Lower adaptive immunity in invasive Egyptian geese compared to sympatric native waterfowls

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Abstract

Successful invasive species increase their spreading success by trading-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 and the regulation of oxidative status. 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 (i) lower investment in immune function, (ii) lower levels of oxidative damage, and (iii) no higher antioxidant defences compared to the native species. We found lower levels of adaptive immune markers (lymphocytes and immunoglobulin Y), in the invasive species compared to the two native species. Reactive oxygen metabolites, a marker of oxidative damage, were also lower in Egyptian geese compared to the other species, while levels of antioxidants were generally similar. Mute swans showed the highest levels of heterophils, lysozymes and haemolysis among the three species, possibly due to its larger body mass. Our results point to a reduced investment in adaptive immune function in the invasive species as a possible resources-saving immunological strategy due to the loss of co-evolved parasites in the new colonised habitats, as observed in a previous study. A lower investment in immune function may benefit other energy-demanding activities, such as reproduction, dispersal, and territoriality.

Keywords: biological invasion, birds, immune system, adaptive immunity, oxidative status, invasive species**,** oxidative stress

Research highlights

Our results point to a reduced investment in adaptive immune function in invasive Egyptian geese compared to native and sympatric waterfowl species, as a possible resources-saving immunological strategy due to the loss of co-evolved parasites in the new colonised habitats**.**

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 & 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 & Notzold, 1995). The EICA hypothesis
70 was refined by Lee and Klasing (2004), who presented an analysis focused on the trade-offs between immune
71 effectors in invasive vertebrates. The revised EICA hypothesis proposed that successful invasive species
72 minimize systemic inflammatory responses that are triggered by strong innate or cell-mediated immune
73 response to infectious agents, as they come at elevated energetic and immunopathological costs. Rather, a
74 successful strategy for invaders to fight novel pathogens might be the increase of humoral defences, which
75 includes both constitutive levels of natural antibodies and adaptive antibody responses (Lee & Klasing, 2004).
76 Studies testing the EICA-hypothesis on vertebrates, however, show differential investment in immune
77 functions between invasive and native species, and between high and low dispersers of an invasive species
78 (revised in Cornet et al., 2016).

79 Maintenance of baseline immune function also requires investment of (micro)nutrients (Koutsos &
80 Klasing, 2014). For example, an experimental study showed that nestling magpies (*Pica pica*)
81 immunostimulated with methionine, a sulphur amino acid that enhances T-cell immune response, had
82 reduced growth rates, but were capable to mount a stronger immune response to an immune challenge
83 during the fledgling stage (Soler et al., 2003). In addition, Tschirren and Richner (2006) showed that nestling
84 great tits (*Parus major*), whose immune system was boosted by extra provisioning of methionine, were able
85 to grow at faster rate when exposed to hen fleas (*Ceratophyllus gallinae*), compared to control birds. These
86 results indicate that the evolution of an optimal host defence is governed by a parasite-mediated allocation
87 trade-off between fitness traits and immune function (Coon et al., 2014; White & Perkins, 2012). Accordingly,
88 invasive species may take advantage of downregulating antibody-mediated responses (i.e. adaptive
89 immunity) because they have lost their co-evolved parasites (Cornet et al., 2016).

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

99 Antioxidants prevent ROS from causing damages to biomolecules or even repair certain types of
100 oxidative damage, and can also be upregulated to prevent disease or to protect the organism from
101 immunopathology induced by the inflammatory response (Jena et al., 2023; Pham-Huy et al., 2008). Thus,
102 upregulated baseline levels of endogenous antioxidants may help hosts to prevent a strong immune response
103 and consequent oxidative stress. For example, white-browed sparrow weavers (*Plocepasser mahali*) with
104 stronger superoxide dismutase (SOD) activity produced a smaller swelling response after an immune

105 challenge (Cram et al., 2015). We are not aware of any studies that compared baseline levels of oxidative
106 status markers between invasive and native species.

107 In this study, we compared the immune-physiological profile of the invasive Egyptian goose to that of
108 two sympatric European waterfowl species. Specifically, we quantified a number of blood-based markers of
109 immune function, oxidative status, and oxygen carrying capacity. The Egyptian goose is a successful invasive
110 species which has been established in Europe since the 1980s and is currently expanding its range (Gyimesi
111 & Lensink, 2012). Previous studies have found differences in immune markers and parasite prevalence
112 between a native population of Egyptian geese (from Namibia) and the invasive population in our study area
113 (Prüter et al., 2018, 2020). The other two waterfowl species included in this study are the mallard (*Anas*
114 *platyrhynchos*) and the mute swan (*Cygnus olor*). They were chosen because resident and native to the study
115 area, where they are sympatric with Egyptian geese, compete with them for territories, and are potentially
116 exposed to the same pathogens. However, these three species also show differences (see Materials and
117 Methods) that need to be considered in the interpretation of results.

118 Since we still have a poor understanding of how variation in immunological traits affects the success
119 of biological invasions, making predictions on singular immune-physiological markers is challenging. We
120 expect to find, however, downregulated immune effectors in the invasive species compared to the native
121 species to save resources to be invested in other fitness traits. We also expect reduced oxidative damage in
122 the invasive species due to its associated fitness costs. We do not expect higher levels of enzymatic
123 antioxidants in the invasive species, compared to the native species, due to the energetic costs required for
124 their synthesis.

125

126 **Materials and methods**

127 **Study species and area**

128 Mallard ducks and mute swans are waterfowl species native to Europe, while the Egyptian goose is native to
129 sub-Saharan Africa and was introduced to Europe in the twentieth century. The invasion of the Egyptian
130 goose started from the Netherlands, where they were introduced as ornamental species in urban parks. From
131 the 1980s onwards, Egyptian geese also invade Germany where its population size increased rapidly.

132 Nowadays, the Egyptian goose is the most common and widespread African waterfowl species in Europe
133 (Gyimesi & Lensink, 2012). Mallard ducks, mute swans and Egyptian geese are resident, territorial and socially
134 monogamous species. While mute swans and Egyptian geese show biparental care, mallards perform female-
135 only parental care. Additionally, mallard populations show adult sex-ratios which are usually male-biased and
136 forced extra-pair copulations frequently occur. Anseriformes are known for their aggressive behaviour
137 towards conspecifics and other species, particularly during the breeding season, to protect their territory,
138 nest and brood (Hohmann & Woog, 2021). All three species live in a variety of water habitats, with a clear
139 preference for freshwater ponds, rivers and lakes (both natural and artificial) sometimes in close proximity
140 to humans.

141 Adult individuals of the study species were live trapped in June and July 2016 in the Rhine and Mosel
142 area in Western Germany (50.4° N, 7.6° E; Fig. S1). All individuals of this study were locally breeding and
143 mostly in the phase of guiding goslings/ducklings at the time of sampling. Birds were caught using either
144 loops or landing nets. Each bird was individually ringed, and body mass and sex were recorded. A blood
145 sample from each bird was collected, either from the vena metatarsalia plantaris superficialis or the vena
146 ulnaris, using a needle of 0.06 mm diameter. Blood samples were kept cool during field procedures, then
147 centrifuged for 15 minutes to separate plasma from red blood cells which were stored separately in liquid
148 nitrogen within 8 hours from blood collection. At the end of fieldwork, samples were stored at -80 °C until
149 laboratory analyses.

150 Sample sizes for each assay were dependent on the total amount of plasma and red blood cells
151 available from each individual, and therefore differ between the tests that were carried out (Table 1).
152 Sampling was authorized in terms of animal welfare by the Landesuntersuchungsamt Rheinland-Pfalz (G 15-
153 20-005) and Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) (84-
154 08.04.2015.A266). The experimental procedures were approved by the Internal Committee for Ethics and
155 Animal Welfare of the Leibniz Institute for Zoo and Wildlife Research (permit #2014-11-03).

156

157 **Laboratory analyses**

158 Several immunological assays were carried out to characterize both the cellular and humoral components of
159 the adaptive and innate immune system of the study species (see supporting information). We used non-
160 specific assays that had previously been applied to samples from a variety of free-living avian species,
161 including different waterfowl (Bourgeon et al., 2010; Matson et al., 2006). Specifically, we counted basophils,
162 eosinophils, heterophils and monocytes as effectors of cellular innate immunity. We quantified the amounts
163 of lysozyme, bacteria killing ability against *E.coli* (BKA), haemagglutination and haemolysis as markers for
164 humoral innate immunity. We measured the total immunoglobulin Y (IgY) concentration and the number of
165 lymphocytes as markers of, respectively, humoral and cellular adaptive immunity (Table 1; Prüter et al.,
166 2020).

167 In addition to the analyses of immune markers, we measured the haematocrit as a proxy for the
168 oxygen carrying capacity of the blood (Ritchison, 2023). Further, we measured the total non-enzymatic
169 antioxidant capacity (OXY-adsorbent assay), and the activity of antioxidant enzymes superoxide dismutase
170 (SOD) and glutathione peroxidase (GPx), to estimate the antioxidant capacity of the blood. We measured the
171 concentrations of protein carbonyls and reactive oxygen metabolites (d-ROMS test) to evaluate blood levels
172 of oxidative damage. More details on the assays used for this study can be found in the Supplementary
173 Material.

174

175 **Statistical analyses**

176 The dataset included 74 actively reproducing individuals of which 28 were Egyptian geese, 10 were mallards,
177 and 36 were mute swans. We collected blood samples from both female and male Egyptian geese (females =
178 12; males = 16) and mute swans (females = 21; males = 15), and only female mallards (Table 1).

179 We performed linear models (LMs) to assess the differences between species in each single marker.
180 Therefore, for each model, we included one marker as a dependent variable and species as an independent
181 variable. We always screened the model for the presence of outliers relying on the Cook's distance. When we
182 detected an outlier, this was removed and the model was run again. Then, we checked for normal distribution
183 of residuals by means of Q-Q plots and Kolmogorov-Smirnov test (Bolker, 2008). If the distribution of model

residuals was not normal, we log-transformed the dependent variables after which we performed again the model diagnostics (Fig. S2).

Subsequently, we implemented LMs testing for the interaction between species and sex on a subset of the original dataset including only Egyptian geese and mute swan (the mallard was excluded because we sampled only females). Following the previous procedures, we removed outliers when detected, we log-transformed the data when necessary; we performed the model diagnostics by visual inspection of Q-Q plots (Fig. S3) and Kolmogorov-Smirnov test. Significant interactions were explored by pairwise comparison through the function *emmeans* of the homonym package of R.

Results

Results of models based on the full dataset (including outliers) are reported in Table S1. Here, we present results of models based on the dataset without outliers, because outliers can affect reliability and robustness of models (Knief & Forstmeier, 2021). Based on model diagnostic results, the distribution of residuals was normal for each model except that for Haemolysis (Kolmogorov-Smirnov test: statistic = 0.18, P-value = 0.03; Q-Q plots are shown in Fig. S2), even after the removal of outliers and log-trasformation of the dependent variable. Based on Knief and Forstmeier (2021), however, linear regression models are robust to violations of normality assumption and violating normality assumption is often unproblematic.

The outcomes of models show downregulated levels of lymphocytes and IgY (i.e. adaptive immunity) in Egyptian geese compared to both other species. Furthermore, Egyptian geese and mallards show lower levels of heterophils, lysozymes, and haemolysis, but higher levels of monocytes and BKA, than mute swans. In addition, Egyptian geese and mute swans show lower level of basophils than mallards (Table 2; Fig. 1).

Regarding markers of oxidative status and haematocrit, mallards show higher levels of reactive oxygen metabolites compared to Egyptian geese and mute swan, and Egyptian geese show higher haematocrit compared to mute swan. We did not detect any differences among species for protein carbonyls and antioxidant markers (Table 2; Fig. 2).

Finally, models testing for the interaction between species and sex showed a significant interaction for lysozymes only (Table S2). Pairwise comparison revealed higher levels of lysozymes in mute swans

211 compared to Egyptian geese for both sexes, but a significant difference was on only detected for females
212 (Table S3).
213

214 **Discussion**

215 In this study we compared the baseline immune-physiological profile of an invasive species, the Egyptian
216 goose, to that of two sympatric European waterfowl species, the mallard and the mute swan. We found lower
217 levels of adaptive immune markers, i.e. lymphocytes and IgY, in the blood of the invasive species compared
218 to the two native species. Our results also show a general similarity in the innate immune profile between
219 Egyptian geese and mallards, with the only exception of basophils which are significantly lower in the invasive
220 species. By contrast, mute swans show higher levels of heterophils, lysozymes and haemolysis compared to
221 both Egyptian geese and mallards, and also the lowest levels of monocytes and BKA. Mallards also show the
222 highest levels of reactive oxygen metabolites among the species (lowest levels were found in Egyptian geese,
223 but the result was statistically significant only in respect to mallards).

224 **Adaptive immune effectors**

225 Lymphocytes are one of the most abundant leucocytes in birds (Davis et al., 2008; Ritchison, 2023) and play
226 important roles in both cell- and antibody-mediated adaptive immunity. Specifically, T-lymphocytes attack
227 infected or abnormal cells inducing inflammation, and B-lymphocytes produce immunoglobulins (Guriec et
228 al., 2018). Immunoglobulin Y (IgY) is the most abundant circulating immunoglobulin in birds and are released
229 by B-lymphocytes (Ritchison, 2023). Thus, the lower levels of constitutive IgY in Egyptian geese are in
230 agreement with the reduced number of lymphocytes, which point to a downregulated antibody-mediated
231 immunity. According to Cornet et al. (2016), invasive hosts that are no more in contact with co-evolved
232 (specialized) parasites should take advantage of downregulating antibody-mediated adaptive responses. In
233 accordance, an experimental study on house finches (*Haemorhous mexicanus*) found higher levels of IgY in
234 populations that co-evolved with the inoculated virus (*Mycoplasma gallisepticum*) compared to unexposed
235 populations (Gates et al., 2021).

236 In accordance with our findings, recent studies found that German populations of Egyptian geese
237 show lower prevalence of infections for a variety of blood parasites and macro-parasites compared to
238 conspecifics from their native habitats and show lower seroprevalences of some common waterfowl viruses
239 (Prüter et al., 2018, 2020). Such findings support the hypothesis that downregulated adaptive immunity in
240 Egyptian geese in their invasive range may be due to reduced prevalences of infectious agents, which would
241 be compatible with the predictions of the evolution of increased competitive ability hypothesis. On the other
242 hand, a reduced investment in the less expensive antibody-mediated immunity is opposite to the predictions
243 of the revised EICA-hypothesis. Further experimental studies are necessary to test the immune response of
244 invasive Egyptian geese compared to that of native species.
245
246 **Innate immune effectors**
247 We found that innate immunity effectors were generally similar between Egyptian geese and mallards.
248 Differently, mute swan had significantly higher levels of heterophils, lysozymes and haemolysis, and lower
249 levels of monocytes and BKA compared to the other species. These differences might be partly due to intrinsic
250 characteristics of the species. For example, a recent multi-species study showed that avian heterophils
251 increase with the body mass (Ruhs et al., 2020), and this might explain why we found higher levels of
252 heterophils in mute swans compared to the other species. On the other hand, large species, such as mute
253 swan, might be more exposed to parasites, and this can justify a different immune regulation (Downs et al.,
254 2019).
255 Heterophils are the major phagocytic leucocytes and important mediators of acute inflammatory
256 responses in birds (Scanes et al., 2022). Their higher levels in mute swans might indicate high prevalence of
257 infections than in Egyptian geese and mallards. This result would agree with higher levels of
258 haemagglutination and haemolysis in the mute swans (Table 1; Fig. 1). Haemagglutination and haemolysis
259 reflect responses to foreign antigens driven by natural antibodies and the complement system, respectively
260 (Matson et al., 2006). Natural antibodies are produced in absence of antigenic stimulation, independently
261 from previous infections, whereas the complement system can be activated directly by pathogens or

262 indirectly by immunoglobulins (Ochsenbein & Zinkernagel, 2000). Natural antibodies and the complement
263 system are part of the innate immune system and act as a first line of defence against infections. Therefore,
264 lower levels of haemagglutination and haemolysis might indicate that Egyptian geese and mallards have
265 reduced prevalence of infections compared to mute swans.
266 Mallards showed the highest level of basophils among the three species. Avian basophils, differently from
267 those of mammals, are involved in inflammation reactions (Ritchison, 2023). The abundance of basophils in
268 the blood of birds exhibits considerable variation among species, but Vinkler et al. (2010) found that basophil
269 counts were higher in birds infected with blood parasites (Haemoproteus). Therefore, higher levels of
270 basophils in mallards might be due to higher prevalence of haemosporidian infections other than different
271 immune strategies compared to the other species. Interestingly, Egyptian goose is the only species showing
272 no increase in any immunological mediator of inflammatory reactions. This observation supports the EICA-
273 revised hypothesis, but further studies are needed to understand how baseline levels of circulating leukocytes
274 might underlie different immunological strategies to fight pathogens.
275 Finally, our results show higher plasma BKA in Egyptian geese and mallards compared to mute swans.
276 The mechanisms that allow plasma to kill bacteria are not well understood, but might be strongly dependent
277 on antibacterial plasma proteins and peptides (Dugovich et al., 2019; Gao et al., 2015). Accordingly, Egyptian
278 geese and mallards might invest relatively more energy in antimicrobial proteins and peptides, which come
279 to lower (i) energetic costs compared to immune cells (e.g. heterophils) and (ii) fitness costs compared to
280 inflammatory effectors.

281 **Oxidative status and haematocrit**

282 We found higher levels of oxidative damage (reactive oxygen metabolites) in mallards compared to Egyptian
283 geese and mute swans. In our study region, mallards are subject to increased competition and aggressive
284 behaviour due to the presence of Egyptian geese (Hohmann & Woog, 2021), which might result in increased
285 metabolic activity and ROS generation (Quque et al., 2022). It might also be that the higher pace of life of
286 mallards compared to Egyptian geese and mute swans determines higher baseline generation of ROS.
287 Egyptian geese exhibit the lowest levels of reactive oxygen metabolites (although only marginally significant

288 as compared to mute swans). This result might indicate that invasive Egyptian geese are less prone to
289 oxidative stress than native species. Reduced levels of oxidative stress may induce important benefits for the
290 invasion success, such as higher reproductive success and longevity (Blount et al., 2016; Xia & Møller, 2018).
291 Finally, we found lower haematocrit in mute swans compared to the other species. Thus, variation in
292 haematocrit does not seem to be relevant to achieve a successful invasion. Rather, our results are in
293 agreement with previous studies that found lower haematocrit in larger bird species (Kostelecka-Myrcha et
294 al., 1993).
295
296 **Conclusions**
297 Our comparative analysis of multiple immune-physiological traits suggests that invasive Egyptian geese might
298 strategically reduce their investment in adaptive immunity compared to sympatric related native species,
299 because they are likely no longer exposed to the pathogens they encountered in their natural habitats. Thus,
300 invasive species might invest less energy to sustain adaptive immune function, which might come at the
301 benefit for other energy-demanding host traits, such as reproduction, dispersal, and territoriality. Besides
302 energetic and nutritional costs, maintaining relatively higher innate immunity is beneficial during dispersal,
303 or establishing in novel habitats, since invasive species mainly encounter novel pathogens. Future work is
304 needed to understand how baseline differences in immunological parameters translate into trade-offs with
305 fitness traits, such as reproduction or survival.
306 The results of our work also indicate species-specific differences in baseline immune status,
307 supporting previous findings on the relationship between species' body mass and heterophils. In addition,
308 we also recognize that mallards show important behavioural, morphological, and pace-of-life differences with
309 the other two species, which might contribute to among species variation in the regulation of oxidative status.
310 On the other hand, physiological plasticity is an important driver of local acclimation and success of invasion
311 (McCann et al., 2018). In accordance with Prüter et al. (2020), high levels of physiological plasticity in Egyptian
312 geese might be one main mechanism underlying their successful invasion to Europe. Determining immune-
313 physiological traits underlying the phenotype of successful invaders will foster our understanding of the
314 proximate mechanisms through which species can successfully colonise novel environments and help to

better predict novel alien species and potential mitigation strategies (Lennox et al., 2015). Future work should include further species that live simpatrically with the invasive Egyptian geese in order to better tease apart the effects of life-history variation from those induced by parasite exposure.

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Conflict of interest

The authors declare no conflicts of interest.

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470 **Tables and figures**

471 Table 1 – Sample size (n), mean and standard deviation (SD) for immunological effectors, and for haematocrit
472 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	6.98±4.26	10	11.17±11.27	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
Non-enzymatic antioxidant capacity	27	197.47±46.99	10	180.61±46.22	32	207.69±43.73
Reactive Oxygen Metabolites	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
Glutathione peroxidase	28	0.29±0.11	10	0.27±0.16	36	0.38±0.21
Superoxide dismutase	28	2.05±0.61	10	2.4±0.53	36	2.11±0.68

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475 Table 2 – Results of LMs with outliers removed. We detected no outliers for lymphocytes, lysozymes, OXY
476 and protein carbonyls, therefore results presented here for those markers are equal to those presented in
477 Table S1. ALEG = Egyptian goose; ANPL = mallard; CYOL = mute swan. Significant P-values are indicated in
478 bold.

Marker	Variable tranformation	Contrast	Estimate	SE	t value	P-value
Basophils	Log	ALEG - ANPL	-0.51	0.11	-4.68	**<0.01**
		ALEG - CYOL	-0.08	0.08	-0.97	0.34
		CYOL - ANPL	-0.44	0.11	-4.17	**<0.01**
Eosinophils	None	ALEG - ANPL	-6.59	5.99	-1.10	0.28
		ALEG - CYOL	1.79	3.77	0.47	0.64
		CYOL - ANPL	-8.38	5.85	-1.43	0.16
Heterophils	None	ALEG - ANPL	-1.03	2.94	-0.35	0.73
		ALEG - CYOL	-9.71	1.93	-5.03	**<0.01**
		CYOL - ANPL	8.69	2.86	3.04	**<0.01**
Lymphocytes	Log	ALEG - ANPL	-0.18	0.07	-2.74	**0.01**
		ALEG - CYOL	-0.16	0.04	-3.66	**<0.01**
		CYOL - ANPL	-0.02	0.06	-0.24	0.81

Monocytes	Log	ALEG - ANPL	-0.02	0.10	-0.21	0.83
		ALEG - CYOL	0.19	0.06	3.04	**<0.01**
		CYOL - ANPL	-0.21	0.09	-2.27	**0.03**
Haematocrit	None	ALEG - ANPL	3.52	2.12	1.66	0.10
		ALEG - CYOL	7.29	1.41	5.15	**<0.01**
		CYOL - ANPL	-3.76	2.12	-1.77	0.08
Lysozymes	None	ALEG - ANPL	-0.82	0.97	-0.85	0.40
		ALEG - CYOL	-4.00	0.68	-5.90	**<0.01**
		CYOL - ANPL	3.17	0.94	3.36	**<0.01**
Haemagglutination	Log	ALEG - ANPL	-0.03	0.03	-1.09	0.28
		ALEG - CYOL	-0.07	0.02	-3.30	**<0.01**
		CYOL - ANPL	0.04	0.03	1.40	0.17
Haemolysis	None	ALEG - ANPL	-0.24	0.50	-0.48	0.63
		ALEG - CYOL	-1.30	0.36	-3.60	**<0.01**
		CYOL - ANPL	1.06	0.47	2.28	**0.03**
IgY	None	ALEG - ANPL	-0.20	0.08	-2.58	**0.01**
		ALEG - CYOL	-0.30	0.05	-5.64	**<0.01**
		CYOL - ANPL	0.10	0.07	1.36	0.18
Bacteria killing ability	None	ALEG - ANPL	-7.36	8.80	-0.84	0.41
		ALEG - CYOL	36.00	6.22	5.78	**<0.01**
		CYOL - ANPL	-43.36	8.80	-4.93	**<0.01**
Non-enzymatic antioxidant capacity	None	ALEG - ANPL	16.86	16.80	1.00	0.32
		ALEG - CYOL	-10.22	11.86	-0.86	0.39
		CYOL - ANPL	27.08	16.44	1.65	0.10
Glutathione peroxidase	Log	ALEG - ANPL	0.04	0.09	0.48	0.63
		ALEG - CYOL	-0.07	0.06	-1.22	0.23
		CYOL - ANPL	0.12	0.09	1.28	0.21
Superoxide dismutase	None	ALEG - ANPL	-0.43	0.22	-1.93	0.06
		ALEG - CYOL	-0.13	0.15	-0.88	0.38
		CYOL - ANPL	-0.29	0.21	-1.37	0.17
Reactive Oxygen Metabolites	None	ALEG - ANPL	-0.34	0.08	-4.18	**<0.01**
		ALEG - CYOL	-0.08	0.05	-1.74	0.09
		CYOL - ANPL	-0.25	0.08	-3.22	**<0.01**
Protein carbonyls	None	ALEG - ANPL	0.02	0.48	0.03	0.97
		ALEG - CYOL	0.55	0.34	1.64	0.11
		CYOL - ANPL	-0.54	0.45	-1.19	0.24

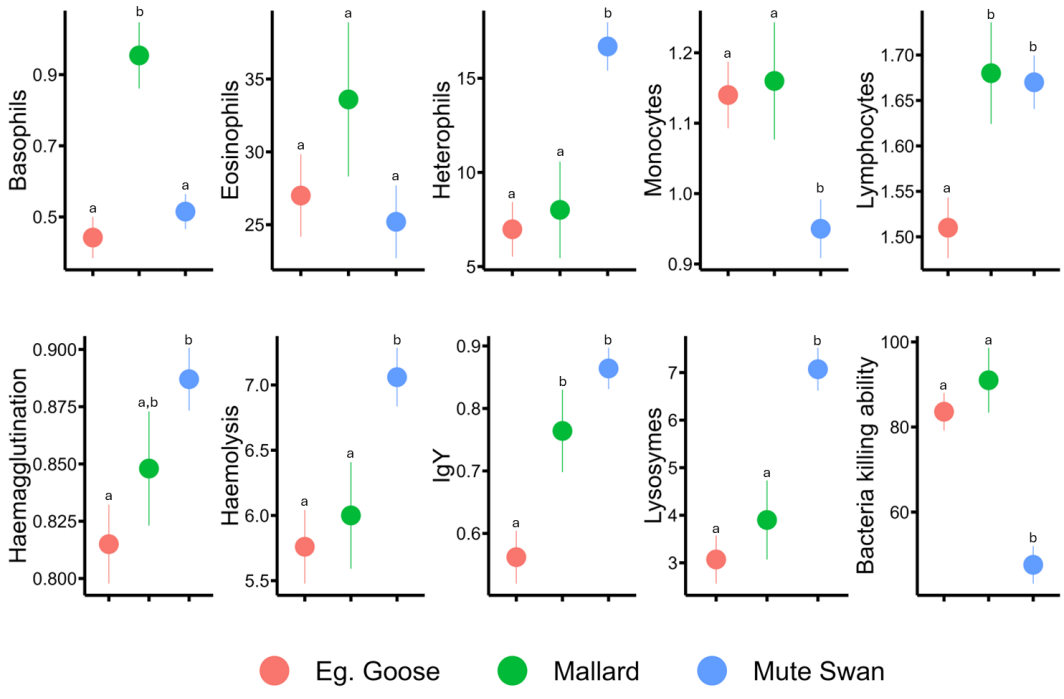

Fig. 1 – Estimated marginal means ± standard error of the mean immune markers level between species. Different superscripts (a and b) represent significant differences ($p < 0.05$).

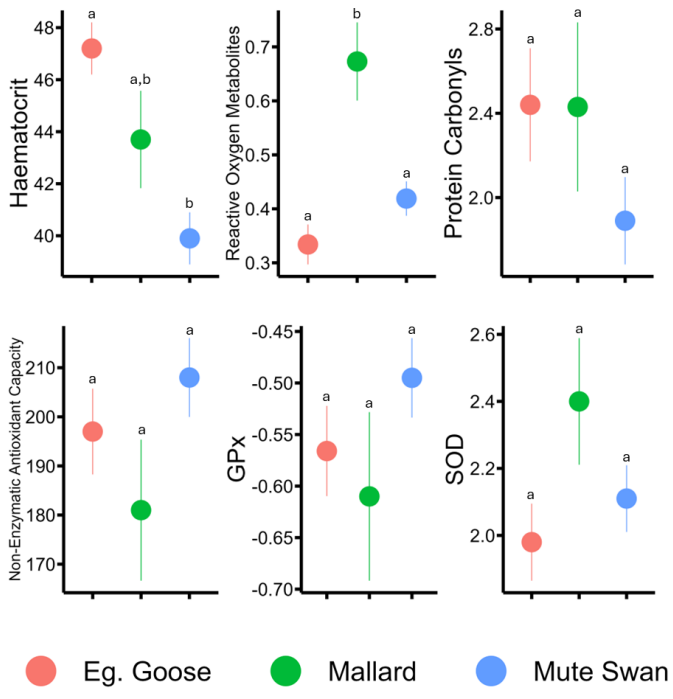

Fig. 2 – Estimated marginal means ± standard error of the mean immune markers level between species. GPx = glutathione peroxidase; SOD = superoxide dismutase. Different superscripts (a and b) represent significant differences ($p < 0.05$).