Lower baseline immunity in invasive Egyptian goose compared to sympatric native waterfowls

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Abstract

Successful invasive species out compete native species for vital resources. To increase their spreading success, invasive species need to trade-off nutritional and metabolic resources allocated to reproduction and range expansion with other costly body functions. One proposed mechanism for the reallocation of resources is a trade-off with the immune function. According to the evolution of increased competitive ability hypothesis, a reduced investment in immunity would be favoured by parasite loss in the colonised habitats. It is also suggested that invaders would reallocate resources among different immune effectors depending on the new pathogens they encounter in the new habitat. Reallocation of resources may also involve the regulation of oxidative status given its fundamental link with the immune system. Relying on a panel of blood-based markers of immune function and oxidative status quantified in an invasive species (Egyptian goose) and two native competing species (mallard and mute swan) in Germany, we tested the hypothesis that the invasive species would have a lower investment in immune function compared to the native species. We predicted lower levels of baseline immune markers associated with systemic inflammatory response, and higher levels of certain low-cost immunological effectors (e.g. humoral effectors) in the invasive species compared to the two native species. If Egyptian geese reduced their investment in immune function, we would also expect that geese generated less oxidative damage and had a lower expression of antioxidant defences than native species. We found lower levels of several immune markers associated either with inflammatory response or humoral innate and adaptive immunity in the invasive species compared to the two native species. Bacteria killing ability of plasma was the only immune marker that was upregulated in Egyptian geese. Markers of oxidative status were higher in mallards compared to the other species. The results of our study point to an overall reduced investment in immune function in the invasive species as a possible energy-saving immunological strategy due to the loss of parasites in the new colonised habitats. Thus, a lower investment in immune function may benefit other energy-demanding activities, such as reproduction, dispersal, and territoriality.

52 **Introduction**

53 The occurrence of biological invasions is increasing worldwide as a consequence of ongoing globalization.
54 Successful invasive species engage in competition for vital resources with native species, sometimes even
55 replacing native species in their natural habitat, and thus concurs with the ongoing biodiversity crisis (Mollot
56 et al., 2017). Moreover, invaders can disrupt ecosystem function and facilitate the distribution of novel
57 pathogens, including some with zoonotic potential, generating massive economic costs and challenges for
58 public and animal health (Henry et al., 2023; Turbelin et al., 2023; Zhang et al., 2022).

59 Non-native introduced species need to establish and spread over the new region to become invasive.
60 High population growth rate and superior dispersal ability are two main life-history traits that promote
61 successful invasions (Sakai et al., 2001). However, increased need for nutritional and metabolic resources for
62 particularly high reproductive investment and dispersion means that invaders need to trade-off those
63 resources with other important body functions. Since invasive plants and animals may experience a loss of
64 parasites during the invasion process (Dunn et al., 2012; Torchin et al., 2003), a reduced investment in the
65 immune function might concur with the reallocation of resources (White and Perkins, 2012).

66 The immune system of vertebrates involves different pathways that require relatively higher or lower
67 energetic costs. The evolution of increased competitive ability (EICA) hypothesis predicts that a loss of natural
68 parasites in a habitat enables invasive species to colonise with reduced investment in defence functions and
69 thus allocate more resources towards fitness-related traits (Blossey and Notzold, 1995). However, as an
70 overall reduction of the immune response of invading hosts might increase their susceptibility towards
71 pathogens specific to the novel habitat, it has been proposed that the reallocation of resources between
72 different immune effectors would reduce the immune costs while keeping the system functioning at the same
73 time (Lee and Klasing, 2004; White and Perkins, 2012).

74 The EICA hypothesis was refined by Lee and Klasing (2004), who presented an analysis focused on the
75 trade-offs between immune effectors in invasive vertebrates. The revised EICA hypothesis proposed that
76 successful invasive species minimize systemic inflammatory responses that are triggered by strong innate or
77 cell-mediated immune response to infectious agents, as they come at elevated energetic and
78 immunopathological costs. Rather, a successful strategy for invaders to fight novel pathogens might be the

79 increase of humoral defences, which includes both constitutive levels of natural antibodies and adaptive
80 antibody responses (Lee and Klasing, 2004).

81 Studies on invasive populations of different vertebrate species show mixed evidence for trade-offs
82 between immune effectors and host traits. For example, invasive cane toads (*Rhinella marina*) moving further
83 distances showed decreased bacteria-killing ability and phagocytic activity, but higher swelling response
84 compared to their more sedentary conspecifics, suggesting trade-offs between dispersal distance and
85 immunity (Brown and Shine, 2014). An immune challenge experiment found minor metabolic changes and
86 reduced movement in American invasive house sparrows (*Passer domesticus*) compared to less-invasive tree
87 sparrows (*Passer montanus*). Additionally, immune-challenged tree sparrows had lower reproductive success
88 compared to house sparrows indicating greater immune and fitness costs in the tree sparrows (Lee et al.,
89 2005). Recently, a study on Egyptian geese (*Alopochen aegyptiaca*) found higher baseline values of
90 haemolysis, heterophil granulocytes and lymphocytes in the German invasive population compared to a
91 native population (Prüter et al., 2020). Although we still have a poor understanding of how variation in
92 immunological traits affects the success of biological invasions, multi-trait comparative studies might provide
93 valuable information on the mechanisms involved.

94 Energy-based trade-off models provide valuable insight into the regulation of immune function in
95 invaders. There are, however, other relevant physiological costs that can underlie immune investment. One
96 important physiological mechanism linking immune response to individual fitness is the regulation of cellular
97 oxidative status (Costantini and Møller, 2009; Hasselquist and Nilsson, 2012). Oxidative status is determined
98 by levels of reactive oxygen species (ROS) generation, oxidative damage caused by ROS to biomolecules, and
99 antioxidant molecules. During the phagocytosis of microbes, there is a rapid increase of ROS (respiratory
100 burst) that enables phagocytic cells to degrade internalized particles and bacteria. Such an increase of ROS
101 can induce oxidative stress if not sufficiently counterbalanced by antioxidants, with potential negative effects
102 for the host fitness (Costantini, 2022).

103 Antioxidants act as free radicals scavengers by preventing and repairing damages caused by ROS, and
104 can be upregulated to prevent disease or to protect the organism from immunopathology induced by the

105 inflammatory response (Jena et al., 2023; Pham-Huy et al., 2008). Thus, upregulated baseline levels of
106 endogenous antioxidants may help hosts to prevent a strong immune response and consequent oxidative
107 stress. For example, white-browed sparrow weavers (*Plocepasser mahali*) with stronger superoxide
108 dismutase (SOD) activity produced a smaller swelling response after an immune challenge (Cram et al., 2015).
109 We are not aware of any studies comparing baseline levels of oxidative status markers between invasive and
110 native species.

111 Important behavioural and life-history traits of successful invasive species, such as increased
112 dispersal, endurance, and high reproductive rate may require particularly high levels of circulating oxygen.
113 Haematocrit is an indirect marker of oxygen carrying capacity of blood (Ritchison, 2023) and thus, it might be
114 upregulated in invasive species. This hypothesis was not supported by a study that compared cane toads
115 (*Rhinella marina*) from the invasion front (spreaders) with cane toads from the core range (resident; Tracy et
116 al., 2012). To our knowledge there is no study that compares levels of oxygen carrying capacity of blood
117 between invasive and non-invasive species.

118 In this study, we compared the immune-physiological profile of the invasive Egyptian goose to that of
119 two sympatric European waterfowl species. Specifically, we quantified a number of blood-based markers of
120 immune function, oxidative status, and oxygen carrying capacity. The Egyptian goose is a successful invasive
121 species which has been established in Europe since the 1980s and is currently expanding its range (Gyimesi
122 and Lensink, 2012). The other two waterfowl species included in this study are native to the study area and
123 share the same habitat with the Egyptian goose: the mallard (*Anas platyrhynchos*) and the mute swan (*Cygnus*
124 *olor*).

125 In accordance with Lee and Klasing (2004), we predicted lower levels of baseline immune markers
126 associated with systemic inflammatory response (i.e., granulocytes, macrophages, and lymphocytes) and
127 higher levels of humoral and low-cost immunological effectors (i.e., lysozymes, immunoglobulins, bacteria
128 killing ability, haemolysis and haemagglutination) in the invasive species compared to the native species. In
129 addition, if Egyptian geese have reduced inflammatory responses, we might expect either (i) lower investment
130 in endogenous antioxidant defences to save energy to allocate in other fitness traits or (ii) higher investment

131 in antioxidant defences for a better control of inflammatory responses compared to the other two species.
132 Baseline levels of oxidative damage are expected to show an opposite pattern to that of antioxidant defences.
133 Finally, we expect higher levels of oxygen carrying capacity in Egyptian geese compared to mallards and mute
134 swans.
135

136 **Materials and methods**

137 **Study species and area**

138 Mallard ducks and mute swans are waterfowl species native to Europe, while the Egyptian goose is native to
139 sub-Saharan Africa and was introduced to Europe in the twentieth century. The invasion of the Egyptian
140 goose started from the Netherlands, where they were introduced as ornamental species in urban parks. From
141 the 1980s onwards, Egyptian geese also invade Germany where its population size increased rapidly.
142 Nowadays, the Egyptian goose is the most common and widespread African waterfowl species in Europe
143 (Gyimesi and Lensink, 2012). Mallard ducks, mute swans and Egyptian geese are resident, territorial and
144 socially monogamous species. While mute swans and Egyptian geese show biparental care, mallards perform
145 female-only parental care. Additionally, mallard populations show adult sex-ratios which are usually male-
146 biased and forced extra-pair copulations frequently occur. Anseriformes are known for their aggressive
147 behaviour towards conspecifics and other species, particularly during the breeding season, to protect their
148 territory, nest and brood (Hohmann and Woog, 2021). All three species live in a variety of water habitats,
149 with a clear preference for freshwater ponds, rivers and lakes (both natural and artificial) sometimes in close
150 proximity to humans.

151 Adult individuals of the study species were live trapped in June and July 2016 in the Rhine and Mosel
152 area in Western Germany (50.4° N, 7.6° E; Fig. S1). All individuals of this study were locally breeding and
153 mostly in the phase of guiding goslings/ducklings at the time of sampling. Birds were caught using either
154 loops or landing nets. Each bird was individually ringed, and body mass and sex were recorded. A blood
155 sample from each bird was collected, either from the vena metatarsalia plantaris superficialis or the vena
156 ulnaris, using a needle of 0.06 mm diameter. Blood samples were kept cool during field procedures, then
157 centrifuged for 15 minutes to separate plasma from red blood cells which were stored separately in liquid

158 nitrogen within 8 hours from blood collection. At the end of fieldwork, samples were stored at -80 °C until
159 laboratory analyses.

160 Sample sizes for each assay were dependent on the total amount of plasma and red blood cells
161 available from each individual, and therefore differ between the tests that were carried out (Table 1).
162 Sampling was authorized in terms of animal welfare by the Landesuntersuchungsamt Rheinland-Pfalz (G 15-
163 20-005) and Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) (84-
164 08.04.2015.A266). The experimental procedures were approved by the Internal Committee for Ethics and
165 Animal Welfare of the Leibniz Institute for Zoo and Wildlife Research (permit #2014-11-03).

166

167 **Laboratory analyses**

168 Several immunological assays were carried out to characterize both the cellular and humoral components of
169 the adaptive and innate immune system of the study species. We used non-specific assays that had previously
170 been applied to samples from a variety of free-living avian species, including different waterfowl (Bourgeon
171 et al., 2010; Matson et al., 2006). Specifically, we counted basophils, eosinophils, heterophils and monocytes
172 as effectors of cellular innate immunity. We quantified the amounts of lysozyme, bacteria killing ability,
173 haemagglutination and haemolysis as markers for humoral innate immunity. We measured the total
174 immunoglobulin Y (IgY) concentration and the number of lymphocytes as markers of, respectively, humoral
175 and cellular adaptive immunity (Table 1; (Prüter et al., 2020)).

176 In addition to the analyses of immune markers, we measured the haematocrit as a proxy for the
177 oxygen carrying capacity of the blood. Further, we measured the total non-enzymatic antioxidant capacity
178 (OXY-adsorbent assay), and the activity of antioxidant enzymes superoxide dismutase (SOD) and glutathione
179 peroxidase, to estimate the antioxidant capacity of the blood. We measured the concentrations of protein
180 carbonyls and reactive oxygen metabolites (d-ROMS test) to evaluate blood levels of oxidative damage. More
181 details on the assays used for this study can be found in the Supplementary Material.

182

183 **Statistical analyses**

184 The dataset included 74 actively reproducing individuals of which 28 were Egyptian geese, 10 mallards, and
185 36 mute swans. We collected blood samples from both female and male Egyptian geese (females = 12; males
186 = 16) and mute swans (females = 21; males = 15), and only female mallards. Sample sizes varied slightly for
187 different immunological markers due to limited amounts of blood for certain individuals (Table 1).

188 We performed a principal component analysis (PCA) on the correlation matrix between all markers
189 of the immune system and of oxidative status to reduce the number of variables by identifying the main
190 sources of variation. Because PCAs do not accept missing data, we imputed missing values through the R
191 package 'missMDA' (Husson 2016) and performed a PCA on the completed dataset using the *prcomp* function
192 (R Core Team 2023). Then, we extracted the first three main axes of the PCA (PC1, PC2, and PC3) and tested
193 their correlations with values of each immune and oxidative status marker to assess how markers loaded on
194 each of the three axes. Correlations above the threshold value of 0.5 were considered as main correlations
195 (Table S1; Cohen, 2008).

196 To test for species differences in immune-physiological traits, we ran three linear regression models
197 (LMs) including each principal component (PC) as a response variable and species as the predictor variable.
198 Initially, we also included the interaction between sex and species to test for intraspecific and interspecific
199 differences between sexes. However, the interaction showed no significant effects and was removed from the
200 models.

201 **Results**

202 The first three PCs of the PCA explained 47.2% of the total variance in markers of the immune system and
203 oxidative status (Fig. S2). In particular, PC1 captured 22.85% of the total variance and reflected both cellular
204 innate immunity and adaptive immunity: the immune markers that strongly correlated with PC1 were
205 heterophil granulocytes (corr. = 0.63), lysozymes (corr. = 0.68), haemagglutination (corr. = 0.66), haemolysis
206 (corr. = 0.72), bacteria killing ability (corr. = -0.61), lymphocytes (corr. = 0.58) and IgY (corr. = 0.67). PC2
207 captured 12.62% of the total variance and was mainly associated with the basophil granulocytes (corr. = -
208 0.77), eosinophil granulocytes (corr. = -0.58), and two markers of the oxidative status, superoxide dismutase

209 (corr. = -0.64) and reactive oxygen metabolites (corr. = -0.54; Fig. 1). PC3 captured 9.56% of the total variance
210 and was associated with haemagglutination (corr. = -0.60) and haemolysis (corr. = -0.55; Table S1, Fig. S3).
211 ANOVA tests of the LMs showed significant differences among species in their immune characteristics
212 (Table 2). Pairwise comparisons of estimated marginal means showed that Egyptian geese had lower levels
213 of both cellular innate and adaptive immune markers compared to mallards and mute swans. Specifically,
214 mute swans showed the highest levels of immune function among the three species, while mallards showed
215 intermediate value. The bacteria killing ability was the only marker negatively correlated with the Principal
216 Component and is thus higher in Egyptian geese compared to mallards and mute swans. We also found
217 significantly higher levels of eosinophil and basophil granulocytes, superoxide dismutase and oxidative
218 damage (ROMs) in mallards compared to both Egyptian geese and mute swans. Those markers correlate
219 negatively with PC2, which means their values were higher for negative values of the PC2. No other significant
220 differences between species were found (Fig. 2; Table 2).
221

222 **Discussion**

223 In this study we compared the baseline immune-physiological profile of an invasive species, the Egyptian
224 goose, to that of two sympatric European waterfowl species, the mallard and the mute swan. As expected,
225 we found lower levels of cell-mediated immune markers in the blood of the invasive species compared to the
226 two native species. Specifically, the Egyptian goose showed lower levels of heterophil granulocytes and
227 lymphocytes. Surprisingly, we also found reduced levels of humoral defences, represented by non-specific
228 natural antibodies (haemagglutination), complement activity (haemolysis) and immunoglobulins-Y (IgY), but
229 higher level of plasma bacteria killing ability (BKA), in Egyptian geese compared to mute swans and mallards.
230 In addition, we found higher levels of basophil and eosinophil granulocytes, superoxide dismutase and
231 reactive oxygen metabolites in mallards compared to Egyptian geese and mute swans.

232 **Immunological effectors**

233 Heterophils and lymphocytes are the most abundant leucocytes in birds, comprising up to 95% of all white
234 blood cells (Davis et al., 2008; Ritchison, 2023). Heterophils are the major phagocytic leucocytes and

important mediators of acute inflammatory responses (Scanes et al., 2022). Lymphocytes play important roles in both cell- and antibody-mediated adaptive immunity. Specifically, T-lymphocytes attack infected or abnormal cells inducing inflammation, and B-lymphocytes produce immunoglobulins (Guriec et al., 2018). According to White and Perkins (2012), lower levels of leucocytes might be due to lower parasitic prevalence and/or reduced immune resistance to infections in the invasive species compared to the native species.

Recent studies found that German populations of Egyptian geese show lower prevalence of infections for a variety of blood parasites and macro-parasites compared to conspecifics from their native habitats and show lower seroprevalences of some common waterfowl viruses (Prüter et al., 2018, 2020). Such findings support the hypothesis that lower levels of leucocytes in Egyptian geese in their invasive range may be due to reduced prevalences of infectious agents, which would be compatible with the predictions of the evolution of increased competitive ability hypothesis. In addition, invasive hosts may tolerate certain levels of parasitic infections because it reduces the overall cost associated with the active removal of parasites (i.e., resistance) and associated sickness behaviours (Lennon et al., 2023; White and Perkins, 2012). Thus, Egyptian geese might benefit from reduced levels of heterophil granulocytes and lymphocytes compared to sympatric species, because it might prevent strong inflammatory responses in case of infection. Reduced cellular and inflammatory response were also found in other successful invasive species, such as the European paper wasp (*Polistes dominula*) and the house sparrow (*Passer domesticus*), compared to sympatric congeneric species (Lee et al., 2005; Wilson-Rich and Starks, 2010).

A previous study on this invasive population of Egyptian geese found higher levels of heterophils and leucocytes than Egyptian geese that were sampled in their native habitat, but higher variance of immune markers (Prüter et al., 2020). This might indicate that immune plasticity may be a key factor for successful invasions (Poirier, 2019; Pyšek and Richardson, 2010; White and Perkins, 2012). Invasive Egyptian geese might defend efficiently against novel pathogens by upregulating plasma BKA while simultaneously investing less energy in other costly immune functions. Our results point to reduced overall costs of immunity in the invasive population of Egyptian geese compared to mallards and mute swans in the same habitat. Therefore, invasive

260 Egyptian geese might have more energy to allocate towards reproduction, dispersal, territoriality, and survival
261 than their competitors.

262 Contrary to our predictions, we found lower levels of humoral immune markers, such as
263 haemagglutination, haemolysis, lysozymes and IgY, in Egyptian geese compared to mallards and mute swans.
264 The only exception was represented by the plasma BKA, that was effectively higher in the invasive species.
265 The mechanisms that allow plasma to kill bacteria are not well understood, but might be strongly dependent
266 on antibacterial plasma proteins and peptides (Dugovich et al., 2019; Gao et al., 2015). Accordingly, Egyptian
267 geese might invest relatively more energy in antimicrobial proteins and peptides, which come to lower (i)
268 energetic costs compared to immune cells and (ii) fitness costs compared to inflammatory effectors.

269 Haemagglutination and haemolysis reflect responses to foreign antigens driven by natural antibodies
270 and the complement system, respectively (Matson et al., 2006). Natural antibodies are produced in absence
271 of antigenic stimulation, independently from previous infections, whereas the complement system can be
272 activated directly by pathogens or indirectly by immunoglobulins (Ochsenbein and Zinkernagel, 2000).
273 Natural antibodies and the complement system are part of the innate immune system and act as a first line
274 of defence against infections. Therefore, lower levels of haemagglutination and haemolysis are in accordance
275 with previous studies (Prüter et al., 2020, 2018) that Egyptian geese have a reduced prevalence of infections
276 compared to mallards and mute swans.

277 IgY is the most abundant circulating immunoglobulin in birds (Ritchison, 2023). As every other
278 immunoglobulin, IgY are produced by B-lymphocytes. Thus, the lower levels of constitutive IgY in Egyptian
279 geese are in agreement with the reduced number of lymphocytes. Furthermore, native species often show
280 higher levels of IgY and immune resistance due to the co-evolution between hosts and parasites (Koskella,
281 2018). For example, an experimental study on house finches (*Haemorhous mexicanus*) found higher levels of
282 IgY in populations that co-evolved with the inoculated virus (*Mycoplasma gallisepticum*) compared to
283 unexposed populations (Gates et al., 2021). Since higher resistance to infections leads to higher costs to host
284 fitness (Rigby et al., 2002), invasive species may benefit from minor resistance to infections for reducing
285 fitness costs (Medzhitov et al., 2012). This might explain why invasive species, which are mainly exposed to

286 novel pathogens, produce less immunoglobulins. Further studies are, however, needed to explore this
287 hypothesis in more detail.

288 **Oxygen carrying capacity and oxidative status**

289 We found no evidence for higher baseline oxygen carrying capacity in Egyptian geese compared to the other
290 species. It might be that differences in oxygen carrying capacity emerge only during the phase of range
291 expansion of invasive species as the haematocrit is rather sensitive to current metabolic requests (Ritchison
292 2023). All Egyptian geese included in our study were reproductively active individuals holding a territory, so
293 their immune-physiological function might differ from that of geese at the invasion front (Gyimesi and
294 Lensink, 2012). While there are studies showing different regulations of the immune system between
295 individuals living at the population core versus those at the front of invasion (Brown and Shine, 2014; Romeo
296 et al., 2023), further studies are required to assess the role of oxygen carrying capacity in individuals that are
297 at the invasion front.

298 Finally, we found that mallards had higher levels of eosinophils, basophils, oxidative damage (ROMs),
299 and activity of the antioxidant enzyme superoxide dismutase compared to Egyptian geese and mute swans.
300 In our study region, mallards are subject to increased competition and aggressive behaviour due to the
301 presence of Egyptian geese (Hohmann and Woog, 2021), which might result in increased metabolic activity
302 and ROS generation (Quque et al., 2022). It might also be that the higher pace of life of mallards compared
303 to Egyptian geese and mute swans determines higher baseline generation of ROS.

304

305 **Conclusions**

306 Our comparative analysis of multiple immune-physiological traits suggests that invasive Egyptians geese
307 might strategically reduce their investment in immune function compared to sympatric related native species
308 because of their lower parasite exposure. Native species that co-evolved with parasites in their natural habitat
309 might exhibit a stronger immune response aiming to clear the infection (i.e., resistance). By contrast, invasive
310 species are likely no longer exposed to the pathogens they encountered in their natural habitats. Moreover,
311 if they are exposed to novel pathogens in the invaded habitat, they might benefit from a less strong immune

312 response (i.e., more tolerant) to avoid immunopathological effects. Thus, invasive species might invest less
313 energy to sustain immune function, which might (i) come at the benefit for other energy-demanding host
314 traits, such as reproduction, dispersal, and territoriality, and (ii) bear minor risks of metabolic and behavioural
315 impairments caused by acute inflammatory responses.

316 The results of our work also indicate that constitutive immune cells involved in acute inflammatory
317 response might be the main downregulated effectors of immune function. This finding supports the
318 hypothesis developed by Lee and Klasing (2004) that invasive species reduce the risk of systemic inflammatory
319 responses as this is energetically and pathologically expensive and therefore ecologically maladaptive. Future
320 work is needed to understand how baseline differences in immunological parameters translate into trade-offs
321 with fitness traits, such as reproduction or survival. We also recognize that some variation in markers among
322 species might also reflect species-specific differences that are not due to their history of colonisation. This is
323 particularly true given that mallards show important behavioural, morphological, and pace-of-life differences
324 with the other two species, which might contribute to among species variation in the regulation of oxidative
325 status and immune function (i.e., relatively higher importance of eosinophil and basophil granulocytes as
326 immune effectors, and upregulated oxidative status). On the other hand, physiological plasticity is an
327 important driver of local acclimation and success of invasion (McCann et al., 2018). In accordance with Prüter
328 et al. (2020), high levels of physiological plasticity in Egyptian geese might be one main mechanism underlying
329 their successful invasion to Europe. Determining immune-physiological traits underlying the phenotype of
330 successful invaders will foster our understanding of the proximate mechanisms through which species can
331 successfully colonise novel environments and help to better predict novel alien species and potential
332 mitigation strategies (Lennox et al., 2015).

333 .

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Tables and figures

Table 1 – Sample size (n), mean and standard deviation (SD) for immunological effectors, and for haematocrit and markers of oxidative status.

Immunological effectors	Egyptian goose		Mallard		Mute swan	
	n	mean±SD	n	mean±SD	n	mean±SD
Lysozymes	27	3.07±2	10	3.89±1.85	34	7.06±3.17
Haemagglutination	21	6.66±1.42	10	7.1±0.99	34	7.67±1.49
Haemolysis	21	5.76±1.13	10	6±0.94	34	6.94±1.59
IgY	23	0.55±0.19	10	0.81±0.22	36	0.86±0.2
Bacteria Killing Ability	27	83.61±24.77	10	84.01±25.77	27	47.61±23.04
Basophils	28	3.29±2.72	10	10.53±6.33	36	3.98±2.44
Eosinophils	28	26.99±13.97	10	33.14±19.78	36	25.21±15.48
Heterophils	28	6.97±4.25	10	11.17±11.26	36	16.69±9.83
Lymphocytes	28	35.18±14.61	10	51.38±19.6	36	49.87±18.99
Monocytes	28	15.1±6.2	10	15.57±10.01	36	10.89±7.24
Haematocrit and oxidative status						
Haematocrit	24	44.79±8.5	7	40.57±8.81	21	39.9±4.51
OXY	27	197.47±46.99	10	180.61±46.22	32	207.69±43.73
ROMS	23	0.33±0.15	8	0.68±0.26	32	0.44±0.22
Protein Carbonyls	18	43.23±17.97	8	40.27±14.61	30	35.31±18.05
GPx	28	0.29±0.11	10	0.27±0.16	36	0.38±0.21
SOD	28	2.05±0.61	10	2.4±0.53	36	2.11±0.68

Table 2 – Between-species comparisons of estimated marginal means obtained from three ANOVA models applied on the Principal Components (PC). Significant results are shown in bold. ALEG = Egyptian geese; CYOL = mute swans; ANPL = mallards; f = females; m = males.

	Contrast	Estimate	SE	df	t.ratio	P-value
PC1	ALEG – ANPL	-1.64	0.50	71	-3.25	**<0.01**
	ALEG – CYOL	-2.92	0.35	71	-8.46	**<0.01**
	ANPL – CYOL	-1.28	0.49	71	-2.61	**0.03**
PC2	ALEG – ANPL	2.05	0.45	71	4.52	**<0.01**
	ALEG – CYOL	-0.19	0.31	71	-0.61	0.82
	ANPL – CYOL	-2.23	0.44	71	-5.09	**<0.01**
PC3	ALEG – ANPL	0.28	0.46	71	0.61	0.81
	ALEG – CYOL	0.26	0.31	71	0.82	0.69
	ANPL – CYOL	-0.02	0.45	71	-0.05	1.00

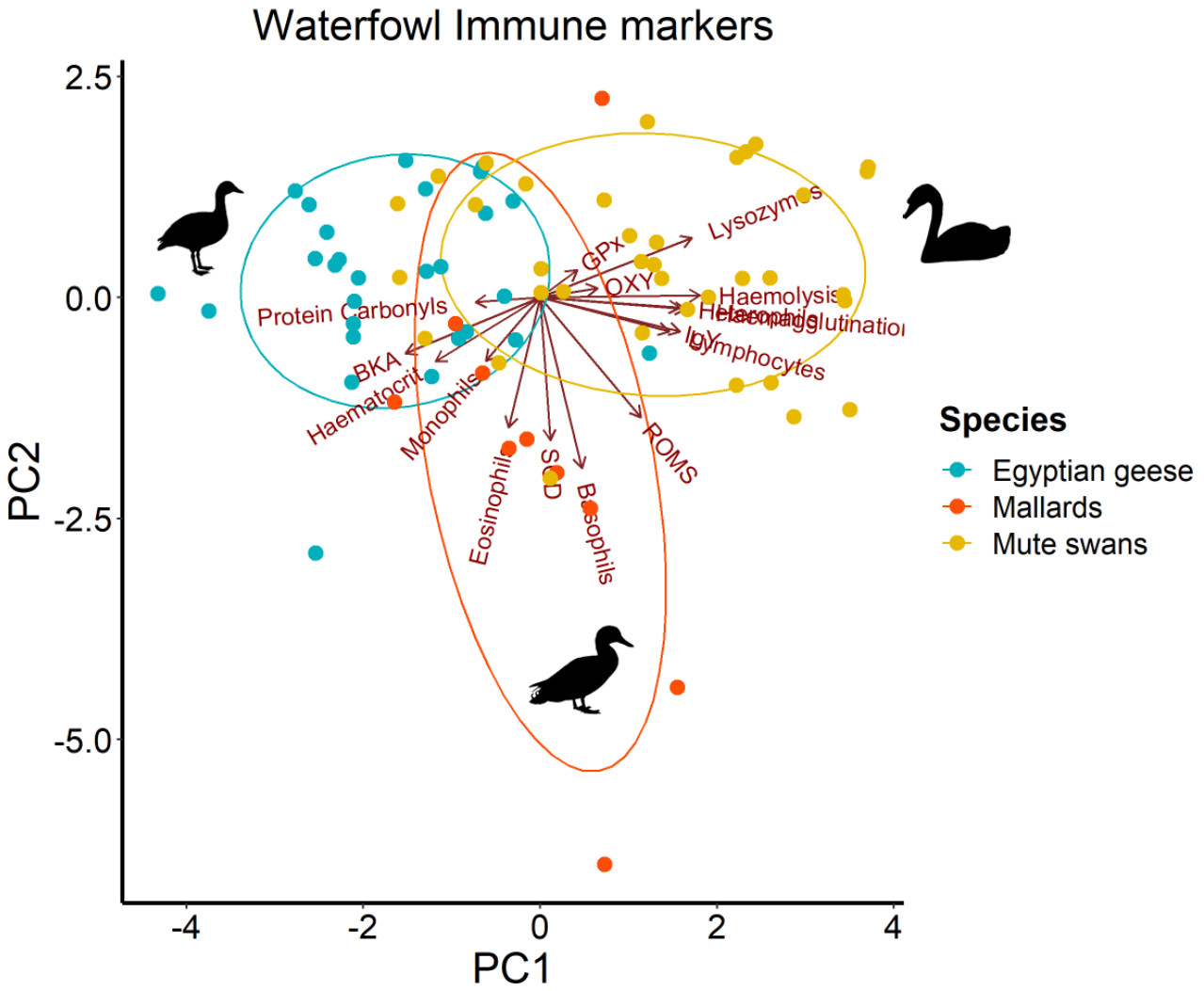
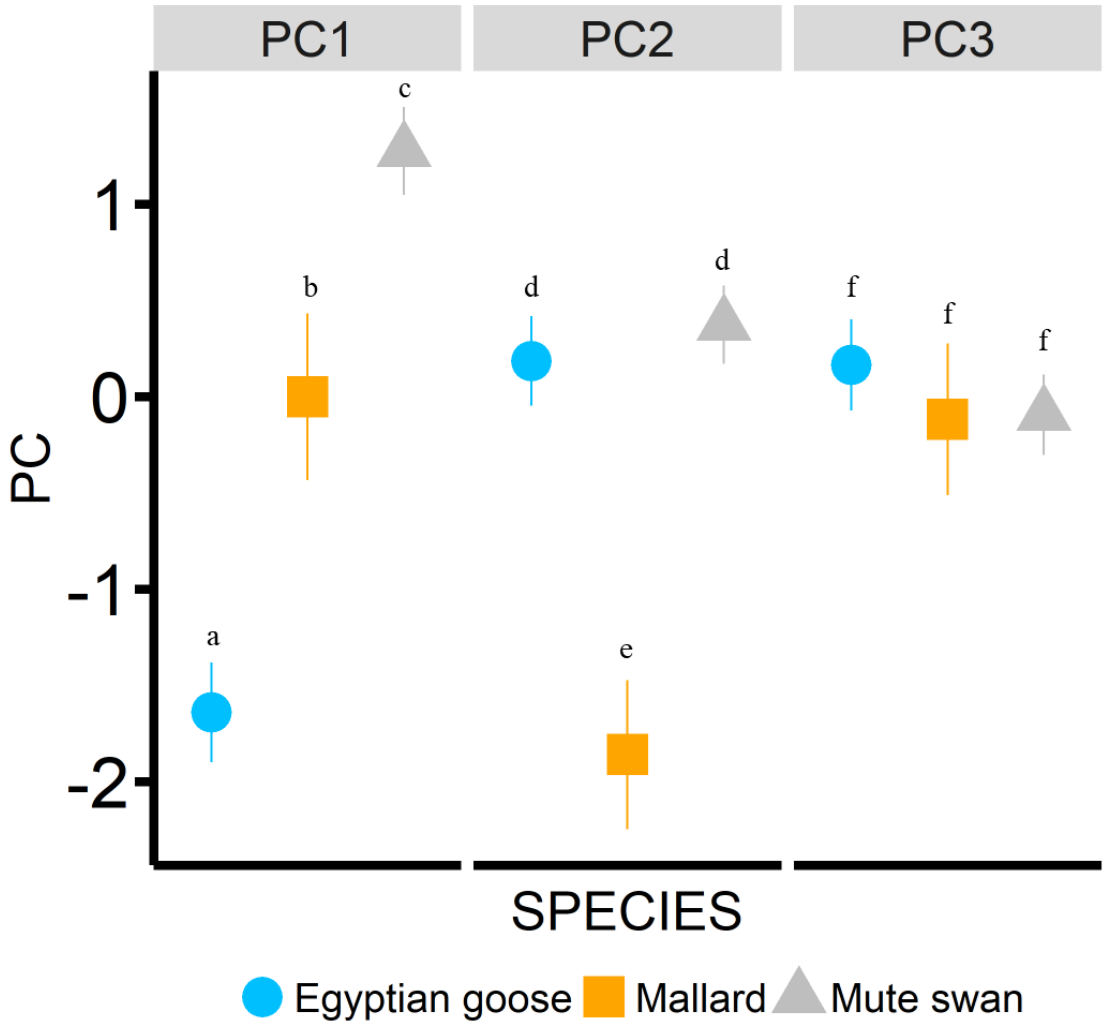


Fig. 1 – **Immune-physiological differences between the study species.** The position of each species is projected into the space defined by the first two Principal Components (PC) of a Principal Components Analysis performed on 16 parameters of immunity and oxidative status. Arrows represent the contribution of each parameter to PC1 and PC2. Species clusters are based on 95% confidence ellipses. Silhouettes of mallard and mute swan were sourced from PhyloPic. Each silhouette is available under CC BY-NC-SA 4.0 Deed licence, with credits to Maija Karala and Andy Wilson.



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493 Fig. 2 – Estimated marginal means ± standard error (SE) of the ANOVA models based on Principal Components
494 (PC). Note that immune markers that are negatively correlated with the PC must be interpreted as higher
495 mean values (e.g., bacteria killing ability is negatively correlated with PC1, thus is higher in the Egyptian goose
496 compared to the other species). See Table S1 for results of correlations between the scores of PCs and the
497 immune markers. Significant results are indicated by different superscripts letters.