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Volcanic ash in the water column: Physiological impact on the suspension-feeding bivalve *Mytilus chilensis*

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ABSTRACT

Ashes settling into the sea from volcanic explosions expose suspension-feeding species to reduced seston quality. Adults and juveniles of the mussel *Mytilus chilensis* were exposed for 15 days to the phytoplankton *Isochrysis galbana* together with various concentrations of ashes. We then quantified impact on survival and physiology. Although no individuals died during the experiment, by the end of the study clearance rates and oxygen consumption rates had decreased substantially, and tissue weight of mussels exposed to the highest ash concentrations declined substantially. Gills showed no physical damage, but did show abundant mucus secretion in response to ash particles. Moreover, as the relative proportions of microalgae to ash in the diet decreased, individuals showed increasing preferential ingestion of microalgal particles. Increased ash content in the diet altered physiological rates and activated distinct particle selection with a high production of pseudofeces and high energy costs, with potential long-term consequences.

1. Introduction

Periodic volcanic eruptions are natural phenomena that can produce global catastrophic effects (Self, 2006), due to the emission of large quantities of gas and ash particles into the atmosphere. Depending on the magnitude of the eruption, the volcanic ashes can remain for several weeks in the troposphere and for months in the stratosphere (Klűser et al., 2013), with high dispersion around the world (Durant et al., 2012; Vernier et al., 2013). The physical and chemical properties of the elements expelled in these events, can lead to negative effects on the physiology of plants (Hirano et al., 1995), arthropods (Buteler et al., 2011; Wolinsky et al., 2013), and fishes (Newcomb and Flagg, 1983), as well as on human respiratory health (Horwell and Baxter, 2006).

When ashes rain down along the coasts, they can cause displacement and massive mortality of benthic organisms (Jewett et al., 2010). Suspended in the water column, ashes become a particularly serious problem for many organisms, through abrasion (e.g. physical damage) or by altering various physiological processes, such as respiration (Newcomb and Flagg, 1983) or feeding (Shirakawa et al., 1984). With juvenile salmon, for example, ashes adhere to the gills, damaging the gill epithelium and making it difficult for the animals to obtain oxygen (Newcomb and Flagg, 1983). For freshwater zooplankton, ingestion of ashes has been shown to damage the digestive tract, again through abrasion (Wolinsky et al., 2013). To our knowledge, similar studies have not been previously reported for marine suspension-feeding invertebrates, even though the volcanic ash can obviously enter the water column and become part of the available particulate material in suspension (seston). Suspension-feeding organisms– especially bivalve molluscs—are therefore likely to be strongly affected, since they are forced to interact directly with the abrasive ash particles as they circulate seawater in their mantle cavity during the process of feeding and/or respiration. In these animals, changes in the concentration and quality of seston can strongly modify physiological processes, including clearance rates, which are typically reduced at high seston concentrations (Newell et al., 2001). Moreover, high particle concentrations–particularly of inorganic particles (Bayne et al., 1993)–can cause particles to be rejected as pseudofeces (Navarro and Velasco, 2002), a phenomenon with clear energetic costs: in addition to the normal costs of suspension-feeding there are now added costs generated by the selection and removal of particles in the form of mucous aggregations (Beninger and St-Jean, 1997a, 1997b; Beninger et al., 1999; Beninger and Dufour, 1996; Kiørboe et al., 1980).

Alterations in diet particle composition may also affect rates of oxygen consumption (Hutchinson and Hawkins, 1992; Bayne and

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Thompson, 1970), which, together with changes in other physiological responses, may affect both growth (Bayne and Widdows, 1978; Bayne and Newell, 1983) and survival (Irlandi et al., 1997; Akberali and Trueman, 1985). Pre-ingestion particle selection has been previously demonstrated in some suspension-feeding molluscs (Beninger et al., 2007; Levinton et al., 2002; Bougrier et al., 1997; Chaparro et al., 2013), although which particles are ingested and which are rejected as pseudofeces is in many cases still not clearly established (Rosa et al., 2013). However, a high concentration of inorganic particles can activate pseudofeces production, thereby improving the quality of the particles that are actually ingested in comparison to what is available (Jørgensen, 1996). In this way, suspension-feeding organisms can regulate ingestion rates and improve the nutritional quality of ingested material relative to what is available (Navarro and Widdows, 1997; Ward and Shumway, 2004). Thus, faced with the persistent presence of suspended volcanic particles, suspension-feeders could possibly distinguish between nutritive particles and volcanic ash particles and expend extra energy in eliminating the latter as pseudofeces.

The Southern-Austral part of Chile is widely exploited for obtaining mussel spat (*Mytilus chilensis*) and doing massive culture of this sessile, suspension-feeding mussel (231,659 tons of these mussels were harvested in 2014; SERNAPESCA, 2015). In addition to the economic importance of this species, it also plays an important ecological role in structuring macrofaunal communities (Acuña et al., 2012). The recent volcanic eruptions in the area (especially that of the recent Calbuco volcano eruption, April 2015, Delgado et al., 2017, Vidal et al., 2017) have led to serious questions about the impact of ash particles on the survival and physiological functioning of *M. chilensis*. Suspension-feeding mytilids direct incoming particles towards the pallial cavity, where they are retained by the gill filaments and then moved to the ventral food grooves where they become embedded in a mucous cord. Particles are then displaced along the food groove by cilia, and moved towards the anterior region of the animal (Garrido et al., 2012; Navarro et al., 2011). Once they reach the labial palps, the mucous cords are disaggregated and particles are then either directed towards the mouth for ingestion or rejected as pseudofeces (Beninger et al., 1999; Beninger and St-Jean, 1997a). In this process, the labial palps play a major role in determining which particles get ingested and which get rejected (Garrido et al., 2012). Thus it is possible that by actively participating in collecting abrasive volcanic material from the seston, the gills could suffer some level of physical damage, which may in turn explain any variations seen among individuals in clearance rates, changes in physical condition (e.g. tissue content), or survival.

In the present research we studied the impact of diets varying in volcanic ash content on adult and juvenile survival of *Mytilus chilensis* over a 2 week period of exposure. We also monitored individual variation in oxygen consumption and clearance rates along with changes in individual biomass for animals fed the different diets. We also quantified the capacity of these mussels to select between microalgae and volcanic ash particles for ingestion or rejection, to assess their ability to thereby improve the quality of the ingested food in comparison to what is available in the water column. Electron microscope studies also allowed us to identify possible physical damage to the gills, caused by the abrasiveness of the volcanic material retained during the clearance process.

2. Material and methods

2.1. Obtaining the experimental material

Adults and juveniles of *Mytilus chilensis* were obtained by divers in the Caleta Amargo sector, Los Ríos Region, southern Chile (39° 52′ 30″ S, 73° 25′ 34″ W) during the southern hemisphere summer of 2016, and taken to the laboratory, where they were kept for a maximum period of 1 week before being used in the experiments. The specimens were maintained in a 200 L aquarium with circulating seawater, taken from the natural environment.

Volcanic ash obtained from the Calfuco volcano explosion of April 2015 was collected by hand in July 2015 near Puente Chico, in the Lakes Region of southern Chile (41° 27′ S, 72° 49′ W) and taken to the laboratory. The ashes were forced through an 80 μm sieve, and particles retained by the sieve were then discarded. The smaller particles that passed through the sieve were then subdivided into different size classes. For this, we suspended the particles in distilled water and by using different times of decantation the particles were subdivided into a number of size classes. The size fractions thus obtained were then dried for 48 h in an oven at 60 °C. In this way we obtained a fraction with ash particles 3–20 μm in diameter; this fraction was used in our experiments (see supplementary material 1).

2.2. Survival and physiology of M. chilensis exposed to diets containing volcanic ash

2.2.1. Survival of M. chilensis

An experiment was conducted to identify potentially lethal impacts of volcanic ash particles on *M. chilensis*. Adults (n = 180 individuals, 5.2 ± 0.3 cm shell length) and juveniles (n = 180 individuals, 2.1 ± 0.3 cm shell length) were both tested. Both received as food a mixture of phytoplankton (*Isochrysis galbana*), at a standard concentration of 50,000 cells mL^{-1} (equivalent to 1.5 mg dry weight; Navarro and Chaparro, 2002) and one of several different concentrations of volcanic ash: 0 mg ashes L^{-1} (control), 30 mg ashes L^{-1}, 60 mg L^{-1}, 90 mg L^{-1}, 120 mg L^{-1} and 150 mg L^{-1}. The maximum ash concentration tested in this study corresponded to the mass that was found in a freshwater lake (Pire Lake) close to the Puyehue-Cordón Caulle volcano following the 2012 eruption (Balseiro et al., 2014). For our experiments we used 18 forty-liter aquaria for adults and 18 twenty-liter aquaria for juveniles, filled with seawater taken directly from the natural environment (14 ± 1 °C and 32 ± 1 PSU) and filtered as described below. The seawater was forced through filters of 20 μm, 5 μm and 1 μm and then sterilized with UV light before being added to the aquaria. The seawater was changed in all aquaria each morning. Food was added once each morning and again in the afternoon; any settled feces and detritus was removed during the next morning before the new food was added.

Control adults and juveniles were distributed among 3 aquaria and fed only a unialgal diet (*I. galbana*) for the full 15 day period. For all treatments, particles were kept in suspension within the aquaria by using a submersible pump and constant aeration.

2.2.2. Effects on oxygen consumption rates (OCR) and clearance rates (CR)

OCR and CR were determined at the end of the 15-day survival study, to document physiological changes caused by exposure to volcanic ash.

2.2.2.1. Oxygen consumption rate (OCR). At the end of the 15-day survival assessment study, OCR was determined for 12 adults and 12 juveniles subsampled from each treatment (72 adults and 72 juveniles examined in total). Specimens were individually installed in hermetic glass chambers filled with filtered seawater (14 ± 1 °C and PSU 32 ± 1) that had previously been saturated with oxygen by bubbling. The volume of seawater in each chamber varied between 150 mL and 1000 mL, depending on the size of the individuals being tested. The aerated seawater was allowed to sit undisturbed for 15 min before animals were added, to facilitate the escape of any remaining air bubbles. The animals were then individually placed inside the respiration chambers while the chambers were submerged under water, to prevent the entry of air; the chambers were then sealed while still underwater, for the same reason. Oxygen concentrations were determined non-invasively every h for up to 3 h, using a PSt3 optimal oxygen sensor (PreSens Precision Sensing GmbH). Oxygen