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The eldest sibling is the lousiest in an obligate brood-reducer seabird

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ABSTRACT

Previous studies on vertical transmission of lice on terrestrial birds have shown that the intensity of parasitism can vary within broods in relation to the hatching order of chicks. In species where the hatching order has an important effect on chick survival, lice would benefit from living on the host with the highest survival prospects. In the present work, we test this hypothesis using as model lice from the Imperial Shag, an obligate brood reducer. Our specific aims were to investigate how infestation by *Eidmanniella pellucida* varies in relation to the age, the order of birth and the hatching date of chicks. We observed that chicks were infested by their parents during their first days of life, mainly by lice in nymphal stage. The first chick to hatch was the most infested. Infestation was also affected by hatching date, increasing as the season progressed, reaching a maximum at the peak of the breeding season and decreasing gradually after that. The age of the chick was unrelated to the intensity of infestation. Our results suggest that lice would prefer the chick that is more likely to survive and highlights the adaptive strategies of lice according to the ecology of their hosts.

Introduction

Chewing lice (order Phthiraptera) are wingless insects, living as permanent and obligate ectoparasites of birds and mammals (Kim 1985). These lice cannot survive for long periods of time off their hosts and the opportunities for dispersal are restricted mainly to those periods of direct physical contact between hosts at chick-rearing and mating stages (Galloway 2012). Therefore, transmission can be either vertical (i.e. from parents to offspring), or horizontal (i.e. between siblings or during mating) (Brooke 2010).

There are relatively few studies about lice transmission patterns and most of them have been performed on terrestrial birds (see Lee and Clayton 1995; Galloway 2012). These studies report that transmission is in most cases vertical, which means that lice are transmitted principally from parents to chicks (Clayton and Tompkins 1994; Lee and Clayton 1995; Galloway 2012). These investigations also concluded that the intensity of parasitism can vary within broods in relation to the hatching order of chicks, with emerging patterns differing according to life history traits of hosts and selective pressures acting on parasites (Christe *et al.* 1998; Brooke 2010). In species where hatching order has a minor effect on chick survival, lice may preferentially aggregate on the latest chick due to

their immunologically depressed system as a consequence of lower access to parental resources (Christe *et al.* 1998). However, if hatching order has a marked influence on chick survival, parasites would benefit from living on the host with higher survival prospects (Brooke 2010).

The Imperial Shag (*Leucocarbo atriceps*) offers a particularly interesting model to answer this question. It is a colonial ground-nesting seabird where both parents play an active role in the care and feeding of chicks throughout the breeding cycle (Johnsgard 1993). Imperial Shag females usually lay three eggs that hatch asynchronously over 4–5 days (Svagelj and Quintana 2011; Giudici *et al.* 2017). The above-described hatching pattern results in age and size asymmetry between chicks, where the probability of fledging drastically decreases with hatching order (Giudici *et al.* 2017). This species exhibits obligate brood reduction where last-hatched chicks from three-hatchling broods usually starve to death within the first week of life, with successful breeding attempts producing one- or two-chick broods (Svagelj and Quintana 2011; Svagelj *et al.* 2012; Giudici *et al.* 2017).

In the present study we document, for the first time, patterns of vertical transmission of a louse infesting a diving seabird under natural conditions. The specific

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goal of this study is to describe patterns of infestation of *Eidmanniella pellucida* in Imperial Shag chicks. In particular, we investigate how lice infestation varies in relation to age, birth order and hatching date. Our ultimate objective is to shed light on the influence of the host reproductive behaviour in determining infestation patterns.

Material and methods

Data collection

The study was undertaken at Punta León colony (43° 03′ S, 64°27′ W), Chubut, Argentina, during the 2014 breeding season. Punta León is a mixed seabird colony (Yorio et al. 1994) where approximately 5000 pairs of Imperial Shags breed (F. Quintana unpub. data).

Sampling of lice

A total of 48 chicks from 27 nests were sampled during the reproductive season. For each chick, we firstly determined whether it was the elder A-chick or the younger B-chick. Lice were collected using forceps and fixed in 96% ethanol in the field. Manipulation stopped when no more lice were seen. We consider that this technique was reliable to address the goals of the present study because the small size and bare body of chicks (for details see Malacalza and Navas 1996) allows the collecting of all the lice. Once in the laboratory, lice specimens were classified into nymphs (N) and adult male (M) or female (F) stages.

Statistical analyses

Infestation parameters were estimated following Rózsa et al. (2000). Prevalence was defined as the frequency of lice occurrence, expressed as a percentage. To set 95% confidence intervals for prevalence we used Sterne's exact method (Reiczigel 2003). Mean intensity was defined as the mean number of individuals per host in the total sample of infested hosts. We used a bootstrap procedure with 20 000 replications to set 95% confidence intervals of mean intensity (Rózsa et al. 2000). Prevalence was compared between chicks with Fisher's exact test and intensity with a Kruskal–Wallis test.

To achieve our main objective of understanding the factors that affect the infestation of *E. pellucida* in Imperial Shag chicks, we performed generalised linear mixed models (GLMM) (Zuur et al. 2009) on the intensity of infestation. As representation of the natural aggregation of parasites, a negative binomial

distribution of the data was considered (Crofton 1971). The explanatory variables selected to model the intensity of the infestation of lice (i.e. number of parasites per host) were: hatching sequence within the brood (*ORD*), age of the chick (*AGE*, in days), and hatching date (*DAT*). In this analysis, we considered only two-chick broods ($n = 21$) where A- and B-chicks represented the eldest and youngest chicks in the brood, respectively. We standardised hatching date, entering it as a deviation from the median hatching date (subtracting the median date of hatching from the actual date) and dividing the result by the standard deviation. Allowing for non-linear relationships between predictor and response variables, we considered linear and quadratic terms of the chick's age and date of hatching. We incorporated nest identity (a unique code for each nest) as a random effect. To determine which model best fitted the data we calculated the Akaike's Information Criterion corrected for small sample size (AICc). Model comparisons were made with ΔAICc, which is the difference between the lowest AICc value (i.e. best of suitable models) and AICc from all other models. The AICc weight of a model (w_i) indicates the relative likelihood that the specific model is the best of the suite of all models. Because there was substantial uncertainty in model selection, parameter estimates from predictor variables were calculated using multiple model inference (Burnham and Anderson 2002). To evaluate the support for parameter estimates, 95% confidence intervals were calculated using unconditional variances (Burnham and Anderson 2002).

Statistical analyses were performed using glmmADMB (Skaug et al. 2016) and MuMIn (Bartoń 2015) packages in R software, v. 3.4.0 (R Development Core Team 2017), and Quantitative Parasitology software, v. 3 (Reiczigel and Rózsa 2005). Values are reported as $\bar{x} \pm$ SE except where noted, and for null hypothesis testing, statistical tests were considered significant at $P < 0.05$.

Results

Ninety-eight per cent of the Imperial Shag chicks were infested with *E. pellucida* and the prevalence was similar between A- and B-chicks, i.e. chick A 100% (CI 95% 88–100%) and chick B 95% (CI 95% 77–99%) (Fisher's exact test, $P = 0.44$). However, A-chicks were more infested (i.e. higher mean intensity) by *E. pellucida* than B-chicks (mean intensity 29.6, IC 95% 22.1–42.4 for chick A and 8.7, IC 95% 6.2–12.4 for chick B, Bootstrap t-test $t = 3.81$, $P < 0.01$) (Figure 1(A)). The range of intensity was 1–119 for A-chicks and 1–28 for
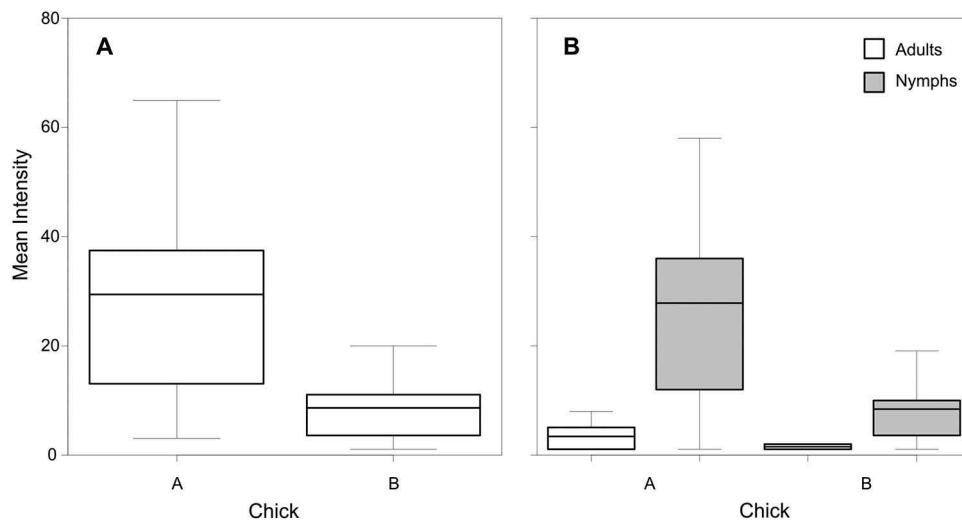
Figure 1. (A) Mean intensity of *Eidmanniella pellucida* infesting A and B Imperial Shag chicks, and (B) mean intensity of adults and nymphs of *E. pellucida* infesting A and B Imperial Shag chicks.
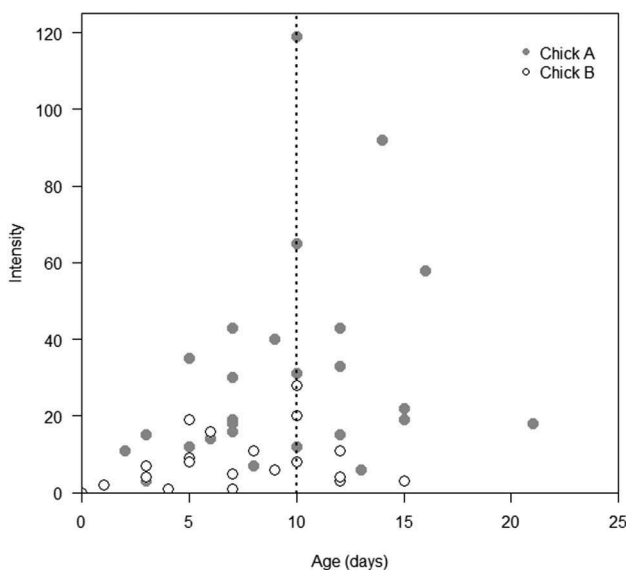

Figure 2. Intensity of *Eidmanniella pellucida* according to age in A-chicks (grey circles) and B-chicks (white circles). The dotted line indicates the date on which down feathers emerge.

Table 1. Summary of model-selection results for models explaining variation in intensity of Imperial Shag chicks by *Eidmanniella pellucida*, in relation to: *ORD*, which refers to hatching order of the chick; *AGE*, expressed as days from hatching; and *DAT*, the time during the reproductive season. See 'Material and methods' for details. Models are listed in decreasing order of importance

Candidate models	K	ΔAICc	w_i
ORD DAT DAT^2	6	0.0	0.508
ORD	4	2.2	0.170
ORD DAT	5	2.8	0.125
ORD AGE	5	3.7	0.078
ORD AGE AGE^2	6	4.3	0.058
ORD AGE DAT	6	5.5	0.032
ORD AGE AGE^2 DAT DAT^2	8	5.8	0.028
DAT DAT^2	5	20.4	0.000
AGE	4	21.5	0.000
DAT	4	21.5	0.000
AGE AGE^2	5	21.6	0.000
Null model	3	24.8	0.000

B-chicks (Figure 2). The mean hatching asynchrony between A- and B-chicks was 2.7 ± 1.2 days ($n = 21$).

Chicks were infested mainly by nymphs (Friedman test, $\chi^2 = 81.6$, df = 2, $n = 48$, $P < 0.001$; Wilcoxon test, adults vs. nymphs $z = -5.9$, $P < 0.001$). We also found differences in the population structure between chicks; A-chicks were more infested by adults and nymphs and B-chicks were infested mainly by nymphs (Figure 1(B)).

The intensity of infestation was affected by both the hatching sequence within the brood and the hatching date. The best model describing the intensity of infestation included the hatching sequence and the linear and quadratic terms of the hatching date ($w_i = 0.508$;

Table 1). All variables included in the model were important predictors of lice infestation and had the highest likelihood parameter values (Table 2). The intensity of infestation was higher in A-chicks than in B-chicks and varied along the reproductive season (Table 2; Figure 3). The intensity of *E. pellucida* increased along the breeding season until it reached a maximum and then decreased (Figure 3). As indicated by low parameter likelihood values and CI including zero, the linear and quadratic terms of age were not related to the variation in the intensity of infestation (Table 2).

Discussion

This is the first study that seeks to understand the patterns of vertical transmission of a species of louse to seabird chicks under natural conditions. Previous

Table 2. Parameter likelihoods, estimates, SE and 95% confidence interval limits (CL) for explanatory variables describing variation in the intensity of infestation of Imperial Shag chicks by *Eidmanniella pellucida*.*ORD* refers to the hatching order of the chick, *AGE* refers to the age of the chick (as days from hatching), and *DAT* refers to the time during the reproductive season. Explanatory variables with CL excluding zero are in bold. See 'Material and methods' for details

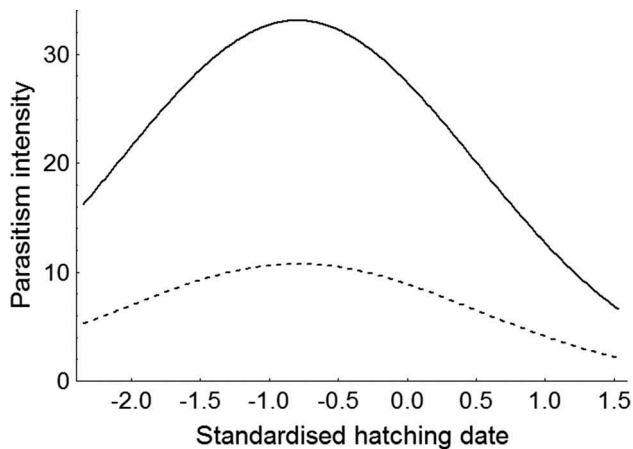| Explanatory variable	Parameter likelihood	Parameter estimate ± SE	CL Lower	CL Upper
Intercept		**3.13 ± 0.33**	**2.35**	**3.90**
ORD	**1.00**	**−1.13 ± 0.19**	**−1.50**	**−0.76**
DAT	**0.69**	**−0.41 ± 0.20**	**−0.82**	**−0.01**
DAT^2	**0.54**	**−0.30 ± 0.13**	**−0.55**	**−0.04**
AGE	0.20	0.07 ± 0.10	−0.14	0.28
AGE^2	0.09	−0.01 ± 0.01	−0.02	0.01

Figure 3. Relationship between the intensity of infestation of *Eidmanniella pellucida* and standardised hatching date for A-(continuous line) and B-chicks (dashed line) of the Imperial Shag. Parameters of the adjusted curves were estimated from generalised linear mixed models with a negative binomial distribution (see Table 1).

works on lice infestation describe that in seabirds the infestation should be characterised by high prevalence and low intensity (Eveleigh and Threlfall 1976; Rivera-Parra *et al.* 2014), which is consistent with the predictions made by Felsó and Rózsa (2006). These authors suggest that the characteristics of the infestation would be affected by the hosts' diving behaviour and that, compared to those from terrestrial hosts, lice infesting diving hosts are exposed to adverse conditions that reduce their probability of survival and transmission (Felsó and Rózsa 2006, 2007).

Our results indicate that vertical transmission of *E. pellucida* starts shortly after a chick hatches and is very effective. This is an interesting result if we consider that down feathers of Imperial Shag chicks appear *ca.*10 days after hatching and juvenile feathers need several weeks to develop (Malacalza and Navas 1996). Eveleigh and Threlfall (1976) estimated a similar time of transmission from parents to chicks in auks which, in contrast to shags, hatch as downy chicks. However, studies on

lice from terrestrial altricial birds described how infestation in nestlings is usually delayed until the feathers emerge (Clayton and Tompkins 1994; Lee and Clayton 1995). The particular pattern of transmission observed in the Imperial Shag (i.e. lice colonising a bared host), would probably respond to an evolutionary process. Both patterns (i.e. lice colonising a bared vs. a downy host) show that vertical transmission may involve complex interactions between the host, the louse and the life history of each one.

The most likely explanation for the observed patterns of lice transmission seems to be an adaptation to the amphibious life of the host. The evolutionary relevance of the association between diving vertebrates and lice was addressed mainly for pinnipeds (Murray 1976) but there is no information about the restriction that lice from seabirds faced during their evolution. As was described for lice from other amphibious hosts, we assume that lice reproduction can occur only during host reproduction because seawater affects the first nymphal stage (Leonardi and Lazzari 2014). Therefore, these feather lice have a brief window of time to reproduce and the quicker they colonise the newly hatched chick the sooner they can start their own reproductive cycle. In this sense, Murray (1976) argued that only lice from penguins could be considered as marine insects because these seabirds are 'truly divers'. However, this author (Durno Murray) also considered that lice from penguins are not really in contact with marine conditions since penguins maintain an air layer within their feathers even when they dive (Murray 1976). Thus, lice from a diving seabird with wettable plumage such as shags may be used as an interesting model to study both the ecological and evolutionary pressures that lead to the adaptation to the marine environment.

Imperial shags from Punta León colony perform dives that last more than 3 min and reach depths of more than 60 m (Gómez-Laich *et al.* 2012). Such exposure to marine conditions would probably affect lice, especially immature stages. Lice need feathers to attach

the eggs, and thus ovoposition must occur on adult shags because feathers are not fully developed on chicks. After hatching, nymphs would migrate to the chicks where they continue their development to adults.

One of the commonest explanations of how parasites choose their host is given by the Tasty Chick Hypothesis (TCH; Christie *et al.* 1998). The TCH postulates that parasites choose the smallest and youngest chick in a brood because it is less immunocompetent than its siblings. However, several studies have demonstrated that there are other factors that would influence how parasites choose their host. For example, Valera *et al.* (2004) demonstrated that flies from European bee-eaters prefer larger nestlings and suggested that factors such as body size, the host life cycle and the ecological or physiological requirements of parasites can drive the selection of a host.

Our data show differences in the mean intensity of *E. pellucida* according to the hierarchical order of chicks; however, chick order did not have an effect on the prevalence. As we had predicted, the mean intensity of *E. pellucida* was higher in A- than in B-chicks and the level of infestation was independent of the age of the chicks. If both chicks get their louse infestation from their parents, they would have the same probability of becoming infested. However, the fact that A-chicks were more infested than B-chicks would indicate a preference of lice for the elder chick. A similar result was reported by Brooke (2010), who found that older nestlings of the Blackbird (*Turdus merula*) were more parasitised by lice than younger nestlings.

Brooke (2010) presents three non-exclusive hypotheses that could explain why the elder chick would be more parasitised. The first one states that lice prefer older chicks because they would have more time to breed and disperse; this seems not to be the case for Imperial Shags because chicks hatch with only a couple of days of difference (Giudici *et al.* 2017). The second hypothesis states that older chicks are more fully feathered, which is not the case for Imperial Shags, as explained above. Alternatively, lice could remain in adult shags until the feathers of the chicks were fully developed. However, the presence of lice in bared chicks seems to support our hypothesis of the negative effect of marine conditions on nymphal survival. The final hypothesis states that lice choose the older chick as a strategy to increase their likelihood of survival. Our results seem to support this hypothesis. In the Imperial Shag population of Punta León, clutches hatch asynchronously over 4–5 days (Giudici *et al.* 2017). This hatching pattern yields an important age and size asymmetry between chicks, where the probability of fledging decreases drastically with hatching order (Giudici *et al.* 2017).

We found that independently of age, A-chicks were more infested than B-chicks (Figure 3), which suggests that lice prefer the chick with the higher survival prospects. This result raises the question of whether or not lice are able to determine host probabilities of survival. Until now there has been no empirical evidence on this issue; however, the fact that lice are able to synchronise their reproductive cycle with that of the host (Marshall 1981), together with the long co-evolutionary history of lice and seabirds, seems to support this idea.

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References

Bartoń, K. (2015). 'MuMIn: Multi-Model Inference. R Package, Version 1.15.6.' Available at https://CRAN.R-project.org/package=MuMIn

Brooke, M. D. L. (2010). Vertical transmission of feather lice between adult blackbirds *Turdus merula* and their nestlings: A lousy perspective. *Journal of Parasitology* **96**, 1076–1080. doi:10.1645/GE-2513.1

Burnham, K. P., and Anderson, D. R. (2002). 'Model Selection and Multimodel Inference: A Practical Information Theoretic Approach.' (Springer–Verlag: New York.)

Christie, P., Møller, A. P., and De Lope, F. (1998). Immunocompetence and nestling survival in the house martin: The tasty chick hypothesis. *Oikos* **83**, 175–179. doi:10.2307/3546559

Clayton, D. H., and Tompkins, D. M. (1994). Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London Series B-Biological Sciences* **256**, 211–217. doi:10.1098/rspb.1994.0072

Crofton, H. D. (1971). A quantitative approach to parasitism. *Parasitology* **62**, 179–193. doi:10.1017/S0031182000071420

Eveleigh, E. S., and Threlfall, W. (1976). Population dynamics of lice (Mallophaga) on auks (Alcidae) from Newfoundland. *Canadian Journal of Zoology* **54**, 1694-1711. doi:10.1139/z76-197

Felsó, B., and Rózsa, L. (2006). Reduced taxonomical richness of lice (Insecta: Phthiraptera) in diving birds. *Journal of Parasitology* **92**, 867–869. doi:10.1645/GE-849.1

Felsó, B., and Rózsa, L. (2007). Diving behaviour reduces genera richness of lice (Insecta, Phthiraptera) of mammals. *Acta Parasitologica* **52**, 82–85. doi:10.2478/s11686-007-0006-3

Galloway, T. D. (2012). Ectoparasites of rabbits and hares in Manitoba, Canada, with observations on age-specific dispersal in *Haemodipsus setoni* (Phthiraptera: Anoplura: Polyplacidae). *The Canadian Entomologist* **144**, 439–446. doi:10.4039/tce.2012.44

Giudici, P. I., Quintana, F., and Svagelj, W. S. (2017). The role of hatching asynchrony in a seabird species exhibiting obligate brood reduction. *Waterbirds* **40**, 221–232. doi:10.1675/063.040.0304

Gómez-Laich, A., Quintana, F., Shepard, E. L. C., and Wilson, R. (2012). Intersexual differences in the diving behaviour of Imperial Cormorants. *Journal of Ornithology* **153**, 139–147. doi:10.1007/s10336-011-0714-1

Johnsgard, P. A. (1993). 'Cormorants, Darters and Pelicans of the World.' (Pennsylvania: Smithsonian Institution Press.)

Kim, K. C. (1985). 'Coevolution of Parasitic Arthropods and Mammals.' (Wiley: New York.)

Lee, P. L. M., and Clayton, D. H. (1995). Population biology of swift (*Apus apus*) ectoparasites in relation to host reproductive success. *Ecological Entomology* **20**, 43–50. doi:10.1111/j.1365-2311.1995.tb00427.x

Leonardi, M. S., and Lazzari, C. (2014). Uncovering deep mysteries: The underwater life of an amphibious louse. *Journal of Insect Physiology* **71**, 164–169. doi:10.1016/j.jinsphys.2014.10.016

Malacalza, V. E., and Navas, J. R. (1996). Biología y ecología reproductiva de *Phalacrocorax albiventer* (Aves: Phalacrocoracidae) en Punta León, Chubut, Argentina. *Ornitologia Neotropical* **7**, 53–61.

Marshall, A. G. (1981). The sex ratio in ectoparasitic insects. *Ecological Entomology* **6**, 155–174 doi:10.1111/j.1365-2311.1981.tb00602.x

Murray, M. D. (1976). Insect parasites of marine birds and mammals. In 'Marine Insects.' (Ed L. Cheng.) pp. 79–96. (North Holland: Amsterdam.)

R Development Core Team. (2017). 'R: A Language and Environment for Statistical Computing.' (R Foundation for Statistical Computing: Vienna, Austria.) ISBN 3–900051–07–0. Available at http://www.r-project.org/

Reiczigel, J. (2003). Confidence intervals for the binomial parameter: Some new considerations. *Statistics in Medicine* **22**, 611–621. doi:10.1002/(ISSN)1097-0258

Reiczigel, J., and Rózsa, L. (2005). Quantitative parasitology 3.0. Available at http://www.zoologia.hu/qp/ [Verified 13 April 2013].

Rivera-Parra, J. L., Levin, I. I., and Parker, P. G. (2014). Comparative ectoparasite loads of five seabird species in the Galapagos Islands. *Journal of Parasitology* **100**, 569–577. doi:10.1645/12-141.1

Rózsa, L., Reiczigel, J., and Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228–232. doi:10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2

Skaug, H., Fournier, D., Nielsen, A., Magnusson, A., and Bolker, B. (2016). 'Generalized Linear Mixed Models Using AD Model Builder. R Package Version 0.8.3.3.'

Svagelj, W. S., and Quintana, F. (2011). Breeding performance of the Imperial Shag (*Phalacrocorax atriceps*) in relation to year, laying date and nest location. *Emu* **111**, 162–165. doi:10.1071/MU10062

Svagelj, W. S., Trivellini, M. M., and Quintana, F. (2012). Parental investment theory and nest defence by imperial shags: Effects of offspring number, offspring age, laying date and parent sex. *Ethology* **118**, 251–259. doi:10.1111/eth.2012.118.issue-3

Valera, F., Hoi, H., Darolová, A., and Kristofik, J. (2004). Size versus health as a cue for host choice: A test of the tasty chick hypothesis. *Parasitology* **129**, 59–68. doi:10.1017/S0031182004005232

Yorio, P., Quintana, F., Campagna, C., and Harris, G. (1994). Diversidad, abundancia y dinámica espacio-temporal de la colonia mixta de aves marinas en Punta León, Patagonia. *Ornitologia Neotropical* **5**, 69–77.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. (2009). 'Mixed Effects Models and Extensions in Ecology with R.' (Springer-Verlag: New York.)