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Assessing potential impacts of deep-sea mining on dispersal and population connectivity of the vent mussel *Bathymodiolus azoricus*

Mónica Alexandra Andrade Silva

This dissertation is submitted for the degree of

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"You never know how strong you are
until being strong is the only choice you have . . . "

Abstract

Hydrothermal vents are unique environments of extreme physical–chemical characteristics and biological species composition. *Bathymodiolus azoricus* is a deep-sea Mytilid bivalve that dominates hydrothermal vent sites along the Mid-Atlantic Ridge (MAR). Very little is known about the genetic structure and connectivity of this species. Thus, the aim of this study was to identify the spatial scales and patterns over which populations are connected, in order to predict how this and other vent species are likely to be affected by SMS mining in the area.

A population genomics analysis of samples collected from three MAR vent sites (Menez Gwen, Lucky Strike, Rainbow) using RAD sequencing and Single Nucleotide Polymorphism (SNP)genotyping highlighted two important aspects: (1) When all SNPs were used no genetic structure was revealed, suggesting that most of the markers do not contribute to any structure among population samples or are simply not informative; (2) when analyzing only the most discriminant sets of SNP markers, a clear pattern of genetic structure was uncovered, corresponding to expectations of the spatial distribution of samples.

This study makes a novel contribution to the understanding of genetic structure at hydrothermal vent populations on the MAR, providing, for the first time, evidence of subtle genetic structure of hydrothermal vent fauna along the northern MAR. This observation is not incompatible with pronounced genetic connectivity, and some care must be taken when drawing conclusions. Possible explanations for this pattern include limited larval exchange between vent fields, but also local adaptation. Both scenarios will impact connectivity and thus have consequences for post-mining recolonization. Recolonization of impacted sites by larvae dispersing from remote sources may be possible. Most of these larvae would, however, not be locally adapted, which might constrain the response to selection, population expansion, and ultimately recolonization rates.

More studies are needed with as many representative taxa as possible in order to obtain a wider perspective of population connectivity on hydrothermal vents. Moreover,

there is a considerable gap in our knowledge of the mechanisms influencing connectivity patterns, which makes it difficult to draw conclusions. Finally, population genetic studies should be coupled with models of larval dispersal in order to identify potential barriers to dispersal.

Resumo

Os campos hidrotermais são caracterizados por serem ambientes únicos, por terem características físico-químicas extremas, assim como composições únicas de espécies biológicas. *Bathymodiolus azoricus* é um bivalve de mar profundo predominante dos campos hidrotermais ao longo da Crista Médio-Atlântica (CMA). Ainda muito pouco é conhecido sobre a estrutura genética tanto como a existência de conectividade desta espécie. Desse modo, o objetivo deste estudo foi identificar escalas e padrões espaciais sobre conectividade entre populações, a fim de prever a suscetibilidade de esta e de outras espécies, uma vez que a área em estudo poderá ser afetada pela mineração.

Uma análise à genómica populacional das amostras, que foram recolhidas em três diferentes locais (Menez Gwen, Lucky Strike, Rainbow) e foram sujeitas a genotipagem "RAD sequencing" e Polimorfismo de um Único Nucleotídeo (SNPs), destacaram dois aspetos importantes: (1) Quando todos os SNPs foram analisados, nenhuma estrutura genética foi revelada, sugerindo assim que a maioria dos marcadores não contribuem para qualquer estrutura entre amostras populacionais ou simplesmente não são significativas; (2) Quando analisados apenas os conjuntos discriminantes dos marcadores de SNPs, um padrão de estrutura genética foi claramente revelado, correspondendo assim às expectativas de distribuição espacial das amostras.

Este estudo proporciona uma contribuição inovadora para a compreensão da estrutura genética nas populações hidrotermais do CMA, fornecendo assim, pela primeira vez, evidências de uma estrutura genética relativa à fauna existente nos campos hidrotermais na zona norte da CMA. Estas observações não são incompatíveis com a conectividade genética pronunciada, e alguns cuidados devem ser tomados em conta quando se sugerem conclusões. Uma possível explicação para este padrão inclui troca larvar insuficiente entre campos hidrotermais, mas também uma adaptação local. Ambos os casos irão, sem dúvida, afetar a conectividade, e assim, ter consequências na recolonização após a mineração. A recolonização em locais de possível impacto por dispersão larval a partir de fontes remotas pode ser possível. A maioria dessas larvas seriam, no entanto, localmente não adaptáveis, o que pode restringir a resposta à seleção, expansão populacional e taxas de recolonização. Mais estudos com um maior

número de taxas representativas são necessários, de modo a obter uma perspetiva mais clara da conectividade populacional nos campos hidrotermais. Além disso, existe uma lacuna considerável de conhecimento sobre os mecanismos que influenciam os padrões de conectividade, dificultando o estabelecimento de conclusões. Finalmente, estudos de genética populacional deverão ser acoplados a modelos de dispersão larval, a fim de identificar possíveis barreiras à dispersão.

Palavras-Chave: Fontes hidrotermais, *Bathymodiolus azoricus*, genética populacional, RAD sequencing, mineração no mar profundo, conservação dos ecossistemas.

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Chapter 1

Introduction

The deep sea is the largest biome on Earth, comprising around 90% of the world's oceans. It has some unique characteristics that distinguish it from other marine ecosystems, such as its role in driving nutrient regeneration and global biogeochemical cycles. These are essential for sustaining primary and secondary production in the oceans. However the deep-sea remains mostly unknown (Ramirez-Llodra et al., 2010; Thurber et al., 2014). Since the 20th-century, numerous technologies developed for oceanographic research (e.g. Remotely Operated Vehicles and Autonomous Underwater Vehicles), have been used for exploration, sampling and experimentation in the deep sea. This technological evolution enabled the discovery of unique and charismatic habitats, such as hydrothermal vents (Ramirez-Llodra et al., 2010).

Scientists first discovered hydrothermal vents in 1977, describing them as underwater hot springs and mineral rich fluid from within the Earth (Craw, 2013). Hydrothermal vents are the result of seawater percolating down through fissures in the ocean crust in the vicinity of subduction zones (Van Dover, 2000). Their faunas are like nothing else on Earth. Vent organisms possess adaptations which allow them to thrive in an hostile environment. Vent are transient habitats with limited longevity (spanning from decades to centuries) and geographically isolated from each other. Community composition and structure are affected by linking and isolating mechanisms between vent fields, by local conditions and by instability of venting, which induces extinction-colonization dynamics (Desbruyères et al., 2001).

Temporal fluctuations in venting activity and abrupt changes in the geochemical environment may affect local populations by creating divergent selective regimes and gaps in organismic distributions along a ridge axis. The embryos and larvae of some vent animals are negatively buoyant and may be transported in near-bottom currents

along the ridge axis (Won et al., 2003b) or move from one vent to another. In regions where vent fields are disturbed frequently, larval dispersal is essential for regional persistence of a population (Mullineaux et al., 2013).

The deep sea is under increasing threat from human activities, such as overfishing (Watson and Morato, 2013), littering (Pham et al., 2014), chemical waste disposal (Ramirez-Llodra et al., 2011), oil and gas development, land-based pollution (greenhouse gases) (Mengerink et al., 2014) and potentially deep seabed mining (Van Dover, 2014). In the last 4 years, the International Seabed Authority has granted 18 contracts for mineral exploration on the deep seabed (Wedding et al., 2015), with continental shelves and Economic Exclusion Zones (EEZ) mining (Rosenbaum, 2011). Thus, deep-sea communities will be vulnerable to physical disturbances given their fragile habitat and extremely slow recovery rates (Craw, 2013).

Nautilus Minerals Inc. is one of the companies that commercially explore the seafloor for massive sulphides, as potential sources of high grade copper, gold, zinc and silver. This company has shown a particular interest in the Seafloor Massive Sulfide (SMS) deposits found on the high seas (The Clarion CLipperton Fracture Zone) as well as in several EEZs such as Papua New Guinea, Tonga, Fiji, Vanuatu, the Solomon Islands, Italy, and the Azores (Nautilus, 2012). The Clarion-Clipperton Fracture Zone (CCZ), in the Pacific, is being targeted by the mining industry owing to high concentrations of polymetallic nodules on its seabed. Considerable efforts are, therefore, being made to better understand the deep-sea communities in this area, in order to implement conservation measures prior to the start of mining activites. The range of impacts that could be related with nodule mining in the CCZ can affect 100 to 1000 species per km^2 per mining operation per year (Wedding et al., 2015).

Seafloor Massive Sulfide deposits are areas of hard substratum with high base metal and sulfide content that are formed through hydrothermal circulation and are commonly found at hydrothermal vent sites (Boschen et al., 2013). Seemingly, these areas will be highly sought for mining, thus posing great potential physical threat to hydrothermal vents and their biological communities. Extraction of ore deposits will result in selective removal of the substratum and the production of a particulate plume. Mining of SMS deposits consists of three stages, (1) prospecting (search for SMS deposits - size, distribution, composition and value), (2) exploration (that follows the prospecting - analysis of defined deposits, mining equipment and facilities and undertaking environmental, technical, economic and commercial studies) and (3) exploitation (recovery for commercial purposes of SMS and the extraction

of the minerals contained - construction and operation of mining, processing and transportation systems) (Boschen et al., 2013). Some organisms will therefore be killed directly by mining machinery while, others may face the risk of smothering by material settling from the particulate plume (Dando and Juniper, 2001).

Several impacts on the diversity and density of biological communities is expected on mined areas, resulting in changes to the food web, with potential impacts on ecosystems and populations of unknown duration (Craw, 2013). These impacts hydrothermal vent ecosystems can fall in two broad categories: causal physico-chemical changes and biological response or consequence (Van Dover, 2014). Mining will surely reshape the seafloor, removing vertical edifices and altering the texture of the substratum (Van Dover, 2011), compromising all benthic organisms inhabiting the substratum, with any high-turbidity, and potentially toxic sediment plumes (Boschen et al., 2013).

There is a need to provide tools (e.g. spatial management, marine reserves) to conserve and protect the marine biodiversity, and vulnerable marine ecosystems (Craw, 2013; Green et al., 2014). Most marine species have a dispersive stage, typically the pelagic larval stage in which the larvae can be transported by ocean currents (Cowen et al., 2000). The term "connectivity" is used when populations in different parts of a species range are linked by exchange of larvae, recruits, juveniles, or adults (Palumbi, 2003). Knowledge of dispersal scales and marine populations connectivity is vital to understand metapopulation dynamics, as well as to establish the size and placement of marine protected areas, for example to assess the movement of adults and larvae into and out of a reserve (Cowen et al., 2000; Palumbi, 2003). In the context of deep-sea mining, understanding scales of connectivity will alow to predict recovery and recolonization in a post-mining scenario. So far, much of the effort has focused on quantifying dispersal or connectivity in shallow water and very little is known in the deep ocean.

This thesis aims to investigate genetic of *Bathymodiolus azoricus* among hydrothermal vents in the Azores, in order to identify the spatial scales and patterns over which populations are connected. To accomplish this goal, state-of-the-art next-generation sequencing techniques were used to characterize thousands of markers in a set of individuals from Menez Gwen, Lucky Strike and Rainbow and employed bioinformatics tools to investigate three possible scenarios: 1) large-scale connectivity, or panmixia, among all vent populations; 2) limited connectivity due to restricted dispersal; 3) Highly structured populations with low connectivity among vents. Research on this

charismatic vent species will enable to predict how this and other vent species are likely to be affected by SMS mining in the area, thus contributing to the delineation of conservation measures.

Chapter two of this thesis presents background information on the distribution, chemical environment and ecology of hydrothermal vents along the Mid-Atlantic Ridge, with emphasis on the Menez Gwen, Lucky Strike and Rainbow vent fields. This chapter also synthesizes relevant information on the biology and ecology of *Bathymodiolus azoricus*, one the most abundant species in these fields, including information on how their larvae are thought to disperse and populations are connected. Finally, it provides a brief description of the kind of impacts that deep-sea mining will likely cause on these ecosystems. Chapter three will describe the methods used in this study which rely on state-of-the-art RAD sequencing techniques and population genomic analyses. Chapter four presents the results obtained and chapter five will discuss these results in the light of current knowledge of deep-sea connectivity, the design of marine protected areas in the deep sea and the establishment of conservation measures on areas targeted for future mining.