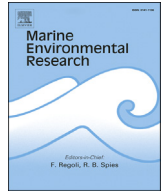




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Aliens in Paradise. Boat density and exotic coastal mollusks in Moorea Island (French Polynesia)

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ABSTRACT

Pacific islands are particularly vulnerable to the effects of invasive species. After habitat destruction or modification, invasive species are responsible for more biological extinctions than any other cause. Further, the rate of extinction of native species has been higher on islands than anywhere else in the world. Invasive species have also degraded native ecosystems. In order to detect exotic intertidal mollusk species, an extensive sampling around Moorea Island, a more or less unspoiled island surrounded by a rich coral reef habitat, has been developed considering that sampled points have different characteristics in wave exposure, algae coverage, type of substrate, distance to ports, distance to freshwater, distance sewage and boat traffic. Samples were DNA barcoded for unequivocal species assignment.

The presence of five NIS among 26 species seems an important signal of introduction of alien biota in Moorea Island coast. However they were represented by a total of 38 individuals among 1487 mollusks (2.55%). While the distance to relatively big ports influenced directly species richness, the intensity of maritime traffic measured as boat density near sampling points was significantly associated with the frequency of exotic species. Other environmental factors did not show significant correlation with the frequency of exotics, suggesting that in an environment without big discontinuities, with little habitat modification, local boat traffic is the most influential factor in the spread of exotic species. This could be mitigated relatively easily by reducing boat density in local zones of ecological interest.

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1. Introduction

Coral reefs are complex and fragile ecosystems extremely sensitive to environmental changes and are currently endangered by a variety of problems, from climate change to acidification to pollution (e.g. Wilson et al., 2006; Hoegh-Guldberg et al., 2007). Introduction of alien species can be one of those factors because it is a well recognized threat for marine ecosystems (reviewed by Molnar et al., 2008), but the impacts of non-indigenous species on coral reefs are not well known (Coles and Eldredge, 2002).

There are different gateways for introduction of exotic species into a habitat. Fouling of maritime vessels is likely the most important (e.g. Gollasch, 2002; Godwin, 2003; Ruiz et al., 2009).

International shipping is indeed important for transferring marine species, but intraregional transport has also been reported to be a major factor of alien species dissemination (Wasson et al., 2001). Aquaculture is other way of introduction of exotic species (Naylor et al., 2001), although its particular impact on coral reefs has not been studied yet.

Nearshore coral reefs are of high conservation value, and, as usual in interconnected ecosystems, they are heavily influenced by their proximity to the land, where most human activities are developed. Mollusks and sessile benthic communities from nearshore reefs are particularly sensitive by human impacts associated with land (e.g. Smith et al., 2008). Intertidal areas are the interface between terrestrial environments and nearshore reefs, and their integrity is therefore crucial for the conservation of the latter. Intertidal species, by their capacity of surviving both submerged and covered, are highly adaptable. Some of them can be carried by maritime transport very far from the native habitat, spreading to exotic geographic settings where they can become invasive. The

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intertidal zone could therefore be considered a gateway for the introduction of aliens or not indigenous species (NIS) into near-shore coral reefs, NIS using intermediate steps to reach new habitats (e.g. Apte et al., 2000).

Mollusks are good model species for studying the role of maritime transport in shaping nearshore coral reefs diversity. They constitute an important part of coral reef communities (e.g. Augustin et al., 1999; Zuschin et al., 2001), and can also be dangerous elements contributing to degrading coral ecosystems (Hutchings, 1996). Mollusks are easily transported via ships. Sessile adults can attach to hulls and pelagic larvae can be contained in the ballast water, so they can be transported in different life stages increasing their likelihood to settle into new habitats. Some mollusks are paradigmatic examples of colonizers via ships. This is the case of various gastropoda and bivalvia carried by vessels to Hawaii, of European origin and now evolved to an American invader (Godwin, 2003), and many other species (Molnar et al., 2008).

On the other hand, alien species are particularly dangerous for island ecosystems, where they impact in an extent not well known yet. A paradigmatic example is the invasion of the Moorea Island (French Polynesia) by foreign land snails (e.g. Reaser et al., 2007), which endangered native flora and fauna. Measures of biological control such as the introduction of a predator were taken to eradicate or at least stop the invasion (Civeyrel and Simberloff, 1996), although the efficacy of those measures has been questioned. This island, which possesses an extremely valued lagoon and coral reef around almost all its perimeter, will be the geographical setting of the present project.

In this study we will determine different diversity indices of intertidal mollusk assemblages from Moorea, sampled at different distances from ports and ship harbors around the island. Association of diversity indices with the distance to ports, as well as with boat traffic, will be examined taken also into account other factors like the type of substrate and proximity to freshwater and sewage sources, if any. The objective is to explore the impact of maritime transport on mollusk diversity in intertidal areas connected with nearshore coral reefs, using Moorea as an example. This study will also allow to detecting exotic mollusks because the target gene will be the COI employed in Barcoding projects, which is extremely robust for species identification, and the taxonomic identification was confirmed with a second marker, 16S rRNA.

2. Material and methods

2.1. Study area and sampling

Moorea Island is situated 17°32' South and 149°50' West, 25 km distant from Tahiti in the Society archipelago in French Polynesia. It is a high island, entirely surrounded by a coral reef rim, cut by eleven passes. Like most of the high islands, the Moorea coral reef complex is divided into four "ensembles" or units: outer slope, barrier reef, lagoon and backreef channel and fringing reef (Battistini et al., 1975). Each unit includes zones and biotopes characterized by coral reefs communities (Faure, 1985).

Samples were taken from 16 points (Table 1) located in different ecosystems within the coral reefs that surround the island (Richard, 1985): from water channels in Papetoai, Maharepa and Tema'e; from deep lagoon in Vai'are, Farehau, and Afareaitu; from fringing reef in Tiahura, Maatea, Atiha, Vai'anae, Haapiti, Tiki and Hauru, and finally from bay environment in Opunohu, Paopao and Entre-Deux-Baies. Eight of the sampling points (Hauru, Tiahura, Entre-Deux-Baies –also named Entre2Baies, Maharepa, Tema'e, Farehau, Maatea and Haapiti) were chosen within each of the eight marine protected areas present around the island (Walker, 2003).

Sampling was carried out picking (at random within species)

mollusk specimens from the intertidal range (upper to lower), which is quite short in Moorea (maximum tidal range of 0.40 m) between the 26 August and the 10 September 2011 (Fig. 1). A total of 100 samples were obtained from each site (Table 1). An effort was made for obtaining representative samples, proportional to the abundance of each species. The environmental characteristics considered are reported in Table 1. For the distance to ports, there is only one big port in the island located in Vai'are, where both cargos and passenger ferries arrive. The rest of ports are local fishing and docking ports, with adjacent marinas in most cases. As a proxy of local maritime traffic intensity, the number of boats (fishing and/or recreational) of any size visible from each sampling location was counted for half an hour in each of three different days (one weekend and two working days). For each sampling point distance to freshwater (watercourse mouth) and sewage discharge, degree of substrate artificiality (from natural to totally artificial), algae coverage and exposure to open waves was recorded. A Likert scale was employed for quantifying each factor whenever possible for reasons of data normalization and statistical treatment. We used a 1–5 scale from the minimum to the maximum expression of each factor in the ecosystems sampled in the island.

The specimens sampled were photographed and visually identified employing taxonomic guides and voucher specimens from the collection of the CRIOBE, in Moorea (French Polynesia). Tissue samples were stored in absolute ethanol until analysis.

2.2. Classification of species as NIS

The species found in this study were classified as native or NIS according to current inventories of Moorea fauna and the native distribution of each species (World Register of Marine Species, www.marinespecies.org; Encyclopedia of Life, available from <http://www.eol.org>, accessed July 2015).

2.3. Genetic analysis

Total DNA was extracted from a small piece of tissue following the standard protocol described by Estoup et al. (1996), employing Chelex[®] resin (Bio-Rad Laboratories). The E.Z.N.A Mollusc DNA kit (IOMEGA, bio-tek) was used for the species with high content of mucopolysaccharides in muscle tissues, following manufacturer's instructions. In both cases, the tubes were stored at 4 °C for immediate DNA analysis, and aliquots were frozen at –20 °C for long-term preservation.

A fragment of partial COI gene was amplified by polymerase chain reaction (PCR), employing the primers described by Geller et al. (2013). The amplification reaction was performed in a total volume of 40 µl, with Promega (Madison, WI) Buffer, 1x, 2.5 mM MgCl₂, 0.25 mM dNTPs, 20 pmol of each primer, approximately 20 ng of template DNA and 1 U of DNA Taq polymerase (Promega), and the following PCR conditions: initial denaturing at 95 °C for 5 min, 35 cycles of denaturing at 95 °C for 1 min, annealing at 48 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min.

Additional sequencing of the mitochondrial 16S rRNA gene with the primers described by Palumbi (1996) was carried out for some samples in order to confirm the taxonomic identification with a second marker. The amplification reaction was performed in a total volume of 40 µl with the same conditions described above for the COI gene and the following PCR conditions: initial denaturing at 95 °C for 5 min, 30 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 7 min.

PCR products were visualized in 2% agarose gels with 3 µl of 10 mg/ml ethidium bromide. Sequencing was performed with the

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