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# Contrasting decay rates of freshwater bivalves' shells: Aquatic versus terrestrial habitats

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ABSTRACT

Freshwater flow regimes are particularly vulnerable to global climate change with changes to the volume and regime of water contributing to global declines in freshwater biodiversity. Droughts or floods can cause massive mortalities of freshwater bivalves, facilitating the accumulation of shells in the aquatic but also in adjacent terrestrial habitats. In order to fully understand the long term impact of these massive mortality events, it is important to assess how bivalve shells persist in the environment. Given that, the present study aimed at studying the shell decays of four different bivalve species (*Anodonta anatina*, *Corbicula fluminea*, *Potomida littoralis* and *Unio delphinus*) in aquatic (i.e. river) versus terrestrial (i.e. sand soil) habitats. Shell decay rates were significantly different among species and habitats. In the aquatic habitat the shell decay rates varied among species, with the native species *A. anatina*, which have the largest and thinnest shell, showing the highest decay rate. Alternatively, in the terrestrial habitat the shell decay rates were more even among species and not related to a particular shell feature or morphology, with the native *U. delphinus* showing the fastest decay. The shell decay rates were 6 to 12 times higher in aquatic than in the terrestrial habitat. These results suggest that bivalve shells can persist for long periods of time on both habitats (but mainly in terrestrial), which may perhaps trigger significant changes on the ecosystem structure and functioning.



## Introduction

Rivers by being highly fragmented in a predominantly terrestrial landscape facilitate the exchange of resources between the terrestrial and aquatic habitats; these subsidies can have important implications in terms of productivity and ecosystems processes for both habitats (Polis et al., 1997). In recent decades many studies called the attention for the movement of nutrients, detritus, prey and consumers between the land-water interfaces (Norlin, 1967; Hunt, 1975; Siegfried, 1981; Wetzel, 1990; Polis and Hurd, 1996; Polis et al., 1997). Nevertheless, a large proportion of studies focus on subsidies from terrestrial to aquatic ecosystems, primarily the importance of leaf litter resources and their use, maintenance or recycling on food-webs (Gessner et al., 1999; Lecerf et al., 2007). Subsidies in the opposite direction (aquatic to terrestrial) are not as well studied (neither receiving the same amount of attention when compared to leaf litter); most of the studies addressing this topic focused in the fundamental importance of emergent insects from the aquatic to adjacent terrestrial ecosystems, emphasizing their ecological significance for the riparian communities (Kato et al., 2003; Nakano and Murakami, 2001; Baxter et al., 2005).

One mechanism which can facilitate subsidies from aquatic to terrestrial are major floods or droughts, which can contribute to massive mortalities of freshwater bivalves (Hastie et al., 2001; Sousa et al., 2012; Bódis et al., 2014a), facilitating the accumulation of dead animals both in the aquatic and in the adjacent terrestrial ecosystems. Some studies have already showed that the biomass resulting from these die-offs may be massive (reaching dozens of kg per $m^2$; Sousa et al., 2012; Bódis et al., 2014a), with part of this carrion being consumed by higher trophic levels and the other part entering the detritus food-web (Ilarri et al., 2011; Sousa et al., 2012, 2014). Since extreme climatic events, such as floods, droughts and heat waves are expected to occur more often in the near future in response to temperature and precipitation regime shifts (Daufresne et al., 2003; Mouthon and Daufresne, 2006; Poff and Zimmerman, 2010) the frequency of these bivalve die-offs may also increase in

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the future (Sousa et al., 2012). However, to date very few studies assessed the longer term impacts of these mortality events. Unlike soft tissue, which quickly decompose down, shells may persist for longer periods of time (Gutiérrez et al., 2003; Mincy, 2012). The input of shells can lead to profound consequences at different ecological levels, from individuals to ecosystems (e.g. Gutiérrez et al., 2003; Strayer and Malcom, 2007), given that the physical structure of shells can provide habitat to a myriad of organisms (Werner and Rothhaupt, 2008) and play an important role in the carbon cycling (Gutiérrez et al., 2003; Sousa et al., 2009).

The newly emerging conditions resulting from these extreme climatic events represent a new challenge in ecology. Until now, just a few studies attempted to investigate the influence of extreme climatic events on bivalve species (but see Sousa et al., 2008; Ilarri et al., 2011; Sousa et al., 2012; Bódis et al., 2014a). Furthermore, these studies only quantified the density and biomass resulting from these mortality events, not assessing the possible fate of shells provided by these species on the aquatic and adjacent terrestrial habitats, which may be of particular importance considering that bivalve shells can persist for several years (Palacios et al., 2000; Strayer and Malcom, 2007). In this context, it is important to assess shell persistence in different habitats (aquatic vs. terrestrial). For this, the present study aimed at: (a) examining if different bivalve species (*Anodonta anatina*, *Corbicula fluminea*, *Potomida littoralis* and *Unio delphinus*) have different shell decay rates; and (b) comparing the shell decay rates of the selected bivalve species between the aquatic and terrestrial habitats. Due to differences in shell structure, size and robustness we hypothesized interspecific differences both in aquatic and terrestrial habitats with species with hard and larger shells being more resistant to decay. In the same vein and due to completely different abiotic conditions between the aquatic and terrestrial habitats we hypothesized much larger decay rates in aquatic habitats mainly due to influence of the current velocity.

## Material and methods

### Study area

The experiment was conducted in the River Minho (NW of the Iberian Peninsula). This river originates in the Serra de Meira, (Spain), with most of its hydrological basin (95%) located in Spain and approximately 5% in Portugal. It has a length of 310 km and a maximum width of two km near the mouth, flowing NNE-SSW into the Atlantic Ocean. The study was performed near the city of Monção (Portugal), nearly 40 km from the river mouth. The study area selected for the assessment of the decaying rates in aquatic habitats was located near the margins (42°04′36.81″N, 8°31′00.25″W). This area has a high density of the Asian clam *C. fluminea*, with more than 2000 ind m$^{-2}$, being also colonized by the native species *A. anatina*, *P. littoralis*, *U. delphinus* and several species from the *Pisidium* genus. These native species have very low densities (less than 1 ind m$^{-2}$) (Sousa et al., 2005). In this area the current velocity varied from moderate (summer and fall conditions) to strong (winter and spring conditions) throughout the year, with variations also occurring in a daily basis due to operations of a dam located 30 km upstream. Furthermore, it is a very shallow area (no more than 1 m depth during summer) with permanent freshwater conditions and colonized by sparse submerged vegetation (Fig. 1a). The study area selected to assess decay rates in terrestrial habitats was located 250 m inland from the River Minho margin (42°04′28.12″N, 8°31′29.14″W) and 1 km downstream from the area used to evaluate the shell decay underwater. This area is characterized by sandy sediments and by the dominance of extensive forest areas formed mainly by acacia, pine, eucalyptus and oak trees (authors personal observation). This area was chosen because during the great flood of 2001 large quantities of bivalves were washed away from the main river channel and were deposited in this site. After more than 12 years shells of the selected species still persist in the area (Fig. 1b).

### Field observations

The densities of empty shells in the aquatic habitat were assessed through a Van Veen grab and in the terrestrial habitat through a quadrat; both sampling devices had an area of 0.05 m$^2$. Six replicates were taken for each habitat.

### Experimental design and laboratory procedures

To study the bivalve's shell decays in aquatic and terrestrial habitats, empty shells that were intact (i.e. of recently dead organisms) of four species were used, namely *A. anatina*, *C. fluminea*, *P. littoralis* and *U. delphinus*. Shells not connected at the hinge of the four bivalve species were manually cleaned, in order to remove any traces of soft tissue, dried for 48 h at 50 °C, weighed, and measured to the nearest 0.1 mm before placing in individual, sealed nylon net bags with 10 mm of mesh size. Each bag had only a single shell. To test the shell decays in the aquatic habitat, a total of 64 net bags (16 per species) were tied with a string in a stake and placed underwater (ca. 70 cm below the lowest water level) about ten meters away from the river margin. The same procedure was used for the terrestrial habitat; however, in this case the stake was placed on land being shells deposited in the sediment surface. Shells remained for 12 months (from July 2012 to July 2013) in both aquatic and terrestrial conditions, in order to experience the environmental pressure of an entire year cycle. At the end of the experiment, the shells were dried (following the same procedure described previously), measured and weighted. The methodology used in the present study did not distinguish the decay rates of the mineral versus organic contents separately.

### Data analysis

The instantaneous rate of shell loss ($k$, year$^{-1}$) was calculated following the method developed by Strayer and Malcom (2007) as:

$$k = \left(\frac{1}{t}\right)\left[\ln\left(\frac{\text{mass final}}{\text{mass initial}}\right)\right]$$

where $t$ is the length of time (in years) that the shells were in water or land. The instantaneous rate of shell loss was selected to facilitate comparison of results to other studies.

Permutational Multivariate Analysis of Variance (PERMANOVA) was carried out to compare the shell decay rates of bivalve species in two different habitats (aquatic and terrestrial). This method analyzes the variance of multivariate data explained by a set of explanatory factors on the basis of any chosen measure of distance or dissimilarity, thereby allowing for a wide range of empirical data distributions (Anderson, 2001). The shell decay and the percentage of shell decay per year, and the instantaneous rate of shell loss were statistically tested using a two-way PERMANOVA (type-III), with treatment (four levels: *Anodonta*, *Potomida*, *Unio*, *Corbicula*) and habitat (two levels: aquatic and terrestrial) as fixed factors. Prior to the two-way PERMANOVA analyses, all variables were always normalised without data transformation and a resemble matrix based on the Euclidean distances was calculated.

In all PERMANOVA tests, the statistical significance of variance ($\alpha$ = 0.05) was tested using 9999 permutations of residuals within a reduced model. When the number of unique permutations was lower than 150, the Monte Carlo $p$-value was considered. Pairwise comparisons were also performed for all PERMANOVA tests.