



# **Mediterranean Marine Science**

Vol 18, No 3 (2017)



Where have all the youngest gone? The postlarval and young stages of the Mediterranean endangered limpet Patella ferruginea Gmelin, 1791

J. GUALLART, J. B. PEÑA, Á. A. LUQUE, J. TEMPLADO

doi: 10.12681/mms.2076

# To cite this article:

GUALLART, J., PEÑA, J. B., LUQUE, Á. A., & TEMPLADO, J. (2018). Where have all the youngest gone? The postlarval and young stages of the Mediterranean endangered limpet Patella ferruginea Gmelin, 1791. *Mediterranean Marine Science*, *18*(3), 385–392. https://doi.org/10.12681/mms.2076

# Where have all the youngest gone? The post-larval and young stages of the Mediterranean endangered limpet *Patella ferruginea* Gmelin, 1791

#### JAVIER GUALLART<sup>1</sup>, JUAN B. PEÑA<sup>2</sup>, ÁNGEL A. LUQUE<sup>3</sup> and JOSÉ TEMPLADO<sup>4</sup>

<sup>1</sup> Laboratorio de Biología Marina, Departamento de Zoología, Universitat de València, E-46100 Burjassot, Valencia
<sup>2</sup> Instituto de Acuicultura de Torre de la Sal (C.S.I.C.). C/ Ribera de Cabanes, s/n. 12595 Ribera de Cabanes, Castellón
<sup>3</sup> Laboratorio de Biología Marina, Departamento de Biología, Universidad Autónoma, C/ Darwin, 2, 28049 Madrid
<sup>4</sup> Museo Natural de Ciencias Naturales (C.S.I.C.), José Gutiérrez Abascal, 2, 28006 Madrid

Corresponding author: angel.luque@uam.es

#### Handling Editor: Marco Oliverio

Received: 11 February 2017; Accepted: 27 April 2017; Published on line: 20 October 2017

#### Abstract

Recruits of *Patella ferruginea* are hardly seen in their habitat; hence, the precise level where they settle and the morphology of the earliest growth stages remain unknown. The earliest post-larval stages of this species are described here for the first time, based on specimens obtained both through controlled reproduction and in its natural environment in Chafarinas Islands (North Africa). Young post-larval specimens of 290 µm in length settled slightly below the water level on floating Petri dishes in the aquaculture tank. Specimens of approximately 4 mm were found in the natural habitat at the level of the vermetid gastropod *Dendropoma lebeche*, whereas those larger than 8 mm were also found at this level, but also in the upper midlittoral, at the *Chthamalus* spp. fringe.

The characteristic star-like shell and colour pattern of young specimens, with alternating concentric dark and light bands, appeared at approximately 8 mm in length. Smaller specimens have a more or less oval shell and dark radial bands. This information is useful for future non-invasive studies on the recruitment of this endangered species, but further information about the post-larval stages of the other three Mediterranean patellid species is needed to distinguish specimens smaller than 3 mm.

Keywords: Patella ferruginea, Mollusca, Gastropoda, Patellidae, post-larval stages, Mediterranean, endangered species, conservation.

#### Introduction

The ferruginous limpet (*Patella ferruginea* Gmelin, 1791) is an endemic western Mediterranean species that is considered one of the most endangered marine invertebrates (Laborel-Deguen & Laborel, 1991a; Templado, 2001).

At the end of the 19th century, Patella ferruginea was distributed throughout most of the western Mediterranean coasts, suffering an important decline during the 20th century (Laborel-Deguen & Laborel, 1991a; Porcheddu & Milella, 1991; Templado et al., 2004; Espinosa et al., 2013). Currently, it is considered extinct on the European continental coasts except for some localities of southern Spain (Templado et al., 2004; Espinosa, 2009; see Espinosa et al., 2013 for an updated review of global distribution). The main populations are currently found along the North African coasts, from Tunisia to Morocco (Rivera-Ingraham et al., 2011; Guallart et al., 2013c; Guallart & Templado, 2016; Zarrouk et al., 2016). The decline of this species is attributed to human impact, such as overexploitation, habitat degradation, development of coastal infrastructures, and marine pollution (Templado, 2001; Paracuellos et al., 2003; Moreno & Arroyo, 2008).

For all these reasons this limpet has been included in Annex II of the Berna Convention, Annex II of Barcelona Convention and Annex IV of the "Habitats" Directive, and also in the Spanish Catalogue of Endangered Species as "in risk of extinction" since 1999 (BOE, 1999). A Spanish national strategy to protect this species was approved in 2008 (MMAMRM, 2008), being the first strategy to protect a marine invertebrate in this country.

*Patella ferruginea* is a large limpet that may reach more than 100 mm of maximum shell diameter (MD) and lives in the upper midlittoral zone (Templado, 2001). Like other limpets, it is a broadcast spawner and is a sequential protandrous hermaphrodite showing alternating sex reversal (Guallart *et al.*, 2013b). It is assumed that this species has a low fecundity, a short planktonic larval phase with limited dispersal ability (Laborel-Deguen & Laborel, 1991b; Guallart *et al.*, 2010), and a slow growth rate (Espinosa *et al.*, 2008; Guallart *et al.*, 2012b). However, nothing is known about the larval phase or on the recruitment processes.

Smaller specimens of *Patella ferruginea* are hardly seen in their habitat (Frenkiel, 1975; Laborel-Deguen & Laborel, 1991b). The latter authors suggested that this may be due to the fact that the first benthic stages probably refuge in the upper infralittoral zone, at the level of the *Cystoseira* belt, where they would be very difficult to find.

Shell morphology of young specimens (MD 20-30 mm) is well known and quite characteristic, with a starlike outline due to prolongation of axial ribs and a colour pattern of concentric dark and light bands. They are common in the midlittoral zone of localities with dense populations of adults (Frenkiel, 1975; Espinosa, 2009; Espinosa *et al.*, 2011; Rivera-Ingraham *et al.*, 2011; Guallart *et al.*, 2013b; Guallart & Templado, 2016). Nevertheless, several authors considered unfeasible the identification of young specimens less than 20 mm (Mari *et al.*, 1998; Giudicelli *et al.*, 1999; Meinesz *et al.*, 2001; Pascal, 2002) or 10 mm (Coppa *et al.*, 2012) on the basis of the external shell characters, and thus did not include them in censuses.

As far as we know, the smallest specimen of *Patella ferruginea* illustrated is that of 7 mm by Scaperrotta *et al.* (2011, p. 20). Laborel-Deguen & Laborel (1991a) briefly described specimens less than 5 mm shell diameter from Corsica as having an elevated conical shell, no visible radial ribs, and ten white radial bands standing out against the dark background of shell. According to these authors, radial ribs appear gradually in specimens larger than 5 mm, which have the characteristic colour pattern of concentric beige bands forming a star-like outline when crossing the ribs.

Casu *et al.* (2010) used genetic (and presumably lethal) methods to identify two juveniles of *Patella* of approximately 2.5 mm found on the shell of an adult specimen of *Patella ferruginea* since "species' diagnostic features may not be apparent" in such small specimens.

Therefore, almost nothing is known about the precise habitat and the morphology of the earliest growth stages of *P. ferruginea*. The early post-larval stages still remain undescribed despite that detailed information about recruitment success and young survival rates are essential for conservation purposes. Morphologic and non-invasive identification criteria for the young stages of this endangered species are therefore necessary.

Accordingly, our aim is to describe here for the first time the sequence of evolution of shell morphology and colour pattern of *Patella ferruginea* from early post-set-tlement stages until 20 mm MD.

## **Materials and Methods**

Data presented here come from two sources: specimens studied in its natural environment (Chafarinas Islands, North Africa) and those obtained through controlled reproduction.

Chafarinas Islands house one of the most important and healthy existing populations of this species, estimated at more than 42,300 adults (Guallart & Templado, 2016). Recruitment, although variable, has been regular in this archipelago during the last years, sometimes exceptionally successful with up to 50 recruits/meter (Guallart *et al.*, 2012a). This facilitated the observations of recruits of different sizes. As a result of thorough censuses carried out in these islands between 1999 and 2013, a total of 7,922 specimens smaller than 20 mm MD were found; among them, 74 specimens had a size of less than 5 mm MD. Nevertheless, we were unable to find specimens smaller than 3.8 mm, due to the difficulty of locating them in a heterogeneous substrate with dense biological coverage.

On the other hand, controlled reproduction under laboratory conditions at the "Instituto de Acuicultura de Torre de la Sal", Castellón (IATS-CSIC), Spain, allowed obtaining newly settled post-larval specimens in 2012 and 2013. The description of the first stages of growth up to 2.5 mm MD is based upon 42 specimens obtained from in vitro fertilisation using eggs and sperm from reproductive individuals coming from the Chafarinas Islands (Guallart et al., 2012c, 2013d). Growth monitoring in the laboratory of these specimens provided data on sizes up to 25 mm MD and larger, consistent with those observed in the natural habitat. Most observations come from specimens kept alive in aquaria but some post-larval shells were obtained dead from the filters at the bottom of the aquaculture tanks. Descriptions of the inner surface of the shell are based on specimens which died during laboratory studies; a representative series of 14 voucher shells in the range 5.6-28.7 mm have been deposited at Museo Nacional de Ciencias Naturales, Madrid (MNCN 15.05/60855).

Scanning Electron Micrographs were taken at MNCN under a low vacuum mode on a FEI QUANTA 200 SEM and at high vacuum mode on a JSM-840 SEM.

# Results

The smallest studied post-larval specimens were found on the algal biofilm covering some Petri dishes placed at the air-water interface, floating in the culture tanks, and were observed nine days after *in vitro* fertilization of eggs. In its natural habitat, specimens longer than 3.8 mm MD were found at the level of the vermetid gastropod *Dendropoma lebeche* Templado, Richter & Calvo, 2016 and the crustose coralline red algae *Neogoniolithon brassica-florida* Harvey (Setchell & Mason, 1943) (Fig. 1A), whereas those larger than 8-10 mm were mainly found in the upper midlittoral, at the *Chthamalus* spp. fringe, together or even on adult specimens (Fig. 1B, C).

The smallest settled specimen measures 267 µm in total length (Fig. 2A), showing a translucent teleoconch (212 µm in maximum diameter). The protoconch (embryonic shell or protoconch I) measured in 12 post-larvae ranged between 210 and 245  $\mu$ m in length (mean 232.7  $\pm$ 12.1  $\mu$ m). It is smooth, symmetrical, but somewhat lefttilted with regard to the longitudinal teleoconch axis (Fig. 3A, B). The protoconch can remain in specimens up to 3 mm or even more in juveniles hatched and maintained in aquaria, although probably disappear much earlier in natural conditions due to erosion. At this time, the teleoconch shows a dense sculpture of fine radial lines, and remains translucid up to nearly 400 µm in length. Postlarval specimens of 267-290 µm and juveniles around 2 mm long were repeatedly observed scraping the algal film on the surface where they were settled with the radula (Supplementary material, S1, S2).



*Fig. 1: Patella ferruginea.* Young specimens in their habitat. A, 3.8 mm limpet on the *Dendropoma lebeche / Neogoniolithon brassica-florida* level. B, four young specimens of approx. 10-18 mm (arrows) and one adult of approx. 55 mm, at the *Chthamalus* level. C, one specimen of approx. 18 mm (arrow) on an adult of nearly 75 mm, at the *Chthamalus* level.

Young specimens of 1-2 mm MD have an ovate shell and a distinct colour pattern of radial bands alternating light (whitish) with 8-12 dark (brown to blackish), most of them forking near the margin (Fig. 2B, C). Some of these forked bands appear later to be grouped in pairs in larger juveniles (Fig. 2 D, E). In 1-2 mm specimens, the initial teleoconch, at approximately 500  $\mu$ m in diameter, shows an almost uniformly dark brown colour. The inner shell shows the same radial banded pattern by transparency (Fig. 5A). This pattern remains in specimens up to 3-4 mm length, both observed in the natural environment and reared in laboratory (Fig. 2D).

Specimens between 5 and 8 mm show dark spots near the shell edge (Fig. 2E). These spots begin the development of the first dark ring of a series of concentric bands, which are characteristics of specimens larger than 10 mm MD (Fig. 2F, G). The banded radial pattern of the smaller specimens often disappears in larger specimens



*Fig. 3: Patella ferruginea.* A SEM micrograph of the shell of a recently settled post-larval specimen 0.99 mm in length. B Detail of the protoconch of the same specimen. Scale bars: A,  $400 \mu$ m; B,  $100 \mu$ m.

(25-30 mm) due to shell erosion in its natural habitat, but remains in laboratory specimens up to 40 mm MD. Figure 2H-N represents the schematic evolution of shell morphology and colour pattern from new settled juveniles 0.5 mm in length until 17 mm.

The shell inner surface in individuals more than 5 mm up to at least 20 mm is darker, predominantly reddish brown and dark blue (Fig. 4A-J). The pattern is illdefined, with blue under the radial ribs and the concentric dark bands of the external surface. Sometimes, the growth edge is more evident due to its whitish colour. The inner surface of the shell becomes gradually whitish in specimens from 20-25 mm (Fig. 4K-N).

The colour pattern of the soft parts differs between young and adult specimens. Adults (>30 mm MD) show the foot sole a yellowish cream colour tarnished with grey; dark grey or blackish foot sides, head and cephalic tentacles; and translucent pallial tentacles (Fig. 5C). Smallest specimens are paler, showing a translucent whitish foot sole, head, and cephalic tentacles at a length of 2 mm (Fig. 5A). Cephalic tentacles are the first to become darker, being grey at 10-12 mm MD (Fig. 5B); head, foot sole, and sides have a light cream, almost yellowish colour. Specimens more than 25 mm have dark grey or blackish cephalic tentacles and the head becoming greyish, while the foot sole and sides remain light cream to sizes over 30 mm. Foot sides darken to grey or blackish colour at approximately 40-50 mm.



*Fig. 4: Patella ferruginea.* External and internal views of the shells of some young specimens stored at MNCN as voucher material. A-B 5.6 mm. C-D 7.0 mm. E-F 7.9 mm. G-H 12.5 mm. I-J 15.2 mm. K-L 20.6 mm. M-N 25.0 mm. Scale bar: 10 mm.



*Fig. 5: Patella ferruginea.* Ventral views of living animals. A 2.56 mm. B 12 mm. C 30 mm.

## Discussion

According to our laboratory observations, the youngest post-larvae settled just at the air-water interface, and the subsequent early stages live slightly above this level, probably to avoid desiccation. The youngest specimens were found in the natural habitat at the Dendropoma lebeche belt (which coincides with the mean sea level), and only specimens more than 8-10 mm long climb to the upper Chthamalus spp. level (upper midlittoral), sharing this habitat with adults. Our observations on the location of the recruits partially agrees with the suggestion of Laborel-Deguen & Laborel (1991b) that the very first benthic stages live at lower levels than adults, but we consider it unlikely that they settle at the Cystoseira spp. belt (infralittoral). Instead, our observations suggest that they could settle at the lower midlittoral, on the irregular surface covered by the Dendropoma lebeche-Neogoniolithon brassica-florida belt. Indeed, Guallart & Templado (2016) found a significant association between maximum values of density of Patella *ferruginea* and the presence of a more or less continuous belt of D. lebeche-N. brassica-florida crusts with moderate vertical development in Chafarinas Islands, and suggested that a more detailed study of this association is needed.

The protoconch of P. ferruginea is illustrated and described here for the first time. Little information is available on the protoconchs of other Mediterranean-Atlantic limpet species. The protoconch of Patella caerulea Linné, 1758 is approximately 200 mm in length according to measurements made on Fig. 4 from Wanninger et al. (1999). It shows also a symmetrical but not sculptured embryonic shell (protoconch I); the right part of the juvenile teleoconch apparently grows faster than the left part, also giving a lefttilted protoconch with respect to the longitudinal axis of teleoconch (Wanninger et al., 1999). Nevertheless, the early teleoconch of P. caerulea lacks the radial sculpture of P. ferruginea. Thompson (1912) described a similar asymmetry with the posterior apex of the protoconch being to the left of the teleoconch axis in Acmaea sp. (Acmaeidae). Similarly, the teleoconch of Lottia digitalis (Rathke, 1833) and L. asmi (Middendorf, 1847) (Lottiidae) was initially symmetrical, but in slightly older post-larvae the protoconch appeared to be displaced slightly clockwise relative to the long axis of the teleoconch (Kay & Emlet, 2002).

Smith (1935) observed that post-larvae of *Patella vulgata* Linné, 1758 loss the protoconch at approximately 0.5 mm length, while in specimens studied here, the protoconch remained in specimens up to 3 mm in length, and even longer in laboratory-cultured juveniles.

Post-larvae and young specimens of *Patella ferruginea* under 5 mm MD are described and illustrated in this work for the first time. They show a remarkably different shell morphology and colour pattern when compared to larger specimens, and can be recognised from a young age. The 7 mm specimen of *Patella ferruginea* illustrated by Scaperrotta *et al.* (2011, p. 20) from Corsica shows an identical shell shape and colour pattern to those we have studied of similar sizes.

*Patella ferruginea* shares its upper midlittoral habitat with *Patella rustica* Linné, 1758. This species lives in a slightly higher level, but no studies about its youngest stages have been published. We have not studied specimens of *P. rustica* under 5 mm MD, but at this size, *P. rustica* has a more regular profile of the shell margin and a greater number (12) of more or less discontinuous radial dark bands than *P. ferruginea*, which are not grouped in pairs: thus both species can be easily differentiated. Scaperrotta *et al.* (2011, p. 21) illustrated a 5.5 mm specimen of *P. rustica* also showing 12 radial black bands. According to Lima *et al.* (2006), small (*ca.* 5 mm) specimens of *Patella rustica* show the characteristic black spots decorating the shell of this species.

Casu et al. (2010) compared the sequences of the cytochrome c oxidase subunit I (COI) of two young limpets of approximately 2.5 mm in length found attached to an adult shell of P. ferruginea from Isole Le Camere (NE Sardinia, Italy), that they considered morphologically unidentifiable (although they were not described). These authors concluded that COI may be useful as DNA barcoding to differentiate Mediterranean species of Patella, and identified one of these two specimens as P. rustica and the other as P. ferruginea. They also stated the unfeasibility of a confident identification of young individuals, "even sacrificing the specimen, a practice not to be encouraged when dealing with a species threatened with extinction". Although they used a tissue sampling protocol by excising samples of foot muscle and gill (Casu et al., 2006), there is no comment on the survival of the two young specimens after this treatment. According to our own experience (unpublished observations) it is unlikely that such small specimens could have survived that procedure, in contrast to the high survival rates observed in adults by Casu et al. (2006) and by our sampling protocol (Guallart et al., 2013a) that did not include such very small specimens. Therefore, this invasive, expensive, and slow genetic method seems to be inapplicable in practice to report the abundance of very young individuals in ecological surveys or in censuses of this species facing risk of extinction, and thus must be discarded.

Although further information is needed about specimens smaller than 5 mm of *P. rustica* (and also of the other two Mediterranean patellid limpets, *P. caerulea* and *P. ulyssyponensis* Gmelin, 1791), the present paper provides information with which to identify with confidence young specimens of *Patella ferruginea* at least 3 mm MD exclusively on the basis of the external shape and colour pattern, a non-invasive and far preferable method than genetic ones.

## Acknowledgements

This work was funded by the project "Action plan for viability proposals of the endangered limpet, *Patella ferruginea*" (Project 0 of the Spanish Research Council– CSIC- Foundation) and former grants awarded by the Organismo Autónomo Parques Nacionales and the Dirección General de Conservación de la Naturaleza of the Spanish Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA). We thank the latter institutions and also Ainhoa Pérez Puyol and the staff of División para la Protección del Mar (Dirección General de Sostenibilidad de la Costa y del Mar, MAGRAMA) for permission to study 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 islands for facilities provided during fieldwork. The authors would also like to thank Iván Acevedo, Marta Calvo, Annie Machordom, and Patricia Cabezas (MNCN), Josu Pérez-Larruscaín (Instituto de Investigación y Tecnología Agroalimentarias, IRTA), and Juanjo Villalón (Melilla) for their valuable contribution to logistics, field or laboratory work.

#### References

- BOE, 1999. Orden de 9 de junio de 1999 por la que se incluyen en el Catálogo Nacional de Especies Amenazadas determinadas especies de cetáceos, de invertebrados marinos y de flora y por la que otras especies se excluyen o cambian de categoría. *Boletín Oficial del Estado*, 148, 23921-23922.
- Casu, M., Casu, D., Lai, T., Cossu, P., Curini-Galletti, M., 2006. Inter Simple Sequence Repeat markers revealed strong genetic differentiation among populations of the endangered mollusc *Patella ferruginea* (Gastropoda: Patellidae) from two Sardinian Marine Protected Areas. *Marine Biology*, 149, 1163-1174.
- Casu, M., Sanna, D., Cristo, B., Lai, T., Dedola, G.L. et al., 2010. COI sequencing as tool for the taxonomic attribution of *Patella* spp. (Gastropoda): the case of morphologically undistinguishable juveniles settled on a *Patella ferruginea* adult. Journal of the Marine Biological Association of the United Kingdom, 90 (7), 1449-1454.
- Coppa, S., De Lucia, G.A., Massaro, G., Magni P., 2012. Density and distribution of *Patella ferruginea* in a Marine Protected Area (western Sardinia, Italy): Constraint analysis for population conservation. *Mediterranean Marine Science*, 13 (1), 108-117.
- Espinosa, F., 2009. Populational status of the endangered mollusc *Patella ferruginea* Gmelin, 1791 (Gastropoda, Patellidae) on Algerian islands (SW Mediterranean). *Animal Biodiversity and Conservation*, 32 (1), 19-28.
- Espinosa, F., González, A.R., Maestre, M.J., Fa, D., Guerra-García, J.M. *et al.*, 2008. Responses of the endangered limpet *Patella ferruginea* to reintroduction under different environmental conditions: survival, growth rates and life-history. *Italian Journal of Zoology*, 75 (4), 371-384.
- Espinosa, F., Rivera-Ingraham, G., García-Gómez, J.C., 2011. Influence of habitat structure and nature of substratum on limpet recruitment: Conservation implications for endangered species. *Estuarine, Coastal and Shelf Science*, 94, 164-171.
- Espinosa, F., Rivera-Ingraham, G., Maestre, M., González, A. R., Bazairi, H. *et al.*, 2013. Updated global distribution of

the threatened marine limpet *Patella ferruginea* (Gastropoda: Patellidae): an example of biodiversity loss in the Mediterranean. *Oryx*, 48 (2), 266-275.

- Frenkiel, L., 1975. Contribution à l'étude des cycles de reproduction des Patellidae en Algérie. *Pubblicazione della Stazione zoologica di Napoli*, 39 (suppl.), 153-189.
- Giudicelli, S., Recorbet, B., Frisoni, G.F., 1999. Inventaire de la population d'Arapèdes géantes (*Patella ferruginea*) de la base marine d'Aspretto et ses abords à Ajaccio (Corse du Sud). *Travaux Scientifiques Parc Naturel Régional de Corse et Reserves Naturelles*, 59, 91-119.
- Guallart, J., Calvo, M., Cabezas, P., 2010. Hermafroditismo en la lapa ferruginosa (*Patella ferruginea*) (Mollusca, Patellidae), especie catalogada "en peligro de extinción". p. 150. In: *Libro de Resúmenes del XVI Simposio Ibérico de Estudios de Biología Marina, Alicante, 6-10 September 2010*. Universidad de Alicante, Departamento de Ciencias del Mar y Biología Aplicada, Alicante.
- Guallart, J., Acevedo, I., Calvo, M., 2012a. Reclutamiento de la lapa amenazada *Patella ferruginea* (Mollusca, Patellidae) en las Islas Chafarinas (Mediterráneo SW). p. 15. In: *Libro* de Resúmenes, III Simposio Internacional de Ciencias del Mar, Cádiz, 24-26 January 2012. Universidad de Cádiz, Facultad de Ciencias del Mar y Ambientales, Cádiz.
- Guallart, J., Acevedo, I., Calvo, M., 2012b. Estimas de edad y crecimiento de la lapa amenazada *Patella ferruginea* (Mollusca, Patellidae) en las islas Chafarinas (Mediterráneo occidental). *Revista de Investigación Marina* (XVII Iberian Symposium on Marine Biology Studies, Donostia-San Sebastián, Spain), 19 (6), 181.
- Guallart, J., Templado, J., Calvo, M., Acevedo, I., Bonilla, E. et al., 2012c. Avances científicos en la conservación de Patella ferruginea. Lychnos, Cuadernos de la Fundación General CSIC, 5, 40-45.
- Guallart, J., Acevedo, I., Calvo, M., Machordom, A., 2013a. Protocolo no letal para la obtención de muestras de tejido (para estudios genéticos) en la lapa amenazada *Patella ferruginea* (Mollusca, Patellidae). *Iberus*, 31 (2), 171-174.
- Guallart, J., Calvo, M., Acevedo, I., Templado, J., 2013b. Twoway sex change in the endangered limpet *Patella ferruginea* (Mollusca, Gastropoda). *Invertebrate Reproduction* & *Development*, 57 (3), 247-253.
- Guallart, J., Luque, Á.A., Acevedo, I., Calvo, M., 2013c. Distribución y censo actualizado de la lapa ferrugínea (*Patella ferruginea* Gmelin, 1791) en el litoral de Melilla (Mediterráneo suroccidental). *Iberus*, 31 (1), 21-51.
- Guallart, J., Peña, J.B., Pérez-Larruscaín, J., Calvo, M., Acevedo, I., 2013d. Estrategias para la obtención mediante técnicas de acuicultura de juveniles de *Patella ferruginea* (Mollusca, Patellidae), especie "en peligro de extinción", y valoración de su uso para la conservación. p. 240–241. In: *Libro de Resúmenes XIV Congreso Nacional Acuicultura, Gijón, 23-25 September 2013.* Sociedad Española de Acuicultura, Gijón.
- Guallart, J., Templado, J., 2016. Distribución, abundancia y selección del hábitat de *Patella ferruginea* (Mollusca, Gastropoda) en las islas Chafarinas (Mediterráneo suroccidental). *Iberus*, 34 (2), 127-172.
- Kay, M.C., Emlet, R.B., 2002. Laboratory spawning, larval development, and metamorphosis of the limpets *Lottia digitalis* and *Lottia asmi* (Patellogastropoda, Lottiidae). *Invertebrate Biology*, 121 (1), 11-24.

- Laborel-Deguen, F., Laborel, J., 1991a. Statut de *Patella ferruginea* en Méditerranée. pp. 91-103. In: *Les espèces marines à protéger en Méditerranée*, Boudouresque, C.F., Avon, M., Gravez, V., (Eds.). GIS Posidonie publ., Marseille, France.
- Laborel-Deguen, F., Laborel, J., 1991b. Nouvelles observations sur la population de *Patella ferruginea* Gmel. de Corse. pp. 105-117. In: *Les espèces marines à protéger en Méditerranée*, Boudouresque, C.F., Avon, M., Gravez, V., (Eds.). GIS Posidonie publ., Marseille, France.
- Lima, F.P., Queiroz, N., Ribeiro, P.A., Hawkins, S.J., Santos, A.M., 2006. Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, 33, 812-822.
- Mari, X., Meinesz, A., De Vaugelas, J., 1998. Répartition de Lithophyllum lichenoides (Rhodophyta), de Cystoseira amentacea var. stricta (Chromophyta), de Patella ferruginea (Mollusca) et des zones polluées par les hydrocarbures de l'ile Lavezzu (réserve naturelle des Lavezzi, Corse). Travaux scientifiques du Parc naturel régional et des réserves naturelles de Corse, 57, 145-162.
- Meinesz, C., Soulet, C., Bottin, L., Cottalorda J.-M., Markovic, L. et al., 2001. Evolution des espèces médiolittorales dans la zone de protection intégrale de la réserve naturelle de Scandola (Corse). Ed. Laboratoire ECOMERS. Université de Nice Sophia-Antipolis, 71 pp.
- MMAMRM, 2008. *Estrategia para la conservación de la lapa ferruginea (*Patella ferruginea) *en España*. Ministerio de Medio Ambiente y Medio Rural y Marino, Gobierno de España, Madrid, 49 pp.
- Moreno, D., Arroyo M.C., 2008. Patella ferruginea Gmelin, 1791. p. 308-319. In: Libro Rojo de los Invertebrados de Andalucía. Barea-Azcón, J.M., Ballesteros-Duperón, E., Moreno D. (Eds). Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, Spain.
- Paracuellos, M., Nevado, J.C., Moreno, D., Giménez, A., Alesina, J.J., 2003. Conservational status and demographic characteristics of *Patella ferruginea* Gmelin, 1791 (Mollusca, Gastropoda) on the Alboran Island (Western Mediterranean). *Animal Biodiversity and Conservation*, 26 (2), 29-37.
- Pascal, P.-Y., 2002. Comparaison inter-annuelle et inter-modale des effectifs et de la structure de la population de *Patella ferruginea* de l'Ile Lavezzu (Corse-du-Sud). *Biologos*, 1, 1-5.
- Porcheddu, A., Milella, I., 1991. Aperçu sur l'écologie et sur la distribution de *Patella ferruginea* (L.) Gmelin, 1791 en mers italiennes. p. 119-128. In: *Les espèces marines à protéger en Méditerranée*. Boudouresque, C.F., Avon, M., Gravez, V. (Eds). GIS Posidonie publ., Marseille.
- Rivera-Ingraham, G.A., Espinosa, F., García-Gómez, J.C., 2011. Conservation status and updated census of *Patella ferruginea* (Gastropoda, Patellidae) in Ceuta: distribution patterns and new evidence of the effects of environmental parameters on population structure. *Animal Biodiversity and Conservation*, 34 (1), 83-99.
- Scaperrotta, M., Bartolini, S., Bogi, C., 2011. Accrescimenti. Stadi di accrescimento dei molluschi marini del Mediterraneo. Volume III. L'Informatore Piceno, Ancona. 184 pp.
- Smith, F.G.W., 1935. The development of *Patella vulgata*. *Philosophical Transactions of the Royal society of London, Series B, Biological sciences*, 225, 95-125.

- Templado, J., 2001. Patella ferruginea Gmelin, 1791. p. 41-50. In: Los Invertebrados no insectos de la "Directiva Hábitat" en España. Ramos M.A., Bragado D., Fernández J. (Eds). Ministerio de Medio Ambiente, Dirección General de Conservación de la Naturaleza, Serie Técnica, Madrid.
- Templado, J., Calvo, M., Garvía, Á., Luque, Á.A., Maldonado, M., Moro, L., 2004. *Guía de Invertebrados y peces marinos protegidos por la legislación nacional e internacional.* Naturaleza y Parques Nacionales, Serie Técnica, Ministerio de Medio Ambiente, Madrid, 214 pp.
- Thompson, W.F., 1912. The protoconch of *Acmaea*. *Proceed*ings of the Academy of Natural Sciences of Philadelphia, 64, 540-544.
- Wanninger, A., Ruthensteiner, B., Dictus, W.J.A.G., Haszprunar, G., 1999. The development of the musculature in the limpet *Patella* with implications on its role in the process

of ontogenetic torsion. *Invertebrate Reproduction and Development*, 36 (1-3), 211-215.

Zarrouk, A., Romdhanea, M.S., Espinosa, F., 2016. Usefulness of marine protected areas as tools for preserving the highly endangered limpet, *Patella ferruginea*, in the Mediterranean Sea. *Marine Biology Research*, 12 (9), 917-931.

# **Supplementary Material**

Video footages of two young stages of *Patella ferruginea*, scraping the transparent substrate where they settled.

**S1.** Post-larva of 267  $\mu$ m in total length, 15 days after fertilisation, seen in dorsal view.

**S2.** Young specimen 2.1 mm MD, approx. 5 months old, seen in ventral view.