Two-way sex change in the endangered limpet *Patella ferruginea* (Mollusca, Gastropoda)

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(Rceived 12 May 2012; final version received 21 November 2012)

Direct observations of sex change were made on the endangered limpet *Patella ferruginea* in Chafarinas Islands (Alboran Sea) between 2006 and 2011. Individuals of the species were sexed and tagged during spawning season for subsequent monitoring to determine possible sex changes. Mortality was minimized by following a carefully designed sexing protocol. Out of 49 tagged specimens (41–88 mm in size) that could be sexed in successive years (28 males and 21 females), 16 males changed into females between consecutive years (50.0% of males smaller than 70 mm and 100% of males larger than this size), while two females changed sex to males between consecutive spawning seasons (both were smaller than 70 mm). Overall, 36.7% of the limpets monitored changed sex between consecutive years: 57.1% of males became females and 9.5% of females changed to males. These observations confirm the occurrence of two-way sex change, or reverse sequential hermaphroditism, in *P. ferruginea*. Our findings unveil this sexual strategy in this endangered limpet and provide new direction for studies designed to address the mechanisms and factors that determine sex change and its effects on population dynamics.

**Keywords:** reproduction; hermaphroditism; Chafarinas Islands; Alboran Sea

**Introduction**

Patellid limpets are broadcast spawners that show no external sexual dimorphism. They are mainly protandrous sequential hermaphrodites, but normally the assumption of protandry is mostly based on sexual dimorphism in size–frequency distribution and/or on the occurrence of specimens bearing both male and female gonads in intermediate size classes (Branch 1981).

As for other limpets, the endangered Mediterranean species *Patella ferruginea* Gmelin, 1791 was initially described as a protandrous hermaphrodite based on the results of indirect methods (Frenkiel 1975; Laborel-Deguen and Laborel 1991; Espinosa et al. 2006). These latter authors attributed observed increasing proportions of female specimens with size to protandric hermaphroditism, while others warned that this argument was insufficient because other factors (e.g. higher rate of growth or survival of females compared to males) could be the cause of this unequal distribution (Smith 1935; Branch 1981). Hence, Guallart et al. (2006) pointed out the need to determine if size distribution by sex in *P. ferruginea* could really reflect their hermaphroditism. In effect, Espinosa et al. (2009) recently reported the direct observation of sex change (male to female) in two specimens of this species.

At an earlier date, Laborel-Deguen and Laborel (1991) had described that individuals of *P. ferruginea* reach sexual maturity as males at a size of about 24 mm, and then, change sex when they are about 40 mm long. These data were not based on their own results, but referred to prior data reported by Frenkiel (1975) in a study conducted on the coast of Algeria. Nevertheless, after careful reading of these works (Frenkiel 1975 and Laborel-Deguen and Laborel 1991), it emerges that the latter authors misinterpreted the information provided by the former, as was clarified by Guallart et al. (2006) and Espinosa et al. (2006). In fact, Guallart et al. (2006), in a study of the population of this limpet inhabiting the Chafarinas Islands, and Espinosa et al. (2006), in a similar study along the coast of Ceuta, highlighted the inaccuracy of this previously assumed information. In the populations studied by these authors, males were found even within the largest size groups, although it is true that the proportions of males compared to females tended to decrease with size. Recently, Rivera-Ingraham et al. (2011) stressed the high variability in the size or age at which sex change occurs in this species and suggested that it is driven by environmental factors, such as density or population structure.

There are two ways to verify the existence of hermaphroditism in limpets: (1) to detect specimens in a transition stage (simultaneously bearing male and female gonads) and (2) to detect individuals that change sex in successive reproductive cycles through continuous monitoring and sexing of tagged specimens. The former
option has revealed the existence of hermaphroditism in some patellid limpets (e.g. Gemmill 1896; Smith 1935; Bacci 1947; Dodd 1956; Creese et al. 1990). The second option requires a method of sexing specimens over time. Given that limpets display no external sexual dimorphism, sexing normally requires the sacrifice of the specimens to examine their gonads. However, Wright and Lindberg (1982) proposed a non-lethal technique for sexing limpets, in which the sex of the specimens was determined by taking a biopsy of the gonad with a hypodermic needle inserted into the gonad through the mantle. Using this technique combined with tagging specimens for subsequent monitoring, hermaphroditism has been confirmed in some species of patellogastropods. Thus, Wright and Lindberg (1982) demonstrated the existence of protandrous hermaphroditism in L. gigantea Sowerby, 1834 and Le Quesne and Hawkins (2006) in P. vulgata Linnaeus, 1758, the latter authors also observing a hardly known reverse change from female to male. Applying this technique in P. ferruginea on the coast of Ceuta, Espinosa et al. (2009) more recently noted that two of four tagged specimens that were initially male had changed into females on second observation.

The aim of the present study was to use direct observations to examine the occurrence of sex change by the limpet P. ferruginea inhabiting the Chafarinas Islands (southwest Mediterranean). In this enclave, this limpet shows a high density (Aparici-Seguer et al. 1995) and a regular recruitment over the past decade (Guallart et al. 2012) that has determined an optimum state of conservation of this population (Guallart et al. 2011).

Materials and methods

To examine sex change patterns in P. ferruginea, field observations were conducted between 2006 and 2011 on a population of specimens inhabiting the Chafarinas Islands (35°10′N, 2°25′W), a small archipelago south-east of the Alboran Sea, about 2 miles off the Mediterranean coast of North Africa. The non-lethal gonad biopsy technique (Wright and Lindberg 1979) was used to determine sex and some sexed specimens were tagged for monitoring in subsequent years.

Given the fairly high mortality rates previously observed for this method (Wright and Lindberg 1982; Le Quesne and Hawkins 2006; Espinosa et al. 2009), it could be considered unsuitable for endangered species. However, to minimize mortality, we designed the following field protocol for sexing individuals:

1. Specimens were collected from their rock habitat as they moved away from their “home-scar”. This occurs at high tide and especially when they are beaten by waves (Guallart and Acevedo 2006). Care should be taken to avoid damage to the foot when limpets are removed from the substratum (see Guallart et al. 2010).
2. The position and orientation of each specimen at the time of capture were recorded, such that they could be returned after biopsy to this exact position.
3. The gonad tissue biopsy was conducted when the limpet was somewhat extended (not retracted) by dipping upside down in a container with sea water or in a tidepool. As the limpet tries to recover its normal position, it will extend the foot.
4. The biopsy was performed using a 1 ml syringe fitted with a 13 mm long hypodermic needle of 0.3 mm inner diameter to puncture the gonad through the posterior mantle.
5. The handling process should be quick (specimens are returned within 15 min).
6. To tag the limpets, a small plastic label was adhered to the shell using a quick-drying cyanoacrylate adhesive.

In total, 428 limpets were sexed in the spawning season (autumn) from 2006 to 2011 to estimate percentages of immature males and females according to size range. Some captured limpets (n = 303) were sexed after their sacrifice to examine other aspects of the biology of the species, such as the reproductive cycle. Other sexed (biopsied) specimens (n = 125) were tagged and returned to the shore for further monitoring. The maximum diameter (MD) of the shell of all tagged limpets was measured at the beginning and at the end of each sexing procedure.

Results

The percentages of immature, male and female specimens according to size range are provided in Figure 1. All limpets smaller than 20 mm MD were immature and all limpets larger than 35 mm MD were mature in the spawning season. The largest immature limpet measured 32.2 mm MD, while the smallest mature specimen (male) measured 24.7 mm MD. Thus, sexual maturity in P. ferruginea in the Chafarinas islands is reached within the size range 24.7–33.2 mm MD. The size at which 50% of specimens reach sexual maturity is about 28 mm MD. Mature male limpets were observed within the range 25–97 mm MD, and females within the range 40–96 mm MD. For the whole set of mature adults, the ratio females: males was 1:1.87 (n = 419). The percentage of males decreased with increasing size: 75.2% of males in the size range 40–60 mm MD (n = 125), 47.0% in the size range 60–80 mm MD (n = 202) and 27.7% of males larger than 80 mm MD (n = 65).

The survival rate after three months of the specimens sexed and tagged using the procedure described was 90.1% (at least 67.2% after one year). Of these
specimens \((n=125)\), only 49 individuals could be resexed in the following year. Of the 76 remaining specimens failed to be resexed, part of them \((n=21)\) was because the second year was strongly attached to their home-scar during sampling; and, in accordance with established protocol, we decided not to sex them to avoid damage. In other 14 specimens, could not determine their sex because they probably had already spawned, and the other 46 tagged specimens were not found in consecutive years.

The sex change results are provided in Table 1. Of the 49 specimens that could be sexed in successive years (size range 41.0–87.9 mm MD during first year), 28 were initially male and 21 female. The following year (next reproductive cycle), 36.7% (18 out of 49) of the surveyed tagged limpets had changed sex. Thus, 57.1% of the males (12 out of 18) changed sex to female and 9.5% of the formerly females (two out of 21) changed sex to male in the interval between consecutive spawning seasons (Figure 2).

According to size (larger or smaller than 70 mm MD), 50% of the males smaller than 70 mm (12 out of 24) and 100% of males larger than this (four out of four) changed sex to female (Figure 2). Conversely, 25% of females smaller than 70 mm (two out of eight) and no females larger than this size (0 out of 13) reversed sex to male.

**Discussion**

The results of this study support the conclusion that *P. ferruginea* is a sequential protandrous hermaphrodite showing alternating sexuality (sensu Hoagland 1984), all reaching adulthood as the same sex (males), and then, having the capacity to repeatedly change sex. According to the data obtained, all individuals of this species seem to first occur as males and later at least some of them change into females under certain unknown circumstances, and may even reverse back to males. In any case, it should be taken into consideration that in some sex changing fish species, there is a proportion of individuals who mature early as the second sex (termed as “early matures” or EMs). EMs can arise either directly from the juvenile stage, having neither passed through the “first sex” (Fennessy and Sadovy 2002) or they can arise by pre-maturational sex-change (de Girolamo et al. 1999). Although these data were obtained in fish species, it must be in borne mind for invertebrate species.

The percentage of *P. ferruginea* individuals changing sex in consecutive spawning seasons was high (36.7%) for specimens larger than 40 mm, being particularly high for males (57.1%). Hence, the sex change pattern may be related, among other factors, to specimen size. On the other hand, 25% of females smaller than 70 mm (2 out of 8) and no females larger than this size (0 out of 13) reversed sex to male. These data suggest that small females show a sex-reversal. It could be related to their reproductive fitness, in the sense that small females would produce a few oocytes and they would have a greater reproductive fitness as males. This sex-reversal could be determined by the presence of large females that would induce the sex-reversal on the smallest females.

Based on the fact that over 57% of the males examined changed sex to female from one year to the next, and that 100% of these former males were larger than 70 mm, we might predict that virtually all larger limpets will eventually be female. However, our sex-by-size distributions for *P. ferruginea* do not show this pattern, and a considerable percentage of males can be found among the larger size classes (Figure 1). To explain this observation, Espinosa et al. (2009) proposed that some specimens never changed sex, following suggestions by other authors (e.g. Orton 1928, Branch 1981), but also considered the possibility of two-way sex change. In effect, the present data suggest that reverse sex changes in *P. ferruginea* may be a common, rather than a rare or occasional, phenomenon. This capacity to change sex in both directions could help regulate the sex ratio or to maximize the reproductive fitness under different environmental conditions.

Departures from the expected Fisher 1:1 sex ratio in dioecious species are common in sequential hermaphrodites (Charnov and Bull 1989). Theoretical models for protandric species predict a male-biased sex ratio and such an excess of males is frequent in protandric patellid and calyptraeid gastropods (Choquet 1971; Branch 1974; Charnov and Bull 1989; Creese et al. 1990). In *P. ferruginea*, here considered a sequential hermaphrodite with alternating sexuality (first male), the sex ratio is
also male biased, as has already been noted by other authors (e.g. Rivera-Ingraham et al. 2011).

Although most prosobranchiate gastropods are dioecious (Heller 1993), sequential hermaphroditism (mostly protandric) is common in some phylogenetic groups including Patellogastropoda, Cocculiniformia, Eulimoidea, Velutinoidea and Calyptraeoidea (see Heller 1993; Calvo and Templado 2005). The occurrence of protandry in different evolutionary lines of prosobranchiate gastropods suggests this sexual strategy, rather than determined phylogenetically, is a versatile adaptive strategy. Hence, sequential hermaphroditism has evolved independently under many different circumstances and in different lineages, and life history theory has sought to identify the conditions under which sex change is favoured over dioecy (Collin 1995). Sex allocation theory explains the way in which organisms allocate resources to male and female functions. Sex-changing animals have a greater diversity of strategies to increase their reproductive success and these different strategies all fulfil the criterion that an individual will change sex when this increases its reproductive efficiency (Munday et al. 2006). Sex change theory predicts that natural selection will favour genes coding for sex change in circumstances where male and female reproductive values are closely related to size or age (Warner, 1988). The direction of sex change (protandrous or protogynous) may be determined by the relative fitness returns over the course of a lifetime for the two sexes, not directly with the size. If male fitness increases at a slower rate than female fitness when young or small, and accelerates above that of the females later on in life when larger sizes or older ages are reached, sex change will be probably protogynous. The reverse holds for protandrous sex change. The evolution of such a sex change would occur in circumstances where male and female fitness are more closely related to size or age in different ways.

Table 1. Sexes and sex changes recorded in tagged specimens of *P. ferruginea* from 2006 to 2011. Numbers in italics indicates specimens changing sex from 1 year to the next.

<table>
<thead>
<tr>
<th>Study period</th>
<th>Initially</th>
<th>Finally</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>2006–2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>2 males</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 female</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3 specimens</td>
<td>1</td>
</tr>
<tr>
<td>2007–2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>7 males</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5 females</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>12 specimens</td>
<td>4</td>
</tr>
<tr>
<td>2008–2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>1 males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 females</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4 specimens</td>
<td>0</td>
</tr>
<tr>
<td>2009–2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>6 males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 females</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11 specimens</td>
<td>2</td>
</tr>
<tr>
<td>2010–2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>12 males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 females</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>19 specimens</td>
<td>7</td>
</tr>
<tr>
<td>2006–2011</td>
<td>Initially</td>
<td>One year later</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>28 males</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>21 females</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>49 specimens</td>
<td>14</td>
</tr>
</tbody>
</table>
allocation mechanism enables the organisms to maximize their genetic contribution to the next generation by being the best sex for the prevailing circumstances (see Warner 1984).

In contrast, according to the so-called “low-density model”, hermaphroditism is evolutionarily advantageous for species with low population densities or low motility, since under such conditions, hermaphroditism increases the likelihood of successful fertilization. Both these models predict the adaptive forces that favour hermaphroditism, but possible cues controlling the timing of sex changes in species featuring sequential hermaphroditism remain to be elucidated. In many species, sex change is determined genetically and occurs mainly after the first reproductive period (Fretter et al. 1998). Nevertheless, in other species, sex change is triggered by specific external cues. In effect, environmental control of sex change is a well-known phenomenon in protandric gastropods like eulimids (Lützen 1972; Warén 1980, 1983), calyptraeids (Hoagland 1978; Warner et al. 1996; Collin 2000), giant territorial limpets (Lindberg and Wright 1985; Wright 1989) and coralliophilids (Chen and Soong 2002; Richter and Luque 2004). In these gastropods, the presence of females or other larger conspecifics delays or inhibits sex change in males (Hoagland 1978). These gastropods are also variable in size and, depending on social factors, sex changes and their male and female size distributions overlap considerably (Hoagland 1978, Wright 1989, Warner et al. 1996, Collin 2000, Guallart et al. 2006, Rivera-Ingraham et al. 2011). Further, labile sex expression (i.e. the presence or absence of females determining sex expression in recruits) has been suggested in the coralliophilid Quoyula monodonta (Blainville, 1832) (Soong and Chen 2003). Thereby, the social control of sex change seems to be common in species that live in small aggregations or patches.

Our results and those of Le Quesne and Hawkins (2006) indicate that at least P. ferruginea and P. vulgata may be capable of two-way sex change. We propose that this capacity is likely to be more extended within the patellid limpets. At this point, we cannot predict the number of sex reversal cycles that could occur in a single individual. While common in some groups of bivalves (e.g. Ostreaeidae and Pholadidae) (Hoagland 1984), two-way sex change among gastropods has only been reported in some epitoniids (Melone 1986), in Janthina (Laursen 1953), in some eulimids (Warén 1980, 1983) and in the vermetid Serpulorbis arenarius (Linnaeus 1767) (Calvo and Templado 2005). However, this sexual condition might be more widespread than believed. Confirming alternating sexuality is difficult, requiring detailed study of the organism’s life cycle along with a large sampling effort, as conducted here. This could explain the low reported incidence of this sexual strategy.

In conclusion, the results presented here unveil a novel perspective of the reproductive biology of P. ferruginea and provide new direction for research into the mechanisms and factors driving sex change and its effects on the population dynamics of this endangered limpet.

Acknowledgements

The authors thank Patricia Cabezas for her valuable contribution to field work in the Chafarinas Islands and Ana Burton for editorial assistance. This work was funded at its late stage by the project “Action plan for viability proposals of the endangered limpet, Patella ferruginea” (Project 0 of the Spanish Research Council –CSIC- Foundation) and in former stages by grants awarded by the Organismo Autónomo de Parques Nacionales and the Dirección General de Conservación de la Naturaleza of the Spanish Ministerio de Medio Ambiente. We thank these institutions for permission to examine a species protected by Spanish law. We are also indebted to Javier Zapata and the staff of the Biological Station of the Chafarinas islands, the Spanish Ministerio de Defensa and the military personnel on the island for facilities provided during the fieldwork. Lastly, we deeply appreciate the comments and suggestions of the reviewers, which have improved the manuscript considerably.

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