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Biogeochemical and hydrological drivers of the dynamics of *Vibrio* species in two Patagonian estuaries

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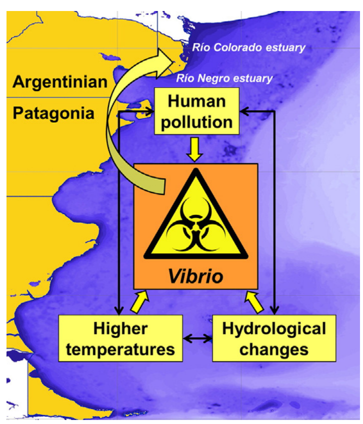
HIGHLIGHTS

- *Vibrio* species with virulence genes were reported in the Argentinian Patagonia.
- Salinity and ammonium were important factors explaining bacterial distribution.
- Salinisation and eutrophication of estuaries favour *Vibrio* abundance.
- Changing baselines are expected to increase the *Vibrio* impacts on ecosystem health.
- Adaptation strategies should sustain a good water quality.

GRAPHICAL ABSTRACT



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ABSTRACT

The ecology of the most relevant *Vibrio* species for human health and their relation to water quality and biogeochemistry were studied in two estuaries in Argentinian Patagonia. *Vibrio cholerae* and *Vibrio parahaemolyticus* were reported in >29% of cases at the Río Colorado and Río Negro estuaries. Neither the pandemic serogroups of *Vibrio cholerae* O1, *Vibrio cholerae* O139 nor the cholera toxin gene were detected in this study. However, several strains of *V. cholerae* (not O1 or O139) are able to cause human disease or acquire pathogenic genes by horizontal transfer. *Vibrio vulnificus* was detected only in three instances in the microplankton fraction of the Río Negro estuary. The higher salinity in the Río Colorado estuary and in marine stations at both estuaries favours an abundance of culturable *Vibrio*. The extreme peaks for ammonium, heterotrophic bacteria and faecal coliforms in the Río Negro estuary supported a marked impact on sewage discharge. Generally, the more pathogenic strains of *Vibrio* have a faecal origin. Salinity, pH, ammonium, chlorophyll *a*, silicate and carbon/nitrogen ratio of suspended organic particulates were the primary factors explaining the distribution of culturable bacteria after distance-based linear models. Several effects of dissolved organic carbon on bacterial distribution are inferred.

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Cholera
Changing baseline

Global change is expected to increase the trophic state and the salinisation of Patagonian estuaries. Consequently, the distribution and abundance of *Vibrio* species is projected to increase under future changing baselines. Adaptation strategies should contribute to sustaining good water quality to buffer climate- and anthropogenic- driven impacts.

1. Introduction

Within the *Gammaproteobacteria*, the genus *Vibrio* comprises several well-known species that are threats to animal and ecosystem health. *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus* are the most important species responsible for emerging or re-emerging infectious disease in humans (Harvell et al., 1999; Morens et al., 2004; Wetz et al., 2014). *Vibrio cholerae* generally enters the human host via contaminated food or water and causes intense watery diarrhoea, which leads to severe dehydration and even to death in certain cases of cholera gravis. *Vibrio parahaemolyticus* provokes acute gastroenteritis through haemolysin production and is generally characterised as a seafood-borne disease. *Vibrio vulnificus* can follow a gastrointestinal route but is also widely known for wound infection and septicaemia in humans. These bacteria are native to estuarine and coastal environments and are hazards for coastal ecological and socioeconomic systems, principally in developing countries (Grimes, 1991; Harvell et al., 1999; Binsztein et al., 2004; Costa et al., 2010). Despite these water safety implications, *Vibrio* species play a key role in biogeochemical processes of aquatic ecosystems.

The ecology of microorganisms is intimately linked to the dynamics of organic and inorganic nutrients. The interactions between organic matter and bacteria are crucial for biogeochemical cycles in aquatic systems (Jiao et al., 2010; Amaral et al., 2016). Bacteria incorporate, metabolise and produce organic matter, changing its chemical properties and bioavailability. Organic matter has a strong effect on *Vibrio* ecology and cell metabolism (Grimes et al., 2009; Lara et al., 2011; Neogi et al., 2011). Heterotrophic bacteria can directly take up inorganic nutrients for metabolic processes (Bradley et al., 2010; Hitchcock and Mitrovic, 2013), and some inorganic nutrients have a direct effect on *Vibrio* abundance, cell motility, stationary phase changes and survival (Jahid et al., 2006; Lara et al., 2009). Likewise, nutrients have a bottom-up effect on the abundance and distribution of planktonic organisms.

Close interactions have been found between plankton abundance and pathogenic *Vibrio* in the aquatic environment (Epstein, 1993; Lipp et al., 2002; Seeligmann et al., 2008; Martinelli Filho et al., 2011). Bacteria, phytoplankton and zooplankton are temporally and spatially associated. The relation of *V. cholerae* with zooplankton has been inferred to be a factor in the transmission of human epidemic cholera. Zooplankton not only forms a suitable hard substrate for *Vibrio* biofilm formation but also offers protection against environmental stress and is an important source of nutrients (Thomas et al., 2006; Lara et al., 2011). Moreover, a variety of physicochemical parameters, such as water temperature, salinity and turbidity affect *Vibrio* abundance and distribution (Neogi et al., 2011; Johnson et al., 2012; Mookerjee et al., 2014; López-Hernández et al., 2015). The numerous implied biotic and abiotic factors, as well as their interactions, increase the complexity of studies on *Vibrio* ecology in changing estuaries.

Estuaries are areas of high productivity that provide habitat to a large number of species, their wetlands offer several ecosystem services such as nutrient retention, sediment accretion and environmental stabilisation. As areas of organic matter production, estuaries also play an important role in carbon cycling and export to the oceans (Wu et al., 2007; Canuel and Hardison, 2016). Several highly populated megacities are located at coastlines, and human activities are changing their hydrological and biogeochemical features. Eutrophication and environmental pollution, as a consequence of industrial, agricultural and domestic runoff, are major threats to coastal and estuarine ecosystems (Nixon, 1995; de Jonge et al., 2002; Fricke et al., 2016). The combination of anthropogenic and climate driven alterations shifts coastal ecosystem baselines and affects environmental restoration (Duarte et al., 2009; Kopprio et al., 2015).

Cholera invaded Argentina in epidemic waves during the second half of the 19th century (Carbonetti and Rodríguez, 2007). Penna (1897) reported the first cases in a regiment from an Indian ship at the Bahía Blanca estuary, near the northern limit of the Patagonian region. The epidemic form of cholera (serogroup O1) reappeared dramatically at the end of the 20th century in South America and several hundred cases were reported in the north of Argentina. *Vibrio cholerae* has been detected in the Río de la Plata Estuary and rivers of Tucumán in the central and northern regions of Argentina (Binsztein et al., 2004; González Fraga et al., 2007; Seeligmann et al., 2008). Recently, two cases of human deaths by *V. vulnificus* infections were reported for two elderly men after recreational activities in Uruguayan waters of the Río de la Plata Estuary. Studies contributing to the understanding of links between hydrological factors and ecological disease agents are urgently needed.

To date, there is no investigation on *Vibrio* abundance and its potential biogeochemical drivers in the temperate estuaries of Argentinian Patagonia. We hypothesise that *V. cholerae* and *V. parahaemolyticus* are present in the studied systems and that *Vibrio* abundance and distribution are strongly influenced by temperature, salinity and nutrient concentrations. The aims of the present study are as follows: I) to identify *Vibrio* species and their relationship to environmental factors in two contrasting Patagonian estuaries, II) to evaluate the origin and fate of organic matter and inorganic nutrients and their links with culturable bacteria and anthropogenic impacts, and III) to estimate the effect of global change on the ecology of *Vibrio* and ecosystem health based on the current baseline of Patagonian estuarine systems.

2. Methods

2.1. Study areas

The Río Colorado and Río Negro rivers (Fig. 1) present a nival regime altered by the presence of several dams. They originate at the confluence of upland tributaries, and both watercourses traverse >600 km across Northern Patagonia to flow into the Southwestern Atlantic Ocean. The Río Colorado and Río Negro rivers are vital resources for this semiarid region and suffer from anthropogenic impacts, mainly driven by agricultural activities and urban settlements (Kopprio et al., 2015). More than half of the human population of Argentinian Patagonia, which comprises a terrestrial surface of >800,000 km^2, is located in the Río Negro basin. The annual mean discharge of Río Negro is 930 $m^3\ s^{-1}$; while the discharge of Río Colorado is about four-fold lower, at 150 $m^3\ s^{-1}$. The Río Colorado estuary is characterised by a microtidal regime and has been significantly modified over decades by irrigation works, which shaped its current deltaic form and changed its hydrological characteristics, including an increase in salinity. The Río Negro is a macrotidal estuary with sand banks and marsh islets. The wetlands of both estuaries, particularly the southern wetland of Río Negro, offer several ecosystem services and are crucial for fish, crustaceans, birds and marine mammals. Moreover, the discharge of both rivers influences the marine protected area of Bahía San Blas, a bay with important fisheries and a nursery area and refuge for endangered species.

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