the second male of a pair to change and males paired with females changed after more than 300 d. In the solitary treatment, all males became female.

Daily growth rates were significantly affected by size (ANCOVA $p < 0.05$; Fig. 4), and marginally by sex (ANCOVA; $p = 0.06$). Daily growth rates for transitionals were significantly higher than for periods before and after the transition (paired t -test $\text{mean}_{\text{male}} = 0.06 \text{ mm d}^{-1}$; $\text{mean}_{\text{transitional}} = 0.11 \text{ mm d}^{-1}$; $\text{mean}_{\text{female}} = 0.07 \text{ mm d}^{-1}$; $p < 0.01$). When standardized for size (ANCOVA) all 3 categories were significantly different and growth (mm d^{-1}) was still greater for transitionals than for males and females ($p < 0.05$).

Snails that had changed sex before Day 77 (the date by which half had changed) were significantly larger (11.8 mm) on that day than those that had not changed (9.2 mm) (t -test $p < 0.0001$). Those that changed first were of smaller size at the sex change (8.2 mm) than those that had not changed by this date (10.2 mm) (t -test, $p < 0.005$). There was a significant effect of growth rate on the probability of changing sex ($p < 0.05$, $r^2 = 0.68$), and initial size did not have a significant effect ($p > 0.2$).

Among paired males, the larger individual always changed sex first ($n = 31$).

Bostrycapulus calyptraeformis

Samples of *Bostrycapulus calyptraeformis* had a male-biased sex ratio, with only 37% females in the population and very few (4%) transitional individuals (Fig. 6). The estimated L_{50} was 14.6 mm and fell between the smallest observed female (12.7 mm) and the largest male (19.7 mm). Transitionals were of smaller size (9.3 to 14.6 mm).

When individuals were raised alone, 24 of the 27 surviving animals passed through a male phase with a distinct, well-developed penis; 3 individuals appeared to bypass the male phase (or this was too brief to be observed within our 3 wk sampling periods). Development of the penis was slower than in *Crepidula incurva*. The penis was generally visible after 60 d and was often retained for more than 90 d. Growth rates were generally slower in this species (Fig. 4).

Because growth and sex change were so slow, we were not able to obtain sample sizes large enough to examine males paired with females. However, there was no significant difference in the size at sex change for solitary males and those paired with males (t -test; $p > 0.1$; $n = 50$). Solitary males and those paired with males changed sex after a similar number of days (Fig. 3).

For solitary males there were no significant differences in growth rates before, during or after sex change ($p > 0.2$), but the sample sizes were small ($n = 10$ to 20). Individuals that changed sex before Day 194 (the day by which half had changed) were significantly larger (15.3 mm) than those that had not yet changed (14.1 mm) on that day. The last male size was smaller (12.9 mm) for males that changed sex by Day 194 than for those that had not changed by this date (15.5 mm) (t -test, $p < 0.0005$). There was a significant effect of growth rate on the probability of having changed sex by this date ($p < 0.05$ $r^2 = 0.30$), and initial size was not a significant covariate ($p > 0.5$), although growth rate was correlated with initial size.

The larger individual in the paired males was most likely to change sex first (19 of 23 pairs; χ^2 , $p < 0.05$).

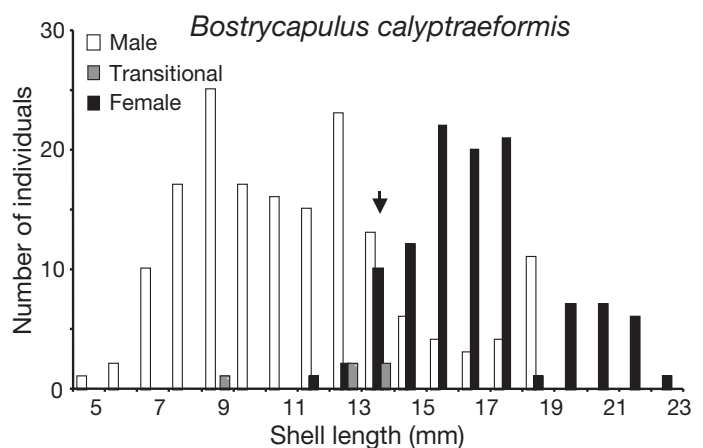


Fig. 6. *Bostrycapulus calyptraeformis*. Size-sex distribution for field-collected individuals. Arrow indicates logistic regression estimate of size at which 50% were male (L_{50}). $n = 544$.

DISCUSSION

Despite the fact that these 3 species of calyptraeids show different development, density and sex ratio, we found that the general patterns of sex change were similar. In all 3 species, (1) isolated juveniles passed through a male phase (although this was somewhat poorly developed and abbreviated in *Crepidula* cf. *onyx*), (2) sex change occurred at the same size for males raised alone and males raised with another male, (3) males that changed sex earlier had higher growth rates and changed at smaller sizes than males that changed later, and (4) the largest male in a pair of males almost always changed before the smaller male. In addition, in 2 out of 3 species, transitionals had higher growth rates than males or females.

Life history theory predicts that there should be an optimal size at sex change (Charnov 1982). For species with labile sex change, it is expected that optimal size at sex change should show less variation among individuals under constant conditions (e.g. laboratory conditions) than in a variable environment (e.g. the field). This does not appear to be the case for the calyptraeid species examined here. The size range between the smallest female and largest male were similar for samples from the field and in the laboratory experiment (Table 2), as was the size range at which transitionals were observed. In fact, the range of size overlap observed for males and females of *Crepidula* cf. *onyx* was actually larger in the laboratory than in field-collected samples. This is surprising as the cue from conspecifics, which is presumably chemical or tactile, must have been highly concentrated in our treatments relative to the field (with the possible exception of *Bostrycapulus calyptraeformis*, which occurs in high densities in the field).

Authors of previous comparisons of sex change among calyptraeids have suggested that the level and types of responses to conspecifics varies among species, and that species with large body size, planktonic larvae and gregarious behavior are more responsive to conspecifics than are less social species with direct development (Coe 1938, Hoagland 1978). Our results, however, show there is little difference in the type of effect of conspecific interactions between *Crepidula incurva*, a somewhat gregarious species with planktonic development, and *C. cf. onyx* a solitary species with direct development. The available evidence also suggests that *Bostrycapulus calyptraeformis*, a gregarious species with planktonic development, shows similar patterns. Warner et al. (1996) also concluded that the direct-developing *C. norrisiarum* (which is large, has direct development, and is usually found in groups of 2 to 13) also modulates sex change in response to associations with conspecifics. It seems clear from this

and previous studies that *Crepidula* species do not fall into 2 groups defined by a suite of life history characters, and that regardless of mode of development or frequency of associations between individuals, they generally have labile sex change which can be modulated by individuals' associations with conspecifics.

We did, however, find a difference between *Crepidula* cf. *onyx* and *C. incurva* in the magnitude of the treatment effect. Associations with conspecifics altered the size at sex change by 3 mm and 32% dry body weight in *C. cf. onyx* and accounted for 34% of the variation in size at sex change. This 3 mm difference is similar to the observed size range of transitionals (4.5 mm) and is similar to the range of size overlap for males and females observed in the experimental treatment (Table 2). In *C. incurva*, however, association with conspecifics altered the size at sex change by only 1 mm or 23% dry body weight and accounted for only 11% of the variation in size at sex change. This 1 mm difference is considerably smaller than the 3.5 mm range for transitionals and 4.5 mm range of size overlap for males and females observed among experimental individuals. This result suggests that while interactions with conspecifics accounts for a reasonable amount of the observed variation in *C. cf. onyx*, the majority of the observed variation in size at sex change is due to other factors in *C. incurva*. The greater responsiveness of the more solitary species to associations with conspecifics compared to the social species could reflect the low predictability of encounters between potential mates, which would select for rapid and sensitive responses to nearby conspecifics.

We found that the male phase is prolonged in the presence of a female both with respect to time and with respect to size. However, contrary to theoretical predictions and the conclusions of Coe (1938), males housed with females neither delayed sex change indefinitely, nor did they reach unusually large sizes before changing sex. In all treatments, those males that did not change sex also remained small. This suggests that sex change cannot be easily uncoupled from body size, and that those factors that increase growth inevitably lead to sex change. A connection between growth rate and sex change is supported by the result that faster-growing individuals changed sex sooner and at a smaller size than slower-growing individuals. Individual growth histories have been predicted to provide cues for other irreversible life history transitions (e.g. metamorphosis, Wilbur & Collins 1973) and in combination with current size, may provide cues that initiate sex change. If this is the case, the slower growth rates of those males kept with females in our experiments may account for their larger size at sex change. However, males in the solitary treatment had higher growth rates than the paired males, yet

changed sex at the same size. Experiments designed specifically to examine this hypothesis are necessary to further elucidate the effects of growth rate on sex change.

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LITERATURE CITED

- Allsop DJ, West SA (2003) Changing sex at the same relative body size. *Nature* 425:783–784
- Alonzo SH, Warner RR (2000a) Female choice, conflict between the sexes and the evolution of male alternative reproductive behaviours. *Evol Ecol Res* 2:149–170
- Alonzo SH, Warner RR (2000b) Allocation to mate guarding or increased sperm production in a Mediterranean wrasse. *Am Nat* 156:266–275
- Baldwin AP, Bauer RT (2003) Growth, survivorship, life-span, and sex change in the hermaphroditic shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Mar Biol* 143:157–166
- Bauer RT, VanHoy R (1996) Variation in sexual systems (protandry, gonochorism) and reproductive biology among three species of the shrimp genus *Thor* (Decapoda: Caridea). *Bull Mar Sci* 59(1):53–73
- Charnov EL (1982) *The theory of sex allocation*. Princeton University Press, Princeton, NJ
- Chen MH, Soong K (2000) Sex change in the hat snail, *Calyptraea morbida* (Reeve) (Gastropoda: Calyptraeidae): an analysis of substratum, size, and reproductive characteristics. *Veliger* 43:210–217
- Chiba S, Goshima S, Shinomiya Y (2003) Male–male competition selects for delayed sex change in the protandrous shrimp *Pandalus latirostris*. *Mar Biol* 142:1153–1157
- Coe WR (1936) Sex phases in *Crepidula*. *J Exp Zool* 72:455–477
- Coe WR (1938) Influence of association on the sexual phases of gastropods having protandric consecutive sexuality. *Biol Bull (Woods Hole)* 75:274–285
- Coe WR (1948) Nutrition and sexuality in protandric gastropods of the genus *Crepidula*. *J Exp Zool* 108:155–169
- Coe WR (1953) Influences of association, isolation and nutrition on the sexuality of snails in the genus *Crepidula*. *J Exp Zool* 122:5–19
- Collin R (1995) Sex, size, and position: a test of models predicting the size at sex change in the protandrous gastropod *Crepidula fornicata*. *Am Nat* 146:815–831
- Collin R (2000) Sex change, reproduction and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae). *Veliger* 43:24–33
- Gould HN (1917) Studies on sex in the hermaphrodite mollusk *Crepidula plana*. 2. Influence of the environment on sex. *J Exp Zool* 23:225–250.
- Gould HN (1919) Studies on sex in the hermaphrodite mollusk *Crepidula plana*. 3. Transference of the male producing stimulus through sea water. *J Exp Zool* 29:113–120
- Gould HN (1947) Conditions affecting the development of the male phase in *Crepidula plana*. *Biol Bull (Woods Hole)* 93:194
- Gould HN (1952) Studies on sex in the hermaphrodite mollusk *Crepidula plana*. IV. Internal and external factors influencing growth and sex development. *J Exp Zool* 119:93–163
- Gould HN, Hsiao SC (1948) New experiments and observations on sexual instability in *Crepidula plana*. *Biol Bull (Woods Hole)* 255–256
- Hoagland KE (1978) Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia* 17:365–391
- Koeller P, Mohn R, Etter M (2000) Density dependant sex change in northern shrimp, *Pandalus borealis*, on the Scotian Shelf. *J Northwest Atl Fish Sci* 27:107–118
- Lutnesky MMF (1994) Density-dependent protogynous sex change in territorial-harem fishes: models and evidence. *Behav Ecol* 5:375–383
- Mackie MC (2003) Socially controlled sex-change in the half-moon grouper, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. *Coral Reefs* 22:133–142
- Munday PL (2002) Bi-directional sex change: testing the growth-rate advantage model. *Behav Ecol Sociobiol* 52:247–254
- Perry AN, Grober MS (2003) A model for social control of sex change: interactions of behavior, neuropeptides, glucocorticoids, and sex steroids. *Horm Behav* 43:31–38
- Petersen CW, Warner RR, Shapiro DY, Marconato A (2001) Components of fertilization success in the bluehead wrasse, *Thalassoma bifasciatum*. *Behav Ecol* 12:237–245
- Robertson DR (1972) Social control of sex reversal in a coral reef fish. *Science* 177:1007–1009
- Ross RM, Losey GS, Diamond N (1983) Sex change in a coral reef fish: dependence on stimulation and inhibition on relative size. *Science* 221:574–575
- Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. WH Freeman & Co, New York
- Soong K, Chen JL (1991) Population structure and sex-change in the coral-inhabiting snails *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Mar Biol* 111:81–86
- Soong K, Chen JL (2003) Sex expression of an immobile coral-inhabiting snail, *Quoyula monodonta*. *Mar Biol* 143:351–358
- St. Mary CM (1993) Sex allocation in a simultaneous hermaphrodite, the blue-banded goby (*Lythrypnus dalli*): the effects of body size and behavioral gender and the consequences for reproduction. *Behav Ecol* 5:304–313
- Warner RR, Swearer S (1991) Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *Biol Bull (Woods Hole)* 181:199–201
- Warner RR, Fitch DL, Standish JD (1996) Social control of sex change in the shelf limpet, *Crepidula norrisiarum*: size-specific responses to local group composition. *J Exp Mar Biol Ecol* 204:155–167
- Wilbur HM, Collins JP (1973) Ecological aspects of amphibian metamorphosis. *Science* 182:1305–1314

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