Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Assessment of the distribution of two sympatric rail species in relation to habitat composition and disturbance on Santa Cruz Island, Galápagos

Hugo Miguel Venceslau Carvalho e Silva

Orientador(es) | Birgit Fessl
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Assessment of the distribution of Galápagos rail in relation to habitat and paint-billed crake on Santa Cruz Island, Galápagos.

Abstract

The Galápagos archipelago is a biodiversity hotspot with relatively few extinctions recorded. However, a negative trend for Galápagos rail's population in Santa Cruz Island was confirmed. We aim to evaluate the species' population status, assess the role of habitat composition on its distribution and describe the first insights about which factors affects its distribution and the paint-billed crake's distribution. Point counts with fixed distance were used in four areas to census both species, using 197 points from 2007 and an additional 111 points. We also assessed the vegetation cover in the tree, shrub, and ground layers. The Galápagos rail detection and abundance significantly increased in Highlands Media Luna since 2007 due to a significant decrease in the occurrence of the red-barked quinine tree. However, the hill blackberry had significantly increased. The Galápagos rail seems to be related with dense ground vegetation. Its population is at the same levels as in 1986, but there is little evidence of about the factors influencing the distribution of both species, based only on habitat requirements.

Keywords: Rallidae, *Laterallus spilonota*, *Mustelirallus erythrops*, birds monitoring, islands conservation, Santa Cruz Island, vegetation composition, habitat disturbance

Avaliação da distribuição de *Laterallus spilonota* em relação ao habitat e a *Mustelirallus erythrops*, na ilha de Santa Cruz, Galápagos.

Resumo

As ilhas Galápagos são consideradas um "hotspot" de biodiversidade onde ocorreram poucas extinções, apesar da população de *Laterallus spilonota* ter reduzido acentuadamente em Santa Cruz. Os nossos objetivos são avaliar o status da sua população e o papel que a composição do habitat tem na sua distribuição, bem como apresentar as primeiras evidências sobre quais os fatores que afetam a distribuição de *L. spilonota* e *Mustelirallus erythrops*. Para realizar os censos de ambas as espécies foram utilizados pontos com distância fixa em 4 áreas de Santa Cruz. Foram usados os mesmos 197 pontos de 2007 e 111 pontos adicionais. Foi também avaliada a cobertura vegetal no estrato arbóreo, arbustivo e herbáceo. A deteção e abundância de *L. spilonota* aumentaram significativamente em *Highlands Media Luna* desde 2007, devido a uma diminuição significativa da ocorrência de *Cinchona pubescens.* Porém, a ocorrência de *Rubus niveus* aumentou significativamente. *Laterallus spilonota* parece ser mais abundante em locais onde a vegetação herbácea é densa, estando a sua população nos mesmos níveis de 1986. Além disso, existem poucos indícios sobre a distribuição das duas espécies apenas com base na composição do habitat.

Palavras-chave: Rallidae, *Laterallus spilonota, Mustelirallus erythrops,* monitorização de aves, conservação em ilhas, Ilha de Santa Cruz, composição da vegetação, perturbação do habitat.

Evaluación de la distribución del Pachay en relación con el hábitat y la Gallareta de Pico Rojo, en la isla Santa Cruz, Galápagos.

Resumen

Las islas Galápagos son un "hotspot" de biodiversidad con pocos registros de extinciones, aunque la población del pachay se ha reducido notablemente en Santa Cruz. Nuestros objetivos son evaluar el estado de su población y el papel que la composición del hábitat tien en su distribución, así como presentar las primeras pruebas sobre qué factores afectan a la distribución del pachay e de la gallareta de pico rojo. Para realizar los censos de ambas especies se utilizaron puntos con distancia fija en 4 zonas de Santa Cruz. Se utilizaron los mismos 197 puntos de 2007 y 111 puntos adicionales. También se evaluó la cobertura vegetal en el estrato arbóreo, arbustivo y herbáceo. La detección y la abundancia del pachay aumentaron significativamente en Media Luna desde 2007, debido a una disminución significativa de la presencia de cascarilla. Sin embargo, la presencia de mora aumentó significativamente. El pachay parece ser más abundante en los lugares donde la vegetación herbácea es densa y su población se encuentra en los mismos niveles que en 1986. Además, hay pocas pruebas sobre la distribución de las dos especies basándose únicamente en la composición del hábitat.

Palabras clave: Rallidae, Laterallus spilonota, Mustelirallus erythrops, monitoreo de aves, conservación en islas, Isla Santa Cruz, estructura de la vegetación, alteración del hábitat.

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1. Introduction

Oceanic islands are listed among the biodiversity hotspots in the world due to their unique environmental conditions which enhance the presence of a high number of endemic species (Myers *et al.*, 2000; Gillespie *et al.*, 2014). These unique areas have been facing large developmental pressures and impacts due to human activities, leading to changes of the natural habitats that threaten their distinctive biodiversity including bird species (Spatz *et al.*, 2017). In fact, over the last two millennia, the birds' communities of nearly all islands of the Pacific Ocean have been significantly altered by the impact of those activities (Steadman, 1995).

Over 90% of bird extinctions have occurred on islands, although only one-fifth of the world's bird species are on these places (Butchart *et al.*, 2006; Szabo *et al.*, 2012). According to IUCN, from the total number of species on islands, 23% are threatened species, representing 39% of threatened birds worldwide. From the threatened bird's species, about one-third are endemic to islands (Fonseca, *et al.*, 2006).

Bird extinctions are commonly related with three main causes: (1) non-native species – specially predators, and invasive plants (Owens & Bennett, 2000), (2) direct persecution by humans – specially bird species with higher body sizes (Clavero *et al.,* 2009), and (3) habitat loss – which reduce niche availability for birds (Owens & Bennett, 2000).

The islands of the Pacific Ocean host many threatened endemic bird species, with more than half of which are classified as endangered or vulnerable (Johnson & Stattersfield, 1990). Large scale bird species extinctions have been documented in several Pacific Islands. For example, in the Hawaiian Islands more than 50 native passerines have become extinct since the arrival of early Polynesian settlers around 10,000 years ago (Riper & Scott, 2001). In addition, many exotic species have invaded these islands: the current bird species composition of Hawaiian Islands comprises over 30 successfully introduced non-indigenous species (Riper & Scott, 2001; Lockwood, 2006). New Caledonia, Fiji and the Marquesas Islands are also examples of archipelagos where bird species extinctions have occurred due to deforestation for agriculture, grazing and invasion of non-native plants and animals (Cuddihy

& Stone, 1990; Cabin *et al.*, 2002; Meyer, 2004; Gillespie *et al.*, 2014). In the Mascarene Islands and New Zealand, 36 bird species have been documented as extinct (Cheke & Hulme 2010; Walters, 2016).

In contrast to many of the Pacific's archipelagos, the Galápagos Islands are largely intact. The islands remained uninhabited by humans until mid-sixteenth century with very few vertebrate extinctions (Tye *et al.* 2002, Phillips *et al.*, 2012), and in modern times only the least vermilion flycatcher, *Pyrocephalus dubius,* has probably become extinct from San Cristóbal Island (IUCN, 2017), although Dvorak *et al.* (2019) have raised the possibility that few birds may have survived in remote parts of the island. The ecological and evolutionary processes from Galápagos are nearly intact, and the archipelago retains 95% of its original species (Bensted-Smith, 2002). The fact that the archipelago has remained in nearly pristine conditions until the present can be explained by its recent discovery, the historical absence of aboriginal populations and the late human colonization of the islands as well as the fact that 97% of the land is under protection as a National Park (Snell *et al.,* 2002; González *et al.*, 2008; Fessl *et al.*, 2010).

Although the Galápagos Islands remain almost pristine relative to other oceanic archipelagos, its unique ecosystems are suffering profound alterations as a result of human activities (Hamann, 1984; Lawesson, 1990; Itow, 2003). Since the arrival of Europeans, the human population on Galápagos Islands has been increasing through migration (Watkins & Cruz, 2007) due to high standard of living, making the islands attractive to immigrants from the Ecuadorian mainland. Despite the demanding conservation policies and legal protection adopted by Ecuador, such as the establishment of the Galápagos National Park in 1959, ecological degradation, local extinctions, and extirpations are occurring at an accelerating rate in the archipelago (Steadman & Ray, 1982; Cruz & Cruz, 1987; Dowler *et al.*, 2000; Bensted-Smith 2002; González *et al.*, 2008).

With colonization and human population growth, the demand for goods and services has been increasing, coming into conflict with the conservation goals (*e.g.* Bensted-Smith 2002). As a result, the geographical isolation of the islands decreased and has favoured the arrival and spread of exotic species through the archipelago (Grenier, 2000). Exotic species such as pigs, rats, goats, and cats, have been present in the archipelago almost since the first settlers, but had become a major threat to the endemic biodiversity, being the primary agents driving biodiversity impacts (Hamann, 1979; Steadman, 1986; Cruz & Cruz, 1987; Loope *et al*., 1988). These disturbances are driving the transformation of natural ecosystems and the depletion of some of native and endemic species' populations (Bensted-Smith 2002).

Vegetation successional stages in Galápagos are typically influenced by natural events such as volcanic eruptions, natural fires, and the *El Niño*-Southern Oscillation (ENSO). But since colonization, ecosystems and vegetation patterns have been subjected to further disturbances, especially on the four inhabited islands (Isabela, Floreana, San Cristóbal, and Santa Cruz) due to invasions by alien species, man-made fires, habitat fragmentation caused by changes in land use, and exploitation of natural resources (Hamann, 2001). In some islands of the archipelago, introduced goats caused massive alterations on vegetation structures and species communities (Schofield, 1989; Mauchamp *et al*., 1998). These changes have impact on native and endemic vegetation decreasing the habitat quality, and reduce the availability of food and water, leading to a decline in Galápagos native fauna populations (Herrero, 1990).

Non-native species are a serious risk to the ecological integrity of the islands, one of the highest priority biodiversity conservation hotspots in the world (Jackson, 1993; Henderson & Dawson, 2009). By 2017, almost 1600 alien species were recorded in the archipelago since its discovery in 1535, with 114 taxa considered invasive and a major threat to native biodiversity (Snell *et al.*, 2002; Jiménez-Uzcátegui *et al.,* 2007; Tye, 2007; Toral *et al.*, 2017). In fact, introduced plant species already outnumber the native ones (Magee *et al.,* 2001). Invasive species are recognized as one of the greatest threats to global biodiversity (Lowe *et al.*, 2000) and Snell *et al*. (2002) documented it as the largest single threat to Galápagos biodiversity in the short term. These authors estimated that since the year 1535 the introduction rate of new species has been approximately 10,000 times the natural rate and has probably increased significantly in modern times. Many populations of endemic and native species are declining, and management actions are needed to slow down this trend.

Our study focused on the Galápagos rail, *Laterallus spilonota*, an endemic bird species that has previously been described to be in decline and for which specific management actions have been carried out at Highlands Media Luna, on Santa Cruz Island. The major reason for the species decline is the invasion of its prime habitat by an invasive plant species, the red-barked quinine tree, *Cinchona pubescens*. In the late 1970s, a control project was initiated by the Galápagos National Park Directory with the aim of controlling the dispersal of the species and reducing its abundance. By 1981 over 30,000 trees had already been destroyed (Van der Werff, 1979; Hamann, 1984) but since then the invasion has continued to spread, leading to a second control programme implemented between 2001 and 2006. Even though the Galápagos rail reacted positively to first management actions, last surveys in Santa Cruz Island carried out in 2000 and 2007 confirmed a negative population trend of the species (Gibbs *et al.,* 2003, Shrivers *et al.,* 2011). As a result, it is critical to update population estimates, evaluate the long-term effect of former habitat restoration efforts and evaluate potential actions within the National Park area and the agricultural zone to stabilize the population in Santa Cruz if deemed necessary.

Our study had three main objectives. First, we focused on the prime habitat of the species to assess trends of the Galápagos rail population in Highlands Media Luna since 2007. In particular, we tested if there are changes on species distribution (presence/absence) and abundance. Additionally, we studied and described the vegetation at Highlands Media Luna, with a focus on invasive species like the red-barked quinine tree and the hill blackberry (*Rubus niveus*) in order to assess if the occurrence of the Galápagos rail can be explained by habitat composition and vegetation characteristics.

Our second objective was to give the first insights about the overlap in distribution between the Galápagos rail and the paint-billed crake, *Mustelirallus erythrops*, a native bird species firstly recorded in the 1950's. Santa Cruz is one of the two islands where both species occurred, and little is known about how these two species may interact and if they overlap in their habitat requirements. For that, we aimed to use vegetation characteristics in Santa Cruz highlands between sites where both species occur and sites where either the Galápagos rail or the paint-billed crake occurs, to predict their distribution. Through this assessment we described the habitat requirements for both species and whether there are characteristics in the vegetation that influence the presence of either species.

Our last objective was to create a long-term monitoring plan for the Galápagos rail, based on the proposal made by Gibbs *et al.* (2003). This plan was presented in a separate chapter, with the idea to be easily applied in the field as a conservation measure for the species. We present detailed methodology that allows a comparison with other studies on the species and help to detect changes in the Galápagos rail's population over a 10-year period. We also presented the field sheets we used to do the Galápagos rail census as well as a budget plan to give an idea of the costs of this plan for the next 10 years.

2. Methods

2.1. Study area

2.1.1 Galápagos archipelago

The Galápagos archipelago (Fig. 1) is located approximately 960 km west of mainland Ecuador and is formed by volcanic islands (Simkin, 1984). It includes 7 major islands with more than 100km^2, 11 smaller islands, and more than 120 islets (González *et al.*, 2008). Only four islands are inhabited by humans: Isabela, Santa Cruz, San Cristóbal, and Floreana, with a resident population of over 30 000 people (Quiroga & Rivas, 2017; Rivas-Torres *et al.*, 2018). From the total area of the archipelago (7995 km^2), 97% is protected by the Ecuadorian government as a National Park since 1959 (González *et al.*, 2008). The Galápagos climate differs from other equatorial islands, having typically two main seasons: a hot season, between January and May, characterized by warmer temperatures and heavy rainfall events that increase with altitude, and a cool season, between June and December, characterized by cooler temperatures with light rainfall and mist - known as *garúa* - in the highlands and almost no rain in the lowlands (McMullen, 1999; Trueman & d'Ozouville, 2010).

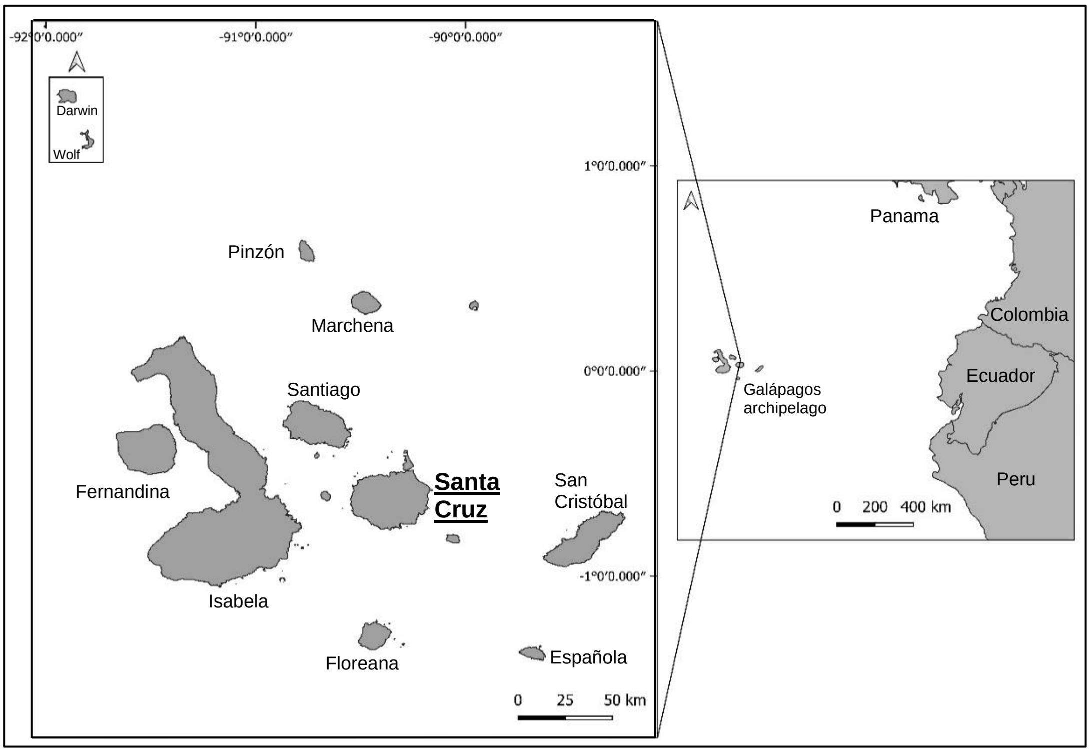


Figure 1 Map of South America with focus on Ecuador on the right, and map of Galápagos archipelago on the left, with focus on Santa Cruz Island. The Darwin and Wolf Islands are not in scale.

2.1.2. Santa Cruz Island

Our study was carried out in Santa Cruz Island which is the second largest island of Galápagos (986 km^2) and is in the centre of the archipelago (00º38' S, 90º20' W). It is a high ocean island (max. altitude of 864 m at Cerro Croker) and 88% of its area is classified as National Park (Moore, 2021; Villegas *et al.*, 2021). Despite have being the last island to be colonized by humans (around 1926), in 2015 Santa Cruz had around 16 000 inhabitants and is the island with the largest human population of the archipelago (Epler, 2007; Dvorak *et al.*, 2012; INEC, 2015). Puerto Ayora is the biggest settlement of Santa Cruz and is where most of the infrastructures and services are, being characterized as an urban area, located on the south coast of the island. Bellavista and Santa Rosa are two small rural areas on Santa Cruz Highlands, with mainly the presence of farms and some points of interest visited by tourists like lava tunnels, ranchos, and lodges.

2.1.3. Vegetation zones

The influence of altitude, exposure, and size of the island along with the unique climate patterns of the archipelago has led to a climatic zonation of the island. The vegetation follows this climatic zonation, being characterized by tropical desert scrub on dry lowlands and subtropical moist forest on humid highlands (Hamann, 1979; Trueman & Ozouville, 2010). In Santa Cruz, 5 different vegetation zones are present: (1) arid zone up to 120 m above sea level, (2) transition zone between 120 – 300 m, (3) *Scalesia* zone between 300 - 650 m, (4) *Miconia* zone between 500 – 680 m and (5) fern-sedge zone from 500 m to the island peak at 864 m (Wiggins & Porter, 1971).

The arid zone is the most extensive of all zones and is extremely dry during most of the year. It is characterized by xerophytic vegetation that drops the leaves during the cool season to reduce water loss. It is comprised of deciduous forest and shrubland with deciduous trees, mainly the palo santo (*Bursera graveolens),* and cacti from the genera *Opuntia* and *Jasminocereus* (Wiggins & Porter, 1971; McMullen, 1999).

The transition zone is a mixture of vegetation from the lower and higher areas. Many of the species are evergreen with trees taller and more closely spaced than in the arid zone and there are many endemic shrub species not found in the arid zone such as the pega pega (*Pisonia floribunda)*, the guayabillo (*Psidium galapageiums)* and the matazarno (*Piscidia carthagenensis).* This zone is hard to pinpoint due to the lack of dominant indicator species (Wiggins & Porter, 1971; Watson *et al.*, 2010; Dvorak *et al.*, 2012; Villegas *et al.*, 2021).

The *Scalesia* zone is largely dominated by species of the endemic *Scalesia* genus, comprising an evergreen forest of *Scalesia pedunculata* where the trees can reach heights up to 20 m. The shrub layer is prominent and uniformly developed. Many epiphytic liverworts, mosses and fern species cover the trunks and the branches of the trees giving a brownish colour to the vegetation (Wiggins & Porter, 1971; Watson *et al.*, 2010; Dvorak *et al.*, 2012). Parts of the *Scalesia* zone was converted into the Agriculture zone (see section 2.1.4.) on the southern slopes of Santa Cruz with the last trees cut in the 1970's. Nowadays, only ~140 ha of *Scalesia* forest is present in Santa Cruz, representing 1% of its original size (Mauchamp *et al.*, 2009), though some patchy planting of *Scalesia* has been done in the last 20 years.

The *Miconia* zone is dominated by the endemic shrub species *Miconia robinsoniana* that can reach heights of 3 to 4 m. The branches of this species are covered with lichens, epiphytic liverworts, and mosses. This zone is also characterized by having a ground layer composed of ferns, lycopods and herbaceous flowering plants (Wiggins & Porter, 1971; Hamman, 1979; Schofield, 1989, Shriver *et al.*, 2011).

The fern-sedge zone is made of low-growing perennial vegetation with narrow leaves above the *Miconia* zone. It has several sedge species of the genus *Cyperus, Eleocharis, Rhynchospora* and *Scleria* and fern species such as the native bracken *Pteridium arachnoideum*. The exception to the low-growing species is the endemic tree fern *Cyathea weatherbyana* that can reach heights of 2 to 3 m. It also has herbaceous and gramineous species as well as presence of lichens covering areas of barren cliffs and exposed rocks (Wiggins & Porter, 1971; Schofield, 1989; Jäger *et al.*, 2007; Jäger *et al.*, 2009; Shriver *et al.,* 2011).

The presence of grazing animals and invasive plant species such as the red-barked quinine tree (*Cinchona pubescens*), the hill blackberry (*Rubus* *niveus*), and the guava tree (*Psidium guajava*) are the main threats to the native vegetation in highland zones (Hamman, 1979; Hamann, 1984). The red-barked quinine tree dominates areas from the *Scalesia*, *Miconia* and fern-sedge zones. It was introduced in the Agriculture zone in the 1940's, and thanks to its winged seeds dispersed by wind, now occupies an area of about 11 000 ha (Hamann, 1984; MacDonald *et al.*, 1988; Lundh, 2006; Jäger *et al.*, 2007; Jäger *et al.*, 2009). Several control programs were carried out mainly by the Galápagos National Park to manage this invasive species (Rentería & Buddenhagen, 2006). The hill blackberry, considered the most aggressive alien plant species in the Galápagos archipelago by several authors (Rentería & Buddenhagen, 2006; Atkinson *et al.*, 2008; Rentería *et al.*, 2012a), is a perennial shrub that forms dense thickets up to 4 m high. It replaces the native vegetation, simplifying the vegetation structure, threatening many rare endemic plants (Rentería & Buddenhagen, 2006), and reducing the breeding success of endemic bird species (Leuba *et al.* 2020). It was introduced in Santa Cruz in the late 1960s for agricultural purposes and had a rapid and aggressive spread through several vegetation zones in Santa Cruz (Renteria *et al.*, 2012b). It can form a large seed bank that persists for up to 10 years, making the control programs even more challenging (Cevallos, 1992; Landázuri, 2002; Soria, 2006).

2.1.4. Agriculture zone

The Agriculture zone comprises areas from the humid and transition zone in the southern side of the island. It covers an area of 114.2 km^2 and is composed of fruit and vegetable farms and grazing land with introduced plant species such as the elephant grass (*Pennisetum purpureum*), the guava tree, the hill blackberry, the red-barked quinine tree, shrub patches, herbaceous plant pastures, and scattered forests (Rosenberg, 1990; Mauchamp & Atkinson, 2010; Hamann, 2011; Dvorak *et al.*, 2012). The limits of the Agriculture zone were defined in 1959 when the Galápagos National Park was established, based on the lands owned by settlers to date (Lundh, 2006). From its natural vegetation, 86% was changed to crops, invasive species, and infrastructures between 1987 and 2006 (Villa & Segarra, 2010). It is possible to identify different land use classes such as (1) fruit and vegetables – smaller farms with crops of *Musa* spp., *Citrus* spp., *Solanum lycopersic*, *Capsicum annuum*, *Cucumis sativus*, *Manihot esculenta*, *Solanum tuberosum* and *Saccharum officinarum* (sugar cane), (2) coffee – medium-sized farms of C*offea* spp., shaded by introduced tree species or banana plants, (3) pasture – large patches of land used for cattle and (4) forest – non-agricultural areas forested by native and non-native tree species (Geladi *et al.*, 2021).

2.1.5. Study sites

We surveyed for rails presence and abundance in 4 different areas of the island: (1) Highlands Media Luna, (2) Perimetral Media Luna, (3) Perimetral Santa Rosa, and (4) Mina Roja (Figure 15). In Highlands Media Luna (Fig. 2a), which encompasses the *Miconia* and fern-sedge zones between 500 and 863 m above sea level, the same points from previous studies were used to monitor both species. In this area we planned a long-term monitoring program for the Galápagos rail (Rosenberg, 1990; Gibbs *et al.*, 2003; Shriver *et al.*, 2011) (see Appendixes). The vegetation is composed of the endemic shrub species *Miconia robinsoniana* and fern species. It was also on this site, where the red-barked quinine tree control program took place in the late 1970’s (see section 1). Furthermore, there is an ongoing rodent control program at this site to protect the Galápagos petrel (*Pterodroma phaeopygia*) colonies, but there is no data available about it.

In Perimetral Media Luna and Perimetral Santa Rosa (Fig. 2b, 2c), points counts were conducted on the perimetral – the boundary between the National Park and the Agriculture zone. This site lies between 300 and 700 m and the vegetation is composed of a mix of introduced species, mainly ground species with no shrub layer, due to the presence of grazing patches and farms. However, it is possible to find some native species from the *Miconia* zone and *Scalesia* forest in the National Park area, close to the border with the Agriculture zone.

In Mina Roja (Fig. 2d), points were counted along a narrow dirt road that connects the Mina Roja to El Puntudo in Highlands Media Luna, between 500 and 800 m above sea level.

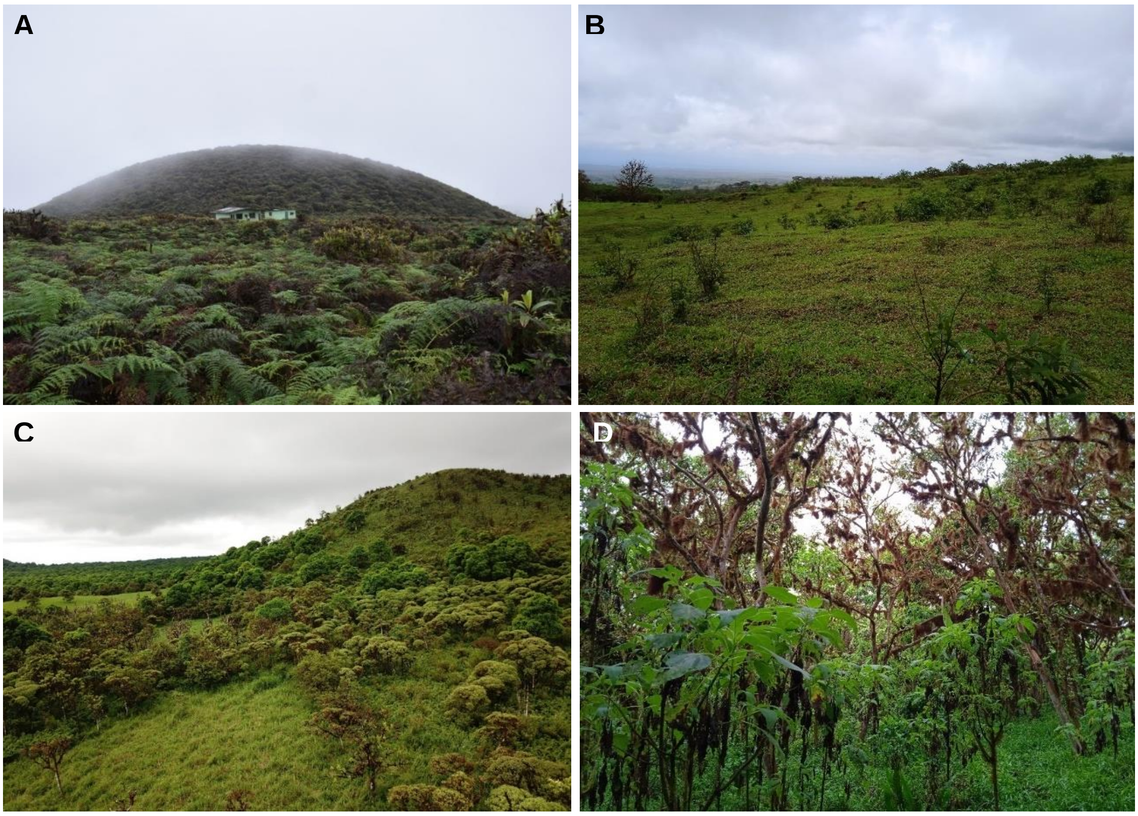


Figure 2 Pictures of the 4 study sites in Santa Cruz Island. A. Highlands Media Luna; B. Perimetral Media Luna; C. Perimetral Santa Rosa; D. Mina Roja. Source: Hugo Silva, Charles Darwin Foundation.

This road (Fig.3) is used by the National Park rangers to access different areas of the *Scalesia* zone. In this site, we also search for rails in 8 plots, where vegetation management for the little vermilion flycatcher, *Pyrocephalus nanus,* is done by the National Park and the Charles Darwin Foundation. Mina Roja has a high variety of different plant species and is characterized by the presence of species from the transition zone and Scalesia zone. Some grasslands are also found as well as patches of invasive species, such as the guava tree and the hill blackberry. In the plots where the vegetation management for the bird species little vermilion flycatcher (*Pyrocephalus nanus)* was carried out, no shrub layer was present due to the control of the invasive species hill blackberry (Fig. 4). In several of these plots, the ground was still covered by dead hill blackberry plants cut by the National Park rangers.

On the tree layer, species such the red-barked quinine tree, the Spanish cedar (*Cedrela odorata)* and *Scalesia pedunculata* were recorded.

Figure 3 Aerial photo of the dirt road in Mina Roja, surrounded by endemic species such as *Scalesia pedunculata* and invasive species such as *Cedrela odorata*. Source: Vegetation Mapping for Ecosystem Restoration project from Charles Darwin Foundation.

Figure 4 *Pyrocephalus nanus* plot in Mina Roja managed by the National Park. No shrub and ground layer were present in the intervened area. Source: Hugo Silva, Charles Darwin Foundation.

2.2. Study species

The Rallidae family has 142 described species divided into 41 different genera and a wide geographical distribution. Despite its distribution, about a third of the species are threatened or endangered, with very fragmented populations (Bennet & Owens, 1997). The *Laterallus* genus has 11 species distributed across the American continent. Recently, Stervander *et al.* (2019) suggested through molecular studies the addition of 3 more species to this genus, thus proving it to be a dynamic genus with the need for future research.

2.2.1. Galápagos rail (*Laterallus spilonota)*

The Galápagos rail is an endemic species from the Galápagos archipelago and was first recorded in 1896 by Charles Darwin, who described it as "*great numbers of a very small water rail* … " (Darwin, 1896). The species was originally found on 7 islands (Franklin *et al.,* 1979), but currently it is only found on 5 islands (Shriver *et al.,* 2011). Recent surveys on different islands have confirmed the probable extinction of the Galápagos rail in Floreana (Dvorak *et al.*, 2017) and San Cristóbal (Dvorak *et al.*, 2019). It is still present in Fernandina, Isabela, Pinta, Santiago and Santa Cruz and a small population was discovered in Pinzón post rat eradication (Fessl, B., pers. comm.). The last species survey in Santa Cruz Island dates from 2007 and it confirms a negative trend for this endemic species (Shrivers *et al.,* 2011). In Santa Cruz, Galápagos rail occurs in the *Scalesia* forest, *Miconia* zone and fern-sedge zone. It is associated with dense ground cover along with freshwater bodies (Taylor & van Perlo, 1998, Donlan *et al.*, 2007). The species also occurs in the Agriculture zone, preferring areas where the vegetation is denser, thus avoiding grazing areas. There are historical records of its occurrence in mangroves on several islands in the archipelago, but the causes of their disappearance from this habitat are unknown (Wiedenfeld, 2006). The only mangrove area in which it can regularly been observed, is the mangroves at Playa Tortuga Negra and Caleta Black, on Isabela Island, home of the last population of the Critically Endangered mangrove finch (*Camarhynchus heliobates)*.

Galápagos rail is a small (< 15 cm) running bird that uses openings in the vegetation for its locomotion, occasionally flying short distances. Adults have a dark bluish-grey head and chest, with small white spots on the dark brown back. The beak is short and black, and the eyes are red (Fig. 5). Chicks have black plumage. During the day, it feeds on small invertebrates and occasionally on seeds, searching through the leaves for ants, dragonflies, beetles, and some terrestrial snails (Franklin *et al*., 1979).

The breeding season for this species happens between December and March (Franklin *et al.,* 1979). It is territorial and builds nests on the ground, in the middle of dense vegetation, covering it with herbaceous plants. It has a long incubation period that lasts between 23 and 25 days. After hatching, the chicks abandon the nests and follow the parents, until they are nearly the size of adults, being it normal to see small groups of individuals together. The species has a high repertoire of vocalizations, including calls for alarm, defence of the territory or reproduction. In addition, it is a species that responds easily to playback, facilitating their census (Franklin *et al.,* 1979).

Figure 5 Adult of Galápagos rail, *Laterallus spilonota,* in Santiago Island. Source: Michael Dvorak, Charles Darwin Foundation.

The species is classified as “Vulnerable” (VU) (IUCN, 2020) and the main threats are the presence of exotic species and the loss of habitat. Former studies have shown its negative association with invasive species, mainly introduced herbivores and the red-barked quinine tree. These species are responsible for changes in the vegetation and composition of the habitat, making it less suitable for native species (Rosenberg, 1990; Gibbs *et al.*, 2003; Donlan *et al.,* 2007; Shriver *et al.*, 2011). Galápagos rail is naturally predated by the two native owl species *Asio flammeus* spp*. galapagoensis* and *Tyto alba* (Rosenberg *et al*., 1990), and probably by the Galápagos hawk (*Buteo galapagoensis)*. Predation is severely accentuated by introduced rodent species and feral former domestic animals such as dogs, cats and pigs (Wiedenfeld & Jiménez-Uzcátegui, 2008).

2.2.2. Paint-billed crake (*Mustelirallus erythrops)*

The paint-billed crake has a wide geographical range from tropical to temperate zones in the American continent with a patchy distribution in South and Central America. Two individuals were recorded in the United States of America and the species was also found in Valdez Peninsula, Argentina. In the Galápagos Islands, the population seems to be recently established (see detailed information below), suggesting it is a very mobile species, probably a migratory bird. However, little is known about the species due to its shy and hidden lifestyle. Additionally, its vocalizations are not well known, leading to misidentifications in the field, slowing the process of understanding its behaviour, abundances, and distribution (Taylor *et al.*, 2020).

The paint-billed crake is bigger than the Galápagos rail (18-20 cm length), with greyish plumage on the face and breast, brown on the back and black and white stripes on the belly and vent. It has bright red legs and a bicoloured red and yellow beak (Fig. 6). The juveniles have pale grey plumage with a greener beak without red at base (Taylor *et al.*, 2020). Immatures are not properly described. It is known that the species feeds on invertebrates from the soil and leaf litter, and seeds, but very little information is available (Taylor *et al.*, 2020). The species can be found in marsh habitats such reedbeds, dry pastures, rice fields and humid woodlands. It can also be found in corn fields, gardens, drainage ditches and bushy areas. In South America, the species seems to prefer dense swamp vegetation and damp secondary woodland in flooded savanna (Taylor *et al.*, 2020). According to IUCN, the species is classified as “Least Concern” (IUCN, 2016) but its status is difficult to assess in some areas because the species is really hard to observe and its distribution, movements and breeding needs more investigation (Taylor *et al.*, 2020).

Figure 6 Adult of paint-billed crake, *Mustelirallus erythrops*, captured on a bird ringing station in Agriculture zone of Santa Cruz Island. Source: Ilke Geladi, Charles Darwin Foundation.

During the collecting expeditions to Galápagos islands carried out between 1835 and 1906, no paint-billed crake was found and therefore it was not included in the comprehensive overview made by Swarth (1931). Only in 1953, two birds were collected for the first time in Santa Cruz Island by Bowman (1960). Currently, the species is expanding its distribution in Galápagos, and it can be found on the 4 inhabited islands: Floreana, Isabela, San Cristóbal and Santa Cruz (Kleindorfer *et al.,* 2022). Harris (1973) was the first to list the species as resident with the presence of nests on Santa Cruz. In 1973-74, another nest was found by Franklin *et al.* (1979) on Santa Cruz, and three birds were recorded in Genovesa Island (Wiedenfeld, 2006). In Floreana Island, 1000 to 2000 territories were estimated (Dvorak *et al.*, 2017) and in San Cristóbal

Island, 11 records were published during a survey of the landbirds of San Cristóbal (Dvorak *et al.*, 2019). There are also records of the species on Isabela Island made by the landbird team of the Charles Darwin Foundation (Fessl, B., pers. comm.). Although there is little information about the species, it appears that a natural colonization of the archipelago occurred during the first half of the 20th century, leading to the establishment of a breeding population in the Galápagos Islands.

2.3. Data collection

Point counts with fixed distance were used to quantitative assess the population of rails in the study area, following protocols used in previous surveys (Rosenberg, 1990; Gibbs *et al*., 2003; Shriver *et al.*, 2011). Sampling points were defined as circular areas with a radius of 25 m, spaced 100 m in Media Luna Highlands and 200 m in Perimetral Media Luna, Perimetral Santa Rosa, and Mina Roja. By doing so, we reduced the probability of counting the same individual more than once, avoiding an overestimation of the population (Shriver *et al.,* 2011).

In each point count, the observer was in the centre of the sampling point for 10 to 14 minutes, based on the rail's responses to the playbacks. The observer noted down the number of individuals of both species within a 25 m radius inside the circular plot. Only birds with more than 1 meter apart were noted, as rails calling simultaneously from the same spot can be hard to distinguish (Gibbs *et al.*, 2003).

At the first minute, the observer noted the sampling condition information such as the point number, GPS coordinates, elevation, time, and weather conditions: (1) % of clouds, (2) precipitation – 0 without rain, 1 drizzle, 2 rain, 3 heavy rain, and (3) wind – 0 no wind, 1 light wind, 2 moderate wind, 3 strong wind. Then, spontaneous calls of both species were noted for 5 minutes. After the first count period, a Galápagos rail's playback was used for 1 minute, 15 seconds in each direction of the main cardinal points. The Bluetooth speaker connected to a smartphone was located approximately 1.5 m above the ground. Then, the observer listened for 3 more minutes and noted any responses from the Galápagos rail or the paint-billed crake. If the paint-billed crake was not heard until after this period, a playback of the paint-billed crake was played for 1 minute, 15 seconds in each direction of the main cardinal points. This was only done in Perimetral Media Luna, Perimetral Santa Rosa and in Mina Roja sites, where the possibility to find both species was higher. In the *Miconia* zone, only the Galápagos rail have been recorded in previous studies. After the second playback, the observer listened for 3 more minutes and noted any responses from each species. Recordings of the species' calls (territorial and primary calls) were obtained from the "Library of Natural Sounds at Cornell University".

For the *Highlands Media Luna* site, the same 197 sampling points censused in 2007 by Shriver *et al.* (2011) were used. The points were in the middle of vegetation, close to narrow foot trails. In Perimetral Media Luna and Perimetral Santa Rosa, 67 points were created along the perimetral, and 44 points were created along the dirt road and plots in Mina Roja. The census was performed between January and March 2022, and we sampled the points between 6:30h to 13:00h.

For vegetation assessment the observer visually estimated the percentage of vegetation cover in a 25-meter radius on each sampling point at 3 levels: tree layer, shrub layer and ground layer. The 25-meter radius was measured with the help of a rangefinder (Nikon Laser 550) in the four cardinal directions. We visually estimated the habitat variables presented in tab. 1. All counts and vegetation assessment were done by the same observer, Hugo Silva.

Table 1 Vegetation variables measured on the field in the three vegetation layers

Layer	Variable	Explanation
Tree	CincS	number of red-barked quinine tree saplings (plants < 50cm)
	CincT	number of red-barked quinine tree trees
	CincD	number of red-barked quinine tree dead trees
	Guava	number of guava trees
	otherT	number of other trees species
Shrub	AHS	average height of shrublayer (cm)
	mico	% of *Miconia robinsoniana* cover
	otherS	% of other shrub species cover (including the hill blackberry)
	rubus	presence of hill blackberry: (**0**) 0% cover, (**1**) < 25% cover, (**2**) between 25 - 50% cover, (**3**) between 50 - 75% cover, (**4**) > 75% cover.
Ground	HGC	% of cover by tall ground vegetation (>30 cm)
	fern	% fern cover
	grass	% of grass cover
	moss	% of moss cover
	vis	% of visibility at a height of 30 cm
	Egrass	% cover by elephant grass
	bare	% cover by bare ground

2.4. Data analysis

Data analysis was divided into three groups, based on the goals established in this study. First, we focused only on data from Highlands Media Luna, where previous studies were performed, analysing the data of the Galápagos rail. Secondly, we did an overall analysis to the data related to both rail species and vegetation in all 4 study sites, to give the first insights about the overlap in the distribution of both rail species. Thirdly, based on our results and experience, we establish the guidelines for a monitoring plan which is presented in this master thesis (see Appendixes). All analyses were performed with R studio software version 202.02.0, and the maps were made in QGIS software version 3.16.5.

2.4.1. Galápagos rail population in Highlands Media Luna

For the Galápagos rail census, we assessed differences in the Galápagos rail presence/absence between 2007 and 2022 using the McNemar’s test (Zar, 1999). For that, we classified the sampling points into 4 groups, based on the combination of their status during the survey in 2007 and 2022: (1) PP – points with detected rails during both surveys, (2) PA – points with detected rails in 2022 and absent in 2007, (3) AP – points with rails absent in 2022 and present in 2007, and (4) AA – points with rails absent in both survey periods. To elucidate differences in the Galápagos rail relative abundance between 2007 and 2022, we used a non-parametric ANOVA (Kruskal-Wallis test). Visually, we described the distribution and abundance of the Galápagos rail in 2007 and 2022 through a map built with data from both surveys.

For vegetation assessment, we analysed the data of invasive plant species, namely the red-barked quinine tree and the hill blackberry. We compared the presence/absent of the red-barked quinine tree between 2007 and 2022 across all sampling points and we looked for differences in dead trees and saplings in the points where this species remained since 2007, using the Wilcoxon test. To visually compare the distribution of the red-barked quinine tree between both surveys, we did a map where we classified the sampling points as: (1) Saplings – if the area of the sampling point was mostly covered by the red-barked quinine tree saplings, (2) Trees - if the area of the sampling point was mostly covered by the red-barked quinine trees, and (3) Dead Trees - if the area of the sampling point was mostly covered by dead red-barked quinine trees. In points where the area was equally covered by 2 of the 3 categories, we considered the point as Tree in the tie between the categories Sapling - Tree and Tree - Dead Tree, and Sapling in the tie between the categories Dead Tree - Sapling. There were no points where the area was covered equally by all 3 categories. For the hill blackberry, we compared the number of points with the hill blackberry in 2007 and 2022 and we described its classification (see section 2.3.). We also looked for differences in presence/absence of the hill blackberry according to the Galápagos rail presence/absent. For that we used a Fisher test to analyse a contingency table. We divided our sampling points into presence/absence of the hill blackberry on the one hand, and those that have and do not have the Galápagos rail on the other. We also created a map to visually compare the distribution of the hill blackberry between both surveys. In 2007 only presence/absent was used to classify the points on the map but in 2022 we used the classification created to classify the points (see section 2.3).

Then we analysed which vegetation variables can explain the abundance of the Galápagos rail in this study site. We built a vegetation model to assess the influence of habitat composition on the Galápagos rail's population, using seven vegetation variables: (1) ExTree – a new variable created by adding the number of all trees and saplings of non-native species counted within 25m of a sampling point (2) HGC – percentage of the sampling point with a vegetation cover taller than 30 cm, (3) vis – percentage of visibility at 30 cm above ground level in the sampling point, (4) fern – percentage of the sampling point covered with fern species, (5) grass – percentage of the sampling point covered with grass species, (6) moss – percentage of the sampling point covered with moss, and (7) AHS – average height of the shrub layer in the sampling point. We chose variables related to ground vegetation because it is known that the Galápagos rail has a relation with this type of vegetation. We also chose a variable related with exotic trees because in Highlands Media Luna, the red-barked quinine tree is the main threat of this species. We did not consider the type of vegetation in shrub layer for this model because in Highlands Media Luna, *M. robinsoniana* dominates relatively to other shrub species. We evaluate the correlation between these variables, using a correlation matrix. Then we used an automated model selection tool in R (dredge function), to check the better-fit distribution to the dataset, choosing the one with the lowest AICc score (Akaike, 1974). Finally, we apply a general linear model (GLM) to the dataset with the selected variables.

2.4.2. Distribution of the two rail species

For rail census, we described the number of birds, percentage of points, range, and we created a map with the sampling points in all 4 study sites. The sampling points were classified according to presence/absent of both species: (1) Galápagos rail – points with only the Galápagos rail present, (2) Paint-billed crake – points with only the paint-billed crake present, (3) Both – both species present, and (4) N/A – both species absent.

For vegetation assessment, we analysed differences in vegetation between all study sites, using a Kruskal-Wallis' test and a Post-hoc Dunn's test. We considered all variables described in section 2.3 except the variable CincDead – number of dead *C. pubescens* trees. We also included a new variable – TotalT: total number of all trees, considering saplings, to include all tree species even the ones with low numbers.

To study the interaction between the two rail species, we performed a principal component analysis (PCA) to visualize the grouping of variables that affects the presence/absent of the Galápagos rail and the paint-billed crake. For that, we classified the sampling points as: (1) AA – absent of both species, (2) PP – presence of both species, (3) PA – presence of only the Galápagos rail, and (4) AP – presence of only the paint-billed crake. We also did a second principal component analysis (PCA) with the same goal as the previous one, without considering the points where both species were absent. Finally, we analysed which vegetation variables can explain the abundance of the Galápagos rail and the paint-billed crake in all study sites. For that, we built a vegetation model for each species to assess the influence of habitat composition on species population. We evaluate the correlation between these variables, using a correlation matrix. Then we used an automated model selection tool in R (dredge function), to check the better-fit distribution to the datasets, choosing the ones with the lowest AICc score. After that, we apply a general linear model (GLM) to the dataset of each species, with the selected variables.

2.4.3. Galápagos rail long-term monitoring plan

In this plan we set its main objectives, we established the methodology needed to perform it in the next 10 years, we present a map to visualise the points and the paths to use in the monitoring plan, and we present a budget for the plan based on current costs (2022).

3. Results

3.1. Galápagos rail population in Highlands Media Luna

3.1.1. Galápagos rail Census

We detected Galápagos rail at 94 points (48%) from the 197 points surveyed at Highlands Media Luna and paint-billed crake was absent from this site, confirming results from previous census data. This is a higher detection rate than found in previous census, namely 16% in 2007 and 29% in 2000 (Tab. 2).

Table 2 Number and % of points where Galápagos rails (*Laterallus spilonota*) was found in 2022, 2007, and 2000. In all three years the same 197 points were sampled. Data from previous years are taken from Gibbs et al., 2003 and Shriver et al., 2011.

Year	Number of points with *L. spilonota*	Detection rate
2022	94	48 %
2007	31	16 %
2000	56	29 %

We detected the species at 76 points where it was absent in 2007 whereas only in 21 points rails had been detected in 2007 but not in 2022. Our data show a significant increase in Galápagos rail detection (McNemar test = 29.26, p-val = 6.328e-08) between 2007 and 2022 (Tab. 3). Although we do not have the presence/absence data for each point from 2000 (Gibbs *et al.,* 2003), data from this year's survey show an increase of approximately. 68% in detectability of Galápagos rail between 2000 and 2022, as in 2022 we detected birds in 38 additional points.

Table 3 Number of point comparisons between 2022/2007, and 2007/2000 using a McNemar test. P = presence, and A = absence of Galápagos rail (*Laterallus spilonota*). The interpretation of P related to A correspond to the year with the same position of the letter. Data from the 2007/2000 group was taken from Shriver et al., 2011. As we do not have the original data with presence/absence for the year 2000, we cannot compare it with the 2022 season.

Group	P/A	A/P	P/P	McNemar test	P-value
2022/2007	76	21	19	29.26	< 0.001
2007/2000	17	42	14	10.91	0.002

Galápagos rail abundance also significantly increased between 2007 and 2022 (χ^2 = 32.55, p-val = 4.005e-07). In 2022, we counted a total of 142 individuals (average = 0.72 ± 0.92 rails/point; range: 0 – 5), while in 2007 only 73 birds were recorded (average = 0.33 ± 0.73 rails/point; range: 0 – 3). Thus, the number of rails almost doubled since 2007. Fig. 7 shows the survey points used in both the 2007 and 2022 surveys.

Visually, it is possible to see differences in abundance and distribution of the Galápagos rail between 2007 and 2022. In 2007, most of the records were in the highest parts of the site close to El Puntudo and Cerro Crocker. In 2022, the species was found almost everywhere at Highlands Media Luna, including Media Luna, El Puntudo and Cerro Croker, with exception of the path that goes from the most eastern counting point to Cerro Croker peak, similarly to 2007. In both years, Cerro Croker was the area with high rail abundance, but in 2022 the path that goes from Media Luna to El Puntudo also had high abundances.

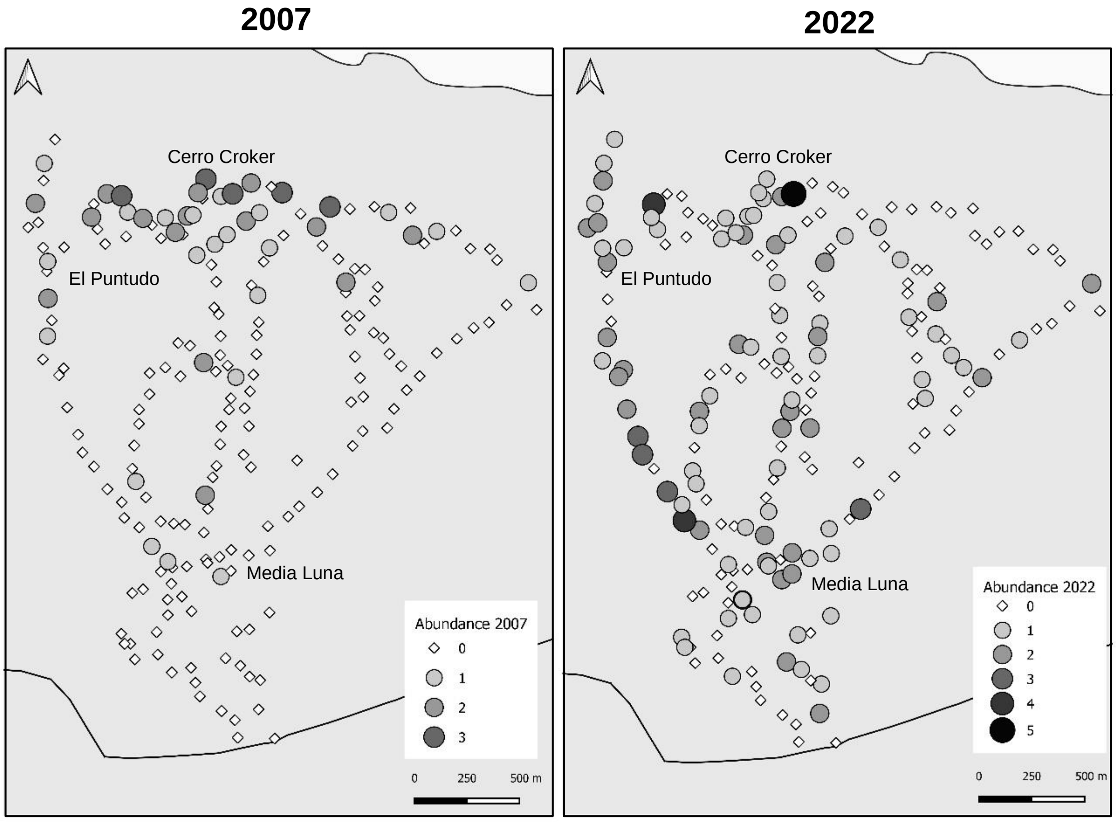


Figure 7 Map of the Media Luna Highlands study site, with the name of the main places, and the recorded individuals of Galápagos rail (*Laterallus spilonota*) in 2007 on the left and 2022 on the right. The white dots represent points where no birds were record.

3.1.2. Vegetation assessment in Highlands Media Luna

The red-barked quinine tree (*Cinchona pubescens*) was identified as a threat to Galápagos rail in Highlands Media Luna due to the reduction in native plant species richness and cover around this plant species (Fig. 8).

Figure 8 Individual of red-barked quinine tree (*Cinchona pubescens*) growing in the middle of ferns on Highlands Media Luna. Source: Hugo Silva, Charles Darwin Foundation.

Fig. 9 shows a map of Highlands Media Luna with the red-barked quinine tree distribution at the sampling points used for bird census. Points are in different colours according to the red-barked quinine tree classification. Each point was classified according to the highest % of cover of the species on that point in 2007 and the highest number of individuals of that point in 2022. We found red-barked quinine tree present on 35.0% of the points (69) in Highlands Media Luna, compared with 84.7% (167) found in 2007.

The presence of red-barked quinine tree between 2007 and 2022 was significantly different (Wilcoxon test = 24, p-value = 0.02), with an increase of points where the species was absent in 2022. In points where the species was present in both years, we found differences in the category *dead trees*, with an increase of the number of dead trees in 2022 (Wilcoxon test = 921.5, p-value =

2.3e-4) and no difference in number of saplings (Fig. 10). As the red-barked quinine tree original data for the year 2007 comes in % of cover, we cannot compare it with the number trees in 2022.

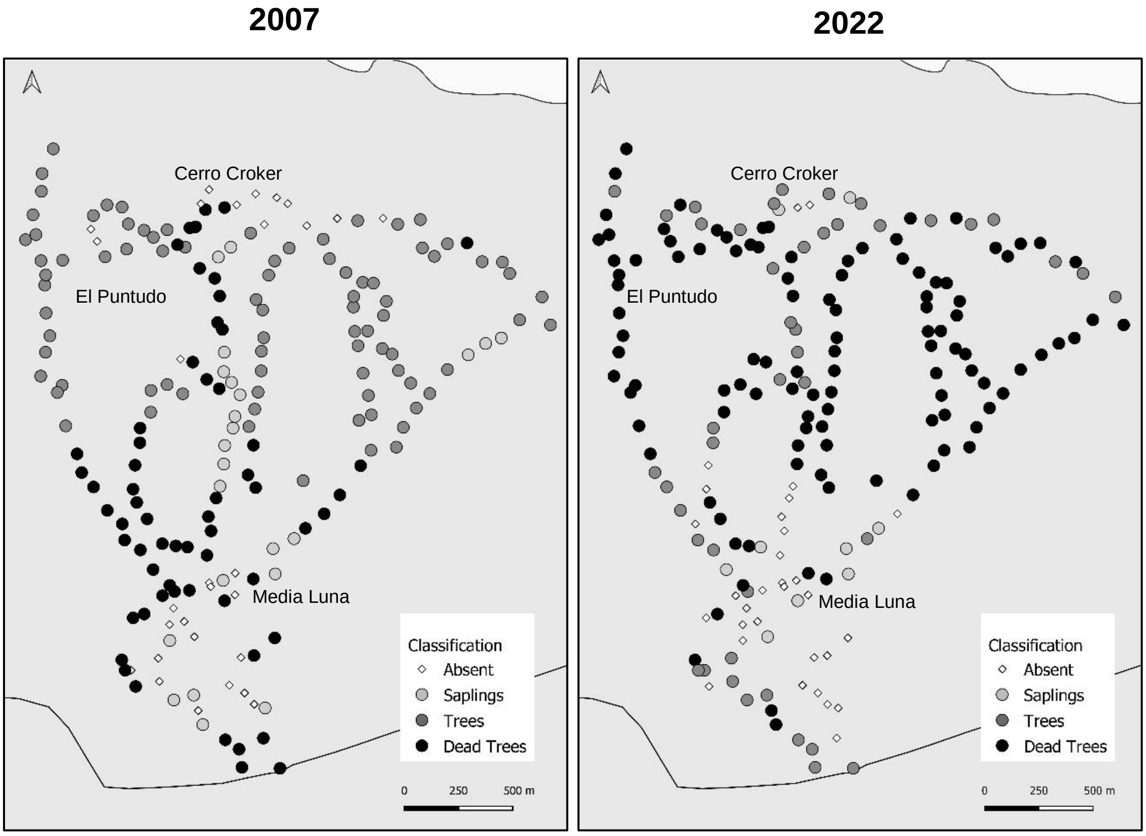


Figure 9 Map of the Highlands Media Luna study site, with the name of the main places and the distribution of the red-barked quinine tree (*Cinchona pubescens*) according to its classification, in 2007 on the left and 2022 on the right. The white dots represent points where no red-barked quinine tree was found.

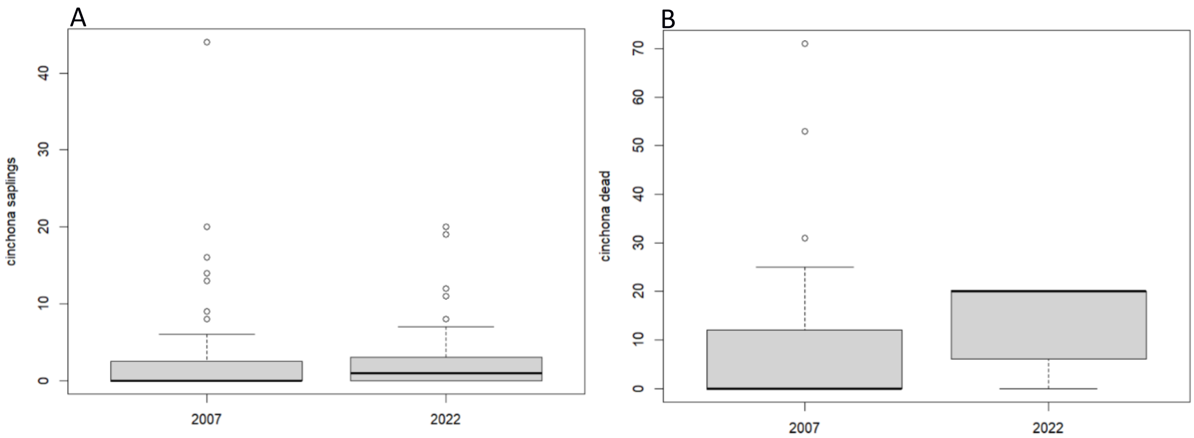


Figure 10 Boxplots with the red-barked quinine tree (*Cinchona pubescens*) occurrence in 2007 and 2022 census. A. Number of saplings. B. Number of dead trees.

The invasive hill blackberry (*Rubus niveus)* is also a threat to biodiversity in the Galápagos archipelago (Rentería & Buddenhagen, 2006), despite missing studies quantifying its impact on Galápagos rail population (Fig. 11).

Figure 11 Hill blackberry (*Rubus niveus*) on a sampling point in Highlands Media Luna. Source: Hugo Silva, Charles Darwin

Its occurrence has significantly increased in Highlands Media Luna. In 2007, it was recorded only in 10 points while in 2022 we found the species in 147 points (χ^2 = 195.85, p-val = 2.2e-16). From these points, 88 points (59.9%) were classified as 1, where the species' cover was less than 25%, 37 points (25.2%) were classified as 2, with patches of adult plants covering between 25 to 50 %, 17 points (11.5%) were classified as 3 with a cover between 50 to 75 %, forming continuous patches of adult plants, and finally, 5 points (3.4%) were classified as 4, with a cover between 75 to 100 %, preventing the growth of native vegetation. Only 50 points (25.4%) were free of hill blackberry (Fig. 12).

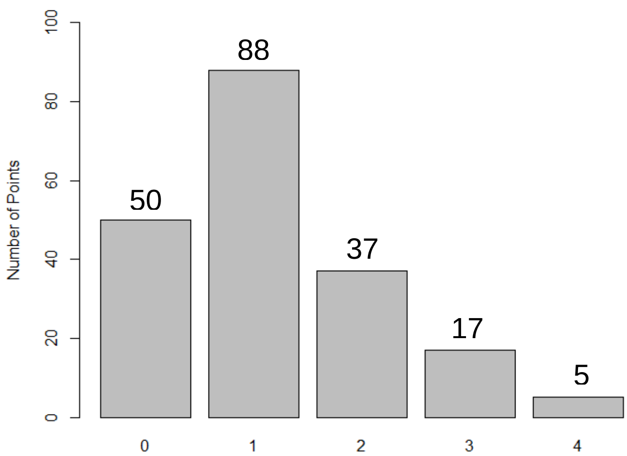


Figure 12 Number of survey points per hill blackberry (*Rubus niveus*) classification.

Fig.13 shows a map of Highlands Media Luna with the species distribution at the sampling points used to count Galápagos rail, both in 2007 and 2022. Points are in different colours according to hill blackberry classification. We did not find significant differences (Fisher test, p-value = 0.5151) in presence/absence of hill blackberry between points where Galápagos rail was detected and points where it was not detected. No rails were counted in the few points that showed extremely dense hill blackberry growth. Thus, we should be cautious concluding that this invasive species does not impact Galápagos rail populations.

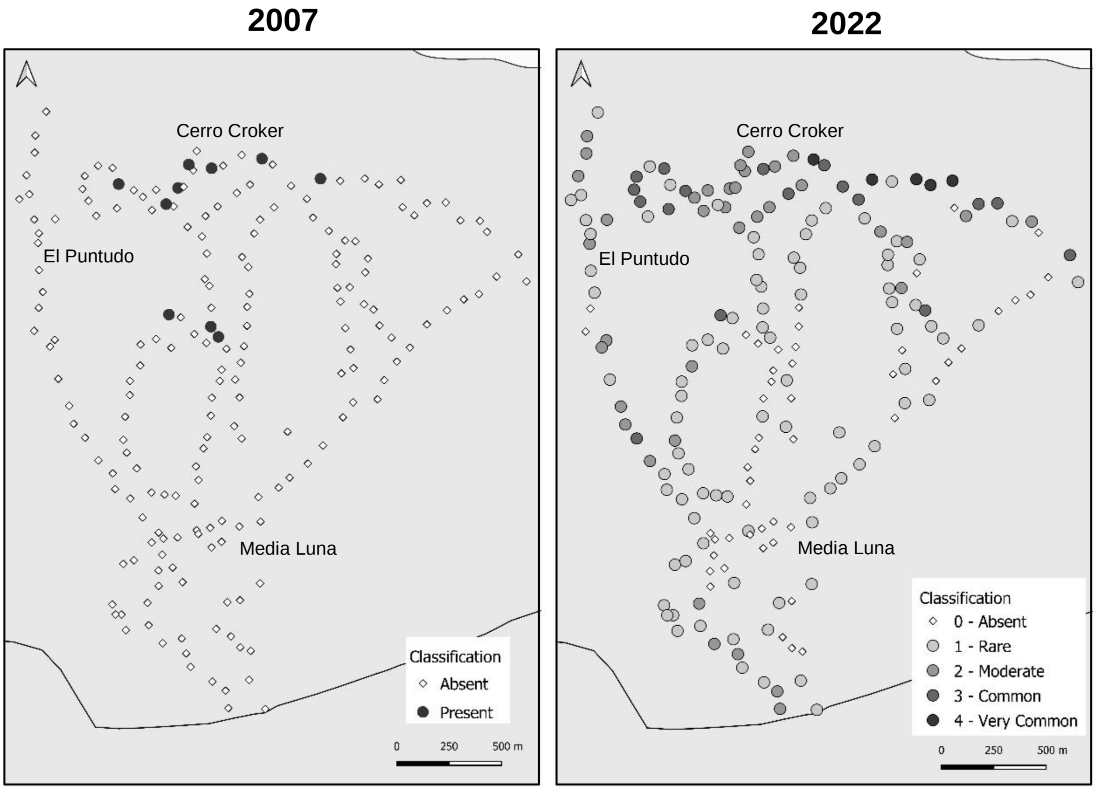


Figure 13 Map of the Highlands Media Luna study site, with the name of the main sites and the distribution of hill blackberry (*Rubus niveus*) according to its presence/absence in 2007 (left) and classification in 2022 (right).

3.1.3. Vegetation model

We did not find strong correlations among the 7 vegetation variables we have chosen for the vegetation model (see section 2.4.) (Fig. 14). Thus, we included all the 7 variables in the model to explain Galápagos rail abundance, according to vegetation characteristics.

						AHS
					moss	0.3
				grass	-0.5	-0.1
			fern	-0.4	-0.1	0.1
		vis	0.2	-0.3	0.3	0.2
	HGC	-0.1	0.1	-0.1	-0.1	0.3
ExTrees	0	0	-0.1	0.1	0	-0.3

Figure 14 Correlation matrix for 7 vegetation variables: Number of exotic trees (ExTrees), % High ground cover (HGC), Visibility (vis), % of fern (fern), % grass (grass), % of moss (moss), and Average Height Shrub layer (AHS).

The General Linear Model (GLM) was selected with an AICc of 436.8, almost 100 units below the second ranked tested model (Table A1-Appendix A).

The best model suggested that Galápagos rail's abundance in Highlands Media Luna can be explained by the percentage of visibility on the point counts. This variable represents the density of the vegetation at 30 cm height. Thus, the Galápagos rail's abundance seems to increase with the density of the vegetation and tends to decrease in points where the vegetation is more open (Tab. 4). We found no evidence that other variables influence the abundance of the species. However, the overall correlation coefficient of the best model is low (r^2 = 0.07) which indicates that the relation we found, despite significant, is weak.

Table 4 Estimates of main effects of vegetation on abundance of Galápagos rail (*Laterallus spilonota*), obtained from the best GLM model identified.

Variables	Estimate	2.5%	97.5%	Adjusted SE	P
% of grass (grass)	0.116	- 0.003	- 0.024	0.078	0.137
visibility (vis)	-0.160	- 0.295	- 0.024	0.069	**0.020***
Exotic trees (ExTrees)	-0.018	- 0.206	- 0.048	0.045	0.692
High ground cover (HGC)	0.004	- 0.090	- 0.167	0.026	0.856
% of moss (moss)	-0.003	- 0.177	- 0.120	0.027	0.905

3.2. Distribution of the two rail species in Santa Cruz

3.2.1. Rail Census

Our 2022 surveys at Santa Cruz Island conducted at four sites with different habitat types and altitudes recorded 163 Galápagos rails (*Laterallus spilonota)* and 36 paint-billed crakes (*Mustelirallus erythrops)* (Tab. 5 and Fig. 15).

Table 5 Number of points, number of individuals (Ind) of Galápagos rail (*Laterallus spilonota*), and paint-billed crake (*Mustelirallus erythrops*), birds per point (birds/point) and % of points where birds were detected (%Points+) at the four study sites sampled in Santa Cruz Island.

Site	Nº points	*Laterallus spilonota*			*Mustelirallus erythrops*		
		Ind	birds/point	%Points+	Ind	birds/point	%Points+
Highlands Media Luna	197	142	0.72	47.7%	0	0	0.00%
Perimetral Santa Rosa	31	9	0.29	22.6%	12	0.39	32.3%
Mina Roja	44	11	0.25	20.5%	14	0.32	22.7%
Perimetral Media Luna	36	1	0.03	2.8%	10	0.28	25.0%
TOTAL	308	163	0.53	52.9%	36	0.12	11.7%

In Perimetral Santa Rosa, Mina Roja and Perimetral Media Luna both species were detected with similar numbers, except for the Galápagos rail at Perimetral Media Luna with only a single record.

Because the four sites differed in their habitat composition and altitude (Tab. 6), it is difficult to depict a clear pattern regarding the distribution of both species. There seems to be a certain split-up in relation to altitude, with paint-billed crake being predominantly detected at lower altitudes compared to Galápagos rail more frequently recorded at higher altitudes. Apparently, this seems to apply for the two perimetral sites. However, other factors may be involved at (1) Highlands Media Luna where no paint-billed crake was detected, and (2) Mina Roja where both species overlap without any clear altitudinal segregation.

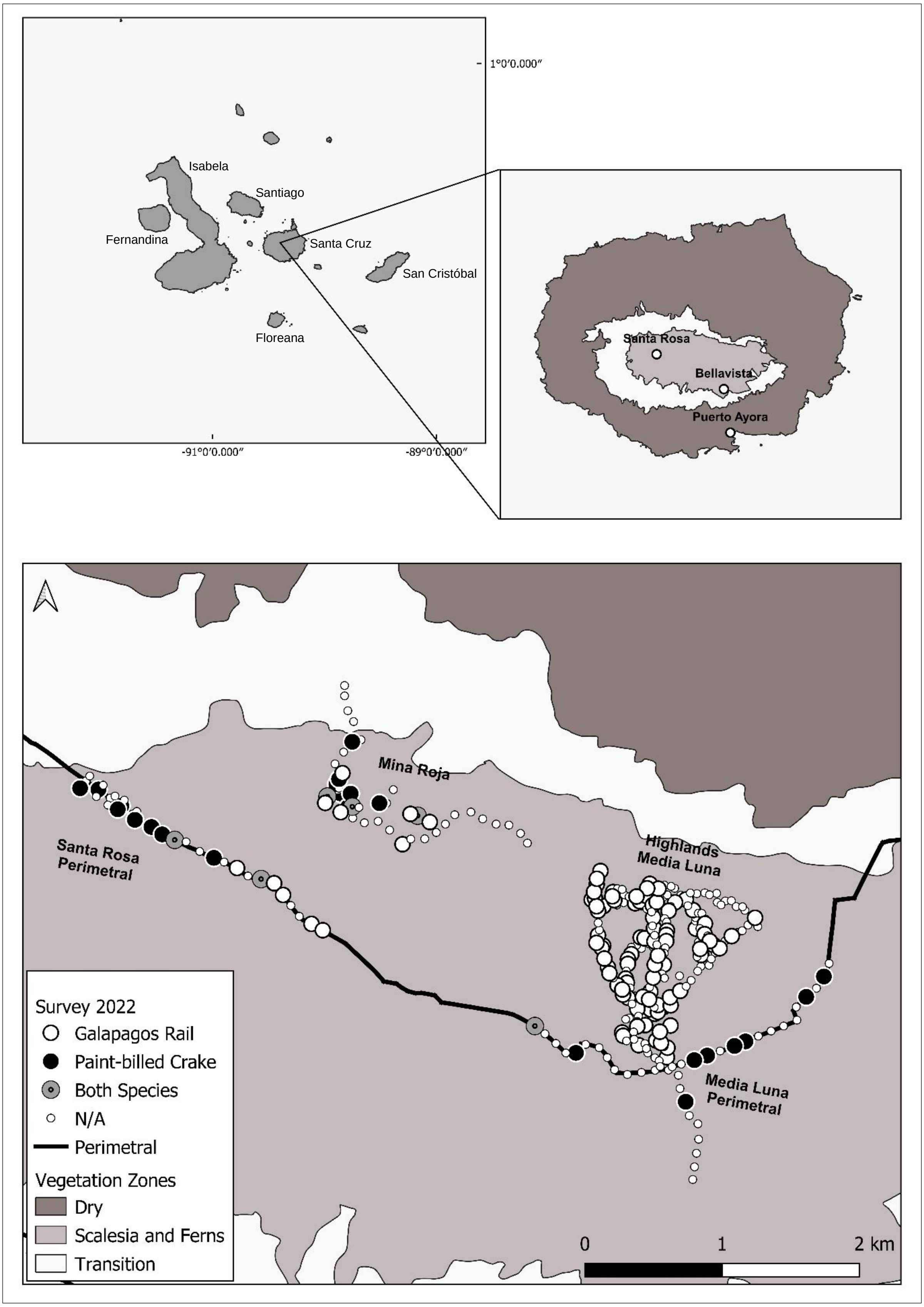


Figure 15 Map of the Galápagos Archipelago with emphasis on Santa Cruz Island on the right and a map of the four study sites with the point counts represented below. The white dots represent points where Galápagos rail (*Laterallus spilonota*) was recorded, the black dots points where paint-billed crake (*Mustelirallus erythrops*) were recorded and grey points where both species were observed. Points with no birds are represented by small white

Tabela 6 Number of individual of Galápagos rail (Gr) and paint-billed crake (Pbc) according to six elevation categories on the four study sites. N = number of points in each category; NA = elevation intervals with no sampling points.

Elevation (m)	Highlands Media Luna			Perimetral Santa Rosa			Mina Roja			Perimetral Media Luna		
	Gr	Pbc	n	Gr	Pbc	n	Gr	Pbc	n	Gr	Pbc	n
301 - 400	NA	NA	0	NA	NA	0	NA	NA	0	0	0	5
401 - 500	0	0	1	NA	NA	0	NA	NA	0	0	2	5
501 - 600	14	0	29	1	11	22	0	0	1	1	8	26
601 - 700	54	0	61	8	1	9	10	9	36	NA	NA	0
701 - 800	43	0	70	NA	NA	0	1	5	7	NA	NA	0
801 - 900	31	0	37	NA	NA	0	NA	NA	0	NA	NA	0

3.2.2. Vegetation assessment

The four study sites differ in vegetation, and this may influence the distribution of the two rail species. In here we describe the differences and similarities for all vegetation parameters measured, starting with ground layer, followed by shrub layer, and tree layer. The average, standard deviation, Kruskal-Wallis test, and p-value for each variable measured are presented in Tab. 7.

3.2.2.1. Ground layer

The highest percentage of visibility (Vis) was recorded in Perimetral Media Luna, and it was significant different to Highlands Media Luna and Mina Roja sites (Fig. 18). Visibility was predicting Galápagos rail abundance for Highlands Media Luna (see section 3.1.3.). The ground layer was characterized by a mix of plant species taller than 30 cm (HGC). Differences were found between Perimetral Media Luna that had a significant lower cover with only 54.3±36.1% compared to Highlands Media Luna and Mina Roja (Fig. 18). In Highlands Media Luna, ferns (Fern) were the type of vegetation with highest percentage of cover followed by moss, with differences between this site and the other three study sites (Fig. 18). The highest percentage of average grass cover (Grass) was 35.3±31.0% in Perimetral Media Luna (Fig. 16) and the lowest was 10.1±17.5% in Highlands Media Luna. It only had a significant difference between these two sites (Fig. 18). Bare ground (Bare) was rather common in Perimetral Media Luna (20.7±22.7%) and Mina Roja (20.6±27.0%). Significant differences with Highlands Media Luna were found, with bare ground almost absent (Fig. 18).

Figure 16 Grassland on Perimetral Media Luna. Source: Hugo Silva, Charles Darwin Foundation.

The invasive elephant grass (Egrass), *Pennisetum purpureum* (Fig. 17) had the highest percentage cover (21.0±31.0%) in Perimetral Santa Rosa. This site had significant differences with Highlands Media Luna and Mina Roja (Fig. 18).

Figure 17 Elephant grass (*Pennisetum purpureum*) field on Perimetral Santa Rosa. Source: Hugo Silva, Charles Darwin

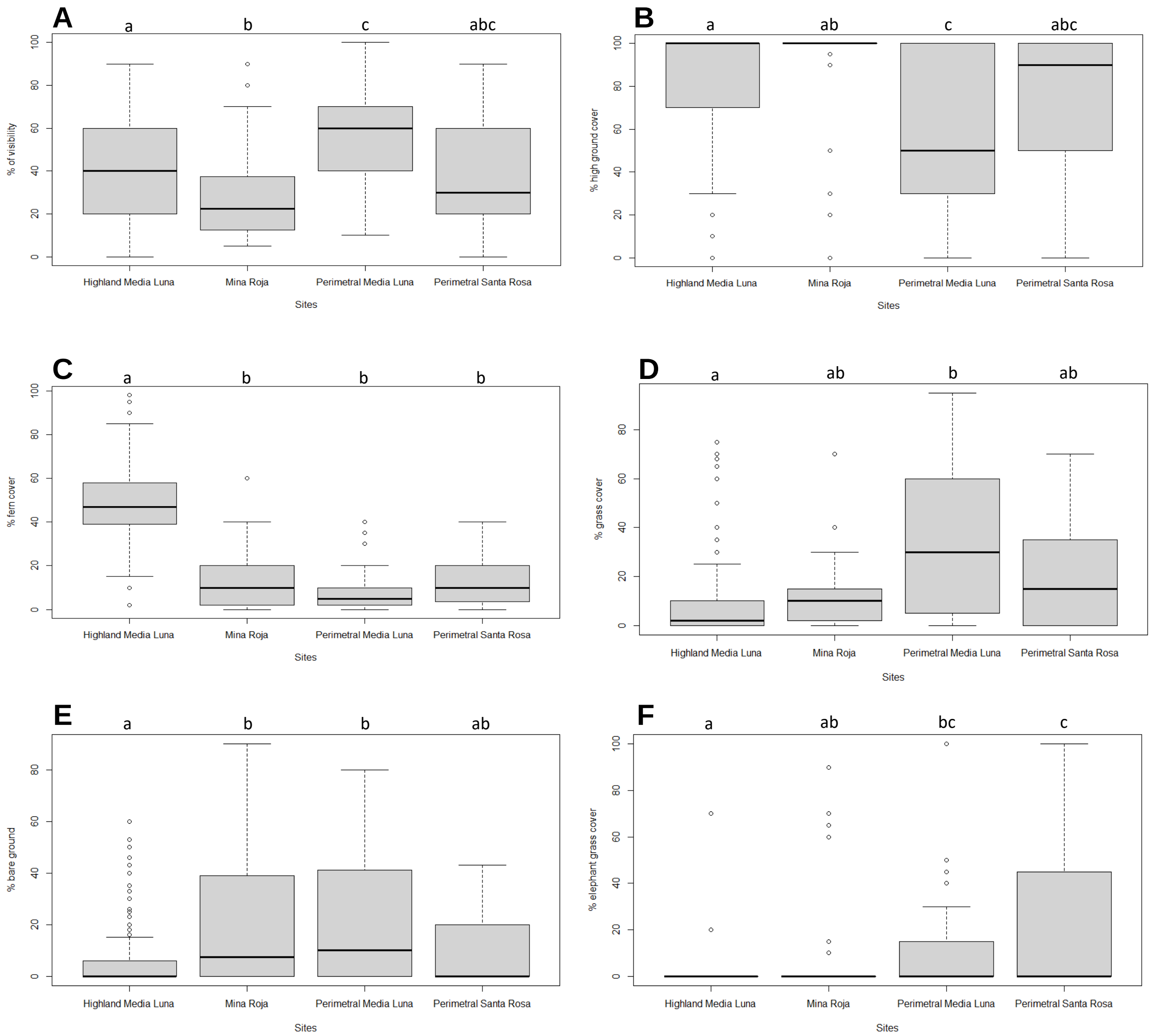


Figure 18 Boxplots with 6 variables related to the ground layer in the vegetation classification, for the four study sites. A. Boxplots of the % of visibility. B. Boxplots of the high ground cover, measured as the % covered by species taller than 30 cm. C. Boxplots of the % cover with fern species. D. Boxplots of the % cover with grass species. E. Boxplots of the % cover with bare ground patches. F. Boxplots of the % cover with elephant grass species. The letters indicate significant differences among sites.

3.2.2.2. Shrub layer

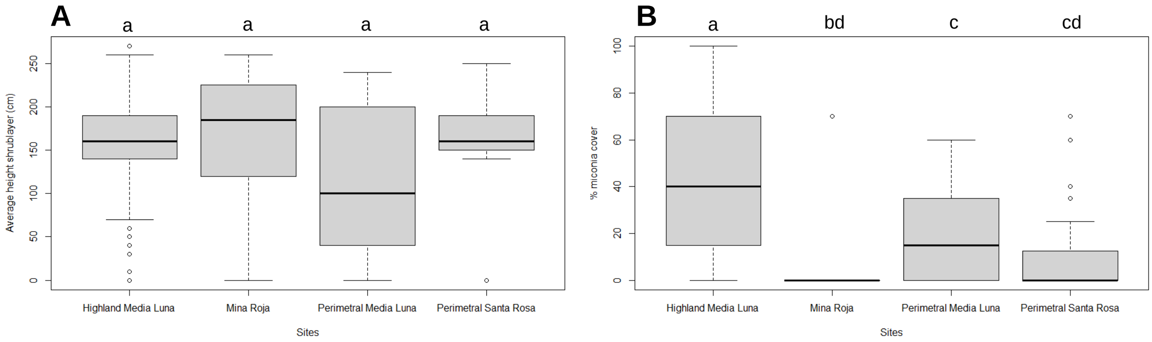
We did not find differences in average shrub layer height (AHS) between the four sites, though Perimetral Media Luna showed the lowest height with 114.6±85.9 cm (Fig. 20).

The endemic species *Miconia robinsoniana* (mico) (Fig. 19) had a average cover of 41.6±29.9% in Highlands Media Luna and was significant different to the other three sites (Fig. 20), mainly because these sites are not in the *Miconia* habitat zone.

Figure 19 *Miconia robinsoniana* in Highlands Media Luna. Source: Hugo Silva, Charles Darwin Foundation.

Other shrub species (OtherS) found in the shrub layer included mainly coffee senna (*Senna occidentalis*), white-haired tournefortia (*Tournefortia pubescens*), *Agave* sp., and the invasive hill blackberry (*Rubus niveus*).

The highest percentage was recorded in Mina Roja with 51.5±38.8%. This site had a significant difference with the other three study sites which reflects its diversity as well found for ground cover (Fig. 20).



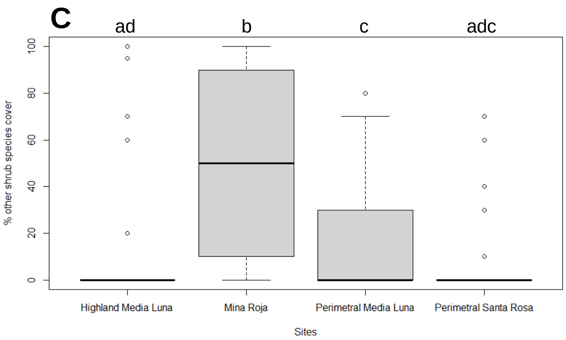


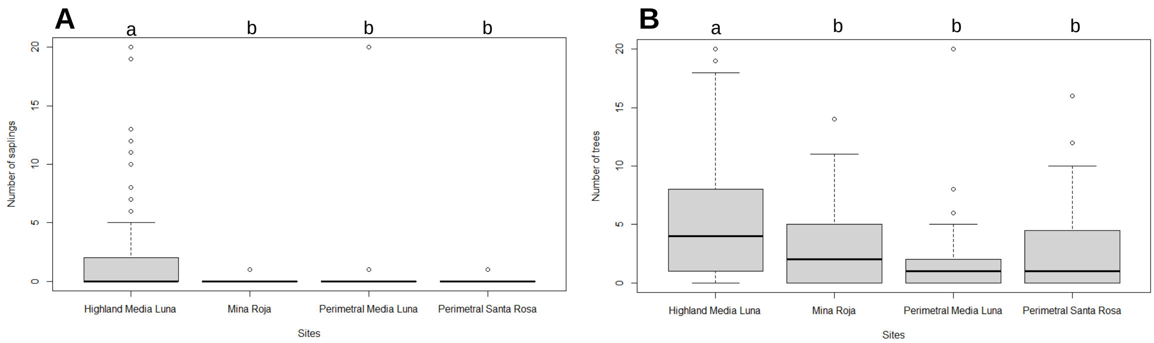
Figure 20 Boxplots with 3 variables related to the shrub layer in the vegetation classification for the four study sites. A. Boxplots of the average height of the shrublayer, in cm. B. Boxplots of the % cover with the endemic shrub Miconia robinsoniana. C. Boxplots of the % cover with other shrub species. The letters indicate significant differences among sites.

3.2.2.3. Tree layer

On the tree layer, we found an average of 1.6±3.0 saplings of the invasive species red-barked quinine tree (CincSap) in Highlands Media Luna and almost no saplings of this species at the other three sites. Similarly, there was a significant difference in the number of red-barked quinine trees between Highlands Media Luna and the other three sites (Fig. 23). The total number of trees (TotalT) was low in Highlands Media Luna. In Perimetral Santa Rosa, the main tree species was the endemic *Scalesia pedunculata* with 6.7±9.4 trees recorded (Fig. 21). In Mina Roja we recorded the highest number of trees (12.5±9.5 trees), mainly the species palo santo (*Bursera graveolens*) in the transition zone, and *S. pedunculata* and the invasive species *Cedrela odorata* in the highland zone (Fig. 22). In Perimetral Media Luna the guava tree (*Psidium guajava)*, also an invasive, was the main species as it is cultivated in this area.

Figure 21 Forest of *Scalesia pedunculata*, with the ground covered by ferns on Perimetral Santa Rosa. Source: Hugo Silva, Charles Darwin Foundation.

Figure 22 Sampling point in Mina Roja, with several different tree species. Source: Hugo Silva, Charles Darwin Foundation.



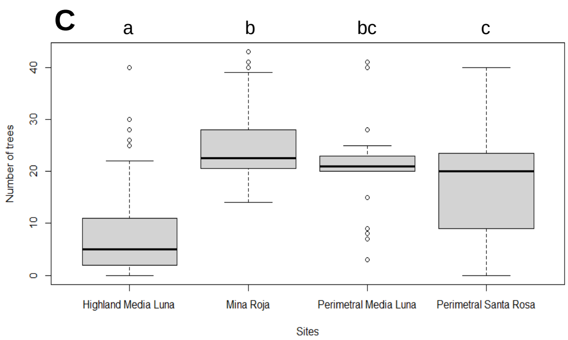


Figure 23 Boxplots with 3 variables related to the tree layer in the vegetation classification for the four study sites. A. Boxplots of the number of saplings of the red-barked quinine tree. B. Boxplots of the number of trees of red-barked quinine tree. C. Boxplots of the total number of trees. The letters indicate significant differences among sites.

Overall, we can state that the Highland Media Luna is a very special site, as dominated by one endemic shrub species, *Miconia robisoniana*, and a rather dense ground cover of ferns and mosses. It is probably the best-preserved area of the four sites studied, especially due to the management actions related to the red-barked quinine tree control. The hill blackberry is present and expanding but seems not having impacts on the rail populations.

The Mina Roja site is probably the second least disturbed area. It has a high variety of different plant species in all 3 layers, even so this includes as well invasive species. However, parts of the forest are heavily invaded and altered by the hill blackberry. Most of the areas visited in this study have had a control of hill blackberry and other invasive plants over the last 3 years as part of a management experiment to stabilize the population of a small passerine, the little vermilion flycatcher, *Pyrocephalus nanus*.

The two perimetral sites are adjacent to the Galápagos National Park area and are more infested by invasive species, including ground (elephant grass), shrub (hill blackberry) and tree layer (guava tree).

Table 7 Average and standard deviation of vegetation variables for the four study sites in Santa Cruz Island.

Variables	Highlands Media Luna	Perimetral Media Luna	Perimetral Santa Rosa	Mina Roja	Kruskal Wallis test
% Visibility (Vis)	39,2 ± 20,9	52,7 ± 25,8	38,4 ± 23,7	27,6 ± 20,2	24.18 P=2.3e-05
% High Ground Cover (HGC)	82,3 ± 29,8	54,3 ± 36,1	74,8 ± 31,6	86,5 ± 31,4	29.63 P=1.65e-06
% of Fern (fern)	46,4 ± 17,8	10,1 ± 12,2	14,4 ± 13,0	14,2 ± 15,3	161.09 P=2.2e-16
% of moss (moss)	28,5 ± 18,3	3,0 ± 5,4	6,1 ± 10,5	2,6 ± 8,0	127.72 P=2.2e-16
% of Grass (grass)	10,1 ± 17,5	35,3 ± 31,0	20,0 ± 22,1	11,5 ± 13,0	34.12 P=1.87e-07
% of Bare Ground (Bare)	5,7 ± 12,2	20,7 ± 22,7	10,4 ± 14,1	20,6 ± 27,0	27.81 P=3.97e-06
% of Elephant grass (Egrass)	10,5 ± 4,7	10,5 ± 21,1	21,0 ± 31,5	6,7 ± 20,6	63.44 P=1.08e-13
Average Height Shrublayer (AHS)	158,2 ± 53,0	114,6 ± 85,9	147,1 ± 78,9	152,6 ± 89,6	5.51 P=0.14
% of Miconia (Mico)	41,6 ± 29,9	19,3 ± 19,4	11,2 ± 20,3	1,5 ± 10,3	105.95 P=2.2e-16
% of other Shrub species (OtherS)	2,4 ± 13,6	15,8 ± 26,4	10,0 ± 21,0	51,5 ± 38,8	128.44 P=2.2e-16
Number Cinchona saplings (CincSap)	1,6 ± 3,0	0,6 ± 3,3	0,0 ± 0,2	0,0 ± 0,2	39.17 P=1.60e-08
Number Cinchona trees (CincTree)	6,1 ± 6,5	2,1 ± 3,7	2,9 ± 4,1	3,0 ± 3,7	24.47 P=1.99e-05
Number total trees (TotalT)	0,1 ± 0,4	3,00 ± 6,9	6,7 ± 9,4	12,5 ± 9,5	134.3 P=2.2e-16

3.2.3. Interaction between the two rail species

In this section we are describing the interactions between the two rail species and describing which variables predict the abundance of Galápagos rail and paint-billed crake, separately. Subsequently, we describe how much the two rail species overlap in their habitat requirements.

In Fig. 24 a PCA with all points is shown. Visually it seems that points with both species, points with only Galápagos rail, points with only paint-billed crake and points with no birds overlap in a very central cloud of bird points. However, the right side of the Galápagos rail cloud seems to be isolated from the other points. In Fig. 25 we present a PCA where points with no birds have been removed and thus, the isolation of the Galápagos rail cloud is clearer. These correspond to points in Highlands Media Luna, where vegetation is different and where this species is very common (see section 3.1.2.).

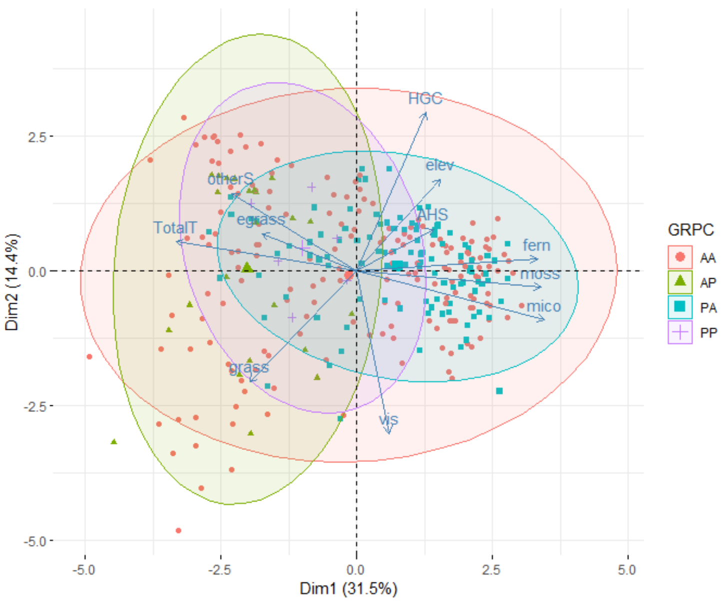


Figure 24 Principal components analysis (PCA) of all relevant vegetation variables measured at each point count, in all four study sites. The presence/absent of the species are coded by colour: Red for points where both species were absence (AA), purple for points where both species where present (PP), blue for points where only Galápagos rail (*Laterallus spilonota*) was present (PA) and green for points where only paint-billed crake (*Mustelirallus erythrops*) was present (AP).

The points that correspond to the presence of Galápagos rail seem to be more influenced by the principal component 1 (dim1) variables. It is also possible to verify that the points corresponding to the presence of paint-billed crake seem to be influenced by the principal component 1 (dim1) and 2 (dim2) variables.

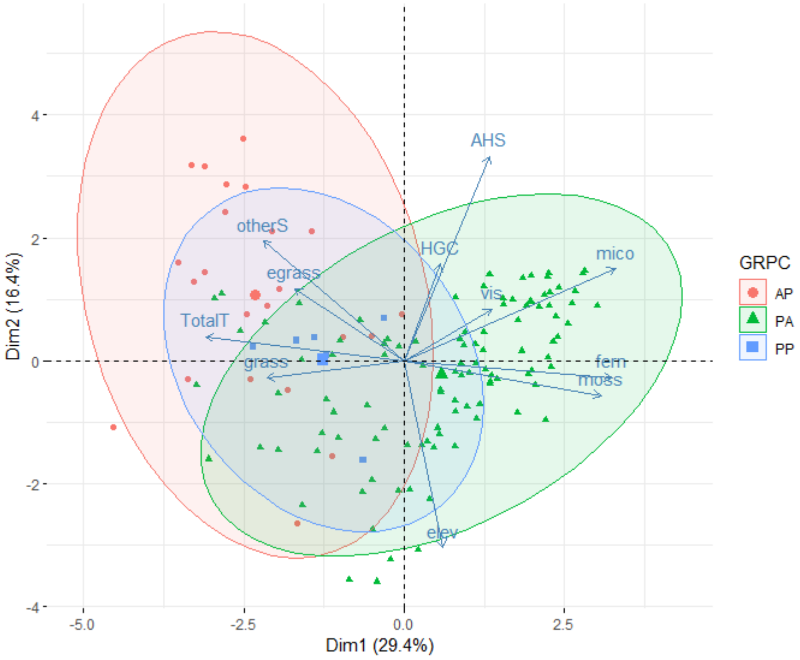


Figure 25 Principal components analysis (PCA) of all relevant vegetation variables measured at each point count, in all four study sites, without the points where both species were absent. The presence/absent of the species are coded by colour: Blue for points where both species where present (PP), green for points where only Galápagos rail (*Laterallus spilonota*) was present (PA) and red for points where only paint-billed crake (*Mustelirallus erythrops*) was present (AP).

To explain Galápagos rail and paint-billed crake abundances with the help of a General Linear Model (GLM), we only considered ground vegetation variables and elevation. Since no strong correlations between the 7 vegetation variables were found (see section 2.4.) (Fig. 26), we included all the variables in the models.

						moss
					egrass	-0.3
				grass	-0.1	-0.4
			fern	-0.4	-0.4	0.4
		vis	0.1	0	-0.1	0.2
	HGC	-0.3	0.2	-0.3	0	0.1
elev	0.2	-0.2	0.3	-0.2	-0.3	0.3

Figure 26 Correlation matrix for 7 vegetation variables: Elevation (elev), High ground cover (HGC), Visibility (vis), % of fern (fern), % grass (grass), % elephant grass (egrass), % of moss (moss), and % other ground species (otherG).

For Galápagos rail the GLM was selected with an AICc of 570.74, almost 200 units below the second ranked tested model (Table A2 – Appendix B).

The best model suggested that species abundance considering the four study sites can be explained by elevation (elev), the percentage of visibility (vis) on the point counts, and the percentage of cover of grass (grass) and fern (fern). As such, the Galápagos rail's abundance seems to increase with the elevation and density of the vegetation and tend to decrease in lower points where the vegetation is more open (Tab. 8).

Moreover, it seems to increase its abundance in points where grass and ferns are denser, decreasing in points with other type of vegetation. Despite that, the presence of the species seems to have a relation with the variables % of moss (moss), % of fern (fern), % of *Miconia robinsoniana* (mico) and Total number of trees (TotalT) in the PCA's, we found no evidence that other variables influence the abundance of the species. However, the overall correlation coefficient of the best model is low ($r^2 = 0.14$) and the estimators' values are small. This indicates that the relation we found, despite significant, is weak.

Table 8 Estimates of main effects of vegetation on abundance of Galápagos rail (*Laterallus spilonota)*, obtained from the best GLM model identified.

Variables	Estimate	2.50%	97.50%	Adjusted SE	P-val
Elevation (elev)	0.001	0.000	0.002	0.0005	**5.23e-03 ****
% fern (fern)	0.008	0.004	0.013	0.002	**4.38e-04 *****
% grass (grass)	0.009	0.004	0.014	0.003	**5.15e-04 *****
% moss (moss)	0.002	-0.002	0.010	0.003	4.78e-01
Visibility (vis)	-0.006	-0.011	-0.002	0.002	**3.53e-03 ****
High ground cover (HGC)	0.0004	-0.002	0.005	0.001	6.89e-01
% elephant grass (egrass)	-3.00e-04	-0.008	0.005	0.002	8.39e-01

For paint-billed crake the GLM was selected with an AICc of -18.74, comparing with an AICc of 205.12 of the second ranked tested model (Table A3 – Appendix B).

The best model suggested that its abundance can be explained by elevation (elev), and the percentage of cover of grass (grass) and fern (fern). Therefore, the paint-billed crake's abundance seems to be higher in lower altitudes (Tab. 9).

Moreover, it seems to increase its abundance in areas were grass and ferns are sparse. Despite the presence of the species seems to be related with the variables % of moss (moss), % of fern (fern), % of *Miconia robinsoniana* (mico), total number of trees (TotalT), % of cover with species taller than 30 cm (HGC), % of visibility (vis), elevation (elev), and visibility (vis) on the PCA's, we found no evidence that other variables influence the abundance of the species.

However, the overall correlation coefficient of the best model is low ($r^2 = 0.09$) which indicates that the relation we found, despite significant, is weak.

Overall, there seems to exist some relationship between the presence of the rail species and the vegetation characteristics of the different study sites, with Galápagos rail preferring more preserved sites with little changes in the native vegetation, such as Highlands Media Luna, and paint-billed crake preferring areas where the vegetation has been altered. The areas shared by both species appear to have a mixture of vegetation characteristics that favour their presence, thus overlapping their geographic distributions and interactions.

Table 9 Estimates of main effects of vegetation on abundance of paint-billed crake (*Mustelirallus erythrops*), obtained from the best GLM model identified.

Variables	Estimate	2,50%	97,5%	Adjusted SE	P-val
Elevation (elev)	-4,40e-04	-0,001	-1,00e-04	1,95e-04	**2,41e-02 ***
% fern (fern)	-0,002	-0,004	-3,00e-05	9,32e-04	**4,63e-02 ***
% grass (grass)	-0,003	-0,005	-5,00e-04	1,12e-03	**1,71e-02 ***
% moss (moss)	0,002	-0,001	0,004	1,25e-03	2,10e-01
Visibility (vis)	6,51e-04	-0,001	0,002	5,91e-04	2,71e-01
High ground cover (HGC)	-9,34e-04	-0,003	0,001	9,96e-04	3,49e-01
% elephant grass (egrass)	-7,03e-04	-0,002	0,001	8,55e-04	4,11e-01

4. Discussion

In recent years, there has been a decline of the Galápagos rail (*Laterallus spilonota)* population in Santa Cruz (Gibbs *et al.*, 2003; Shriver *et al.*, 2011) and it is urgent to evaluate if applied conservation measures were successful or if additional actions are needed to aid the species conservation. In this study, we update the population status of the Galápagos rail for Santa Cruz Island and present data that, to our knowledge, can help to better understand how habitat composition influences its abundance. We also aim to provide the first insights about possible habitat requirements for two rail species, their overlap in distribution, and the need for continued monitoring to evaluate if the rather newcomer paint-billed crake can contest territories with the Galápagos rail.

4.1. Galápagos rail*:* the role of habitat composition and invasive species in Highlands Media Luna

After several declines recorded in previous studies (Gibbs *et al.*, 2003; Shriver *et al.*, 2011), the Galápagos rail has significantly increased its occurrence and abundance in the main study site, Highlands Media Luna, between 2007 and 2022. Using the same sampling effort from Shriver *et al.* (2011), a total of 142 individuals were counted in 2022 compared to 73 individuals recorded in 2007. Based on our results, it seems that after a period of decline the Galápagos rail is roughly at the same population level of 1986 (Rosenberg, 1990). A comparison of current vegetation composition with previous studies suggests that this trend twist is related to the control of the invasive, red-barked quinine tree (*Cinchona pubescens)*. Currently, there are more dead trees of this species in the study area and a lower number of adult trees compared to 2007 (Shrivers *et al*., 2011). In fact, until 2004 the red-barked quinine tree's distribution increased on Santa Cruz Island, reaching a total of 11 000 ha (Buddenhagen *et al.*, 2004). The density of individuals was estimated at 1 individual per ha in 1987 (MacDonald *et al.*, 1988), having a significant increase until 2005 to 1873 individuals per ha (Jäger *et al.*, 2018). Then after, due to management actions, the species range declined. In 2014, the area occupied by the red-barked quinine tree has been quantified at less than 1541 ha (Trueman *et al.*, 2014). The overall reduction of species distribution and numbers is notable at our study sites (especially at the Highlands Media Luna) and is explained by the management actions carried out by the Galápagos National Park Directorate in the highlands of Santa Cruz, namely in the area between Cerro Crocker, Media Luna, Los Picachos, and El Puntudo, covering about 310ha of National Park area (GNPD, 2009). Additionally, recent studies showed a possible natural dieback of the species through an accidentally introduced pathogen, which can also contribute to a reduction of densities and distribution area of the species, but further studies are needed to assess this theory (Jäger, 2018).

The invasion of the highlands in Santa Cruz by this plant species led to profound changes in plant community and habitat structure, reducing native species diversity and cover (Jäger *et al.,* 2007). This reduction was especially pronounced for herbaceous species, with reductions of 89% in endemic species and 82% in native species in the period between 1998 and 2005 (Jäger *et al.*, 2009). Furthermore, the red-barked quinine tree has a significant negative impact on water, light, and nutrient regimes (Jäger *et al.*, 2007, 2009, 2013). Since the red-barked quinine tree has induced several changes in habitats, animal populations that are related to native ground vegetation, namely species such as Galápagos rail or Galápagos petrel (*Pterodroma phaeopygia)*, have been affected in Santa Cruz, with marked population declines (Wiedenfeld & Jiménez-Uzcátegui, 2008).

Through the management plan of the red-barked quinine tree in the Galápagos National Park, it was possible to effectively reduce the area occupied by the species, and the native vegetation recovered quickly within two years (Jäger & Kowarik, 2010), restoring the percentages of cover of herbaceous species, mosses, and ferns. The recovery of the native vegetation seems to have led to an increase in suitable habitat for Galápagos rail, and consequently to a recovery of its population in Highlands Media Luna. In fact, management actions that favoured the recovery of native vegetation on other islands of the archipelago triggered an increase of Galápagos rail (Donlan *et al.*, 2007).

Looking at the current distribution of this rail, we were interested if there were any additional vegetation variables that help to predict its abundance. According to our vegetation model, vegetation density seems to be the variable that most explains rail abundance at Highlands Media Luna. These results are consistent with what we know about this bird's biology, as it builds its nests with herbaceous plants under a dense vegetation cover (Franklin *et al.*, 1979). Thus, a characteristic of suitable habitat for the species can be described as sites with a high density of (native) ground species providing shelter and nesting sites. In addition, dense vegetation composed by mosses and ferns seems to retain moisture, and rails have shown affinity for the presence of water in their habitat (Donlan *et al.*, 2007). Unfortunately, we did not include the variable 'water' in our study. Permanent water points do exist in Highlands Media Luna but are absent from the two Perimetral sites or Mina Roja. When considering the vegetation model including all four study sites, beside visibility, we found percentage of fern and grasses to have a positive effect (the denser it is, the higher the bird abundance is as well) and elevation - with Galápagos rails occurring at higher elevation which is mostly again explained by the many points in Highlands Media Luna. However, the effects of the models are low and are thus not helpful in predicting distribution or abundance of Galápagos rail in different areas.

Predation by alien species is another known limitation for wildlife. Rodents and mainly the black rat (*Rattus rattus)* are widespread in Galápagos and are known to impact the breeding success of several species (Cruz & Cruz, 1996; Cruz-Delgado *et al.*, 2010, Rueda et al., 2019). Dogs, cats, and pigs also impact ground breeding species (Cruz & Cruz, 1996; Cruz-Delgado *et al.*, 2010; Philips *et al.,* 2012). A control of rats through the placement of traps with poisoned bait was developed with the aim of reducing the rodent populations in Highlands Media Luna and increases the reproductive success of Galápagos petrel, a species that shares partly the same inland habitat with the Galápagos rail (Cruz & Cruz, 1987, 1996). Despite not being one of the main causes of the decline of Galápagos rail populations (Franklin *et al.*, 1979), rat control might have reduced predation rate and thus may have also contributed to the increase of the species in Highlands Media Luna. However, since there are no data available on the effect of rat's control, we must face this possibility with caution.

Despite the decreasing threats to the Galápagos rail population in Highlands Media Luna, there may exist other threats. The hill blackberry (*Rubus niveus)* is considered the worst invasive species of the Galápagos archipelago (Rentería *et al.*, 2012a), threatening native plants of the *Scalesia* forest (Rentería &

Buddenhagen, 2006) and bird species such as the little vermilion flycatcher, *Pyrocephalus nanus* (Leuba *et al*., 2020, Mosquera *et al*., 2022) or the Galápagos petrel (Cruz-Delgado *et al.,* 2010). Our data reveal an extraordinary increase in the presence of hill blackberry in Highlands Media Luna, with the species being present in 147 out of 197 sampled points, while in 2007 hill blackberry was detected only in 10 of the same 197 sampled points. Furthermore, this invasive species was classified as abundant or very abundant in about 15% of the points. This is due to the natural dispersal capacity of the species (Landázuri, 2010), but it also expanded into areas where the red-barked quinine trees have been controlled. The manual control actions of the red-barked quinine tree cause disturbances in the surrounding vegetation and soil, facilitating the stabilisation and dispersal of introduced species (Jäger & Kowarik 2010).

Jäguer (2018) shows that the percentage cover of the red-barked quinine tree before control measures is correlated with the hill blackberry cover after 5 years of the control actions. These are worrying results with the fact that the hill blackberry is a transformer species, meaning that it causes major changes in the composition of the native flora (Pyšek *et al.*, 2004). If Galápagos rail's population was severely affected by a drastic change in habitat structure caused by the invasive red-barked quinine tree, the ideal conditions for a new threat posed by the hill blackberry and decline of the Galápagos rail's population might be ahead. However, so far, our data did not show a clear relationship between the hill blackberry density and the Galápagos rail presence. The hill blackberry creates very dense vegetation areas, a factor that can predict the presence of the Galápagos rail. Even so, at points where hill blackberry was very common, no rails were heard or seen. Recently, the Galápagos National Park Directory has been implementing some control measures for hill blackberry, namely the manual control of the species in Santa Rosa and Mina Roja (Anchundia & Fessl, 2021), preventing the expansion of this species in these areas. In the agriculture zone, farmers control it chemically, despite herbicides are not very effective in woody species like the hill blackberry (Jäger *et al*., 2019). It is therefore not possible to conclude on the impact that hill blackberry has on the Galápagos rail populations, and continued monitoring is highly recommended. Further studies are needed on this topic, and several questions arise: is the Galápagos rail tolerant to a certain density of hill blackberry? At what density level can the hill blackberry have an impact on Galápagos rail's population? What are the possible impacts of hill blackberry control on Galápagos rail population? How does the native vegetation regenerate on sites where hill blackberry was controlled?

4.2. Distribution and interactions between the two rail species

Our results show a difference in the total number obtained for Galápagos rail and paint-billed crake, with 163 individuals vs. 36 individuals, respectively. This difference was due to Highlands Media Luna site where 142 individuals of Galápagos rail were recorded and not a single individual of paint-billed crake was heard or seen. As we argued previously, these results demonstrate that Highlands Media Luna site has suitable habitat for Galápagos rail being home to one of the largest populations of the species in the archipelago (Rosenberg, 1990; Gibbs *et al.*, 2003). Though, Galápagos rail seems not to be a habitat specialist but prefers dense vegetation which provides shelter from natural predators, food, and breeding sites (Franklin *et al.*, 1979). Previous data have showed a broader distribution of the species across the archipelago (Franklin *et al*., 1979), where the species used to be present in mangroves and highland habitats, but currently its distribution in Santa Cruz is restricted to highland habitats such as Highlands Media Luna. There is no obvious explanation for this reduction in its distribution.

Little is known about the natural history of the paint-billed crake in the Galápagos Islands. It was listed as "resident" in 1973, when few nests were detected (Harris, 1973), and since then it has expanded its distribution, being present on the four inhabited islands of the archipelago. In Highlands Media Luna, there is no clear indication about the reason why the species is not present. It can be due to habitat conditions, due to aggressive interactions with Galápagos rail, its high density at this site, or altitude. Further studies related to paint-billed crake are needed to understand its current distribution, habitat and food requirements and which factors affect its distribution.

If we only consider the number of birds recorded at the Perimetral sites and Mina Roja, 21 Galápagos rail were recorded vs. 36 individuals for paint-billed crake. This shows that outside Highlands Media Luna the population of Galápagos rail is probably small and with a patchy distribution. At Perimetral Media Luna, the Galápagos rail occurs at very low frequencies, confirming results of previous studies (*e.g.* Gibbs *et al.*, 2003). At this site we observed a degradation of the native habitat due to farming and grazing, favouring the presence of invasive plant species, and consequently reducing the suitability for Galápagos rail. Although Perimetral Media Luna seems to be not a good site for Galápagos rail, paint-billed crake was present in this area, recorded mainly in areas with elephant grass and guava trees, suggesting that probably this bird species is less impacted by alien vegetation.

Both species were present in the Perimetral Santa Rosa and Mina Roja, overlapping in its distribution. Analysing the vegetation data, these sites have patches of native vegetation, typical from *Scalesia* and *Miconia* zones, and patches of non-native vegetation. Galápagos rail seems to use areas with dense vegetation provided by the presence of native vegetation. By contrast, paint-billed crake seems to be less dependent on it, or even avoid this type of vegetation, as it is considerably bigger and cannot move easily through dense vegetation. We found that in Santa Cruz paint-billed crake is present in sites with low elevation, from 300 to approximately 700 m (while Galapagos rail occurred from 400 to 863 m), and in habitats with changes in the natural vegetation structure. Although this can explain its preference for agricultural areas, it is important to mention that with our models we cannot predict in a comprehensive way which factors affect the distribution of both species, based only on vegetation variables.

There are other factors that may influence the distribution and interaction of these two species, and it is important to consider them in future studies. Differences in both species' biology may play an important role for its distribution, namely feeding strategies. We know that Galápagos rail feeds mainly on soil invertebrates like ants, dragonflies, moths, isopods, spiders, amphipods, and snails (Franklin *et al*., 1979; Hill, 2020). However, no data on food availability for Galápagos rail is available, and it would be interesting to verify if food availability affects the distribution of the species. The change in land use with consequent modification of vegetation composition as well as the higher use of herbicides and pesticides in the agricultural zone may impact the soil invertebrate community (Bastida *et al*., 2020; Gunstone *et al*., 2021). Besides, invasive invertebrate species, such as the little fire ant (*Wasmannia auropunctata*), may also have consequences on the invertebrate community (Wetterer & Porter, 2003; Causton *et al*., 2005). These threats could reduce the availability of food for Galápagos rail and influence its distribution. However, we reinforce the lack of data on this subject and studies focusing on invertebrates' community and abundance in highlands of Santa Cruz are needed to take solid conclusions about this topic. Paint-billed crake also feed on soil invertebrates, but we also noted that in Santa Cruz this species feeds on fruits from the agriculture zone. During the fieldwork we talked with local farmers about the species, and they have reported seeing the species flying near banana trees and feeding on these fruits. These apparent differences in biology and ecology between the species can help to explain the differences between the preferred habitats for both species.

It is known that rails defend their territories through vocalizations (Franklin *et al.,* 1979; Stiles & Levey, 1988; Taylor & van Perlo, 1998; Budka & Osiejuk, 2013; Depino & Areta, 2017) and this might be another factor to influence the distribution and interactions between these species. These vocalizations also serve to communicate among individuals either of the same or different species (Jedlikowski *et al.*, 2021). During our fieldwork it was noticeable that Galápagos rail reacted more easily to playbacks than paint-billed crake thus showing a greater detectability. Moreover, outside Highlands Media Luna we noticed both species sometimes replied to playbacks from the other species. In addition, Galápagos rail seems to be more reactive to paint-billed crake playback than paint-billed crake to Galápagos rail. This might be a sign of interspecific territorial behaviour as Depino & Areta (2017) showed on the coast of Río de la Plata with two sympatric *Laterallus* crakes, but only telemetry studies can prove this (e.g. Depino & Areta, 2020). There are examples where rails establish strong bonds with partners during the reproductive season, and defending their territories together (Meanley,1957; Kaufmann, 1983; Wanless & Hockey, 2008), being intolerant and aggressive to other bird species, including rails species (Ciach, 2007). However, and since rails are hard to see and study, there is very limited information about the intra- and interspecific interactions, which means we cannot state if the territorial behaviour can be used to explain Galápagos rail and paint-billed crake distributions and interactions.

To assess this issue, continuous monitoring is important for data collection and creation of robust models to help clarify the distribution and interaction between these species. In Appendix D of this study, a long-term monitoring plan for Galápagos rail is presented, prepared to be applied in the field and collect data for 10 years. We think this data combined with other programs/studies to cope the gaps listed throughout this discussion may support an explanation for this issue.

4.3. Overall recommendations

Recommendations resulting from our study can be divided into two parts. In first, we propose conservation actions that we find useful when applied in the field, with positive results for Galápagos rail population. Secondly, we focus on the missing knowledge we have about these two species and propose future studies that may contribute to the increase of knowledge about these rails in Galápagos archipelago.

Since invasive species are one of the main causes of Galápagos rail decline and because the species has reacted positively to the red-barked quinine tree management actions, we recommend the maintenance of the control of this plant species in Highlands Media Luna, with the goal to ensure that re-sprouting stems as well as saplings are constantly removed manually.

During our study a rapid spread of the hill blackberry distribution in Highlands Media Luna was observed. This may be the next major threat to Galápagos rail and/or Galápagos petrel and we recommend the manual control of this plant species in Highlands Media Luna, uprooting whenever possible, individuals of hill blackberry. These actions will reduce its dispersal, helping to slow down the speed at which the species spreads. In areas where hill blackberry forms dense and continuous patches, we recommend cutting small areas with the minimum of intervention (as for example in the surroundings of the trail in Cerro Croker). After that, the cut plants must be removed from the ground. This will prevent dead plants from creating dead ground cover, and enable the regeneration of native vegetation. In Mina Roja and Perimetral Santa Rosa it is also recommended that the control of this species continues.

However, it is recommended to consider that Galápagos rail is probably affected in the areas that are controlled until the native vegetation grows, as the removal of hill blackberry leads to disturbances in the surrounding vegetation and soil. Although the control of hill blackberry in these areas does not have Galápagos rail as a focus, implementing measures that ensure the regeneration of native vegetation as quickly as possible together with the hill blackberry control may bring benefits to this and other species.

Besides the control of invasive species, it is of utmost importance to disseminate and inform the local community, namely farmers adjacent to the *Miconia* zone, about the results obtained and information about the rail species of Galápagos. This recommendation comes from the fact that during the fieldwork we noticed that there is a general lack of knowledge about the species shown by local people often confusing species identification. Usually people use the term "Pachay", the Spanish common name for Galápagos rail, to refer to any of the rail species, leading to misidentification of the species. Since paint-billed crake has possible impacts in agriculture and is often mistaken with Galápagos rail, farmers can eventually show a negative attitude against the endemic species. It is therefore essential to teach and use the correct terminology when we talk about these species. We can achieve these goals through informal talks, workshops with farmers, or walks in Media Luna to hear the Galápagos rail and explain its biology and ecology to the participants. Also, the distribution of leaflets showing the differences between these two species – like the one we developed during our study – might be useful (see Appendix C). For tourism, it is important to maintain the information panels located around Media Luna in good conditions, to inform and alert tourists about the presence and threats to this endemic species. Finally, it is recommended to implement the long-term monitoring plan for Galápagos rail (see Appendix D) in the next 10 years to ensure a monitoring of the population in Highlands Media Luna and to adjust the resources for its conservation according to the evolution of the population.

We also consider important to carry out studies on the biology, ecology, and phylogeny of paint-billed crake like those conducted for the Galápagos rail (e.g. Franklin *et al.,* 1979, Rosenberg, 1990; Chaves *et al*., 2020). We know that this is a huge challenge, but we believe it is an essential step to increasing the knowledge on these two rail species in Galápagos islands. Related to Galápagos rail, it is important to quantify the impacts of hill blackberry on its population and understand its food requirements to better shape future management plans. Finally, we consider that studies based on the vocal behaviour of Galápagos rail and paint-billed crake may give insights to their territorial interactions, and help to develop more effective methods to monitor the paint-billed crake.

4.4. Final remarks

Our work showed that the population of Galápagos rail is currently stable or even increasing in the *Miconia* zone of Santa Cruz Island, probably due to the efforts made for habitat recovery, namely the control of the invasive red-barked quinine tree. Furthermore, it is shown that vegetation might play a role in the distribution of the two rails species in Santa Cruz. However, we recognize that this is a baseline study about the role of habitat composition and disturbance in the distribution of Galápagos rail and paint-billed crake, and more data is needed to develop a management plan that not only help to maintain Galápagos rail population but also help to include the native paint-billed crake in the conservation's efforts in the archipelago. Nevertheless, our study reinforces the idea that it is imperative to control or eradicate invasive plant species in key areas such as islands (CBD, 2021) and prevent invasions of new potential threats through the enforcement of biosecurity measures, as invasive species are a primary driver of native biodiversity loss worldwide, and the greatest threat to endemic species that depend on native vegetation (Duncan & Blackburn, 2007). Overall, the Galápagos Islands offer great chances for endemic species conservation such as Galápagos rail, an example being where it is possible to increase an ecosystem's resilience and combine nature conservation with human livelihoods.

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6. Appendixes

Appendix A – Goodness-of-fit statistics for parametric distributions fitted to the dataset, to explain Galápagos rail abundance in Highlands Media Luna.

Table A1. Goodness-of-fit statistics for four different distributions, applied to the dataset, with the Chi-square and AICc scores. The lowest AICc score is in bold, indicating a better-fit distribution to the dataset.

Distribution	Chi-square	AICc
Poison	8.53	556.2
Normal	110.2	531.3
Log-normal	145.8	**436.8**
Negative Binomial	8.52	558.2

Appendix B – Goodness-of-fit statistics for parametric distributions fitted to the dataset, to explain Galápagos rail and paint-billed crake abundances in all study sites

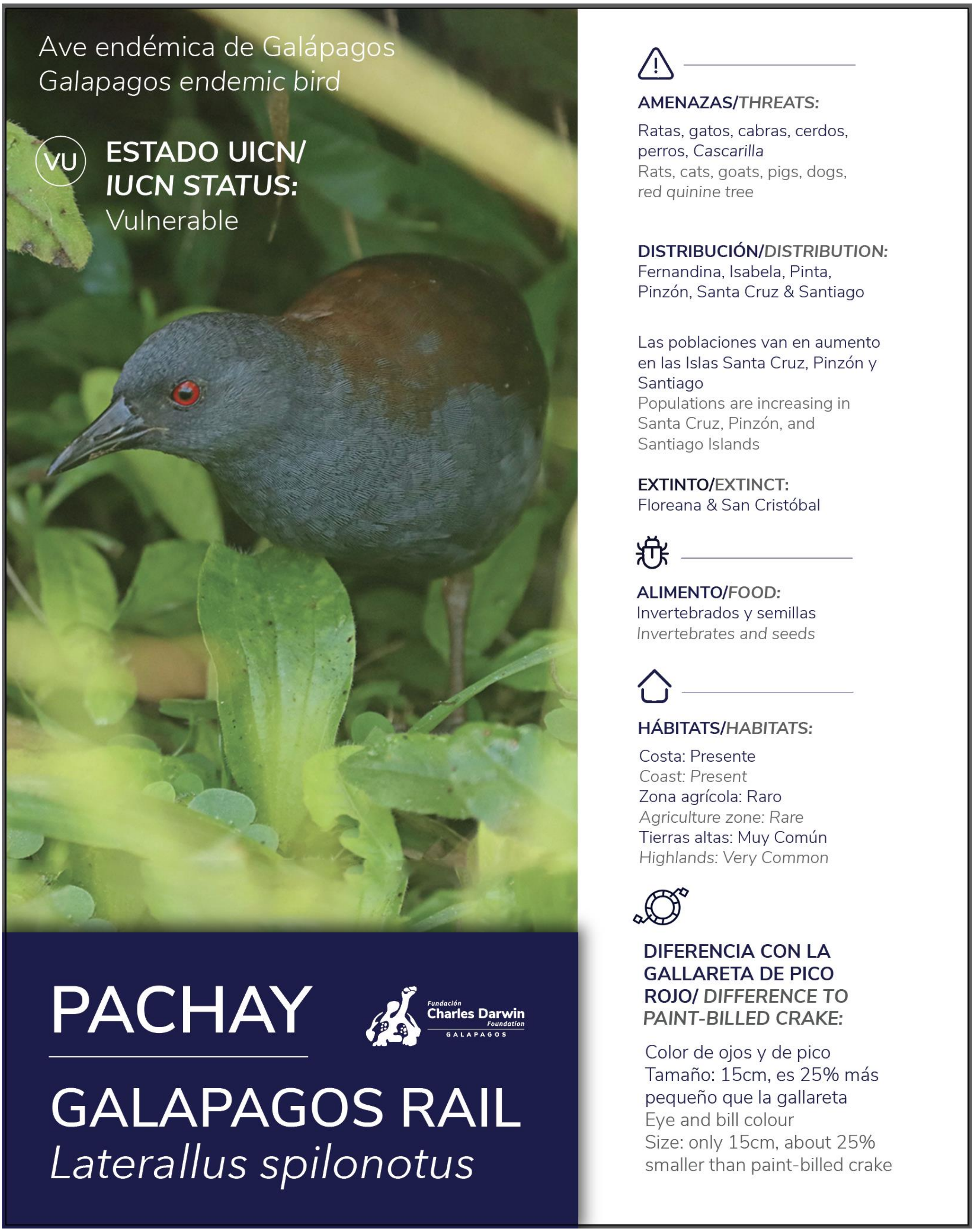
Table A2. Goodness-of-fit statistics for four different distributions, applied to the data set, with the Chi-square and AICc scores. The lowest AICc score is in bold, indicating a better-fit distribution to the dataset.

Distribution	Chi-square	AICc
Poison	15.36	823.27
Normal	227.32	768.85
Log-normal	272.99	**570.74**
Negative Binomial	15.36	825.27

Table A3. Goodness-of-fit statistics for four different distributions, applied to the data set, with the Chi-square and AICc scores. The lowest AICc score is in bold, indicating a better-fit distribution to the dataset.

Distribution	Chi-square	AICc
Poison	65.76	663.59
Normal	100.83	205.12
Log-normal	98.93	**-18.74**
Negative Binomial	65.79	665.59

Appendix C – Flyer with information about the Galápagos rail, developed based on the results of this thesis, used to share it with local people and tourists at Charles Darwin Foundation.



Appendix D – Long-term monitoring plan of Galápagos rail (*Laterallus spilonota*) in Santa Cruz, Galapagos – a Manual

Galápagos rail (*Laterallus spilonota*) long-term monitoring plan in Santa Cruz, Galápagos

Manual

Hugo Miguel Venceslau Carvalho e Silva

Birgit Fessl

Évora

2022

Table of Contents

1. Introduction

The Galápagos Islands remained uninhabited by humans until mid-sixteenth century with very few vertebrate extinctions recorded (Tye *et al.,* 2002, Phillips *et al.*, 2012). Although the islands remained almost pristine relative to other oceanic archipelagos, its unique ecosystems are suffering profound changes due to human activities (Hamann, 1984; Lawesson, 1990; Itow, 2003) with an increasing demand for goods and services, coming into conflict with the conservation goals (*e.g.* Bensted-Smith 2002). Exotic species have been present in the archipelago almost since the first settlers becoming a major threat to endemic biodiversity, and are the primary agents driving biodiversity impacts (Hamann, 1979; Cruz & Cruz, 1987; Loope *et al*., 1988). These disturbances are transforming the natural ecosystems and depleting native and endemic species populations (Bensted-Smith, 2002).

The Galápagos rail *Laterallus spilonota* is an endemic bird that has been described to be in decline and for which specific management actions have been carried out at Media Luna, on Santa Cruz Island (Gibbs *et al*., 2003; Shriver *et al*., 2011). The major reason for the species decline is the invasion of its habitat by the exotic plant species red-barked quinine tree *Chinchona pubescens*. In the late 1970s, a management project was initiated by the Galápagos National Park Directory aiming to control the dispersal of this invasive species and reducing its abundance and by 1981 over 30,000 trees had been destroyed (van der Werff, 1979; Hamann, 1984). Since then, the red-barked quinine tree has continued to spread, leading to a second control programme that was carried out between 2001 and 2006.

Due to the Galápagos rail's population status, classified as Vulnerable by IUCN (IUCN, 2020) along with the threats it faces, monitoring plans can be a useful tool to foresee the trend of its population. Moreover, if monitoring delivers robust data, it is possible to coordinate management actions to maintain or increase rail populations in the future.

The goal of this long-term monitoring plan is to provide data with adequate power to detect trends in Galápagos rail population of Santa Cruz in the next 10 years with a moderate sampling effort, low-cost fieldwork, and high confidence results. In this manual, we describe the steps to be taken to implement the plan.

2. Action plan

- **Target species**

The Galápagos rail (*Laterallus spilonota)* is an endemic species from the Galápagos archipelago and was first recorded by Darwin (1896). It is classified as "Vulnerable" (VU) (IUCN, 2020) and the main threats are the presence of exotic species and the loss of habitat. Currently, it is found on 5 islands (Shriver *et al.,* 2011) namely Fernandina, Isabela, Pinta, Santiago and Santa Cruz and a small population was discovered in Pinzón (Fessl, pers. comm.). Recent surveys confirmed the probable extinction of the species in Floreana (Dvorak *et al.*, 2017) and San Cristóbal (Dvorak *et al.*, 2019) and Shrives *et al*. (2011) confirmed a negative trend in Santa Cruz. In this island the species only occurs in the highlands and agriculture zone, despite historical records of its occurrence in mangroves (Franklin *et al*., 1979; Wiedenfeld, 2006).

The Galápagos rail is a small (< 15 cm) bird living in dense vegetation moving or running through clearings and occasionally flying short distances. Adults have a dark bluish-grey head and chest, with small white spots on the dark brown back. The beak is short and black, and the eyes are red. The breeding season occurs between December and March (Franklin *et al.,* 1979). It is territorial and builds nests on the ground, in the middle of dense vegetation, covering it with herbaceous plants. The species has a high repertoire of vocalizations, including calls for alarm, defence of the territory or reproduction (Franklin *et al.,* 1979).

- **Geographic extent**

This monitoring plan was designed to be applied in Media Luna site, Santa Cruz Island, which encompasses the *Miconia* and fern-sedge zone between 500 and 863 m above sea level. This area is known to be the prime habitat of the Galápagos rail in Santa Cruz. In this area, the vegetation is composed mainly by the endemic shrub *Miconia robinsoniana* and various fern species (Wiggins & Porter, 1971). It was also on this site, where the *Cinchona pubescens* control program took place in the late 1970's.

- **Frequency**

Censuses should be carried out twice a year and exact periods should be established according to the availability of the entity responsible for its implementation. We recommend that at least one of the census periods must cover the species' breeding season. In case the responsible entity is the Charles Darwin Foundation, we also recommend including these activities in the fieldwork plan of the Landbird Conservation Project.

- **Point counts**

We randomly selected 50 sampling points from the same 197 sampling points censused in 2007 by Shriver *et al.* (2011) (Fig. 1). The GPS coordinates of each sampling point are listed in table A1 - Appendix A. We also created a shapefile with the sampling points and foot trails to be used during the census, to facilitate fieldworkers to implement this plan. This shapefile can be easily imported to a GPS device and to the QGIS software to help with data analysis. The shapefile can be accessed online (see appendix B). Following the protocol suggested by Gibbs *et al*. (2003), each sampling point need to be visited 3 times in each census, which requires 6 fieldwork days to complete the censuses (25 points per day). The same points must be used along the 10 years of the long-term monitoring plan.

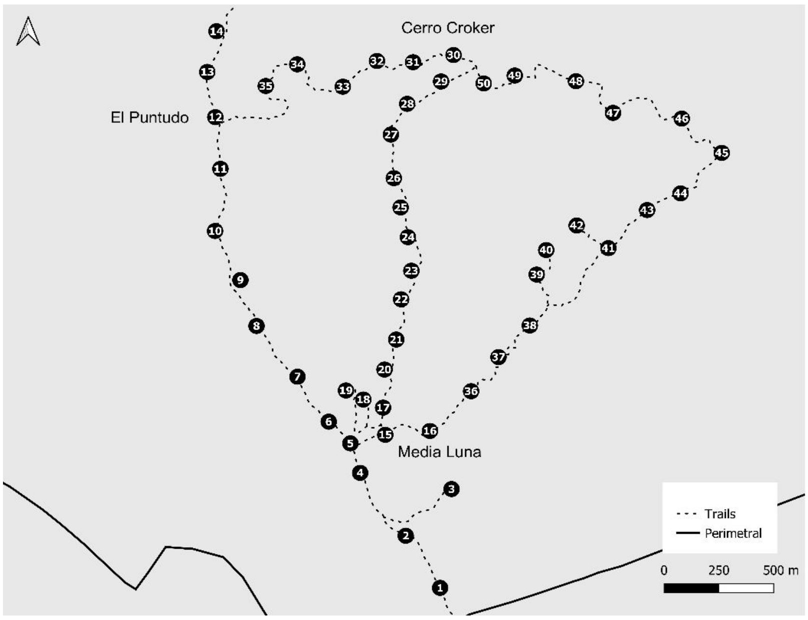


Figure 1. Map of the 50 sampling points used on the Galápagos rail (*Laterallus spilonota)* long-term monitoring plan, and the main trails used to do the census.

- **Rail census**

The census takes 9 minutes per sampling point (Fig. 2). In the first 5 minutes, the observer records spontaneous calls of the species inside the circular plot, defined as the circular area with a radius of 25 meters from the centre of the point, where the observer should be. Only birds detected more than 1 meter apart should be noted, as rails calling simultaneously from the same spot can be hard to distinguish (Gibbs *et al.*, 2003). At the same time, the sampling condition information needs to be record, such as the point number, elevation, time, and weather conditions: (1) % of clouds, (2) precipitation: 0 without rain, 1 drizzle, 2 rain, 3 heavy rain, and (3) wind: 0 no wind, 1 light wind, 2 moderate wind, 3 strong wind. Then, the observer plays a playback of the species for 1 minute, 15 seconds in the direction of each main cardinal points. The Bluetooth speaker connected to a smartphone needs to be located approximately 1.5 meters above the ground. The species' sounds can be obtained in Appendix B. After the playback, responses of the individuals to the playback needs to be noted during the last 3 minutes.

In tab. 1, we present the field sheet to collect data, prepared with all the fields that needed to be filled in. We also made a excel file with the all the same fields as in the field sheet, to help to create a dataset of the long-term monitoring plan (see Appendix B).

For vegetation assessment the observer needs to visually estimate at each sampling points the following variables: (1) CincSap: number of red-barked quinine tree saplings (plants < 50cm), (2) CincTree: number of red-barked quinine tree trees (plants > 50cm), (3) CincDead: number of red-barked quinine tree dead trees, (4) Rubus: presence of hill blackberry (index from 0 to 4: 0 = absent, 1 = rare, 2 = moderate and, 3 = common, 4 = very common), (5) %Rubus: % of cover by hill blackberry, and (6) HGC: % of cover by tall ground vegetation (>30 cm). The 25-meter radius can be measured with the help of a rangefinder in the four cardinal directions. In table 2, we present the vegetation sheet to collect data, prepared with all the fields that needs to be filled in.

Figure 2. Sequence and time of the steps used to monitor the species.

Table 1. Field sheet to use in the Galápagos rail census during the long-term monitoring plan period in Media Luna, Santa Cruz.

Galápagos Rail Long-term Monitoring Plan – Field sheet

Plano largo de monitoreo del Pachay – Ficha de campo

Date/Fecha: Observer/Observador:

Point Punto	**Visit** Visita	**Time** Tiempo	**Elevation** Altitud	Clouds Nubes	Rain Lluvia	Wind Viento	**Nº birds 5 minutes** Nº de aves 5 minutos	**Nº of birds during playback** Nº aves durante el playback	**Nº of birds after playback** Nº aves después del playback	**Notes/** Notas
1										
2										
3										
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Table 1. Cont.

Galápagos Rail Long-term Monitoring Plan – Field sheet

Plano largo de monitoreo del Pachay – Ficha de campo

Date/Fecha: Observer/Observador:

Point Punto	**Visit** Visita	**Time** Tiempo	**Elevation** Altitud	Clouds Nubes	Rain Lluvia	Wind Viento	**Nº birds 5 minutes** Nº de aves 5 minutos	**Nº of birds during playback** Nº aves durante el playback	**Nº of birds after playback** Nº aves después del playback	**Notes/** Notas
26										
27										
28										
29										
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Table 2. Vegetation sheet to use in the Galápagos rail census during the long-term monitoring plan period in Media Luna, Santa Cruz.

Galápagos Rail Long-term Monitoring Plan – Vegetation sheet

Plano largo de monitoreo del Pachay – Ficha de Vegetación

Date/Fecha: Observer/Observador:

Point Punto	**CincSap**	**CincTree**	**CincDead**	Rubus	%Rubus	%HGC	**Notes/** Notas
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Table 2. Cont.

Galápagos Rail Long-term Monitoring Plan – Vegetation sheet

Plano largo de monitoreo del Pachay – Ficha de Vegetación

Point Punto	**CincSap**	**CincTree**	**CincDead**	Rubus	%Rubus	%HGC	**Notes/** Notas
26							
27							
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- **Budget**

The costs to run the long-term monitoring plan are based on expenditure in 2022 (Table 2).

One researcher is needed for 6 days to visit each point 3 times in one census period. Thus, a total of 12 days per year are needed to follow the recommended frequency. Transportation consisted of two trips per day with taxi from Puerto Ayora to Media Luna (Bellavista) and the way back. For field material, we present a budget to buy the necessary material to start the census. However, the equipment needs to be probably replaced once throughout the long-term monitoring program. Note that the total budget was estimated only for one year of the bi-annual census and prices might change over the years.

Table 2. Costs of the long-term monitoring program, considering the 12 days needed to follow the recommended frequency. The costs are in US dollars and were estimated based on the Galápagos rail monitoring expenditures from 2022.

Expenses	Costs for 12 days (USD)
Subsistence (20 US/ day)	240
Transportation (40 US / day)	480
Field Material	
GPS device	180
Rangefinder	300
Speaker	60
Other material (notebook, pencils, boots, …)	200
TOTAL	1544

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5. Appendixes

Appendix A – Points of Long-term monitoring plan

Table A1. Coordinates of the 50 points used for the long-term monitoring plan.

Point	Latitude	Longitude	Point	Latitude	Longitude
1	-0.66496	-90.32565	26	-0.64775	-90.32755
2	-0.66277	-90.32706	27	-0.64592	-90.32767
3	-0.66081	-90.32518	28	-0.64463	-90.32700
4	-0.66013	-90.32893	29	-0.64369	-90.32561
5	-0.65889	-90.32933	30	-0.64258	-90.32510
6	-0.65798	-90.33022	31	-0.64288	-90.32676
7	-0.65609	-90.33150	32	-0.64283	-90.32824
8	-0.65396	-90.33318	33	-0.64391	-90.32964
9	-0.65203	-90.33384	34	-0.64297	-90.33151
10	-0.64997	-90.33488	35	-0.64388	-90.33280
11	-0.64736	-90.33466	36	-0.65670	-90.32438
12	-0.64519	-90.33487	37	-0.65430	-90.32400
13	-0.64329	-90.33520	38	-0.65291	-90.72014
14	-0.64158	-90.33482	39	-0.65180	-90.32169
15	-0.65853	-90.32790	40	-0.65077	-90.32130
16	-0.65837	-90.32607	41	-0.65069	-90.31875
17	-0.65739	-90.32799	42	-0.64974	-90.32005
18	-0.65705	-90.32880	43	-0.64909	-90.31716
19	-0.65700	-90.32928	44	-0.64839	-90.31579
20	-0.65579	-90.32793	45	-0.64669	-90.31410
21	-0.65453	-90.32745	46	-0.64525	-90.31574
22	-0.65284	-90.32725	47	-0.64501	-90.31856
23	-0.65164	-90.32683	48	-0.64368	-90.32008
24	-0.65023	-90.32697	49	-0.64344	-90.32259
25	-0.64898	-90.32727	50	-0.64511	-90.32291

Appendix B – Support files

The shapefile with the 50 sampling points, the species playbacks, and the Excel file to be used in the Galápagos rail long-term monitoring plan can be access in:

https://drive.google.com/drive/folders/1s4O1srQxoKhmcNSJGD0aiROi-vLIKYU3?usp=sharing