Sex change in two Mediterranean species of Coralliophilidae (Mollusca: Gastropoda: Neogastropoda)

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Sex ratio and size–gender distribution of *Coralliophila meyendorffii* and *Babelomurex cariniferus* collected in the field was studied. While sex ratio did not depart from the expected Fisher 1:1 ratio for dioecious species, the size–frequency distributions showed clearly a sexual size dimorphism with males significantly smaller than females. Laboratory monitoring of penis reduction in both species, combined with an anatomical and histological study of the reproductive system of individuals reducing penis revealed the existence of transitional sexual stages, proving the existence of protandry. Penis monitoring also demonstrated a high plasticity in size at sex change, suggesting a socially controlled sex change. In *C. meyendorffii*, where penis monitoring followed an experimental design based on the assumption that protandry existed and that presence of females influenced sex change, results strongly supported a social control of sex change. Evidences for protandry in other coralliophilids are summarized and protandry as a widespread sexual strategy in Coralliophilidae is postulated.

INTRODUCTION

Animals that are largely sedentary as adults but possess dispersal stages and have patchy distributed substrates in time and space have frequently evolved protandry (Hoagland, 1978). Coralliophilidae, a family of marine neogastropods that feed exclusively on anthozoans (Richter & Luque, 2002), fits this category of animals. Coralliophilids have predominantly planktotrophic larval development (Richter & Luque, 2002), their adult phase tends to have low mobility remaining close to or on their hosts or preys (Hayes, 1990; Lalli et al., 1997; Oren et al., 1998; Chen & Soong, 2002), and their food resources are discontinuously distributed in the marine realm. Some coralliophilids (i.e. *Magilus*, *Leptoconchus* and *Rhizochilus* species, *Reliquiaecava robillardi* (Lienard, 1870) and *Coralliophila brevis* (Blainville, 1832)) are sessile at least when they are females or when they attain larger sizes (Gohar & Soliman, 1963; Albergoni & Spada, 1972; Poorman, 1981; Massin, 1987, 1988, 1990, 2000). Therefore, though it has been commonly accepted that coralliophilids have separate sexes (Robertson, 1970; Wells & Lalli, 1977; Oliverio, 1989), it is expected that in coralliophilids protandry might be more widely distributed than dioecy. The fact that all groups of marine gastropods that feed on cnidarians present some type of hermaphroditism (Heller, 1993) also strongly supports this assumption.

However, to date direct evidence of the existence of sex change in coralliophilids is scarce. In the Caribbean *Coralliophila abbreviata* (Lamarck, 1816) and the Indo-Pacific *Coralliophila neritoidea* (Lamarck, 1822), protandry has been proposed to occur on the basis of the observed sexual size-dimorphism, with males smaller than females, presence of penis in females and sex ratios diverging from the expected Fisher 1:1 for dioecious species (Hayes, 1989; Soong & Chen, 1991). Yet, only in *C. neritoidea* the existence of sex change from male to female could be recently demonstrated through direct observation of penis reduction of experimentally controlled individuals (Chen et al., 1998).

Within the framework of a study focusing on the reproductive biology of Mediterranean coralliophilids, the present work addresses the sexual strategy and sex–size distribution of *Coralliophila meyendorffii* (Calcara, 1845) and *Babelomurex cariniferus* (G.B. Sowerby I, 1834). *Coralliophila meyendorffii* is a fairly common Mediterranean infralittoral species that feeds on actiniarians and scleractinians, while *B. cariniferus* is an uncommon infralittoral species that feeds exclusively on scleractinians. A preliminary field study carried out in 1994 (Richter & Luque, 1994) reported the presence of pseudopenis in females of *C. meyendorffii* and *B. cariniferus* and raised the question whether the Mediterranean species are protandric or dioecious.

Previous information on the reproductive biology of *C. meyendorffii* and *B. cariniferus* is limited. Spada (1968) and Ghisotti & Spada (1970) contributed with some notes on the egg-capsules, breeding behaviour and breeding season of *B. cariniferus*. A study carried out on *C. meyendorffii* by Oliverio & Mariottini (2001) showed a sexual size dimorphism with males smaller than females and an ecological variability in shell size of mature females. A study dealing with the anatomy of the reproductive system of both species has been published elsewhere (Richter & Luque, 2003).

MATERIALS AND METHODS

Material and sampling sites

Seventy-one individuals of *Coralliophila meyendorffii* and 42 of *Babelomurex cariniferus* were collected for the present study from the Spanish Mediterranean by SCUBA diving. Date of collection, sampling sites, prey and number of specimens collected are summarized in Table 1.

Table 1. *Number of specimens in each sample, sampling localities, depth, host and date of sample collection.*

Species	N	Locality	Depth (m)	Host	Date
Coralliophila meyendorffii	5	Cape Gata	1–2	*Anemonia sulcata*	10–1993
	7	Cape Gata	0–2	*Actinia equina*	10–1993
	1	Punta de la Mona	1–6	*Astroides calycularis*	3–1994
	21	Cape Gata	0–2	*Actinia equina*	6–1994
	1	Punta de la Mona	14	*Astroides calycularis*	8–1994
	4	El Calón	0–2	*Actinia equina*	10–1995
	6	Escombreras	—	—	11–1995
	16	Cape Gata	0–2	*Actinia equina*	6–1996
	2	Cala Cerrada	0–2	*Actinia equina*	7–1996
	1	Cala Cerrada	1–2	*Anemonia sulcata*	7–1996
	3	El Playazo	1–3	*Anemonia sulcata*	9–1996
	3	Los Escullos	3–4	*Balanophyllia europaea*	9–1996
	1	Los Escullos	1–2	*Anemonia sulcata*	10–1996
Babelomurex cariniferus	14	Punta de la Mona	10–20	*Astroides calycularis*	9–1993
	13	Calahonda	10–20	*Astroides calycularis*	8–1994
	1	Calahonda	10–20	*Astroides calycularis*	5–1995
	3	Punta de la Mona	10–20	*Astroides calycularis*	5–1996
	8	Punta de la Mona	10–20	*Astroides calycularis*	2–1997
	3	Calahonda	10–20	*Astroides calycularis*	7–1997

N, number of specimens; —, no data.

Collected specimens of both species were brought alive to the laboratory, measured with a calliper to the nearest 0.05 mm, sexed, and then installed in marine aquaria for observation. Shell length was used as size variable in *C. meyendorffii*, while aperture height was used in *B. cariniferus*, since in the latter species encrusting algae covered most of the shell spire. Each species was fed with its natural prey. Sex was determined by observing the pallial cavity. If both, a well-developed curved penis and the absence of egg capsules were observed, the specimen was identified as male, and if absence of a penis, presence of a reduced penis (=pseudopenis) or presence of egg capsules were observed, the specimen was identified as female.

Laboratory study of sex change

The presence of a penis in females of *Coralliophila meyendorffii* and *Babelomurex cariniferus* collected in the field, revealed by a preliminary study carried out from autumn 1993 to summer 1994 (Richter & Luque, 1994), suggests that both species are protandric. In order to test the hypothesis of protandry, the pallial cavity of females and males just collected from the field and kept in aquaria were regularly inspected to detect incubation and any change in penis size. The individuals and their initial sex were identified by a mark made on their shell when sexing them. Size and time at which an initially male individual was observed brooding or with a reduced penis was annotated, and individuals with reducing penis were fixed to study their gonads and reproductive system. Fixing of these individuals was undertaken either as soon as penis reduction was observed, or after a period during which they bred at least once.

Since specimens of *C. meyendorffii* were more available than those of *B. cariniferus* and the pallial cavity of the first species was more easily observable, male individuals of *C. meyendorffii* were monitored following an experiment designed to test whether a social control of sex change existed, assuming that the species was protandric. For the experiment, two independent and artificial assembled populations of males were established, one in which females equalled or surpassed males in number (control population), and another in which males were isolated physically and chemically from females (test population). Both populations were maintained in separate marine aquaria from the resting stage of one year until the reproductive season of the next year, and subjected to the same manipulation. The latter consisted of removal of all the individuals with reducing penes during a period extending between two and a half months and three months from the time at which the first penis reduction was detected in the population. Removal of individuals with reducing penis was carried out to avoid contact with other males, and to preclude a possible inhibitory effect by them on sex change of other males.

The experiment was repeated twice, and male individuals used to form the experimental populations were collected the same year in which each experimental run was set off. In each run, the pallial cavity of each individual of each treatment was examined on the same day or within a few days at monthly or bimonthly time intervals. An inspection in shorter time intervals was discarded, because handling the snails made their pallial cavity more vulnerable to parasitic infection. The initial and final composition of each artificial population, starting time and end of each experimental run and the time at which removal of individuals with reducing penis was interrupted, are summarized in Table 3 (see Results).

Individuals of *Babelomurex cariniferus* were examined several times at least every year close to and during the reproductive season. In this species, composition of the laboratory populations during the study varied widely in the course of the experimental study and was not considered for the purpose of this study.
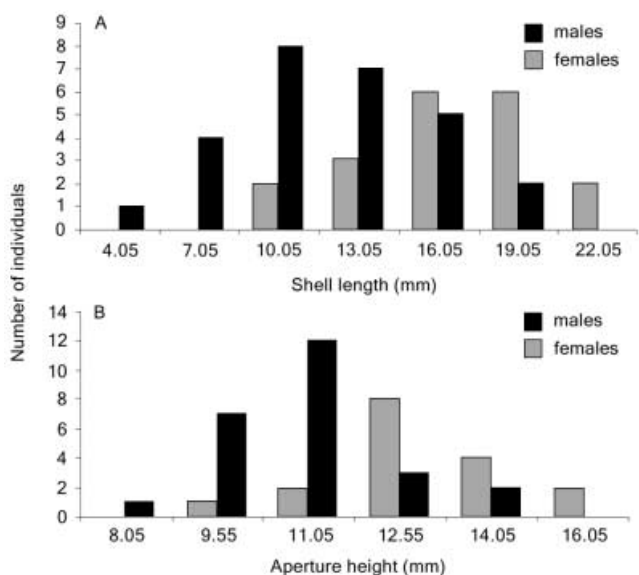
Figure 1. Size–frequency distribution of individuals collected in the field. (A) *Coralliophila meyendorffii* from Gata Cape. (B) *Babelomurex cariniferus* from Granada.

Anatomical study

For the anatomical and histological study of the reproductive system, the specimens were relaxed in $MgCl_2$ isotonic with seawater, fixed in Bouin or 10% formaldehyde for one day and dehydrated in an ascending series of graded ethanol. Then they were submersed in bencilbenzoate for one day maximal, embedded in Paraplast, serially sectioned at 7–10 μm using a Leica microtome, and stained with standard haematoxylin and eosin or Azan.

RESULTS

Sex ratio and sexual size dimorphism

Coralliophila meyendorffii

Forty-four individuals of *Coralliophila meyendorffii* from the stable population of Cape Gata ranged from 5.90 to 23.30 mm in shell length. Eighteen were females (40.9%) and 26 were males (59.1%). This sex ratio did not differ significantly from the expected Fisher 1:1 ratio for dioecious species ($\chi^2=1.45$; $P>0.05$). However, sex ratio changed with size-classes of individuals. Males outnumbered females in the smaller size-classes, whilst in the larger size-classes the opposite occurred (Figure 1A). This resulted in a sexual size dimorphism with females significantly larger than males ($F_s=19.047$; $P<0.001$; df=42). The average female and male shell lengths were respectively 17.90 ±3.61 mm (range: 10.50–23.30 mm; N=18) and 11.70 ±3.42 mm (5.90–20.00 mm; N=26).

The aggregates collected in the field were composed of two to six individuals. The sex ratio of the aggregates varied widely from 33% to 83% males per aggregate, but males tended to outnumber females (80% of the aggregates). In all aggregates collected, the smallest individual was male, while the largest individual was either male or female. Nevertheless, females tended to be the largest member of the aggregates (80% of the aggregates) (Table 2). The size of the smallest or unique female of the aggregates tended to be larger than that of the largest male of the same aggregate (80% of the aggregates). If only aggregates associated with actiniarians were considered, the size of the unique female in the aggregate was positively correlated to the number of members in the aggregates (Spearman $R=1$; $P<0.05$).

Among the solitary individuals, two males and one female were encountered. The smallest solitary individual was male (shell length 7.40 mm), and the largest was female (shell length 21.65 mm).

Babelomurex cariniferus

Forty-two individuals of *Babelomurex cariniferus* measured between 8.80 and 16.70 mm in aperture height. Seventeen were females (40.5%) and 25 were males (59.5%). As in *Coralliophila meyendorffii*, this proportion did not differ significantly from the expected Fisher 1:1 ratio for dioecious species ($\chi^2=1.52$; $P>0.05$). However, males dominated in the smaller size-classes and females in the larger size-classes. Aperture height differed significantly between sexes ($F_s=19.306$; $P=0.0001$; df=40), with female and male aperture height averaging 13.9 ±0.39 mm (range: 11.00–16.70 mm; N=17) and 11.70 ±0.30 mm (range: 8.80–15.30 mm; N=25), respectively (Figure 1B).

Table 2. Coralliophila meyendorffii. *Sex ratio of natural population samples, aggregates or solitary snails collected from the field.*

Locality	Date	N	Female size (mm)	Male size (mm)	Sex ratio (m:f)	Observation
Cape Gata	10–1993	5	20.9–21.75	14.5–18.6	3:2	population sample
Cape Gata	10–1993/6–1994/6–1996	44	11.3–18.1	7.7–11.8	26:18	population sample
El Calón	10–1995	4	23.7	14.0–19.6	3:1	ag
Escombreras	11–1995	6	10.9	10.2–13.2	5:1	ag
Escullos	9–1996	3	10.35–?*	7.4	1:2	ag
Escullos	10–1996	1	21.65	—	0:1	s
Cala Cerrada	7–1996	2	17.9	14.6	1:1	ag
Cala Cerrada	7–1996	1	—	7.8	1:0	s
Calahonda	3–1994	1	—	15.4	1:0	s
El Playazo	9–1996	3	21.3	18.5–?**	2:1	ag

*, second female larger than 10.35 mm; **, second male with a size intermediate between the female size and the smallest male size; ag, aggregate; f, females, m, males; N, sample size; s, solitary.
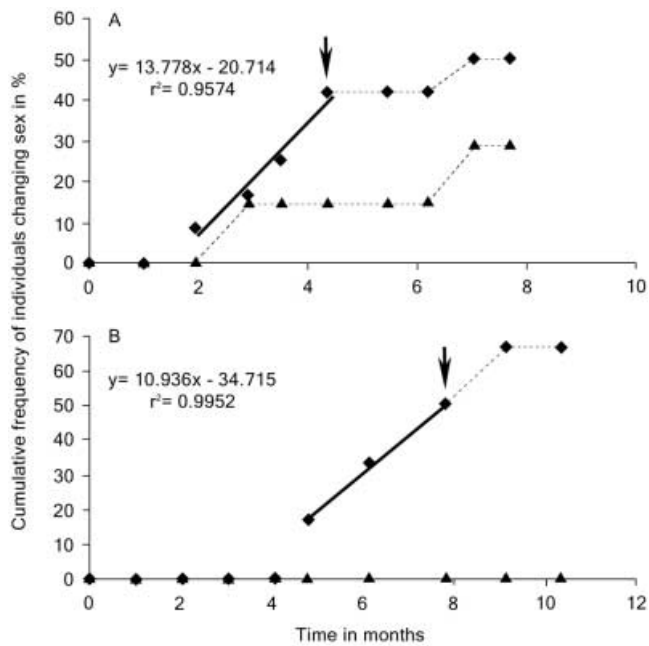
Figure 2. Rate of individuals reducing penis. (A) First experimental run covering period 1994–1995. (B) Second experimental run covering period 1996–1997. ◆, test males reared isolated from females; ▲, control males reared together with females. Continuous line, fitted regression line is significant; arrow, time at which removal of individuals changing sex was interrupted.

Sex change

Coralliophila meyendorffii

A total of 12 male individuals reduced their penes noticeably at a size ranging from 14.10 to 21.20 mm (average shell length: 17.51 ±2.30 mm; N=12) in the course of the experimental study. Penis reduction was irreversible and progressive, and all individuals that were kept alive after penis reduction, seven out of 12, started breeding egg-capsules inside their pallial cavity during the reproductive season. Three individuals that were fixed as soon as penis reduction started were in a transitional sexual phase. Their reproductive tracts consisted of an immature ovary, an atrophied seminal vesicle, the latter sometimes still with remains of eusperm, a reduced penis, and a male pallial reproductive section (two individuals) or a glandular gonoduct looking like a prostate, with an incipient seminal receptacle proximally (one individual) (for anatomical details, see Richter & Luque, 2003).

Penis reduction affected males of the same population asynchronically, and started in each run at a different time of the reproductive cycle. In the first experimental run, one male from the control population, that is from the male population grown together with females, and two from the test population, that is from the male population that was isolated from females at onset of the experiment, reduced their penes near the beginning of the breeding season, which started in March 1995, while five more males, four from the test population and one from the control population, reduced their penes during the reproductive season. The individual that reduced its penis first in the test population did so in January 1995, and in the control population, in February 1995, one month later. The individual that reduced its penis last did so in June

1995 in both treatments. In the second experimental run, all of the males from the test population that reduced their penes, a total of six males, did so during the breeding season. The individual that reduced its penis first did so in February 1997, at onset of the breeding season. The last penis reduction occurred in June 1997. No male of the control population changed sex in the second run.

In both experimental runs, removal of individuals with reducing penis enhanced sex change in the test population when compared to the control population. As shown in Figure 2, in both experimental runs, the cumulative frequency of individuals changing sex, measured as the total number of individuals that reduced their penes at the time of observation divided by the total number of males at onset of the experiment, increased linearly with time in the male population that was isolated from females (test population) during the period in which individuals with reducing penis were removed from the populations, while it remained constant in the male population grown together with females (control population) (Figure 2A,B). In the first run, the cumulative frequency of individuals changing sex increased at a constant rate of 13.78% month^{-1} ($F=44.92$; df=2; $P<0.05$) in the test population during the period extending from January 1995 (time at which the first individual changed sex in this population) to the end of March 1995, while in the control population it remained constant at approximately 14% from February 1995 (time at which the first individual changed sex in this population) to the end of March of 1995 (Figure 2A). In the second run, the cumulative frequency of individuals changing sex increased in the test population at a constant rate of 10.94% month^{-1} ($F=205.3l$; df=1; $P<0.05$) from February 1997 (time at which the first individual changed sex in this population) to May 1997, while in the control population it did not increase at all throughout the whole experimental period (Figure 2B). As the rate at which the cumulative frequency of individuals changing sex increased in the test population did not differ significantly among both runs ($F=4.885$; $P>0.05$), data of both runs were combined to regress cumulative frequency of individuals changing sex on time. The slope of the new fitted regression equation was 12.42% month^{-1} ($F=41.55$; $P<0.0l$; df=3). However, in spite of the fact that the control and the test population responded differently to the removal of individuals changing sex, the proportion of individuals that changed sex at the time removal of individuals with reducing penis was interrupted did not differ significantly among populations (Fisher's exact test with data in table 5, $P=0.0778$).

In both runs, the rate of individuals changing sex in the test population dropped visibly during the period that elapsed from the time removal of individuals with reducing penis was interrupted until the end of the experiment (Figure 2A,B). In both runs, merely a single male reduced its penis in the test population (Table 3) during this time interval. This represented a mean increment in the cumulative frequency of individuals with reducing penis of 2.5% month^{-1} in the first run, and a mean increment of 6.7% month^{-1} in the second run.

Babelomurex cariniferus

Of 25 individuals collected with developed penis, 22 could be re-identified in the course of the laboratory

Table 3. *Initial conditions and results of the experiment on sex change in* Coralliophila meyendorffii.

Population	Experimental period	t_n	Initial composition	Final composition*	N of males that changed sex when removal was interrupted		N of individuals that changed sex at the end of the experiment
					changed sex	did not	
Control	11/1994–7/1995	3–1995	16 f:7 m	16 f:4 m	1	6	2
Test	11/1994–7/1995	3–1995	0 f:12 m	3 f:5 m	5	6	6
Control	9/1996–7/1997	5–1997	2 f:2 m	2 f:2 m	0	2	0
Test	9/1996–7/1997	5–1997	0 f:6 m	2 f:2 m	3	3	4

f, female; m, male; N, number; t_n, time at interruption of removal of individuals with reducing penis; *, without including individuals that died or were removed from the population.

Table 4. *Data relative to sex change for* Babelomurex cariniferus.

t_0	Aperture height at t_0 (mm)	t_n	Aperture height at t_n (mm)	Brooding	Penis reduction	Sex
9–1993	12.00	6–1996	11.90	●	+	female
9–1993	11.65	8–1994	11.65	●	+	female
9–1993	15.30	8–1994	15.30	●	?	female
9–1993	12.35	6–1995	12.35	●	+	female
9–1993	11.00	6–1995	11.00	●	+	female
9–1993	14.10	6–1996	14.15	●	+	female
8–1994	12.00	6–1995	?	●	+	female
8–1994	12.30	6–1996	12.40	⊗	+	transitional female
8–1994	11.40	6–1996	11.40	●	+	female
8–1994	12.70	5–1997	13.00	⊗	+	transitional female
8–1994	11.80	6–1996	11.60	*	+	female
5–1996	13.90	3–1997[1]	14.20[2]	⊗	—	transitional female
2–1997	12.20	10–1997[1]	11.50[2]	⊗	—	transitional male

●, breeding observed at least once during the laboratory study; ⊗, breeding has never been observed before fixing of the individual; *, breeding was inferred from the observation of a postreproductive ovary; +, progressive and sensible penis reduction; —, not appreciable penis reduction; ?, data not available; t_0, date of collection; t_n, date at which incubation and/or penis reduction was detected for the first time; [1], date of fixation; [2], size at time of fixation.

study and were observed regularly. Ten individuals out of these 22 reduced their penis noticeably in the course of the laboratory study. Penis reduction was irreversible and progressive once it started. Eight out of those that reduced their penes became functional females and brooded egg capsules at least once during the laboratory study either in the same reproductive season they first reduced the penis or in that of the following year. The reproductive tracts of these individuals were indistinguishable from those of females collected from the field. The only signs of a previous male phase were a reduced penis, traces of a cephalic sperm duct and a twisted and reddish posterior oviduct close to the kidney (for anatomical details, see Richter & Luque, 2003). The remaining two individuals that reduced their penes were transitional females in different stages of sex change. One of them was fixed as soon as penis reduction was observed in May 1997, close before onset of the reproductive season, and one was fixed in October 1997 at the end of the reproductive season, more than one year after penis reduction was observed. One individual out of the 22 started brooding egg-capsules. Whether it reduced its penis or not could

not be assessed, as it had died and decomposed before its head-foot could be examined.

Eleven individuals did not reduce their penis during the laboratory study. Except for three of them, the rest were functional males with a mature or a spent testis. The remaining three individuals were a functional female with a postreproductive ovary, collected in February 1997 and fixed in July 1997, a transitional female collected in May 1996 and fixed in March 1997 before onset of the reproductive season, and a transitional male collected in February 1997 and fixed in September 1997 at the end of the reproductive season.

Since inspection of the pallial cavity was not carried out monthly in *Babelomurex cariniferus*, the exact time and size at penis reduction could not be assessed. An estimate for the size at sex change was therefore used. This was the aperture height the individuals reached at the time they were observed either brooding for the first time, or with a reduced penis or at the time they were studied anatomically showing a transitional reproductive tract. This estimate was considered a good approach, since 12 of the individuals that showed evidences for sex change and on

Table 5. *Prosobranchs with environmentally controlled sex change.*

	Crepidula fornicata	Crepidula lingulata	Crepidula norrisiarum	Coralliophila neritoidea	Lottia gigantea
Overlap between male and female size	?	37.5% of total size range	≈ 55%[1] of total size range	40% of total size range	≈50%[1] of total size-range
SC inhibited/delayed by presence of females in the aggregates or pairings	+	+	+	+	not applicable[3]
SC controlled by presence of other males in aggregates or pairings	?	—	+	?	not applicable[3]
SC controlled by population sex ratio	+	?	?	?	?
Size at SC positively correlated to population size or age structure	+	?	?	?	—
Size at SC influenced by presence/absence or number of females in aggregates or pairings	?	?	+	+[2]	not applicable[3]
Size at SC positively correlated to female population density	+	?	?	?	?
Size at SC positively correlated to population density	+	?	?	?	+[4]

SC, sex change; + exists; —, does not exist; ?, unknown; [1], estimated here from the histograms of the size-gender distributions; [2], only two categories were considered: one female per aggregate and more than one female per aggregate; [3], not applicable since species is territorial; [4], as long as population is not overcrowed.

which size data were available, suffered negative shell growth or increased their aperture height less than 3% during the period between time of collection and the time at which the first evidence for sex change occurred (Table 4). The estimated aperture height at sex change ranged from 11.00 to 15.30 mm (average 12.53 ±1.34 mm; N=12).

DISCUSSION

Protandry in *Coralliophila meyendorffii* and *Babelomurex cariniferus* has been demonstrated here. In the first species, protandry was previously inferred from the indirect evidence of size–gender distribution (Oliverio & Mariottini, 2001), but direct evidences for sex change were not provided. In the present study, the irreversible and progressive penis reduction during or close to onset of the reproductive season followed by egg-brooding by monitored individuals with initially developed penis, and the fact that individuals reducing penis were transitional sexual stages are direct evidences for sex change from male to female.

In Coralliophilidae, protandry has also been reported in the Indo-Pacific coral dweller *Coralliophila neritoidea* (Chen et al., 1998). In this species, the occurrence of sex change from male to female was previously assumed on the basis of indirect evidences such as the presence of pseudo-penis in small females, a male biased sex ratio and sexual size dimorphism, with males smaller than females (Soong & Chen, 1991), and demonstrated later by direct observation of penis reduction in males (Chen et al., 1998). Protandry has also been previously postulated in the Caribbean *Coralliophila abbreviata* (Hayes, 1989), but never demonstrated. According to Hayes (1989), the variable sex ratio observed points to a special pattern of protandric sex change. In contrast, Wells & Lalli (1977) considered this species as dioecious despite the variable sex ratio and the fact that males were significantly smaller than females.

In *B. cariniferus* and *C. meyendorffii*, sex change apparently starts during the postreproductive resting stage with complete resorption of the testis and its substitution by ovarian tissue, since oocytes and spermatocytes were never observed developing simultaneously in individuals reducing penis and in transitional sexual stages. This is contrary to other protandric prosobranchs (Smith & Breyer, 1983; Creese et al., 1990). Sex change proceeds then with the transformation of the anterior section of the reproductive tract (reduction of penis and cephalic vas deferens, glandular redifferentiation of the pallial gonoduct, new formation of bursa copulatrix). These events parallel the activation and maturation of the oocytes during the prereproductive and reproductive season.

The onset of sex change during the resting stage with complete resorption of the testis is also supported by the fact that in *B. cariniferus*, the individual in the earliest stage of sex change, the transitional male, was encountered after the end of the reproductive season. In *C. neritoidea*, sex change might also start during the resting stage with complete resorption of testis, because individuals with both testis and ovaries were never found during the reproductive season (Lalli et al., 1997; Chen & Soong, 2002). Besides, in the latter species, growth rates of individuals changing sex are significantly higher than those of females and males, thus suggesting that somatic growth during sex change might occur at the expense of gonads, which might be resorbed during the period of sex change (Chen & Soong, 2002). Sex change from male to female during a resting stage has been reported previously in some patellids (Bacci, 1947; Choquet, 1966; Branch, 1974, 1981; Thompson, 1979; Bowman & Lewis, 1986) and in calyptraeids (Bacci, 1951).

In *C. neritoidea*, timing of sex change is labile and influenced environmentally by social interactions, and the same might be true for *C. meyendorffii* and *B. cariniferus*. As revealed by a field study with marked and recaptured individuals, in *C. neritoidea* the presence of females inhibits sex

change in males. The proportion of individuals that changed sex is significantly higher in isolated males than in males paired with females (Chen et al., 1998). In addition, in *C. neritoidea*, size at sex change is plastic and not fixed, as suggested by the large overlap in male and female sizes, and seems to depend on aggregate structure. A study of size–gender distribution and aggregate structure showed that the smallest female size, used as an estimate for size at sex change, is significantly smaller in aggregates with a single female than in aggregates with more than one female (Soong & Chen, 1991).

As in *C. neritoidea*, in *C. meyendorffii* and *B. cariniferus*, size at sex change is highly variable and overlap between the size distribution of females and males is large, covering approximately half of the total population size range. Both facts suggest the existence of a social control on sex change in the Mediterranean species. However, a high plasticity in size at sex change can also be controlled genetically. At least in *C. meyendorffii*, there are further evidences that strongly support the existence of a socially controlled sex change. As revealed by the present study, in *C. meyendorffii*, size at sex change can be correlated to social factors. In aggregates associated to actiniarians, the size of the unique female of the aggregate, which can be used as an estimate of the size at which sex change occurs in each aggregate, is positively correlated to the total number of individuals in the aggregate. A significant correlation between size at sex change and any social factor should not be expected if sex change in *C. meyendorffii* is not environmentally controlled. In addition, presence of females and individuals undergoing sex change apparently influences sex change in males. In artificial populations in which males are isolated from females, the cumulative frequency of males that change sex increases linearly with time once sex change starts if individuals changing sex are removed from the populations, while in likewise manipulated artificial populations in which females equalled or surpassed males in number, the cumulative frequency of males that change sex do not increase linearly with time but remain constant. This indicates an inhibition by females on sex change of males. In addition, interruption of removal of individuals undergoing sex change apparently causes a drop in the rate of sex change in male populations isolated from females. This also points to an inhibitory effect of individuals with reducing penis on sex change of males. The latter is plausible, since individuals with reducing penis had ovaries, and thus could be producing female pheromones. Female pheromones are thought to be responsible for the inhibition of sex change in males of species with a social control of sex change (Hoagland, 1978).

The fact that the difference in the proportion of males that changed sex was not significant among treatments (males isolated from females vs males grown with females) during which individuals with reducing penis were removed ($P=0.078$), does not contradict the existence of a socially controlled sex change in *C. meyendorffii*. This result can be explained by the short duration of the experimental phase during which individuals with reducing penis were removed. If presence of females and individuals undergoing sex change inhibits or delays sex change in males, then the later the removal of individuals with reducing penis is interrupted, the greater will be the

difference among treatments in the cumulative frequency of individuals that change sex, provided that, as shown in the present study, in a population, sex change is an asynchronic process that spans a long period affecting males of the same population at a different time. Asynchrony in sex change has been previously reported in *Crepidula norrisiarum*, where individuals change sex from 36–124 days after onset of experiments (Warner et al., 1996) and in *Coralliophila neritoidea*, where individuals change sex from the first to the fifth month after the onset of the experiment (Chen et al., 1998). In the present study, removal of individuals undergoing sex change was interrupted a few months before the last sex change occurred in each run.

Environmental control of sex change is a well known phenomenon in protandric gastropods like eulimids (Lützen, 1972; Warén, 1980, 1983), stack forming and non-stack forming calyptraeids (Hoagland, 1978; Warner et al., 1996; Collin, 2000), and giant territorial limpets (Wright, 1989). In these gastropods, presence of females or other larger conspecifics delays or inhibits sex change in males (Hoagland, 1978; Warner et al., 1996; Collin, 2000). These gastropods are also highly plastic in size at sex change, which depends on social factors, and their male and female size distribution overlap considerably (Hoagland, 1978; Wright, 1989; Warner et al., 1996; Collin, 2000) (Table 5).

In the stack-forming limpet *Crepidula fornicata*, the proportion of individuals changing sex depends on initial population sex ratio, and population and female population density influence size at sex change. In newly colonizing populations, sex change occurs soon in small sized individuals, while in stable populations with older age-classes, individuals change sex at larger sizes. Size at sex change is also positively correlated with female population and total population density, with size of solitary or mated intermediates increasing with increasing female population density (Hoagland, 1978). In *Crepidula norrisiarum*, size at sex change is highly variable, since, in individuals forming aggregates, sex change is controlled by the size structure of males and the presence/absence of females. Sex change in this species is triggered if females are absent in the aggregates and if individuals attain the largest size of the members of an aggregate regardless of its actual size (Warner et al., 1996). Presence of smaller males also enhances sex change in males when compared to solitary males (Warner et al., 1996). In *Crepidula lingulata*, presence of females inhibits sex change in males, and the overlap between female and male size covers a considerable portion of the total size-range of the population (Collin, 2000). However, whether in *C. lingulata* age or size at sex change depends on social factors (presence/absence of females or other conspecifics) has not been demonstrated experimentally. In the territorial limpet *Lottia gigantea*, sex change seems to be enhanced by low density (Wright, 1989), since age and size of females is positively correlated with population density. But this is only true as long as populations are not overcrowded and individuals exhibit territorial behaviour.

Protandry either with a fixed timing of sex change or a labile environmentally controlled timing may not be restricted to the Indo-Pacific *Coralliophila neritoidea* and the Mediterranean *C. meyendorffii* and *Babelomurex*

cariniferus, but rather be a common phenomenon in Coralliophilidae. This hypothesis is supported by the following facts: (1) the frequent presence of pseudopenis in functional females; (2) the widespread sexual size dimorphism, with males smaller than females; (3) the unusual sex ratios that differ from the Fisher 1:1 sex ratio expected for dioecious species; and (4) the apparently constant positive correlation of female fecundity with size.

Pseudopenis in functional females is usual in protandric caenogastropods (Coe, 1953; Lützen, 1972; Gooding & Lützen, 1973; Hoagland, 1978; Gallardo, 1976; Warén, 1983; Reid, 1986; Bieler & Mikkelsen, 1988; Bieler & Hadfield, 1990; Soong & Chen, 1991; Collin, 2000), which still retain in the female phase a vestige of the male penis after sex change. In Coralliophilidae, apart from the protandric species *Coralliophila meyendorffii*, *Babelomurex cariniferus* (present study) and *C. neritoidea* (Soong & Chen 1991; Lin & Liu, 1995; Lalli et al., 1997), pseudopenis occurs in mature females of *C. abbreviata* (Fioroni et al., 1991), *C. squamosa* (Fioroni et al., 1991; Oehlmann, 1994, both authors as *Coralliophila lamellosa*), *C. costularis* and *C. radula* (Lalli et al., 1997), and *C. brevis* (Richter & Luque, 2003). In *Leptoconchus peronii*, Massin (1990) reported two individuals considered as males with reduced penis. However, since gonads were not examined histologically, the possibility that the individuals were females or transitional sexual stages from male to female cannot be discarded. In *C. abbreviata* (Fioroni et al., 1991) and *C. squamosa* (Fioroni et al., 1991; Oehlmann, 1994), the female pseudopenis was interpreted as a morphological expression of imposex. However, in *C. squamosa*, Oehlmann (1994) could not demonstrate TBT content in tissues due to the small sample size.

Sexual size dimorphism with males smaller than females occurs consistently in protandric gastropods (Bacci, 1947; Lützen, 1972; Gooding & Lützen, 1973; Branch, 1974, 1981; Gallardo, 1976; Smith & Breyer, 1983; Bieler & Mikkelsen, 1988; Wright, 1989; Bieler & Hadfield, 1990; Creese et al., 1990; Warner et al., 1996; Collin, 2000). In Coralliophilidae, sexual size dimorphism with males smaller than females, apart from *Coralliophila meyendorffii* (present study; Oliverio & Mariottini, 2001), *Babelomurex cariniferus* (present study) and *C. neritoidea* (Soong & Chen, 1991; Lalli et al., 1997), has been reported in the Caribbean species *C. abbreviata* and *C. caribaea* (Wells & Lalli, 1977) and in the Indo-Pacific species *C. radula*, *C. costularis* (Lalli et al., 1997) and *Leptoconchus* (Massin, 1988, 1990). In the latter genus, male dwarfism has been described (Massin, 1988, 1990). In *C. squamosa* (Oehlmann, 1994) and *C. brevis* (Richter & Luque, 2003), the smallest individual encountered was male suggesting that sexual size dimorphism with males smaller than females might exist. Nevertheless, it has still to be corroborated statistically.

Departures from the expected Fisher 1:1 sex ratio for dioecious species are usual in species that change sex (Charnov & Bull, 1989). Theoretical models predict for protandric species a male biased sex ratio (Charnov & Bull, 1989), and in fact an excess of males is usual in protandric patellids and calyptraeids (Choquet, 1966; Branch, 1974; Charnov & Bull, 1989; Creese et al., 1990). In Coralliophilidae, departures from the Fisher 1:1 sex

ratio have been reported in *Coralliophila abbreviata* and *C. neritoidea*. In the first species, the sex ratio varies depending on the geographical locality (Wells & Lalli, 1977; Hayes, 1989), and thus does not coincide with theoretical predictions for protandric species. However, variable sex ratios are usual in protandric species with an environmentally controlled sex change (Hoagland, 1978). In *Coralliophila neritoidea*, the sex ratio is male biased (Soong & Chen, 1991). In *Coralliophila caribaea* (Wells & Lalli, 1977), *C. meyendorffii* and *Babelomurex cariniferus* (present study), by contrast, females and males are equal in proportion, and according to Massin (1988), the sex ratio of *Leptoconchus* species are close to 50:50. Such sex ratio, although normal for dioecious species, might also occur in some protandric limpets (Branch, 1981) and calyptraeids (Charnov & Bull, 1989). In the coral-boring coralliophilids *Leptoconchus vangoethemi*, *L. cyphastraeae* and *Reliquiaecava robillardi*, sex ratios are very unusual in that males are very scarce or virtually absent in samples (Massin, 1987). These abnormal sex ratios might be due to the fact that males are generally overlooked (Massin, 1988). Similar situations with males virtually absent in samples seem to be common in semisessile or sedentary protandric gastropods with a mobile male phase, like the sedentary turritellid *Vermicularia spirata* (Bieler & Hadfield, 1990) and the non-gregarious species *Crepidula dilatata* (Gallardo, 1976).

A positive correlation between fecundity and female size should be expected in protandric species, since sex change are shown to be advantageous over dioecy if fecundity of a sex increases with size or age (Warner et al., 1975). In the few coralliophilids in which the relationships between fecundity and size have been studied, a positive correlation between female fecundity and size has been demonstrated. In *Coralliophila abbreviata*, *C. caribaea* (Wells & Lalli, 1977), *C. radula* (Lalli et al., 1997) and *C. neritoidea* (Lin & Liu, 1995; Lalli et al., 1997) mean number of eggs per capsule and total eggs per spawn increase with female shell length. In *C. costularis* mean egg content per capsule increases with female size (Lalli et al., 1997).

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