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Risk-taking behavior relates to *Leucocytozoon* spp. infection in a sub-Antarctic rainforest bird

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Abstract

Parasites inhabit practically all environments of the planet, representing an important selective pressure on different traits, including behavior. However, there is limited knowledge on the relationship between haemosporidian infection and the behavior of birds. In this study, we recorded risk-taking behaviors exhibited during three behavioral tests: struggle in the hand, open-field, and simulated territorial intrusion, in a resident population of thorn-tailed Rayadito (*Aphrastura spinicauda*) located on Navarino Island (55°S), southern Chile. This population shows a high prevalence of the haemosporidian *Leucocytozoon* spp., providing us with an opportunity to explore the connection between this parasite and the risk-taking behaviors of a Neotropical bird species. Our results indicate that birds infected by *Leucocytozoon* spp. exhibit higher activity scores in the behavioral tests, suggesting that individuals infected may be more propensity for risky behaviors. These findings raise intriguing questions about whether behavior precedes infection or results from it.

Keywords Birds · Parasites · Open-field test · Simulated territorial intrusion · Struggle in the hand

Introduction

Parasites comprise a diverse group of organisms, including viruses, bacteria, fungi, helminths, arthropods, and protozoa, all of which maintain a close relationship with their hosts (Arneberg et al. 1998). Parasites inhabit practically every environment on the planet, thriving in a wide range of environments (Frank 1993). This high diversity has been shaped by coevolutionary relationships with their hosts, resulting in an intricate interplay of adaptations strongly associated with behavior (Moore 2002).

Behavioral ecologists have researched how host behavior affects the prevalence of parasites and how this relationship impacts on fitness (e.g., Poulin 2013). This focus has revealed, for instance, that foraging is an important means of transmission for trophically transmitted parasites (e.g., Fenton and Rands 2006). These parasites use the consumption of infected prey as a primary mode of transmission, and hence, increased rates of encounter, predation, and scavenging enhance the proliferation of these parasites (Lafferty 1992). On the other hand, social behavior plays a particularly significant role in transmitting contact-transmitted parasites. Consequently, high contact rates within social animals, such as gregarious herbivores or colonies, offer

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more opportunities for parasites to move from one host to another (Altizer et al. 2003). A case in point is the parasite *Sarcoptes scabiei*, a mite responsible for causing scabies, which spreads rapidly in densely populated and socially bonded populations (Browne et al. 2022). Similarly, mating strategies are strongly associated with the transmission of sexual parasites, particularly in cases involving promiscuity or multiple sexual partners, where the potential for parasite transmission is heightened (Sheldon 1993; Thrall et al. 2000). A classic example is the mating system of the geese, where the number of sexual partners facilitates the spread of mycoplasma (Stipkovits and Szathmary 2012).

Evidence also shows that individuals with a proclivity for risk-taking behaviors are associated with higher prevalence of various parasites. For instance, Klein et al. (2004) observed that rats (*Rattus norvegicus*) with an increased prevalence of Seoul virus displayed higher levels of aggression during a simulated intrusion test. Petkova et al. (2018) found that sticklebacks infected with *Glugea anomala* were more active during open-field tests and exhibited bolder behavior during mirror tests than their uninfected counterparts. Similarly, Boyer et al. (2010) reported that Siberian chipmunks (*Tamias sibiricus*) infected with ticks displayed increased activity levels during open-field tests, and Bohn et al. (2017) found similar finding in chipmunks (*Tamias minimus*). These heightened activity levels are associated with a greater likelihood of encountering parasites and their vectors (Wilson et al. 1993), providing a plausible explanation for the link between risk-taking behaviors and parasite prevalence.

In line with this, Lafferty and Shaw (2013) identified four physiological mechanisms employed by parasites that can affect the behavior of both vertebrate and invertebrate hosts: neural system, endocrine system, neuro-modulatory pathways, and immuno-modulatory responses. These mechanisms have the potential to induce several modifications in host behavior. For instance, host behavior may be altered because of the energetic costs of fighting the infection, a phenomenon known as parasitic "constraints" (Poulin 1994; Kavaliers et al. 1999). These modifications may encompass a reduction in the competitive ability of the hosts (e.g., Barber and Ruxton 1998) and alterations in their foraging (e.g., Cunningham et al. 1994) and reproductive behaviors (e.g., Wiklund et al. 1996).

Host behavior can also be influenced by parasite strategy aimed at increasing the likelihood of infection, a phenomenon known as parasitic "manipulation" (Poulin 1994, 2013). An example can be seen in the case of trematode parasites, such as *Echinostoma californiensis*, which possess a complex life cycle involving killifish (*Fundulus parvipinnis*) as intermediate hosts and birds as final hosts. In this scenario, parasitized killifishes become more susceptible to ingestion by birds during their foraging activities, implying that trematodes induce behavioral changes in killifishes that play a crucial role in trematode transmission (Lafferty and Morris 1996). Furthermore, parasites may induce behavioral changes in their host as a strategy to mitigate the costs of infection (Hart 1990). An example of this can be observed in fish that are parasitized by ectoparasites. These ectoparasites can be removed by other organisms that perform cleaning behavior, as seen in Sikkel et al. (2004). The host's behavioral response to ectoparasites is known as parasitic "resistance," which is a strategy discussed by Grutter (1999). All these modifications are often more pronounced when the parasite imposes significant energetic demands on hosts, as discussed by Barber and Wright (2005).

Considering the varied mechanisms that could potentially underlie the relationship between parasites and host behavior, it is highly likely that reciprocity and bidirectionality characterize this interaction (Ezenwa et al. 2016). Moreover, it is important to note that the strength of the relationship host behavior and parasites can vary from weak to strong and is contingent upon factors such as the pathogenicity of the parasite (Dobson 1988), the condition of the host (Seppälä et al. 2008; Cornet et al. 2014), the host species involved (Sorci et al. 2013), and environmental conditions (Lee et al. 2016).

An interesting association between host behavior and parasites has been observed in bird populations concerning to haemosporidians (*Plasmodium* spp., *Hemoproteus* spp., and *Leucocytozoon* spp.). These are a group of blood parasites extremely widespread across the avian clade, primarily transmitted by blood-sucking arthropods (Valkiūnas 2004). Infection by hemosporidians may cause weight loss, weakness, anemia, and occasionally mortality, especially in populations of naive hosts (Atkinson et al. 2008). In birds, infection by haemosporidians has been associated to a variety of behavioral traits, including the utilization of multiple vertical strata for foraging (e.g., Fecchio et al. 2022), innovation in feeding behaviors (Garamszegi et al. 2007), the presence of cooperative social systems (e.g., Fecchio et al. 2011), and enhanced problem-solving abilities (Dunn et al. 2011). Furthermore, haemosporidian infection also has been linked with a propensity for risk-taking behaviors, characterized by increased exploration levels (Dunn et al. 2011; García-longoria et al. 2014; Remacha et al. 2023), as well as neophilia (Marinov et al. 2017), which refers to preference/exploration of novel environments or objects (Greenberg and Mettke-hofmann 2001). Additionally, it has been correlated with elevated frequencies of agonistic behaviors, such as alarm calls and aggressions during simulated territorial intrusion tests (Dunn et al. 2011; Remacha et al. 2023). Nonetheless, empirical evidence remains mixed, as some studies have not found support for these relationships (Garamszegi et al. 2015; Marinov et al. 2017), suggesting that the links between haemosporidian infection and risk-taking behaviors may vary across populations and host species.

To increase our understanding on the relationship between haemosporidian infection and risk-taking behaviors in neotropical birds, we investigate risk-taking behaviors displayed during three behavioral trials: (1) assay of struggle in of hand, (2) open-field test (OFT), and (3) simulated territorial intrusion (STI). We tested if these behaviors are linked to *Leucocytozoon* spp. infection in thorn-tailed Rayadito (*Aphrastura spinicauda*). If the infection by *Leucocytozoon* spp. is associated with risk-taking behaviors in our study population, we predict that infected individuals will exhibit higher activity scores in the behavioral trials compared to uninfected individuals.

Methods

Species and study site

The thorn-tailed Rayadito (Furnariidae: Passeriformes; Fig. 1) is a small (c. 11 g) insectivorous bird endemic to the South American temperate forests (Remsen 2006), and it is a secondary cavity-nesting bird that occupies nest boxes when available (Moreno et al. 2005; Botero-Delgadillo et al. 2017; Poblete et al. 2021a). Rayaditos are socially monogamous birds without evident sexual dimorphism, and they exhibit biparental care with equal participation of males and females in nest provisioning (Moreno et al. 2007; Espíndola-Hernández et al. 2017). A previous study on the prevalence, diversity, and drivers of haemosporidian parasites in thorn-tailed Rayadito along its distributional range in Chile (~ 3000 km; 30°–56° S) found that *Leucocytozoon* spp. was the haemosporidian with the highest prevalence (25.8%), and its distribution was positively related to decreasing temperatures and increasing precipitation towards higher latitudes (Cuevas et al. 2020). Thus, sub-Antarctic population of thorn-tailed Rayadito located on Navarino Island (55°40 S, 67°400 W; Fig. 1) showed the highest prevalence (55.4%) (Cuevas et al. 2020). The effects of *Leucocytozoon* spp. infection on this population include a decrease in their incubation period (Cuevas et al. 2021). Furthermore, on Navarino Island, early-breeding Rayaditos displayed high activity scores in the OFT, indicating a higher propensity for risk-taking than their late-breeding counterparts (Poblete et al. 2021b). This population thus provides a great opportunity to test whether *Leucocytozoon* spp. infection is associated with risk-taking behaviors.

General field procedures

We monitored 220 nest boxes that were installed in a large young patch of Magellanic forest as part of a long-term study of the breeding biology of thorn-tailed Rayadito (Moreno et al. 2007; Botero-delgadillo et al. 2020b; Poblete et al.

2021a). We captured breeding adults (2013: $n = 30$; 2014: $n = 43$) in their nests when nestlings were 12–14 days old using a manually triggered metal trap (Botero-Delgadillo et al. 2019) and then transported them inside a cloth bag to a field-portable aviary located c. 60 m from the capture site for measuring exploratory behavior (see open field test in the methods section). Subsequently, we performed an assay of struggler in the hand (see assay of struggler in the hand in the methods section) and marked birds with colored plastic bands and individually numbered them with aluminum metal bands. In addition, we measured the tarsus length for each parent to the nearest 0.01 cm with a digital caliper and recorded the age as "yearling" or "adult" according to molt/plumage-based system (WRP system; Pyle et al. 2015). Before release, a small blood sample was obtained by brachial venipuncture (ca. 75 μl) and stored on FTA cards (Whatman®) for subsequent genetic analysis and molecular sexing (Botero-Delgadillo et al. 2017). We determined adult sex by amplifying the CHD locus using the primers P2/P8 (Griffiths et al. 1998).

Characterization of behaviors

Assay of struggle in the hand

The struggle assay is a commonly used technique for replicating predator capture scenarios. This evaluation analyzes how individuals respond when held in the hand, simulating the risk of predation (Møller et al. 2013; Jablonszky et al. 2017). This assay is based on the hypothesis that individuals with superior escape skills would be more prone to take predation risks and, hence, are more likely to attempt to escape when held in the hand. Conversely, individuals with limited escape abilities avoid predation risks in trying to escape less frequently when held in the hand (Møller et al. 2006; Møller and Nielsen 2010). We conducted this assay to 72 individuals (2013: $n = 29$; 2014: $n = 43$). Before blood sampling, birds were placed with their back against the palm of the observer's hand, maintaining approximately 40 cm from the observer's face. The number of struggle attempts (e.g., struggle and pecking) was scored for 20 s. These scores allowed the computation of a struggle activity axis of variation (see Brommer and Kluen 2012; García-longoria et al. 2014 for similar methods).

Open-field test

The open-field test is a frequently used method to assess exploratory behavior, which is characterized by the activity level displayed by individuals in response to novel environments or unfamiliar objects (e.g., Dingemanse and De Goede 2004). This behavior may be linked to risk-taking behavior, as it enables individuals to gather
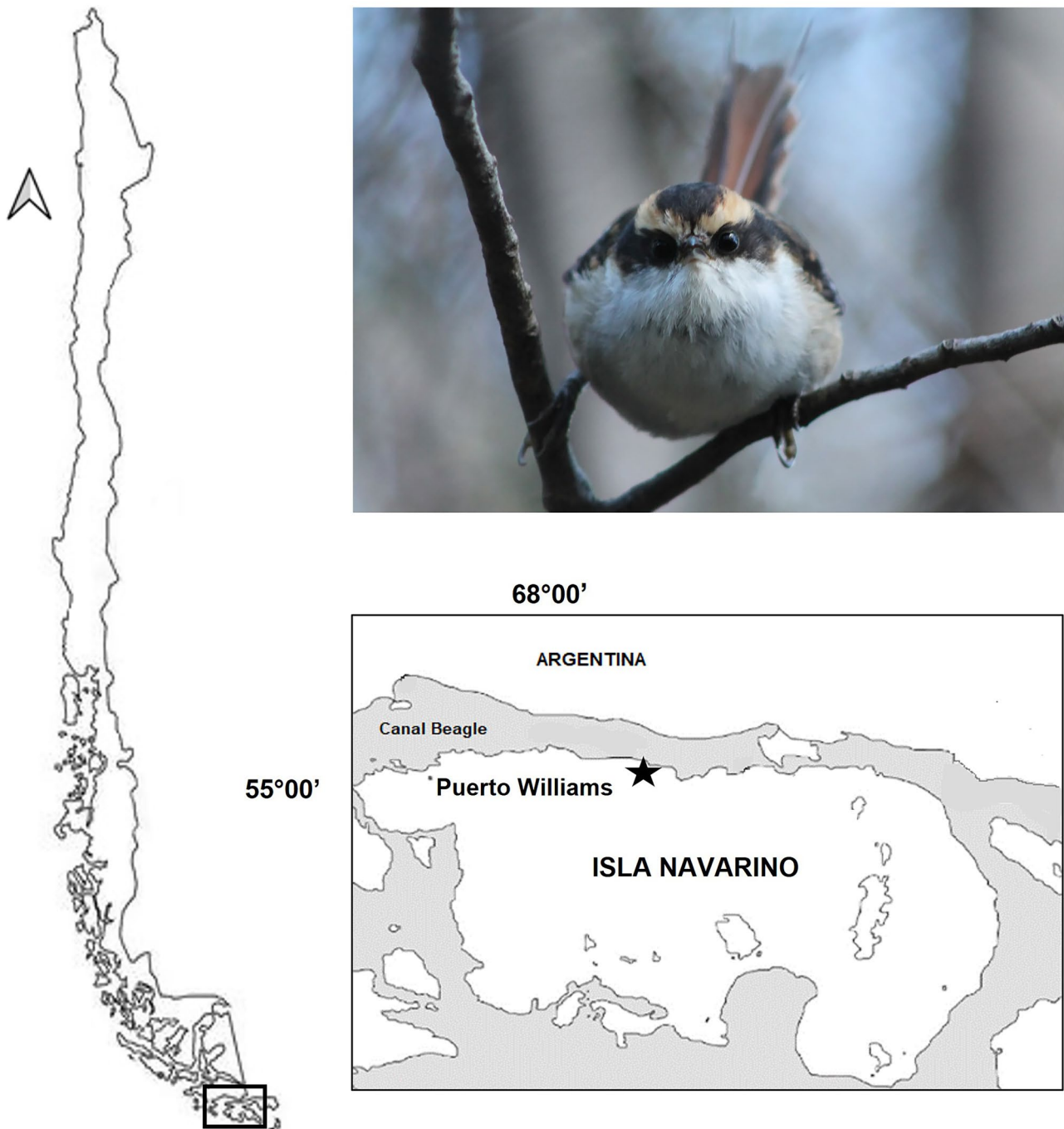
Fig. 1 Location of the study plot (black star), on Navarino Island, southern Chile. An adult thorn-tailed Rayadito is shown during a behavioral trial. Photograph by Yanina Poblete

information about various environmental aspects associated with unknown costs (e.g., Abbey-Lee et al. 2016). We used the OFT to describe activity scores within a novel environment, represented by an experimental aviary (e.g., Botero-delgadillo et al. 2020a; Poblete et al. 2021b, 2023). This test was performed using a field-portable aviary (270 cm long × 150 cm wide × 150 cm high) made of PVC poles and semitransparent black shading cloth. The floor of the cage was divided into four quadrants of equal size, and there were 14 possible perching locations, including four perches (80 cm long, 2 cm in diameter), two at 50 cm and two at 110 cm off the ground. Before the

trial, birds had a 5-min acclimatization period in a small cage (30 cm long × 25 cm wide × 39 cm high) placed in a corner of the larger experimental cage and covered with a black cloth, which was removed at the beginning of the test (Poblete et al. 2018, 2021b; Botero-delgadillo et al. 2020a). Emergence times from the small cage were 0.2 ± 0.7 s (mean ± SD). Behaviors were recorded with a digital camera (Sony DCR-68) during a 10-min period to obtain data on the movement rate of each bird.

A total of 73 videos (2013: $n = 30$; 2014: $n = 43$) were eligible to analysis using the J-Watcher software, and six variables were extracted for describing exploratory behavior: (1) total number of hops; (2) total number of flights; (3) total number of movements (i.e., hops and flights); (4) exploratory speed, defined as the number of perching locations visited per minute; (5) number of different perching locations visited during first min of the test; and (6) proportion of perching locations visited, calculated in relation to the 14 locations available (see Botero-Delgadillo et al. 2020a; Poblete et al. 2021a).

Simulated territorial intrusion test

We conducted the STI to describing activity levels associated with conspecific territorial intrusion. High activity levels in this test are indicative of heightened aggressive territoriality (STI; Wingfield et al. 1987) which in turn can increase vulnerability to predator risk during encounter with conspecific intruders (Garamszegi et al. 2008). The STI was performed in 2014 on 43 breeding individuals that were previously banded 15 days after egg hatching using a simulated territory intrusion by a conspecific male (STI; Wingfield et al. 1987). A stuffed mount was placed 3 m in front of the nest box and played back tape-recorded songs of a male bird for 10 min (Ippi et al. 2013; Botero-Delgadillo et al. 2020a). Two hidden observers at 10–15 m from the nest box recorded all behaviors of each parent on a Sony™ ICD-BX700® digital voice recorder. Recordings were analyzed with the J-Watcher software (Blumstein and Daniel 2007), and five variables were extracted for describing aggressiveness: (1) total number of hops; (2) total number of flights; (3) total number of alarm calls; (4) total number of aggressive interactions with the intruder, including pecking the intruder, flying over the intruder, or hovering close to it; and (5) total time close to the intruder (3 m around nest; see details in Botero-Delgadillo et al. 2020a).

During field observations and video and audio checking, the observers were blinded to the hypothesis being tested (i.e., no knowledge on the infected or uninfected state of the individuals).

DNA extraction and screening for haemosporidian parasites

We extracted DNA from the collected blood that was stored on FTA cards using the salting-out procedure (Aljanabi and Martinez 1997). We performed a nested PCR protocol that amplifies a fragment of approximately 480 bp (excluding PCR primers) of the mitochondrial DNA cytochrome b (cyt b) gene of *Leucocytozoon* spp. parasites using parasite genus-specific primers (Hellgren et al. 2004; see Cuevas et al. 2020 for details of the parasite infections in thorn-tailed Rayadito). Samples were screened at least twice to avoid false negatives. We included two positive controls for parasites and two negative controls (ddH_2O). Contamination was not detected.

Statistical analysis

We used principal component analyses (PCA) to reduce dimensionality in our behavioral dataset and for computing uncorrelated behavioral variables for OFT and STI, respectively. To this end, the observable variables were previously z scores normalized, subtracting the mean (μ) from each data point and then dividing by the standard deviation (σ).

We used ANCOVA to assess the relationship between *Leucocytozoon* spp. infection with the struggle activity and OFT-PC and STI-PC, respectively. For each response variable (struggle activity, OFT, and STI (PCA scores)), we formulated candidate models and selected the more suitable predictive model. The predictive variables considered were *Leucocytozoon* spp. infection ("infected" and "uninfected"), tarsus length (as indicator of body size), age ("yearling" or "adult"), and sexes ("female" or "male"). The models for struggle activity and OFT also incorporated the sampling year ("2013" and "2014") as a cofactor. We used Akaike information criterion corrected for small sampling size (AICc) to select the most parsimonious models (Burnham and Anderson 1998; see Table S1 for model selection). We checked model assumptions for all analyses. For statistical significance, results were considered when $P < 0.05$. All analyses were carried out in R 3.5.3 (R Core Team 2019).

Results

The first principal component from OFT (OFT-PC) with eigenvalues ≥ 1 was extracted, accounting for 44% of the total variance in the OFT (Table 1). This principal component showed high and negative loadings of variables related to activity (e.g., flights, movements, and speed). For ease of interpretation of OFT-PC, all loading and PC scores were reversed (i.e., all trait loadings and all PC

Table 1 Results from principal component analyses (PCAs) on behavioral variables collected from open-field tests (OFTs) (*n* = 73) and simulated territorial intrusions (STIs) (*n* = 43) in a population of thorn-tailed Rayadito. PC loadings, eigenvalues, and estimations of explained variance are presented

Observable behaviors during OFT	OFT-PC	Observable behaviors during STI	STI-PC
Total no. of hops	0.30	Total no. of hops	0.47
Total no. of flights	0.50	Total no. of alarms	0.38
Total no. of movements	0.49	Total no. of flights	0.43
Exploratory speed	0.19	Total time inside test	0.52
Perching locations (1st min)	0.54	Total no. of movements	0.41
Proportion visited locations	0.29		
Eigenvalue	**1.62**		**1.80**
Variance explained (%)	**44**		**62**

scores were inverted; Table 1; Fig. 2A). About STI, the first principal component (STI-PC) with eigenvalues ≥ 1 was extracted, accounting for 66% of the total variance for STI. This principal component presented high and positive

loadings of all measures of agonistic behavior (e.g., total number of agonistic interactions; Table 1; Fig. 2B).

Out of the 73 individuals, 43 (59%) were infected with *Leucocytozoon* spp. (see Cuevas et al. 2020). Based
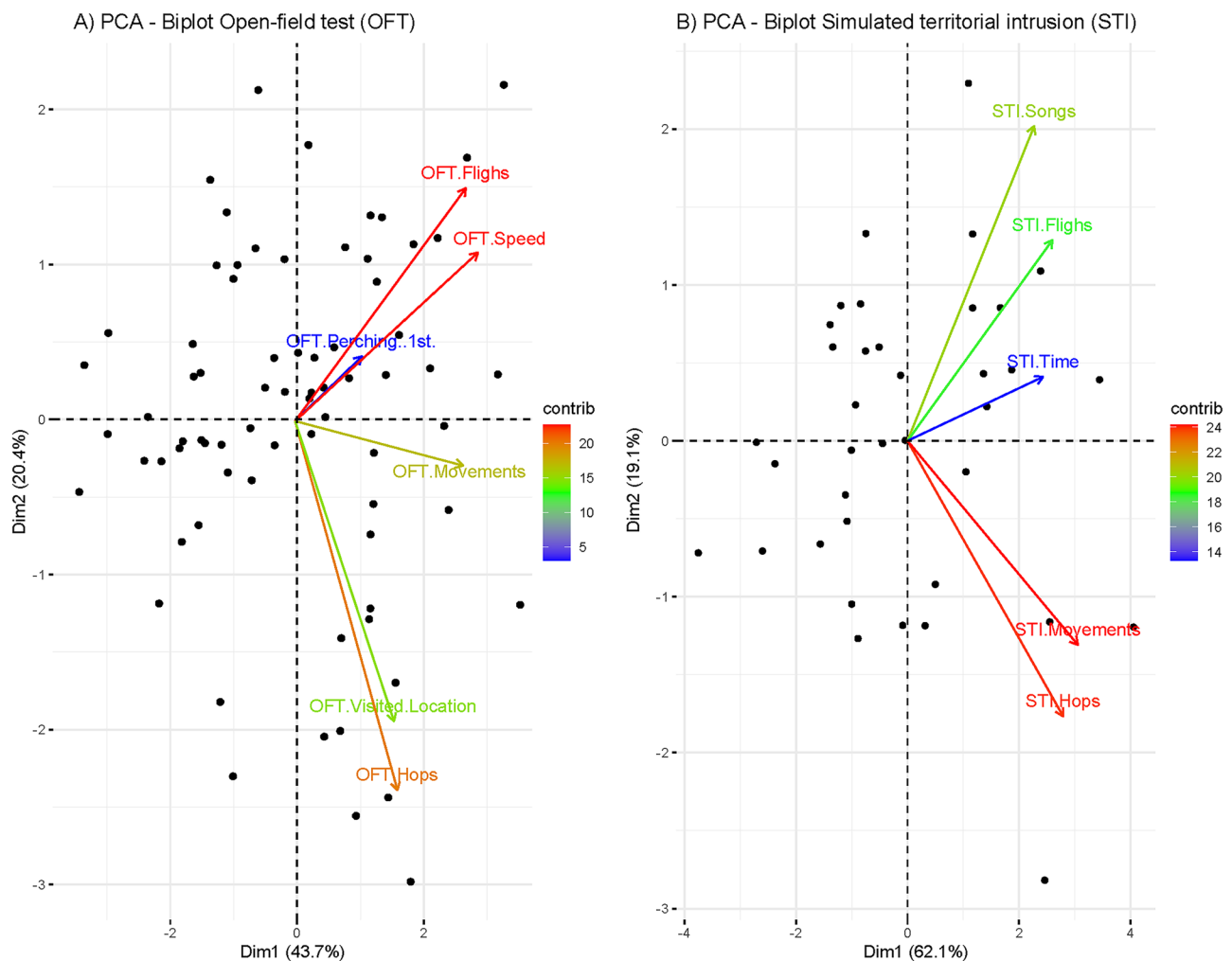

Fig. 2 PCA plot illustrates the multivariate in behavioral variables related to open-field tests (OFTs) in (**A**) and the simulated territorial intrusion test in (**B**). The vectors in the plot represent the direction and strength of each behavioral variable concerning the overall distribution. The colored arrows are associated with the observable behav-iors defined in this study. The first two principal axes explained 44% and 62% of the variance for OFT and STI, respectively. In the plot, the darker red and longer arrows signify a higher contribution, while the darker blue and shorter arrows indicate variables with lower contributions

Table 2 Analysis of variance for testing for differences between infected and uninfected individuals by *Leucocytozoon* spp. in relation to behavioral variation in a population of thorn-tailed Rayadito. Measured behavioral traits are struggle activity (*n* = 72), OFT-PCOFT (PCA scores; *n* = 73), and STI-PC (PCA scores; *n* = 43)

Source	df	*F* value	*P* value
Struggle activity			
Infection status	1	8.28	**0.01****
Body size (tarsus length mm)	1	0.40	0.52
OFT-PC			
Infection status	1	4.85	**0.03***
Body size (tarsus length mm)	1	0.08	0.77
Sex	1	2.42	0.12
STI-PC			
Infection status	1	5.39	**0.02***

Significant codes: 0***; 0.001**; 0.01*

on the analysis, the model that considers infection status and body size as variables (with an AICc value of 189.6) provides the best explanation for the correlation between struggle in the hand assay and *Leucocytozoon* spp. Infected individuals exhibited significantly higher activity levels during the struggle in the hand assay than uninfected individuals ($F_{1,68} = 8.28$; $P = 0.01$; Table 2; Fig. 3), irrespective of their body size ($F_{1,68} = 0.40$; $P = 0.52$; Table 2). Similarly, the model for OFT that considers infection status, sex, and body size as variables (with an AICc value of 275.1) best explains the relationship between OFT and *Leucocytozoon* spp. Infected individuals scored significantly higher in activity during OFT than uninfected individuals ($F_{1,68} = 4.85$; $P = 0.03$; Table 2; Fig. 3), regardless of their sex ($F_{1,68} = 2.42$; $P = 0.12$ Table 2) or body size ($F_{1,68} = 0.08$; $P = 0.77$; Table 2). Lastly, the model for STI (with an AICc value of 147.4) that considers infection status
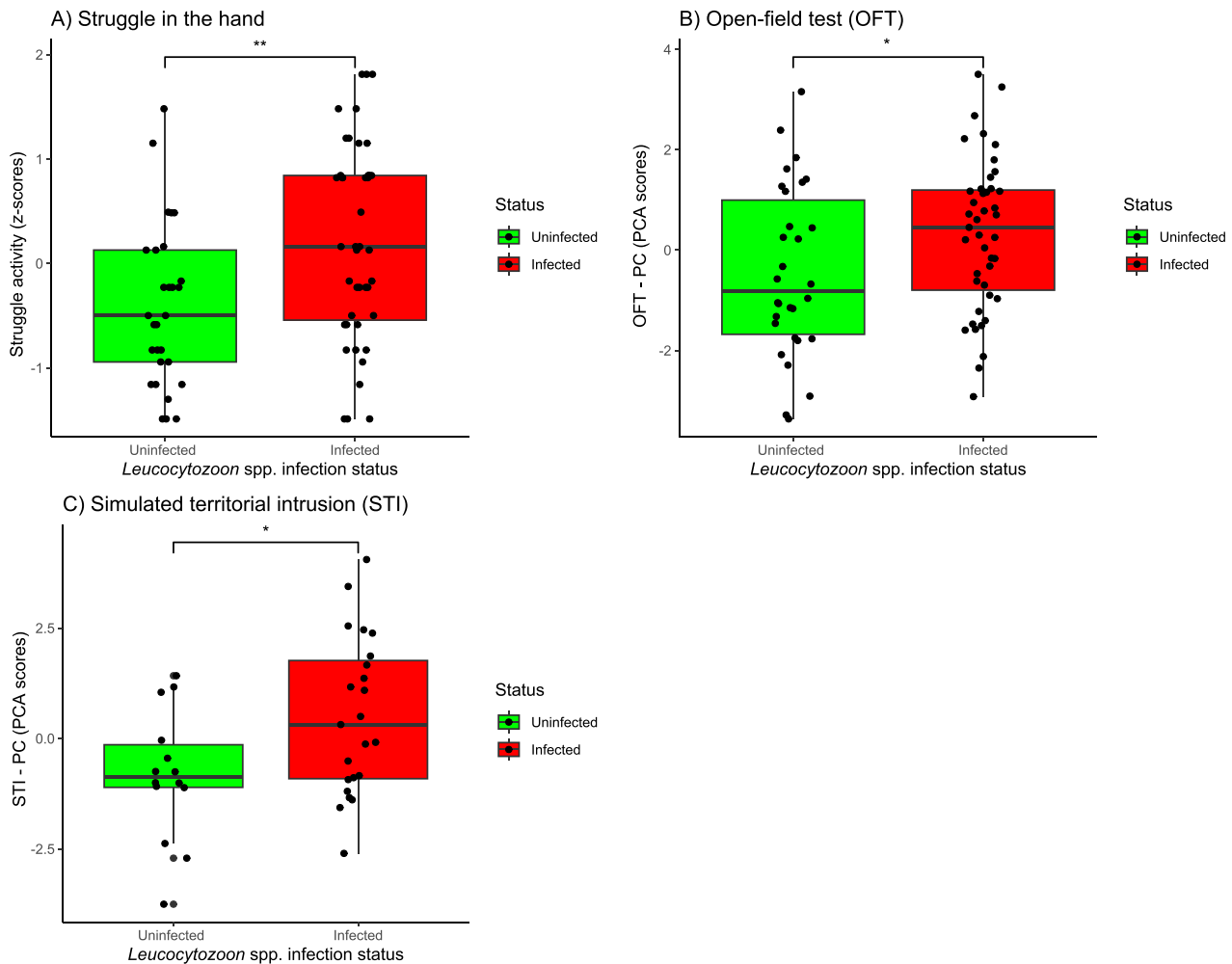

Fig. 3 Differences in struggle activity (*n* = 72), OFT (*n* = 73), and STI tests (*n* = 43) between infected and uninfected individuals by *Leucocytozoon* spp. in a population of thorn-tailed Rayaditos in Navarino Island, Chile. The bold horizontal line inside the box represents the median score for struggle in the hand (**A**), OFT (**B**), and STI (**C**), respectively. Vertical lines indicate maximum and minimum behavioral scores, whereas points show individual data

as a variable provides the best explanation for the relationship between STI and *Leucocytozoon* spp. Infected individuals scored significantly higher in activity during STI than uninfected individuals ($F_{1,35} = 5.39$; $P = 0.02$; Table 2; Fig. 3; see Table S1 for all models).

Discussion

In this study, we found that Rayaditos infected by *Leucocytozoon* spp. exhibited higher activity scores in all behavioral tests, as anticipated. These differences did not significantly vary with sex, age, body size, or sampling year.

Several studies support the idea that birds with high activity scores in behavioral assays tend to be more competitive and often secure higher-quality resources compared to less active birds (see Carere and Maestripieri 2013 and references therein). However, empirical evidence also indicates that birds with high activity scores are more prone to taking risks and, consequently, are more likely to attract predators (Montgomerie and Weatherhead 1988; Møller et al. 2010; Møller and Szép 2011) and hematophagous vectors (e.g., Dunn et al. 2011; García-longoria et al. 2014). Specifically, the activity scores in the OFT are typically interpreted as reflecting exploratory behavior. This behavior shows the activity levels displayed by individuals in response to unfamiliar objects or novel environments (Montiglio et al. 2010). It may be linked to risk-taking behavior, as it allows individuals to obtain information about different environmental aspects associated with unknown cost, such as exposure to predators and parasites (Minderman et al. 2009).

On Navarino Island, Rayaditos with high activity scores in the OFT started their breeding attempts earlier than individuals with lower activity scores. This behavior may be associated with a competitive strategy that helps them secure more suitable nesting cavities than their conspecifics (Poblete et al. 2021b). It may also serve as a means to reduce costly interactions with other cavity-nesting species, as highlighted in previous studies (see Botero-Delgadillo et al. 2015; Poblete et al. 2021a). It seems plausible that Rayaditos with high activity scores in the OFT have an advantage in acquiring suitable nesting sites through increased exploration of their environment, although this could entail costs associated with *Leucocytozoo* spp.

Rayaditos infected with *Leucocytozoon* spp. also displayed high activity scores in the struggle in the hand assay when compared to uninfected individuals. A similar result was reported by García-Longoria et al. (2014), who found that bird species infected by *Leucocytozoon* spp. tend to exhibit more wriggling behavior when held in hand compared to uninfected birds. Activity levels during the struggle in the hand assay are typically interpreted as anti-predatory behavior, as this behavior allows birds to evade predators

once captured (Møller et al. 2011). Evidence suggests that individuals struggling with predators have a higher likelihood of being infected with blood parasites (Navarro et al. 2004), possibly because this behavior attracts secondary predators (Hogstedt 1983; Møller and Nielsen 2010) and potential vectors (Garcia-Longoria et al. 2014). Our finding supports the hypothesis that high activity scores in the struggle in the hand assay are associated with *Leucocytozoon* spp. infection.

Our results also showed that Rayaditos infected with *Leucocytozoon* spp. displayed high activity scores in the STI test. Higher activity scores may indicate more aggressive and territorial individuals, as they vigorously defend their territories against intruders (e.g., Ippi et al. 2013). Such behaviors can be energetically costly, as they require expending energy and potentially exposing the defending individuals to increased predation and parasites infection risk (Moore 2002). On Navarino Island, Rayaditos might exhibit heightened aggression and territoriality in response to resource competition (Botero-Delgadillo et al. 2015). Suitable nest cavities are a limited resource for most secondary cavity-nesting birds, so competition is a common interaction among birds requiring this resource (Winkler 2016). During this study, we documented evidence of competition for nest cavities among Rayaditos, House wrens (*Troglodytes aedon*), and Chilean swallows (*Tachycineta leucopyga*), including a case of lethal aggression by a pair of Chilean swallows towards Rayadito nestling (Botero-Delgadillo et al. 2015). It seems plausible that Rayaditos with high activity scores in STI would have a breeding advantage through increased territorial aggressiveness, although this behavior would entail costs associated with *Leucocytozoon* spp. infection.

Certainly, it would be interesting to assess whether the high activity scores found in this study are the cause of *Leucocytozoon* spp. infection or its consequence. However, this would require controlled experimental infections or comparing behavioral scores in the same individuals before and after infection, which may be methodologically complex to achieve in wild birds. Specifically, in our study population, the recapture rate is too low and includes repeated measurements in the analysis (Poblete et al. 2021b).

Given that haemosporidians can affect an individual's health and access to critical resources (e.g., food or suitable nesting sites), it is possible that infected Rayaditos may adopt more active behavioral strategy in response to resource availability (Dingemanse and Réale 2005). In line with this, Barber and Dingemanse (2010) and Kortet et al. (2010) propose that selection likely favors infected individuals that can survive and breed after acquiring an energetically demanding parasite. These individuals are expected to be physiological and/or genetically more resistant to infection (i.e., parasitic resistance strategy), possibly through factors like the major histocompatibility

complex (MHC), which in turn could be advantageous for parasites' persistence.

Additionally, it is important to note that a previous studio in *Passer domesticus* found that when the prevalence of haemosporidians is reduced in infected individuals treated with primaquine and chloroquine, the biting rate of these medicated sparrows significantly decreased during the struggle in the hand assay. These birds also spent more time in tonic immobility before flying away (García-longoria et al. 2014). These outcomes suggest that experimental reduction of haemosporidians led to a decrease in the intensity of the behavior of their avian host. This phenomenon could also be applicable in our study population, where Rayaditos displaying high activity scores may be a result of parasitic manipulation strategy.

In summary, this study explored the relationship between *Leucocytozoon* spp., and the risk-taking behavior displayed by Rayaditos across three distinct behavioral trials. While our findings raise the intriguing question of whether risk-taking behavior precedes infection or result from it, the practical complexities associated with conducting controlled experiments in this study population have left this question unresolved. Long-term studies that involve the sampling of individuals before and after infection are essentials to shed further light on the underlying cause and consequences of the relationship between risk-taking behavior and *Leucocytozoon* spp. infection in this Rayadito population. Such studies hold the promise of providing valuable insights into the dynamics of this intricate host-parasite interaction.

Declarations

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