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Response of top shell assemblages to cyclogenesis disturbances. A case study in the Bay of Biscay

M. Muñoz-Colmenero ^a, G.-J. Jeunen ^a, Y.J. Borrell ^a, J.L. Martinez ^b, P. Turrero ^c, E. Garcia-Vazquez ^{a, *}

^a Departamento de Biología Funcional, Universidad de Oviedo, Spain

^b Servicios Científico-Técnicos, Universidad de Oviedo, Spain

^c Universidad Nacional de Educación a Distancia, Campus de Gijón, Spain

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ABSTRACT

Cyclones and other climate disturbances profoundly affect coastal ecosystems, promoting changes in the benthic communities that require time, sometimes even years, for a complete recovery. In this study we have analysed the morphological and genetic changes occurred in top shell (*Gibbula umbilicalis* and *Phorcus lineatus*) assemblages from the Bay of Biscay following explosive cyclogenesis events in 2014. Comparison with previous samples at short (three years before the cyclogenesis) and long (Upper Pleistocene) temporal scales served to better evaluate the extent of change induced by these disturbances in a more global dimension. A significant increase in mean size after the cyclogenesis was found for the two species, suggesting selective sweeping of small individuals weakly adhered to substrata. Loss of haplotype variants at the cytochrome oxidase subunit I gene suggests a population bottleneck, although it was not intense enough to produce significant changes in haplotype frequencies. The high populations to recover from disturbances. At a wider temporal scale, cyclogenesis effects seemed to compensate the apparent decreasing trends in size for *P. lineatus* occurred after the Pleistocene –Holocene transition. Considering disturbance regimes for population baselines is recommended when the long-term effects of climate and anthropogenic pressures are evaluated.

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1. Introduction

Marine communities are heavily impacted by different environmental factors, both anthropogenic and natural. Among the latter, "natural disasters" such as tropical storms or hurricanes, despite their relatively infrequent occurrence, can affect the benthic marine biota, from continental shelves to deep and abyssal bottoms (Harris, 2014). Natural disturbances are an important ecological process for benthic ecosystems and might be a key factor controlling the spatial distribution of many species in the marine environment, with mud and sand bottom communities recovering from disturbances faster than gravel and reef benthos (Harris, 2012, 2014).

Although big storms are known to cause profound modifications

E-mail address: egv@uniovi.es (E. Garcia-Vazquez).

in nearshore communities (e.g. Morton, 1988), their effect on local benthic communities is not well known yet, at least in the Bay of Biscay, mainly due to a lack of spatio-temporal baselines for model taxa and species assemblages; this hampers impact assessments in the zone (e.g. Juanes et al., 2007; Puente et al., 2009). In the last years cyclogenesis and storm events are increasing in the region – some are derived from subtropical processes (Liberato et al., 2013) and may be due to global climate change that promotes instability in temperate areas (e.g. Lozano et al., 2004). Storminess is expected to increase the vulnerability of coastal zones in these regions, with more intense erosion and subsequent changes in biodiversity (Lozano et al., 2004).

In this study we focused on the effect of the cyclogenesis that affected the Bay of Biscay in the 2013–2014 winter (cyclone Dirk; see for example http://alert.air-worldwide.com/EventSummary. aspx?e=727&tp=31&c=1, last accessed June 2015), taking the top shells *Gibbula umbilicalis* (da Costa 1778) and *Phorcus lineatus* (da Costa 1778; previously *Osilinus lineatus*) as model species. We have





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^{*} Corresponding author. Departamento de Biología Funcional, C/ Julián Clavería s/ n, 33006-Oviedo, Spain.

chosen top shells because we have a baseline of previous genetic and morphological population data (e.g. Turrero et al., 2014), and because top shells are model species useful for assessing the impact of climate (e.g. Mieszkowska et al., 2006; Hawkins et al., 2009). Rapid alterations in the distributional limits of P. lineatus and G. umbilicalis have been described following climate change in the mid-1980s (Mieszkowska, 2009), and top shells are vulnerable to wave exposure since their sizes change along with this and other factors (e.g. Crothers, 2001; Preston and Roberts, 2007). Following this, we expected them to be sensitive to the effects of the cyclogenesis, which modify different parameters of the environment such as turbidity, nutrients dissolved in the water, intensity of wave action on the coast, etc. (Harris, 2014). Selection against vulnerable size classes, too big or too small for proper anchoring to the substrate under the influence of strong windstorms, would be expected in more exposed beaches, affecting population size distributions and even population genetic diversity if the storms caused very intense mortality. Although the removal of individuals is not necessarily the only effect of these storms, due to the strength of these events in recent years we considered it as one of the factors with most modifying power over these populations. The sequence of events would be: A storm comes to shore. More vulnerable (small and exposed) individuals are swept out. If many individuals are lost from an exposed population, a bottleneck and subsequent genetic drift is expected. These effects are expected to be less intense in less exposed beaches, and therefore differences between exposed (open) and sheltered beaches are expected for both size and population genetic variation (populations from more exposed beaches are expected to be less variable).

2. Materials and methods

2.1. Studied area and post-cyclogenesis sampling

The taxonomic nomenclature we use follows that currently accepted in the World Register of Marine Species (WoRMS, Boxshall et al., 2014). The top shell species *G. umbilicalis* (da Costa 1778) and *P. lineatus* (da Costa 1778) were chosen as models due to the existence of previous observations and genetic data from the region.

The study area was the central coastal part of the Spanish Bay of Biscay, in the region of Asturias (43°40'00"N/42°53'50" N to $-7^{\circ}12'00''W/4^{\circ}32'20''W$; see Fig. 1). Rocky beaches were visited in late March just after the end of the cyclogenesis that occurred during the previous winter (cyclone Dirk in December 2013 and later episodes; see for example http://poleshift.ning.com/profiles/ blogs/north-atlantic-wave-bombs, last accessed June 2015). The type of geological substrate, mainly calcareous, is very similar in all the considered beaches. None of tem is significantly affected by contamination. At least partially sheltered rocky shores were preferred for sampling to minimize wave exposure, although even sheltered beaches were impacted during the cyclogenesis event. This was apparent when sampling the beaches of Andrín and Vidiago (Fig. 1), where no gastropods could be found except for a few G. umbilicalis specimens. Sampling of the two considered species was conducted from three affected (more open) beaches (Otur, Verdicio and Toró), where sand had been removed and more rocks than usual were exposed, and three less affected (more sheltered) beaches, where sand was still in place and rock exposure had not changed noticeably (Perlora, La Griega and Póo).

At each location, samples were obtained at random (i.e. without selection for size) from the intertidal transect, covering an area of approximately 2000 m². For reasons of comparable habitat and sampling effort, naked rocks with <10% algae coverage were targeted for sampling. Sampling effort was roughly 10% (one individual collected per ten individuals observed). The samples were

visually identified based on morphological traits (see for example Crothers, 2003) and stored in 96% ethanol until genetic analyses were performed. The ethanol was changed twice on consecutive days to improve tissue preservation.

2.2. Prehistoric and contemporary (pre-cyclogenesis) baselines

Details of the baselines can be found in Turrero et al. (2014), and can be summarised as follows: remains of the species *P. lineatus* (formerly named *Osilinus lineatus*) were found in 6 archaeological sites (Balmori, Coberizas, Cuetu la Mina, La Lloseta, La Riera and Tito Bustillo) in the archives of the Archaeological Museum of Asturias, and the maximum widths of 1106 archaeological top shells were measured. These were found in strata from three different cultural/ technological phases: Solutrean, from ~20000 years ago (20 ka) to ~17 ka; Magdalenian, from ~17 ka to ~11.5 ka; and Epipalaeolithic, from ~11.5 ka to 6 ka (roughly coincident with the beginning of the Holocene epoch).

Contemporary shellfish were randomly sampled during winter (December to January) from 2009 to 2011, with average temperatures between 9 and 10 °C (comparable to the 10.2 °C average for March 2014 in the region; see A.E.MET, 2015 for weather data), from the accessible intertidal level of rocky coast close to the archaeological sites. This area corresponds to the eastern part of the sampling area of the present study, between La Griega and Toró. A total of 135 top shells were collected from six areas, visually identified and stored in 96% ethanol for further genetic identification employing the Barcoding COI gene (Donald et al., 2012).

2.3. Morphometric analysis

The maximum widths of the shells were measured using a Vernier Caliper (± 0.1 mm). For graphical representation, shell measurements were grouped into mean-centred shell width categories every 2.5 mm.

2.4. Genetic analysis

DNA extraction was carried out following Estoup et al.'s (1996) resin-based Chelex protocol. The region of the mitochondrial gene cytochrome oxidase I (COI) was PCR-amplified with Applied Biosystem's Veriti Thermal Cycler from specimen DNA according to the procedure revised by Geller et al. (2013). The primers jgLC01490 (TITCIACIAAYCAYAARGAYATTGG) and jgHC02198 (TAIACYTCIGGRTGICCRAARAAYCA) were used. PCR was prepared with 4 μ L 5× PCR buffer, 0.2 μ L Taq polymerase (Promega), 2 mM MgCl₂, 0.5 μ M of each primer, 2 μ L of 2.5 mM dNTP and 2 μ L of genomic DNA in 20 μ L reactions. PCR conditions were an initial denaturing step at 95 °C for 5 min; then 35 cycles of 1 min at 95 °C, 1 min at 46 °C, 1.30 min at 72 °C; and a final 7 min at 72 °C. PCR products were examined on a 2% agarose gel stained with ethidium bromide.

The illustraTM ExoStarTM 1-Step GE Healthcare Life Sciences protocol was applied to the PCR products, that were sequenced employing the BigDye Terminator Cycle Sequencing Kit v3.1 and analysed on a 3130 xl Genetic Analyzer (Applied Biosystems) Automated Sequencer at the Unit of Genetic Analysis of the University of Oviedo.

Sequence chromatograms were edited using Seqman Lasergene v7.0.2. (DNASTAR).

DNA sequences were treated using different programs. FASTA files were compiled per species after morphological classification was confirmed by comparison of the DNA dataset with reference sequences using the nBLAST program within NCBI (http://blast.st-va.ncbi.nlm.nih.gov/). Alignment of COI sequences per species

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