High-performance–validated tests are essential for successful epidemiological monitoring, surveillance of parasitic infections, and comparative studies in wildlife populations. The Mini-FLOTAC is a novel flotation–based technique for the sensitive detection and quantification of gastrointestinal parasites that is recently being explored for use in wildlife. A limitation of any flotation-based copromicroscopic method is the selection of the flotation solution (FS), which might influence the performance of the test. However, no study has compared the influence of using different FS in the Mini-FLOTAC technique for parasite detection in wild birds. Here, we evaluated the diagnostic performance of the Mini-FLOTAC in three waterbird host species using two widely used FS: saturated salt (NaCl; specific gravity 1.20) and saturated zinc sulfate (ZnSO₄; specific gravity 1.35). One hundred fresh fecal samples were analyzed for parasite fecal egg counts (FEC). Regardless of the host species, fecal samples evaluated with the Mini-FLOTAC method using ZnSO₄ resulted in a significantly higher detection rate and higher FEC of strongylid, capillarid, cestode, and trematode parasites, than samples analyzed with the NaCl solution. Our concise study demonstrated the importance of using an appropriate FS for the identification of parasite eggs in wildlife species, especially in hosts with an expected aggregated distribution and low parasite load such as waterbird hosts. The higher analytical sensitivity of the Mini-FLOTAC technique achieved with ZnSO₄, and its applicability to fieldwork, highlights this method as a promising tool for the quantitative surveillance of parasite infections in wild bird populations.

**Keywords** Black-necked swan · Brown-hooded gull · Fecal egg count · Flotation solution · Hudsonian godwit

**Introduction**

Precise quantification of parasite infections in wildlife represents a major methodological limitation for the identification of key epidemiological parameters and further ecological consequences of host-parasite interactions in natural populations (Thompson et al. 2010). Moreover, parasite populations are highly aggregated in a small number of hosts (Poulin 1993) limiting the performance of traditional diagnostic methods. Consequently, the use of sensitive, precise, and properly validated tests can greatly contribute to the successful wildlife population-based epidemiological monitoring and surveillance (Stallknecht 2007; Budischak et al. 2015).

In wildlife, parasite identification is mostly performed by post-mortem examination of hosts (e.g., lethal sampling), which involves highly trained professionals but also ethical limitations (Budischak et al. 2015). As a non-invasive alternative, fecal egg–counting techniques are used for the detection of gastrointestinal (GI) parasites, although mainly for qualitative diagnostic purposes, and more recently also for quantification of infection intensities (Coker et al. 2020). Among the quantitative methods, the Mini-FLOTAC is a novel and sensitive flotation–based technique for the detection and quantification of parasitic elements (PE) from GI helminths (i.e., eggs or larvae of nematodes, cestodes, and trematodes) and protozoa (i.e., cysts and oocysts) (Barda
et al. 2013; Cringoli et al. 2017). The use of the Mini-FLOTAC for detection of GI parasites has been validated in several domestic and wild mammals, demonstrating higher diagnostic sensitivity and accuracy than commonly used copromicroscopic methods such as the McMaster method, obtaining a detection limit of 1 egg per gram (EPG) (Cringoli et al. 2017; Catalano et al. 2019). In birds, this new technique has been modified and applied for quantitative parasite diagnosis in domestic species and wild-caught individuals (Borrelli et al. 2015; Bortoluzzi et al. 2018; Carrera-Játiva et al. 2018; Daş et al. 2020) and has only recently been explored for the diagnosis of coccidia and helminths in wild birds (Carrera-Játiva et al. 2018; Coker et al. 2020).

However, there are still some methodological gaps in the use of the Mini-FLOTAC that are needed to improve the detection of parasites in wildlife species, particularly for the use in wild birds.

Because each GI parasite species has PE with a particular specific gravity (s.g.) (Ballweber et al. 2014), one limitation of any flotation-based technique is that the flotation solution (FS) used significantly influences the diagnostic performance of the method (Cringoli et al. 2004, 2010; Barda et al. 2013). Indeed, several FS have been evaluated in mammalian hosts for the Mini-FLOTAC technique, with the most widely used being the saturated sodium chloride (NaCl; s.g. 1.20) and zinc sulfate (ZnSO₄; s.g. 1.35) solutions (Cringoli et al. 2017). In contrast, it is not clear which FS may perform better for the use of the Mini-FLOTAC in birds. Previous studies have detected Macrorhabdus ornithogaster and Isospora sp. in caged songbirds (order Passeriformes) using ZnSO₄ solution (Borrelli et al. 2015), Eimeria maxima oocysts in poultry excreta using sodium nitrate (s.g. 1.20) (Bortoluzzi et al. 2018), and oocysts of coccidia (Eimeria spp.) in brown kiwi (Apteryx mantelli) with magnesium sulfate (s.g. 1.28) (Coker et al. 2020). Recently, Daş et al. (2020) reported that sucrose solution (s.g. 1.32) increases the accuracy of the Mini-FLOTAC technique in comparison with NaCl (s.g. 1.20) for the detection of Ascaridia galli and Heterakis gallinarum eggs in feces of poultry chickens. However, no studies have yet compared the effects of using different FS in the Mini-FLOTAC technique for the detection of PE in fecal samples from wild birds. Therefore, this study aimed to evaluate the performance of the Mini-FLOTAC technique using two different FS for the detection of helminth eggs in fecal samples from different wild bird species.

Methods

Fresh fecal samples were collected from free-living individuals from three waterbird species with expected differences in their gastrointestinal helminth fauna: (a) Brown-hooded gull (Chroicocephalus maculipennis; n = 30), a trophic generalist species that is mainly infected by trophic transmission with several helminth species (Oyarzún-Ruiz and González-Acuña 2021); (b) black-necked swan (Cygnus melancoryphus; n = 34), a strictly herbivorous bird infected mostly by parasites with a direct life cycle (Oyarzún-Ruiz and González-Acuña 2021); and (c) Hudsonian godwit (Limosa haemastica; n = 36), a long-distance migratory shorebird that host helminths species but a low infection rate (Kinsella et al. 2007). Samples were collected between November 2018 and January 2020 in different wetlands in Los Ríos (39° 16′–40° 41′ S; 71° 35′–73° 70′ W) and Los Lagos (40° 13′–44° 33′; 74° 49′–71° 34′) Regions, Chile. Individual samples from gulls and godwits were collected immediately after dropped, whereas samples from swans were directly collected from cloaca during handling. All samples were immediately placed in sterile 15-ml tubes and preserved at 4 °C with 70% ethanol in sufficient quantity to cover the fecal material.

Each sample (n = 100 independent biological replicates in total) was analyzed twice to determine parasite fecal egg counts (FEC) using the Mini-FLOTAC method (Cringoli et al. 2017) either FS with NaCl (s.g. 1.20) or ZnSO₄ (s.g. 1.35). At least 1 g of fecal sample from gulls and swans and 0.5 g from godwits were thoroughly homogenized and mixed with the respective FS at a 1:10 (sample:FS) dilution ratio in the Fill-FLOTAC device (Barda et al. 2013; Cringoli et al. 2017). One milliliter of the filtered fecal suspension with each FS was transferred from the Fill-FLOTAC (equipped with a plastic filter consisting of 250-µm holes) into one (godwits) or two (gulls and swans) chambers of a Mini-FLOTAC reading disk. After 10 min, the reading disks were observed under 400× in a light microscope and the floated PE were morphologically identified based on taxonomic keys and previous reports of gastrointestinal parasites in the studied wild birds (Kinsella et al. 2007; Mehlhorn, 2016; Taylor et al. 2016; Oyarzún-Ruiz and González-Acuña, 2021). Based on morphological keys, the observed nematode eggs were classified as either strongylids or capillarids, whereas trematode and cestode eggs were not further characterized into genus level. The FEC of each sample was expressed as EPG of feces (multiplication factor of 10 for godwits and 5 for gulls and swans).

Differences in the performance of each FS for the detection of parasite eggs in the Mini-FLOTAC were evaluated as follows. The detection rate (number of samples positive to parasite eggs/total number of samples analyzed) of each FS was calculated for each bird species and per parasite egg type, then ordered in 2×2 contingency tables and compared using a McNemar’s Chi-squared (χ²) test with continuity correction tests. The agreement in the detection rate of positive samples between the two FS was evaluated using Cohen’s kappa (κ) statistic. Differences in the FEC detected by the two FS in samples from the same host species were
evaluated using untransformed EPG data (per parasite egg type) in generalized linear models (GLM) with negative binomial distributions. All analyses were performed in R and a level of \( P < 0.05 \) was considered significant. All procedures performed with animals in this study were approved by the Ethical Committee on Animal Research, Universidad Austral de Chile, Chile (385/2020), and the Chilean Agriculture and Livestock Service (4559/2018) in compliance with Chilean laws and regulations.

**Results and discussion**

We identified several helminth eggs in the three bird species, including nematode (i.e., strongylid and capillarid), trematode, and cestode eggs, although with significant differences in the detection rate and FEC between bird species and depending on the FS used (Table 1). Strongylid eggs were detected in all three host species, whereas capillarid and trematode eggs were identified in a brown-hooded gull and black-necked swan. Cestode eggs were only observed in the brown-hooded gull. No further characterization of detected helminth eggs into genus or species was performed, but several species of gastrointestinal nematodes (e.g., *Amidostomum* sp., *Capillaria* sp., *Contracecum* sp., *Viktorocara* sp.), trematodes (e.g., *Stephanoprora* sp., *Echinostoma* sp., *Notoctylus* sp.), and cestodes (e.g., *Tetrabothrius* sp., *Sobolevicanthus* sp., *Echinocotyle* sp.) have been previously identified in the wild bird species investigated (Kinsella et al. 2007; reviewed by Oyarzún-Ruiz and González-Acuña 2021).

Overall, fecal samples evaluated with ZnSO₄ solution resulted in a significantly higher detection rate of any type of parasite eggs (\( n = 51 \)) than when analyzed with the NaCl solution (\( n = 11 \)) in the three bird species (McNemar’s \( \chi^2 = 41.023, \text{df} = 1, P < 0.001 \); Table 1). The Mini-FLOTAC with ZnSO₄ solution detected significantly more infected individuals with strongylid, capillarid, and trematode parasites than with the NaCl solution (\( P < 0.01 \) for all parasites). Furthermore, cestode eggs were only identified by ZnSO₄ solution (Table 1). By host species, the method with the ZnSO₄ solution was consistently more efficient to detect individuals with parasite eggs (\( P < 0.01 \) for all three host species) than those analyzed with NaCl solution. A poor agreement in detection rates between FS was confirmed with low Cohen’s kappa values for detection of individuals with strongylid (\( k = −0.02 \)), capillarid (\( k = 0.11 \)), and trematode (\( k = 0.12 \)) positive samples, as well as low agreement to identify positive samples to all helminth eggs in the brown-hooded gull (\( k = −0.01 \) and black-necked swan (\( k = 0.07 \)). Notably, the Mini-FLOTAC analyses using NaCl were not able to detect any PE in samples from the Hudsonian godwit, whereas we were able to detect nematode (i.e., strongylid) eggs in eight individuals using the ZnSO₄ solution (Table 1). The only previous report in the Hudsonian godwit described the presence of the nematodes *Viktorocara limosa* and *V. capillaris* (Order

### Table 1

|                | Strongylids | Capillarids | Trematodes | Cestodes | All helminths |
|----------------|-------------|-------------|------------|----------|---------------|
| NaCl           | ZnSO₄       | NaCl        | ZnSO₄      | NaCl     | ZnSO₄         |
| n positive (%) | 0           | 7* (23.3)   | 2 (6.7)    | 8 (26.7) | 0             |
| EPG (min–max) | -           | 5.71⁺       | 5          | 6.88⁺    | -             |
| Brown-hooded gull (\( n = 30 \)) | 3 (10) | 6 (20) | 0 | 2⁺ (6.7) | 5 (16.7) |
| Black-necked swan (\( n = 34 \)) | 5 (14.7) | 9 (26.5) | 1 (2.9) | 15⁺ (44.1) | 0 |
| Hudsonian godwit (\( n = 36 \)) | 8 (5) | 5 | 8⁺ | 15 | 14.62⁺ |
| All samples (\( n = 100 \)) | 5 | 24⁺ | 3 | 23⁺ | 4 (19⁺) |

*Significant differences in positive samples between FS analyzed by McNemar’s Chi-squared (\( \chi^2 \)) test with continuity correction tests (\( P < 0.05 \)) or in FEC per parasite egg type between FS following analyses by GLM (negative binomial) of untransformed EPG data (\( P < 0.01 \)). *No statistical analysis were applied (only samples analyzed with ZnSO₄ solution were positive to parasite eggs). Arithmetic mean EPG and min–max EPG were calculated with data from only positive samples.
Spirurida) in their breeding grounds in Alaska and Mani-
toba (Kinsella et al. 2007). Therefore, further research is
needed to confirm our findings, and fully characterize the
parasite fauna hosted by Hudsonian godwits during their
non-breeding season in the Southern Hemisphere.

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tion resulted in a significantly higher detection rate of
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Mini-FLOTAC with ZnSO₄ solution detected significantly
more infected individuals with strongylid, capillarid, and
trematode parasites than with the NaCl solution (P < 0.01
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ples from the Hudsonian godwit, whereas we were able to
detect nematode (i.e., strongylid) eggs in eight individuals
using the ZnSO₄ solution (Table 1). The only previous
report in the Hudsonian godwit described the presence of
the nematodes Viktorocara limosa and V. capillaris (Order
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toba (Kinsella et al. 2007). Therefore, further research is
needed to confirm our findings, and fully characterize the
parasite fauna hosted by Hudsonian godwits during their
non-breeding season in the Southern Hemisphere.

In terms of FEC sensitivity, the Mini-FLOTAC using
ZnSO₄ solution was able to detect higher mean EPG of all
types of helminth eggs in those infected individuals than the
method with NaCl, consistently across all three bird species
(Table 1). Moreover, the Mini-FLOTAC using ZnSO₄ solu-
tion was the only method able to detect strongylid eggs in
the brown-hooded gull and Hudsonian godwit and cestode
eggs in the brown-hooded gull (Table 1). The method with
ZnSO₄ solution detected significantly higher FEC of capil-
larid and trematode eggs in samples from the brown-hooded
gull and black-necked swan (GLM of untransformed EPG
data; P < 0.01; Table 1), than those analyzed with NaCl solu-
tion, whereas FEC of strongylid eggs detected in samples
from the black-necked swan was not significantly different
between both FS.

The number of samples with undetected (0 EPG) or posi-
tive (≥ 5 EPG) FEC of strongylids, capillarids, and trem-
atodes across all bird species (total n = 100 samples) analyzed
by both FS are shown in Fig. 1. Most of the samples ana-
lyzed with both FS had 0 EPG, with strongylid, capillarid,
and trematode eggs being highly aggregated in few indi-
viduals in all bird species. However, while only 3–5% of the
samples analyzed by NaCl were positive to these helminth
eggs, 18–24% of samples analyzed by ZnSO₄ were positive
(Fig. 1). Noticeably, FEC values obtained for all parasite egg
types in all three species of wild birds were very low (<30
EPG) regardless of the FS used, therefore might be unde-
tected by other quantitative methods (e.g., McMaster tech-
nique has a detection limit of ≥50 EPG; Scare et al. 2017,
Daş et al. 2020). Since the accuracy and precision of the
detection of PE is highly correlated with the volume of feces
examined (Torgerson et al. 2012), the microscopic exami-
nation of a larger subsample in the Mini-FLOTAC reading
disk (1–2-ml subsamples analyzed for FEC per fecal sample
vs. 0.15–0.3 ml in the McMaster slide; Cringoli et al. 2004;
Cringoli et al. 2017) partially contributed to minimize the
diagnostic limitation of analyzing a small amount of fecal
material. Furthermore, results from previous studies using
NaCl as a FS, especially those reporting the absence of PEs
in a given bird species, might be considered with caution.

Fig. 1 Number of samples with undetected (0 EPG) or positive (≥ 5 EPG) fecal egg counts of strongylid, capillarid, and trematode eggs across all bird species (total n = 100 samples) analyzed by the Mini-FLOTAC technique using two flotation solutions (NaCl and ZnSO₄)
Our findings highlight the importance of using quantitative diagnostic techniques with higher analytical sensitivity, such as the Mini-FLOTAC and appropriate FS, for accurate quantification of PE from limited volumes of fecal samples that are usually obtained from wild birds. Furthermore, the dynamic of gastrointestinal helminths infection in wild birds could be explored by combining a sensitive copromicroscopic method such as the Mini-FLOTAC with ZnSO₄, and molecular techniques for parasite identification in positive samples as previously explored in other host species (Budischak et al. 2015; Maurelli et al. 2018).

The higher diagnostic sensitivity of the Mini-FLOTAC method using the ZnSO₄ solution compared with NaCl was expected, as the increasing specific gravity of the FS results in the flotation of more PEs. However, studies using different FS with the same specific gravity do not produce the same results (Cringoli et al. 2004), so further analyses are needed to evaluate other FS. Other factors such as the fixative agent (e.g., ethanol) and the time the samples were stored before the microscopic examination (1–3 months) could have limited the detection of more eggs or other PE. Ethanol is widely used as a fixative of fecal samples collected from wildlife for parasite diagnosis when these analyses cannot be conducted immediately (Teichroeb et al. 2009; Froeschke et al. 2010). Here, the potential influence of ethanol on the recovery of helminth eggs or other PE from fixed fecal samples should have uniformly impacted the egg flotation in the Mini-FLOTAC tested with both flotation solutions, as the same (fixed) fecal sample was analyzed with either ZnSO₄ or NaCl. Therefore, the higher egg recovery with the Mini-FLOTAC method using the ZnSO₄ solution seems to be more related with the flotation solution than with the effect of the fixative agent. Future studies should explore the effects of commonly used fixative agents on the recovery of helminth eggs in feces from wild birds, particularly considering previous studies in wildlife reporting that fecal samples fixed in formalin resulted in the sinking of nematode eggs in flotation solutions (Baines et al. 2015). Certainly, further research is needed to perform FEC analysis of fresh fecal samples from wild birds, perhaps directly in the field. In this regard, the Mini-FLOTAC technique does not require a centrifugation step, in contrast to other sensitive quantitative flotation methods (e.g., sensitive centrifugal flotation technique or the FLOTAC technique, Taylor et al. 2016). Therefore, the Mini-FLOTAC could be considered as a sensitive and adaptable method that could be used directly in field with resource-limited laboratory equipment, commonly experienced in the study of wildlife parasites.

In conclusion, we were able to evaluate the performance of the Mini-FLOTAC technique with two commonly used FS with different specific gravities in the detection and quantification of gastrointestinal parasites in three waterbird species, confirming that the use of ZnSO₄ as FS results in a higher detection rate of infected individuals and higher FEC, compared with the NaCl solution. Our concise study demonstrates the importance of using an appropriate FS for the detection of parasite eggs in wildlife, especially in hosts with an expected low parasite load such as wild birds. A higher analytical sensitivity of the method using ZnSO₄ and the applicability of the Mini-FLOTAC in the field highlight this quantitative technique as a promising tool for the surveillance of parasite infections in wild birds, especially studying spatio-temporal dynamics of parasitic infections in migratory species where an improved technique is needed to determine key epidemiological parameters that allow the understanding of further ecological consequences of parasite infections in natural populations.

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Author contributions Claudio Verdugo and Miguel Peña-Espinoza contributed to the study conception and design. All resources were provided by Claudio Verdugo, Miguel Peña-Espinoza and Juan G. Navedo. Material preparation, data collection and analysis were performed by Dante Lobos-Ovalle and Claudio Navarrete. The first draft of the manuscript was written by Dante Lobos-Ovalle and Claudio Navarrete, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Most of the data generated or analyzed during this study are included in this published article. The dataset generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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