Manipulating parental condition affects brood sex ratio, immunocompetence, and early chick mortality in two gull species differing in sexual size dimorphism

Dariusz Bukaciński,[1,*,] **Monika Bukacińska,**[1] **and Przemysław Chylarecki**[2]

[1] Institute of Biological Sciences, Cardinal Stefan Wyszyński University of Warsaw, Warsaw, Poland
[2] Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
* Corresponding author: dbukacinski@op.pl

ABSTRACT

Sex allocation theory predicts that parents should adjust their brood sex ratio to maximize fitness returns in relation to parental investment. Adaptive adjustment of sex ratio may be driven by differential costs of rearing sons and daughters or differential benefits of investing limited resources into offspring of different sex. In both cases, possible sex ratio bias should depend on parental condition. For sexually dimorphic birds with males larger than females, sons may be less likely to fledge since they are more vulnerable to food shortages or because they have impaired immunocompetence due to higher testosterone levels. Poor condition females should thus overproduce daughters to minimize possible reproductive failure. We manipulated the number of eggs laid and the amount of food available to laying females to induce differences in the condition in 2 gull species differing in sexual size dimorphism. In the Black-headed Gull (*Chroicocephalus ridibundus*), sexual size differences are marginal; but in the Mew Gull (*Larus canus*), males are 11% larger. In both species, females forced to lay an additional egg (presumed in worse condition) overproduced daughters, whereas females receiving supplemental food before laying (presumed improved condition) overproduced sons. This sex ratio skew was larger in Mew Gull, a species with larger size dimorphism. Chick immunocompetence at hatching was unrelated to sex, being higher in broods of fed mothers and lower for chicks hatched from last-laid eggs. Chick survival between hatching and day 5 post-hatch was positively related to their immunocompetence, but chicks from last-laid eggs and males of Mew Gull, the more dimorphic species, survived less well. Results indicate that costs of raising larger sex offspring coupled with parental condition shape brood sex ratio in populations studied. Adaptive brood sex ratio adjustment occurs mostly before egg laying and includes differential sex allocation in eggs depending on the probability of producing a fledged chick.

Keywords: brood sex ratio, chick mortality, egg sequence, gulls, immunocompetence, parental effort, sexual size dimorphism

LAY SUMMARY

- We found that Mew Gull and Black-headed Gull change the proportion of sons and daughters in their broods depending on the parental condition during egg laying. Natural selection should favor deviations from parity of sexes among offspring if the costs or benefits of producing sons and daughters are different.
- We tested this by manipulating the condition of gulls before egg laying and found that in both species females in poor condition had more daughters whereas females receiving extra food had more sons.
- These differences were larger in the species where males are 11% heavier than females (i.e. Mew Gull) than in species where both sexes are of similar size (i.e. Black-headed Gull).
- It shows that differential costs of raising sexes shape the brood sex ratio in addition to parental condition.
- Additionally, improved parental condition translated into improved immunocompetence of chicks, which enhanced their chances of surviving the first days of life, irrespective of sex.

Manipular la condición parental afecta el cociente de sexos de las crías, la inmunocompetencia y la mortalidad temprana de los polluelos en dos especies de gaviotas con diferente dimorfismo sexual de tamaño

RESUMEN

La teoría de la asignación por sexo predice que los progenitores deberían ajustar el cociente de sexos de las crías para maximizar las ganancias en términos de adecuación biológica con relación a la inversión parental. El ajuste adaptativo del cociente de sexos puede ser impulsado por los costos diferenciales de criar hijos e hijas o por beneficios diferenciales de invertir recursos limitados en crías de diferente sexo. En ambos casos, el posible sesgo en el cociente de sexos debería depender de la condición parental. Para las aves con dimorfismo sexual con machos más grandes que las hembras, los hijos pueden tener menos probabilidad de emplumar debido a que son más vulnerables a la escasez de alimentos o porque tienen una inmunocompetencia deteriorada debido a niveles más altos de testosterona. Por lo tanto, las hembras en malas condiciones deberían sobre-producir hijas para minimizar un posible fracaso reproductivo. Manipulamos en número de huevos puestos y la cantidad de alimento disponible para las hembras ponedoras, para inducir diferencias en la condición de dos especies de gaviotas con diferente dimorfismo sexual de tamaño. En *Chroicocephalus ridibundus*, las diferencias de tamaño por sexo son mínimas, pero en *Larus canus*, los machos son 11% más grandes. En ambas especies, las hembras que fueron forzadas a poner un huevo adicional (supuestamente en peores condiciones) sobre-produjeron hijas, mientras que las hembras que recibieron alimento suplementario antes de poner (supuestamente en mejores condiciones) sobre-produjeron machos. Este sesgo en el cociente de sexos fue más grande en *L. canus*, la especie con mayor dimorfismo de tamaño. La inmunocompetencia de los polluelos al momento de la eclosión no estuvo relacionada con el sexo, siendo más alta en las crías de madres alimentadas y más baja en polluelos eclosionados de los últimos huevos puestos. La supervivencia de los polluelos entre la eclosión y el día 5 luego de la eclosión estuvo positivamente relacionada con su inmunocompetencia, pero los polluelos de los últimos huevos puestos y los machos de las especies con mayor dimorfismo (*L. canus*) sobrevivieron de peor modo. Los resultados indican que los costos de criar polluelos de sexos más grandes, junto con la condición parental, dan forma al cociente de sexos de las crías en las poblaciones estudiadas. El ajuste adaptativo del cociente de sexos de las crías ocurre principalmente antes de la puesta de los huevos e incluye la asignación diferencial de sexos en los huevos dependiendo de la probabilidad de producir un polluelo emplumado.

Palabras clave: cociente de sexos de las crías, dimorfismo sexual de tamaño, esfuerzo parental, gaviotas, inmunocompetencia. mortalidad del polluelo, secuencia de los huevos

INTRODUCTION

Costs and benefits of producing sons and daughters are often unequal and are known to vary as a function of multiple external and internal factors (Cockburn et al. 2002, West 2009, Komdeur 2012, Merkling et al. 2015). Thus, one of the possible parental responses to the varying social and environmental conditions during reproduction may be the adaptive sex ratio adjustment within a brood (Nishiumi 1998, Nager et al. 1999, Riechert et al. 2013, Cantarero et al. 2018). Generally, parents should bias brood sex ratio toward the sex that maximizes fitness gains per unit cost of parental investment. Here, sexes may differ in fitness returns if offspring reproductive value is related to some environmental condition (e.g., maternal condition, social rank, mate attractiveness) and one sex reaps greater reproductive benefits of being reared under the improved condition (Trivers and Willard 1973, West 2009). For example, in some polygynous ungulates, mothers in better nutritional condition or ranking higher socially increase their parental effort and that preferentially benefits sons, as better nourished males are more likely to win in inter-male contests over females and increase their reproductive success (Clutton-Brock et al. 1986, Cassinello and Gomendio 1996, Cameron et al. 1999; but see Hewison

and Gaillard 1999). While the mechanism first described by Trivers and Willard (1973) is quite general (West 2009, Veller et al. 2016), it does not necessarily mean that sons should be always overproduced by good quality mothers or under favorable nutritional conditions. As offspring fitness integrates also differential survival of sexes until maturity or age-specific reproduction schedules, exact outcomes of the generalized Trivers–Willard model of sex allocation are difficult to predict and are heavily dependent on demography of the population under study (Schindler et al. 2015). Furthermore, sex-specific costs and benefits of offspring philopatry are known to influence adaptive brood sex ratios (reviewed in Cockburn et al. 2002, Komdeur 2012, Li and Kokko 2019). However, most hypotheses invoked to explain variation in avian brood sex ratios focus on unequal costs of rearing chicks of different sexes, rather than on inequality in expected benefits (Myers 1978, Cockburn et al. 2002, Douhard 2017). Where parents are in a poor condition or the food availability is limited, we should expect the "cheaper" sex (i.e. the sex with lower nutritional or energy requirements) to occur at a higher frequency in the brood. It is usually assumed that this is the sex that is smaller in size, which is most often the female. This relationship has been confirmed many times for species with different degrees of sexual size

dimorphism (Cameron-MacMillan et al. 2007, Merkling et al. 2015, Bukaciński et al. 2020a, 2020b). However, the magnitude of sexual size dimorphism may be only weakly correlated in some species (or even not correlated at all) with the actual difference in costs of rearing each sex (Torres and Drummond 1999, McDonald et al. 2005, Townsend et al. 2007).

Parents can influence the sex of offspring at various stages of the offspring's development. Brood sex ratios can be modified right at the start during the first meiotic division, when the sex chromosomes segregate, a stage controlled primarily by females in birds (Rutkowska and Badyaev 2008, Tagirov and Rutkowska 2013). As a result, the brood sex ratio can be skewed by the time of egg laying (primary sex ratio). A similar effect may also be a consequence of sex-dependent mortality of embryos before hatching (secondary sex ratio) or during the chick-rearing period (Krackow 1995, Nager et al. 2000b, Cook and Monaghan 2004). The latter occurs through the maternal effect on the egg size of different sexes and through their laying order (Nager et al. 2000a, Blanco et al. 2003, Bukaciński et al. 2020a, 2020b). After hatching, selective mortality of sexes during the brood reduction phase can lead to further adjustments to the brood sex ratio. In gulls (Laridae), this mechanism may be facilitated through differential sex allocation in the last laid egg in a clutch (egg C). Chicks from these eggs usually hatch last and are smaller than their brood mates (Lundberg and Väisänen 1979, Kilpi et al. 1996, Rubolini et al. 2011), typically making them the first victims of brood reduction during the period of peak chick mortality in the first days of life (Lundberg and Väisänen 1979, Różycki 2014, Bukaciński and Bukacińska 2015a, 2015b). This is particularly evident in periods of low food availability or diminished parental condition when parents provide less food to the brood or are less attentive (Bukaciński et al. 1998, Monaghan et al. 1998, Tveraa et al. 1998).

The main factor favoring the brood sex ratio adjustment during the chick-rearing period is the different susceptibility of each sex to adverse conditions (Griffiths 1992, Emlen 1997). The basis for this sensitivity has been explained by 2 competing hypotheses. The size hypothesis assumes that the difference in body size between chicks of different sexes is the decisive factor. Chicks of larger sex (usually males) require more time, food, and energy during their postembryonic development. This makes them more vulnerable to increased mortality during periods when parents are in poor condition or during food shortages (Clutton-Brock et al. 1985, Clutton-Brock 1991, Krijgsveld et al. 1998). The male phenotype hypothesis assumes that mechanisms independent of body size cause higher rates of mortality in male chicks. According to this hypothesis, higher levels of androgens (mainly testosterone), the hormones responsible for developmental

differences between males and females (Schwabl 1993), in male chicks can lead to higher rates of energy expenditure, and therefore higher nutritional requirements, even in the absence of differences in body size (Buchanan et al. 2001). Androgens can also weaken the immune system, as posed by the immunocompetence handicap hypothesis (Folstad and Karter 1992, Foo et al. 2017), leading eventually to increased mortality in male chicks (Saino et al. 1995, Nolan et al. 1998, Müller et al. 2003). However, to complicate this picture, chicks with higher androgen levels are known to outcompete siblings in intra-brood competition over food delivered by parents (Eising et al. 2001, Müller et al. 2012).

The first empirical testing of the male phenotype hypothesis was conducted on species with reversed sexual size dimorphism (i.e. with larger females). Studies on Blue-footed Boobies (Sula nebouxii; Velando et al. 2002) and Great Skuas (Catharacta skua; Kalmbach et al. 2005) indicated that daughters (the larger-bodied sex) experienced greater mortality in adverse conditions. But their results provided more support to the size hypothesis than the male phenotype hypothesis (Clutton-Brock et al. 1985, Krijgsveld et al. 1998). On the other hand, studies on Common Kestrels (Falco tinnunculus) showed reduced levels of an immune response in male chicks (smaller sex), suggesting a higher risk of mortality in this sex, and providing support for the male phenotype hypothesis (Fargallo et al. 2002).

To test whether the parental condition has an impact on brood sex ratio and if so, how it relates to the 2 hypotheses presented above, we conducted an experimental study on 2 species of gulls (Laridae), a taxonomic group where the male is the larger sex. We compared the brood sex ratio at hatching and 5 days post-hatch for females subjected to experimentally improved and worsened body condition (by supplemental feeding and forcing to lay a single extra egg, respectively), across 2 species differing in the degree of sexual size dimorphism. Here we chose Black-headed Gull (Chroicocephalus ridibundus, hereafter BHG) and Mew Gull (Larus canus, hereafter MG). In the BHG, the differences in body mass and size between females and males are marginal, whereas, in MG, males are apparently heavier and larger than females (Supplementary Material Table S1). Thus, regarding the effects of the parental condition and sexual size dimorphism on brood sex ratio, we sought to test 2 predictions. We expected that (1) the deterioration of the female's condition (laying an extra egg) would bias the brood sex ratio toward females (less expensive sex) and (2) that this tendency would be stronger for the species with greater sexual size dimorphism. More specifically, females in worsened condition should have on average more daughters in a brood, compared to females with improved condition (supplementary fed) in both species, and this difference should be more pronounced in the species with greater sexual size dimorphism.

Although we did not measure the actual energy and physiological costs associated with the production of different sexes (Merkling et al. 2015), we previously found sex ratio skew toward the "cheaper" sex in broods of pairs laying naturally induced replacement clutches (Bukaciński et al. 2020a). That corresponded well with our assumption that smaller sex (females) would also be less costly to rear. Furthermore, we also found that among female gulls forced to lay a replacement clutch, individuals in poor condition skewed offspring sex ratio toward daughters, while those with experimentally improved condition overproduced sons (Bukaciński et al. 2020b). We wanted to know if these results apply to parents in a prime condition, which did not bear the costs of producing 3 eggs (Monaghan et al. 1998, Nager et al. 2000b) prior to their decision to lay a focal clutch.

Our experiment was designed to control for effects of egg position in the laying sequence as well as immunocompetence of newly hatched chicks as possible factors shaping sex ratios and selective sex-dependent mortality of chicks in their first 5 days of life. Thus, in addition to effects of condition and size dimorphism on brood sex ratio at hatching, the between-species comparison of experimental results, combined with measurements of cell-mediated immunity (CMI) in chicks (Smits et al. 1999, details in the section "Methods"), allowed us to verify predictions of both presented above hypotheses explaining sex-dependent chick mortality. If it was the body size that would shape sex differences in the sensitivity of chicks to adverse conditions (size hypothesis predictions), we would expect that (1) in a species with a larger size dimorphism, the differences in chick survival of different sex will be larger (lower survival rates for the larger sex); (2) this relationship will be more evident in the nests of mothers in a worse condition; (3) the condition of the females will affect the level of the immune response of chicks; and (4) we would not find sex-related differences in the offspring immune response, regardless of the female condition (comparisons within species). In the case of male phenotype hypothesis, the 2 key predictions presented in points (1) and (4) will be opposite to those given above. That is, sex-related differences in chick survival will be smaller in species with larger sexual size dimorphism (MG) and male chicks will show a reduced immune response, as compared to females. Additionally, we expected our study gulls to show a less pronounced sex ratio skew than gulls from the same populations that were laying replacement clutch after having produced the first clutch. At first clutch, females should be in better condition than while laying the replacement clutch, as egg laying is costly in gulls (Monaghan et al. 1998, Nager et al. 2000b). Thus, in line with the reproductive cost hypothesis of sex allocation (Myers 1978, Cockburn et al. 2002), birds with experimentally increased reproductive effort should show a lesser skew toward cheaper sex in first clutches analyzed here, than in replacement clutches analyzed in our companion paper (Bukaciński et al. 2020b).

METHODS

Study Sites and Species

The study area covered islands in the middle reaches of Vistula River, central Poland, a large freely flowing lowland river with a wide, braided channel, between Dęblin and the mouth of Pilica River (393–457 km of the waterway; 51.5594°N–51.8625°N, 21.8266°E–21.2829°E). Both MG and BHG nest colonially and simultaneously on these islands within similar habitats and consume the same diet during the breeding season (Bukaciński and Bukacińska 1994, Ostrowska 1995, Bukaciński and Bukacińska 2003, 2015a, 2015b). MG males are 11–15% heavier and 5–9% larger than females (7–8% at the middle Vistula River), whereas BHG males are 3–8% heavier and 2–6% larger than females (Supplementary Material Table S1). Sexual size dimorphism appears already at hatching and is present during the entire period of chick-rearing (i.e. for 4–5 weeks; D. Bukaciński and M. Bukacińska personal communication), like in many other size-dimorphic waterbirds (Weimerskirch et al. 2000, Becker and Wink 2003, Merkling et al. 2012).

Field Data Collection

We conducted our study in April–June 2014. We marked all nests with numbered sticks and checked them every 2 days until clutch completion, when we removed eggs to the incubator (see below). As females of these gull species lay eggs approximately every 2 days (MG: 1.97 ± 0.20, range: 1.5–3 days between the first and second egg and 1–2.5 days between the second and third egg in the clutch; BHG: 1.90 ± 0.20, range: 1.5–2.5 days and 1–2 days, respectively; D. Bukaciński personal observation) we had no problems with determining the egg-laying sequence in the clutch. We marked eggs in clutches as A, B, and C, according to their laying order, with a nontoxic marker (hereinafter referred to as egg A, egg B, and egg C). During incubation, clutches were monitored every 3–5 days. When the first egg in a clutch started to crack slightly in the incubator, we put the clutch back into the parents' nest. Nests were checked daily until all chicks hatched (from 5 to 8 days, including 1–3 days from the hatching of the first to the hatching of the last chick in the brood; usually more synchronously in BHG; D. Bukaciński personal observation). We could unambiguously determine hatching order and from which egg every chick hatched (i.e. chick from egg A, chick from egg B, and chick from egg C).

We banded chicks and took ~40 µL of blood from the tarsal vein on either hatch day or the next day (mean chick age: 0.36 ± 0.49 days and 0.37 ± 0.49 days for MG and BHG, respectively, where 0 is the hatching day). The blood sample was preserved in 1 mL of APS buffer (Arctander 1988) and stored at −20°C until DNA extraction and PCR analysis. We also collected biological material (liver or heart) from dead chicks in the few cases where they died just prior to blood sampling and preserved tissues in 1 mL of Queens Buffer (Seutin et al. 1991) for later molecular analyzes. Most adults were individually marked with colored rings in earlier years. The remaining were trapped on nests and banded during the study year.

The CMI measurements in hatchlings were made according to a standard protocol (Smits et al. 1999, Müller et al. 2003; see also Martin II et al. 2006). Briefly, (1) we measured a ball of the foot with a torque-limiting micrometer (3 repeated measurements, right before blood sampling) in freshly hatched chicks; (2) we injected intradermally sterile 0.05 mL of 1 mg mL^{-1} phytohemagglutinin-P (PHA) dissolved in phosphate-buffered saline (2 mg mL^{-1}) with a disposable syringe into a ball of the foot; and (3) we measured a local swelling of the ball caused by perivascular accumulation of T lymphocytes followed by macrophage infiltration. Three measurements for the same individual were taken on average after 27.15 ± 2.97 hr and 27.20 ± 2.99 hr, for MG and BHG, respectively (range: 24–36 hr; for both species, 50% chicks measured no later than after 26 hr, and 75% chicks measured no later than after 29 hr). After 48 hr the swelling disappeared; the procedure did not affect the health and survival of the chicks. As a measure of CMI, we used the difference between pre-injection and post-injection measurements (mean values of the 3 measurements). In vivo injection of PHA is generally considered a reliable method of CMI investigation (Müller et al. 2003).

Experimental Treatment: A Differentiation of Maternal Condition

Within each species, we randomly assigned pairs to 3 groups. Parental conditions in 2 of the groups were modified using standard methods (Heaney and Monaghan 1995, Nager et al. 1999, Kalmbach et al. 2005) to obtain pairs with an improved condition or pairs with a lowered condition. The third, unmanipulated group served as the control (n = 62 and 55 nests for MG and BHG, respectively). Control nests were visited in the same way as experimental pairs.

The improved condition group (hereinafter referred to as "fed"; n = 40 and 59 nests for MG and BHG, respectively) was fed 150–160 g day^{-1} of fish per pair for 14–18 days (until clutch completion). The fish were naturally available freshwater species, mainly roach (*Rutilus rutilus*). Fish are a valuable, high-protein component of the diet of our study species, and the portion size provided clearly exceeded the daily energy requirements of adult birds of these species (Bolton et al. 1992). Food portions were placed out every morning in the same location close to the nest in the territory of each selected pair. Gulls had no problem in finding a feeding place and would successfully defend it against neighbors. Both parents were usually at the territory during feeding (after 2 or 3 days, birds got used to feeding and waited for extra food near the territory). In such a situation, the majority of portions were eaten by females, while males defended them against kleptoparasites. Even if the majority of the food was eaten by a male while the female was away, the female received her part from the male shortly after, during courtship feeding (Bukaciński and Bukacińska 2003, 2015a, 2015b). MG parents fed with protein (fish) or fat portions (skinless bacon) before egg-laying increase their body mass and protein reserves in pectoral muscles, compared to non-fed birds (Różycki 2014, D. Bukaciński and M. Bukacińska personal communication). We determined the time of the supplemental feeding commencement based on territorial and courtship behaviors, which we knew well from previous studies (Bukacińska and Bukaciński 1994, Bukaciński 1998, Bukacińska 1999). We knew that MGs begin to spend more time together at the territory, make their first copulation attempts, and make several scrapes 2–3 weeks before the start of egg laying (D. Bukaciński and M. Bukacińska personal communication).

The decreased condition group (hereinafter referred to as "removed"; n = 38 and 39 nests for MG and BHG, respectively) was implemented by forcing females to lay one more egg. Within a few hours after laying, the first egg laid in the nest (i.e. egg A) was removed and put into another nest of the same species, ensuring that this foster nest was at the same stage but with an incomplete clutch. Thereafter, we referred to all removed, first eggs as egg A0. Focal females treated in this way continued laying until 3 eggs were in the nest. Monaghan et al. (1998) found that the increase in egg production by 1 egg reduces body mass and protein reserves of Lesser Black-backed Gulls (*Larus fuscus*) by not less than 5–6%. Because parents in these nests were actually rearing chicks from the second through fourth eggs laid, we checked the size difference between egg A0 (the first egg we took to induce females to lay the next 3) and egg A (the second egg laid but oldest egg left in the nest of the removed group) and between the B and C eggs (the third and fourth eggs laid), which could potentially influence the results. In both gull species, the mean volume (calculated after Barth 1967, Lundberg and Väisänen 1979) of the compared eggs in clutches from removed treatment was similar (egg A0 vs A: 49.2 ± 3.1 cm^3 vs 49.0 ± 3.0 cm^3 for MG; 33.6 ± 2.8 cm^3 vs 33.5 ± 2.6 cm^3 for BHG; egg B vs C: 46.3 ± 3.2 cm^3 vs 46.7 ± 3.3 cm^3 for MG, and 32.5 ± 2.5 cm^3 vs 32.4 ± 2.3 cm^3 for BHG) and the differences

were not statistically significant ($P > 0.5$). Throughout the article, we refer to the 3 eggs left in nests of removed treatment parents as A, B, and C to maintain consistency with labels used in the other 2 treatments. This labeling also reflected our assumption that that chick fitness was affected more by hatching asynchrony and sibling competition than by egg size and composition, which may be determined by true laying order. This should be kept in mind while interpreting the results of our study for the removed group.

For analyses, we used breeding pairs that laid 3 eggs and started laying no later than at the peak of the season (i.e. no later than April 30 for BHG and May 10 for MG). Due to frequent river flooding and predation pressure from American mink (*Neovison vison*) and red fox (*Vulpes vulpes*), which cause instant clutch failures to the majority of MG and a large part of the BHG pairs (Bukaciński et al. 2020a, 2020b), we had to ensure that complete 3-egg clutches will survive until hatching. For this purpose, we carried out a method of active gull protection used in Poland (Bukaciński and Bukacińska 2008, Bukaciński 2015, Bukaciński et al. 2018). Shortly after clutch completion, eggs were taken to the incubator, and wooden dummies in the color of the original eggs were put in their place. Shortly before hatching, we put back clutches from incubators into the nests from which we had previously taken them. During this study, as well as during the gull protection activities at the Vistula River islands, we did not observe cases of clutch desertion or less intensive incubation in nests with wooden dummies (Bukaciński 2015, Bukaciński et al. 2018).

At the beginning of the breeding season, fragments of islands with control and experimental nests were surrounded by an electric fence (Bukaciński 2015, Bukaciński et al. 2018). Fences hampered (but not fully prevented) chick movement, which meant that they did not move far away from the territory, at least during the first 5 days of life when their mobility was not high. Above all, however, the electric fence prevented predation by American mink and red fox (i.e. the type of breeding failures that is not related to the quality and/or condition of parents, or their reproductive tactics). We did not include broods for which we were uncertain about chick survival to the fifth day posthatching in statistical analyses (BHG: 1 removed, 3 control, and 1 fed; MG: 1, 3, and 1, respectively).

Molecular Methods

We used GeneMatrix Quick Blood DNA Purification Kit (EURx) for blood samples and GeneMatrix Quick Tissue DNA Purification Kit (EURx) for tissue samples. For sex determination, we applied a method based on the amplification of the CHD1 gene intron (Chromo-helicase-DNA-binding gene) using 2550F and 2718R primers (Fridolfsson and Ellegren 1999, Ležalova et al. 2005). The 25 µL PCR

reaction mixture contained 2.5 µL 10× PCR buffer (EURx), 1.5 µL 25 mM $MgCl_2$ (EURx), 200 µM each dNTP (EURx), 30 pmol of each primer, and 1 U Taq DNA Polymerase (EURx). About 50–150 ng of genomic DNA was used as a template. PCR was performed in Biometra TOptical Gradient 96 thermal cycler. We applied the following program: an initial denaturing step at 94°C for 1 min 30 s, 30 cycles of 48°C for 45 s, 72°C for 45 s, 94°C for 30 s, then 48°C for 1 min, and final elongation at 72°C for 5 min (Griffiths et al. 1998, Ležalova et al. 2005). PCR products were separated for 30 min at 7–10 V cm^{-1} using 3% agarose gel stained with ethidium bromide. Males were identified by 1 band and females by 2 bands. One known adult male and one known adult female were added to the last line of each gel, as well as a blind sample without DNA.

Statistical Analyses

We analyzed factors associated with variation in sex ratio among chicks of MG and BHG at 2 stages: (1) at hatching (i.e. within 12 hr after each chick hatched) and (2) when the first-hatched chick was 5 days old (day of hatching = 0). This interval includes the period of the most intense chick mortality (Bukaciński and Bukacińska 1995, Bukacińska 1999, Buczyński 2000, Bukaciński and Bukacińska 2015a, 2015b). We used sex of the chick (0, female; 1, male) as a binary response to model the probability of a chick being male as a function of 3 predictors: experimental treatment of the parental pair during the laying of the first clutch of the season, egg position in the laying sequence, and species identity. Treatment was coded as a factor with 3 levels: pairs continuing to lay the first clutch after the first egg being removed (removed), control pairs with all initially laid eggs kept intact (control), and pairs receiving supplemental feeding until clutch completion (fed). Egg position in the laying sequence was coded also as a factor with 3 levels: first laid (egg A), second laid (egg B), and third laid (egg C). For removed pairs, this coding refers to the eggs eventually left in the nest (i.e. not removed). Species were coded as another factor with 2 levels: MG and BHG. Only clutches with known chick origin (from eggs A, B, or C) were included in the analysis ($n = 153$ nests and $n = 140$ nests of BHGs and MGs, respectively).

For chicks challenged with PHA injection shortly after hatching ($n = 420$ MGs, $n = 459$ BHGs), we modeled thickness of skin swelling (normally distributed variable, a proxy for the strength of immune response) as a function of experimental treatment, sex, egg position in the laying sequence, and species identity. We also modeled variables linked to variation in chick survival between hatching and fifth day of their life (0, died; 1, survived) as a function of 5 possible predictors: (1) experimental treatment, (2) PHA-induced skin swelling, (3) sex, (4) egg position in the laying sequence, and (5) species identity.

We used generalized linear mixed models (GLMMs) with a binomial error structure and logit link function to model the relationship between the sex of the focal chick (at hatching or on fifth day post-hatch) and 3 candidate predictors, and between the survival of chick and 5 candidate predictors. To model the relationship between PHA-induced swelling response and 4 candidate predictors, we used GLMMs with a Gaussian error structure and identity link. We employed an information-theoretic approach (Burnham and Anderson 2002) to find the most parsimonious model among candidate model set, starting from the most complex model and including all possible simpler models (all subsets; Grueber et al. 2011). We assessed multiple competing models for their fit to the data using Akaike's information criterion corrected for small sample size (AIC_c). Model selection procedure started from the global model that included all fixed predictors as main terms and all their 2-way interactions (Bolker et al. 2009). We did not use higher-order interactions, because they are difficult to interpret. Consequently, we ranked 18 (chick sex on hatching and on day fifth), 113 (chick survival), or 190 (PHA-swelling response) models using AIC_c. Brood identity was used as a random intercept in all models. We used R environment (R Core Team 2019) to fit GLMMs with *lme4* (Bates et al. 2015) and *glmmTMB* (Brooks et al. 2017) packages, and the *MuMIn* package for model selection (Bartoń 2019).

Intensity of chick immune response, as measured by PHA swelling, was strongly related to the feeding treatment of their parents. Treatment category explained as much as a half of variation in the immune response of chicks (repeatability = 0.506, 95% confidence interval [CI]: 0.460–0.561; *rptR* package; Stoffel et al. 2017), so immune response and experimental treatment conveyed largely the same information (although the immune response was a continuous variable, thus providing a more precise analysis than the 3-level treatment variable). Thus, we did not allow immune response and treatment to enter as predictors within a single model. Instead, we assessed their performance as alternative ways to quantify presumed differences in offspring quality.

Models differing by <6 AIC_c units from the top model were considered equally informative (Richards 2008, Richards et al. 2011) and averaged to obtain parameter estimates and their 95% CIs, whereas remaining lower-ranked models were discounted. The natural (conditional) averaging method (rather than zero or full method) was employed here to obtain weighted parameter estimates across the candidate model set. We then used model-averaged estimates and their 95% CIs to support decisions regarding the focal predictor and associated models, particularly models deemed most informative. Predictors with 95% CIs covering zero were considered uninformative. We also used relative variable importance (RVI) as a supplementary tool in identifying uninformative variables in the model selection procedure, although we consider it as a measure of variable criticality rather than true importance (Azen et al. 2001, Galipaud et al. 2017).

In all 4 model selections, the second-best model showed support comparable to that of the top model, with evidence ratios for the top model not exceeding 1.45. Given such a high level of model selection uncertainty, we considered the second-best model as the most informative one if it included variables we are primarily interested in (treatment, species) or variables allowing us to better understand possible mechanisms behind observed patterns. For the most informative model, we used the *effects* package (Fox 2003) to estimate and visualize fixed factors effects (i.e. average model predictions) and their 95% CIs.

We used one-way analysis of variance (ANOVA) to test for differences among treatment groups in the time of breeding, and chi-square tests to test for differences in the frequencies of broods with different chick mortality rates among experimental groups (SPSS 11.0 for Windows; SPSS Inc., Chicago, Illinois, USA). For the laying date, we presented a mean ± SD.

RESULTS

Breeding Phenology
The mean laying date for first clutches for all 153 BHG pairs was 24 April ± 8.3 days and did not differ among treatment groups (one-way ANOVA, $F_{2,150} = 0.68$, $P = 0.51$). Similarly, in the case of MG, we found no differences among treatment groups in the egg-laying date (one-way ANOVA, $F_{2,137} = 0.44$, $P = 0.65$). The mean laying date for the first clutches for all 140 MG pairs was 5 May ± 9.0 days. Due to the fact that we did not record any losses at the incubation stage in our sample, there were 3 eggs in all clutches on the day of hatching. Thus, primary (i.e. during egg laying) and secondary (i.e. at hatching) sex ratios were the same.

Sex Ratio at Hatching and Variation in PHA-Induced Response
Sex ratio at hatching varied with the treatment applied in the study. The model with treatment effect as the sole predictor of sex ratio at hatching ranked first in the model selection procedure (Table 1). Accordingly, pairs of both species receiving supplemental food until completion of the clutch had more males among newly hatched chicks (54.8%) than both control birds (48.4%) and gulls with the first egg removed (37.6%). However, a model assuming that the treatment effect is modified by species identity had comparable support to the treatment-only model ($\Delta AIC_c = 0.35$, evidence ratio = 1.19), and we feel it is justified to consider that species × treatment interaction additionally shaped sex ratio in this study. In support for this, model averaging of 8 top models (with $\Delta AIC_c < 6$) yielded an estimate of

TABLE 1. Summary of model selection results for GLMMs explaining sex ratio at hatching (probability of chick being male), recorded in first clutches of 2 gull species. For each model, k is the number of estimated parameters, LL is the model log-likelihood, AIC_c is the Akaike's information criterion corrected for small samples, ΔAIC_c is the difference between the model's AIC_c value and the minimum AIC_c for the whole set of 18 competing models, and w_i is the Akaike weight for a model. Only models within 6 AIC_c units from the top model are listed, in ascending order of ΔAIC_c. For each model, no. = model number, treatment = feeding treatment, species = Mew Gull or Black-headed Gull, egg = egg position within laying sequence, sex = sex of the chick (0, female; 1, male). All models had the binomial error structure, with the identity of brood entered as a random factor.

No.	Model (fixed parameters)	k	LL	ΔAIC_c[a]	w_i
1	Treatment	4	−600.580	0.00	0.251
2	Treatment + species + treatment:species	7	−597.712	0.35	0.211
3	Treatment + egg	6	−598.948	0.79	0.170
4	Treatment + egg + species + treatment:species	9	596.069	1.14	0.142
5	Treatment + species	5	−600.580	2.02	0.091
6	Treatment + species + egg	7	−598.948	2.82	0.061
7	Treatment + species + egg +egg:species + treatment:species	11	−595.705	4.51	0.026
8	Treatment + egg + treatment:egg	10	−597.358	5.76	0.014

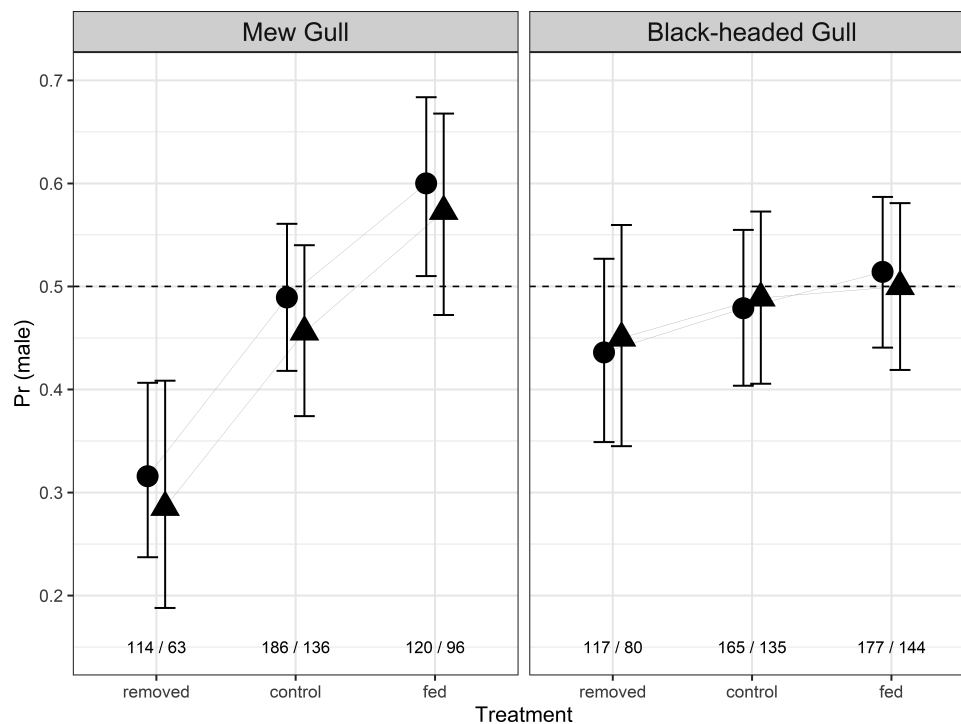[a]The lowest AIC_c was 1,209.2.

FIGURE 1. Probability of a chick being male at hatching (dots) and at the fifth day post-hatching (triangles) in relation to experimental treatment differentiating parental condition and species (panels). Modeled means ± 95% confidence intervals from the second-best model selected for the hatching day (Table 1) and second-best model for the day 5 post-hatching (Table 4) are shown, based on the values obtained from the *effects* package. Figures within panels denote sample sizes (day 0/day 5).

species × treatment effect that did not include zero for the "fed" treatment category (Supplementary Material Table S2). In this model, MG showed generally more pronounced differences in the proportion of males along the treatment-induced condition gradient than BHG, with twice as many males among newly hatched chicks from fed clutches than from removed clutches (60.0% vs 31.6%, Figure 1). Comparable figures for BHG were 51.4% vs 43.6% (Figure 1). For both species, the sex ratio did

not vary with the egg-laying sequence (Supplementary Material Figure S1).

Strength of PHA-induced swelling response among newly hatched chicks varied with experimental treatment, species, and egg position in the laying sequence. Apart from the main effects of these predictors, the best supported model also included egg × species and treatment × species interactions (Table 2). Thickness of skin swelling was not related to the sex of the chick (RVI = 0.53). Experimental treatment was the main driver of

TABLE 2. Summary of model selection results for GLMMs explaining variation in PHA-induced skin swelling recorded for newly hatched chicks subjected to immune challenge, in first clutches of 2 gull species. For each model, k is the number of estimated parameters, LL is the model log-likelihood, AIC_c is the Akaike's information criterion corrected for small samples, ΔAIC_c is the difference between the model's AIC_c value and the minimum AIC_c for the whole set of 113 competing models, and w_i is the Akaike weight for a model. Only models within 6 AIC_c units from the top model are listed, in ascending order of ΔAIC_c. For each model, no. = model number, treatment = feeding treatment, species = Mew Gull or Black-headed Gull, egg = egg position within laying sequence, sex = sex of the chick (0, female; 1, male). All models had a Gaussian error structure, with the identity of brood entered as a random factor.

No.	Model (fixed parameters)	k	LL	ΔAIC_c[a]	w_i
1	Treatment + egg + species + egg:species + treatment:species	12	−2,918.664	0.00	0.189
2	Treatment + egg + species + egg:species + treatment: egg + treatment:species	16	−2,914.862	0.75	0.130
3	Treatment + egg + species	10	−2,921.309	1.20	0.104
4	Treatment + egg + species + sex + egg:species + treatment:species	13	−2,918.319	1.42	0.093
5	Treatment + egg + species + sex + egg:species + treatment:egg + treatment:species	17	−2,914.432	1.99	0.070
6	Treatment + egg + species + sex + egg:species + sex:species + treatment:species	14	−2,917.701	2.26	0.061
7	Treatment + egg + species + sex + treatment:species	11	−2,920.925	2.50	0.054
8	Treatment + egg + species + sex + egg:species + treatment:egg + sex: species + treatment:species	18	−2,913.748	2.72	0.049
9	Treatment + egg + species + treatment:egg + treatment:species	14	−2,918.082	3.02	0.042
10	Treatment + egg + species + sex + sex:species + treatment:species	12	−2,920.345	3.40	0.035
11	Treatment + egg + species + sex +treatment:egg + treatment:species	15	−2,917.590	4.12	0.024
12	Treatment + egg + species + sex + treatment:egg + sex:species + treatment:species	16	−2,916.952	4.94	0.016
13	Treatment + egg + species + sex + egg:sex + egg:species +treatment:species	15	−2,918.065	5.07	0.015
14	Treatment + egg + species + sex + egg:species + treatment:sex +treatment:species	15	−2,918.150	5.24	0.014
15	Treatment + egg + species + sex + egg:sex + egg:species + treatment:egg +treatment:species	19	−2,914.197	5.73	0.011
16	Treatment + egg + species + sex + egg:species + treatment:egg +treatment: sex + treatment:species	19	−2,914.238	5.81	0.010
17	Treatment + egg + species + sex + egg:sex + egg:species + sex:species + treatment:species	16	−2,917.449	5.93	0.010

[a] The lowest AIC_c was 5,861.7.

variation in skin swelling, with offspring of fed birds showing the most pronounced response to PHA challenge, whereas chicks produced by birds in the removed treatment showed the least developed immune response. This relationship was species-specific, with MGs showing a lower level of immune response among chicks in the removed treatment (Figure 2). MGs also showed a less developed swelling response among chicks from eggs C (Figure 3). Additionally, the second-best model suggested a possible effect of an egg × treatment interaction, which was supported by model-averaged estimates (Supplementary Material Table S3; 95% CI did not include zero). Here, the PHA-induced response decreased with egg position in the laying sequence among chicks from the fed treatment group, but not in control and removed pairs (Supplementary Material Figure S2).

Selective Mortality and Sex Ratio During First 5 Days of Chicks' Life

In all experimental groups, the frequencies of broods with different number of chicks lost during the first 5 days of life were different ($\chi^2 = 8.11$–16.16, df = 3, $P < 0.05$ for MG and $\chi^2 = 10.30$–22.39, df = 3, $P < 0.01$ for BHG, across treatment groups). Frequency of chicks lost also differed between experimental groups ($\chi^2 = 15.31$, df = 6, $P < 0.02$ and $\chi^2 = 14.88$, df = 6, $P = 0.02$ for MG and BHG, respectively; Supplementary Material Figure S3). Broods with all 3 chicks surviving the first 5 days after hatching were most often observed in the fed treatment and least often in the removed treatment (55.0% vs 34.2% and 66.1% vs 43.6%, $\chi^2 = 6.33$, df = 2, $P < 0.05$ and $\chi^2 = 6.54$, df = 2, $P < 0.05$ for MG and BHG, respectively; Supplementary Material Figure S3). In both species, broods with no chicks surviving until day 5 post-hatch (total failure) were recorded most often in the removed group, least often in fed group (26.3% vs 5.0% and 17.9% vs 5.1%, $\chi^2 = 11.47$, df = 2 and $\chi^2 = 7.48$, df = 2, $P < 0.05$ for MG and BHG, respectively; Supplementary Material Figure S3).

If parents lost any of their offspring during the first 5 days of their life, it was usually one chick (23.7–35.0%
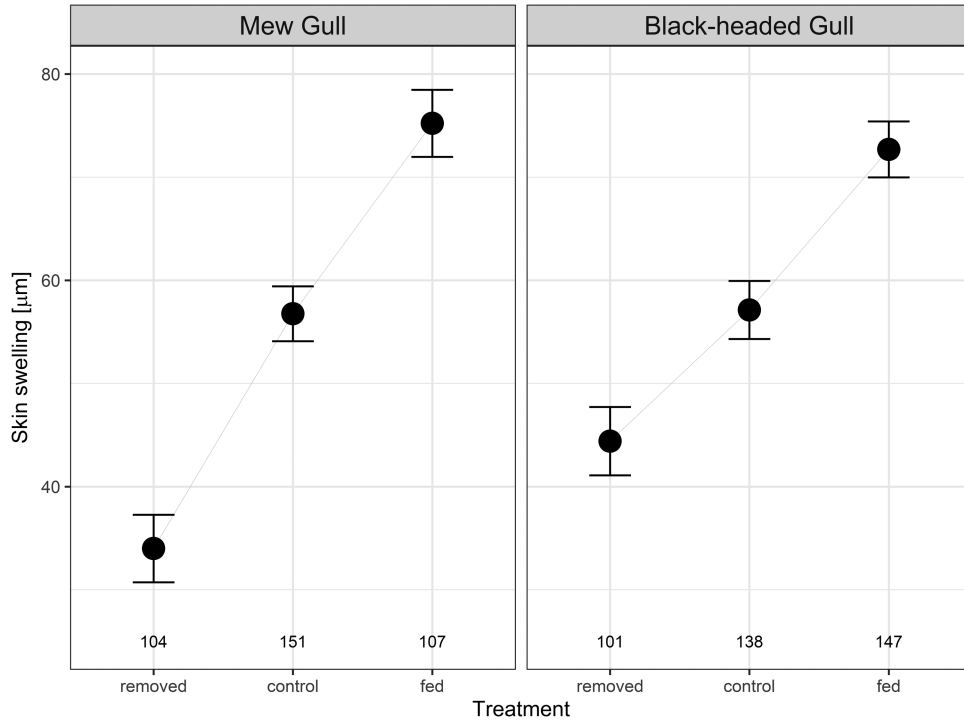
FIGURE 2. Thickness of PHA-induced swelling response in relation to experimental treatment differentiating parental condition and species (panels). Modeled means ± 95% confidence intervals from the best model selected (Table 2) are shown, based on the values obtained from *effects* package. Figures within panels denote sample sizes.
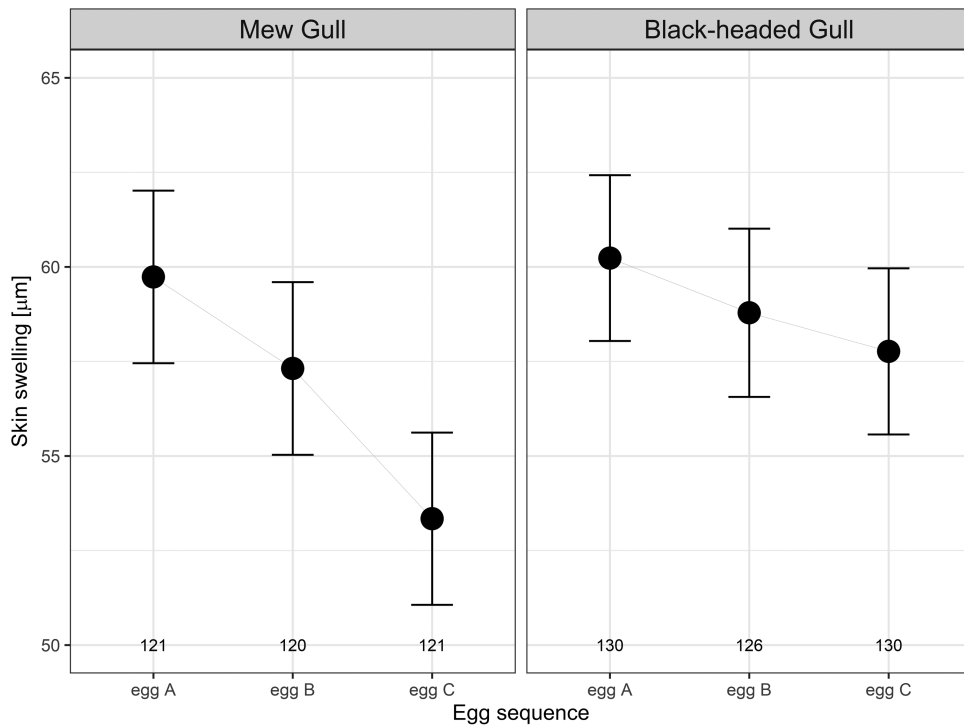

FIGURE 3. Thickness of PHA-induced swelling response in relation to egg position in the laying sequence (egg A = first laid egg, egg B = second laid, egg C = third laid) and species (panels). Modeled means ± 95% confidence intervals from the best model selected (Table 2) are shown, based on the values obtained from the *effects* package. Figures within panels denote sample sizes.

and 16.9–35.9% of all MG and BHG broods, respectively, according to treatment; Supplementary Material Figure S3). In MG broods, this chick originated most often from egg C (77.8–90.0% of all such broods), whereas in BHGs, the percentage of broods with a lost chick from egg C was significantly lower and varied between 40.0% and 57.1% (χ^2 = 3.65–4.94, df = 1, $P < 0.05$, according to treatment; Supplementary Material Figure S3). In broods with 2 chicks lost, usually one of them hatched from the last laid egg (in each species 80.0–100% of all broods, according to treatment; Supplementary Material Figure S3).

Almost three-quarters (74.4%, 95% CI: 71.4–77.2) of 879 chicks that hatched survived until the fifth day of life. In a subset of 748 chicks that were challenged immunologically at hatching, the proportion of chicks surviving to the day fifth was effectively the same (72.9%, 95% CI: 69.5–76.0). In the latter group, model selection revealed that survival was generally much better explained by PHA-induced swelling response than by experimental treatment per se (RVI: 1.00 vs 0.01). Here, the most parsimonious model explained offspring survival as an interaction of strength of immune response, species, and sex of the chick (Table 3). High immunocompetence, as measured by the amount of PHA-induced skin swelling, enhanced survival for all chicks. There was a tendency for a more pronounced effect of immune response on survival for chicks hatching from eggs C, but that was not supported in model-averaged estimates (Supplementary Material Table S4), suggesting that the second-best model (lacking this interaction and having comparable support to the top model; evidence ratio 1.17) may better reveal critical predictors of chick survival. Survival was lower for MG male chicks in general (Figure 4) and for chicks hatching from egg C, and these effects were stronger for MG chicks (Figure 5).

Although models including immune response performed better than those including experimental treatment, we also briefly refer to the latter models. The most parsimonious model of chick survival among those including treatment rather than skin swelling has the same structure as the second-best model with immune response (Table 3, cf. model 16 with model 2). Survival was best explained here by treatment, species, egg sequence, and sex, with species effects being modified by egg sequence and by sex. Fed and control chicks survived better than those from the removal treatment, irrespective of species (Figure 6). Survival was lowered for male MGs and for chicks hatching from the last laid egg, particularly among MGs (Supplementary Material Figures S4 and S5). Fixed effects in the best model including treatment explained 11% of the variance in chick survival, whereas in the second-best model overall, including PHA-induced immune response in place of treatment, they explained 44% of the variance in chick survival. Despite the lack of convincing evidence to support the inclusion of egg × experiment interaction as an informative parameter (Table 3, model 16), we present this relationship in Supplementary Material Figure S6. It provides a useful background to explain the mortality rate of chicks of different sexes in the early chick period (see further in the section "Discussion").

Among 5-day-old chicks, variance in sex ratio across treatment categories mirrored that found at hatching. Models including treatment as the only predictor were best supported (Table 4), with the second-best model differing by the inclusion of a species × treatment interaction and having only marginally lower support (ΔAIC_c = 0.48, evidence ratio = 1.28). Model-averaged estimates supported species-specific effects of treatment (Supplementary Material Table S5). Thus, for all chicks that survived the first 5 days, we concluded that the treatment × species interaction was the most important predictor of sex ratio (Figure 1).

DISCUSSION

The Impact of Parental Condition on Brood Sex Ratio Skews in BHG and MG

The islands located in the middle course of a large, untrained, lowland river, the Vistula, inhabited by the studied gull populations, form a challenging habitat for ground-nesting birds. Frequent floods during the breeding season, mass outbreaks of black flies, and strong predation pressure of American minks and red foxes mean that gulls typically have to lay more than 3 eggs in a breeding season (Bukaciński and Bukacińska 1994, 2000, 2003, 2015a, 2015b). A large number of birds, after completing the first clutch of the season, lay 3 eggs in a replacement clutch. A weakened condition of the parents significantly affects the primary sex ratio and chick mortality rate during an early-chick stage in replacement clutches (Bukaciński et al. 2020a, 2020b). Some losses, mainly related to short-term (2–3 days) river flooding in May, occur during the egg-laying period. Parents typically lose only the first or first and second eggs in these early flooding events. In such situations, gulls usually lay next 3 or 2 eggs, respectively, into the same nest, usually without the typical interval of few days between the first and replacement clutch (Różycki 2014, Bukaciński and Bukacińska 2015a, 2015b; a phenomenon termed "continuous laying," Arnold et al. 2002).

Our results indicate that the extra effort to produce only one egg more than usual affected the primary sex ratio in both BHG and MG broods. In such broods (removed treatment), females were the prevailing sex among chicks shortly after hatching, while a predominance of males was recorded in broods of gulls receiving supplemental food. Although the laying of an additional egg by MG

TABLE 3. Summary of model selection results for GLMMs explaining probability of chick surviving until the fifth day post-hatching, recorded in first clutches of 2 gull species. For each model, k is the number of estimated parameters, LL is the model log-likelihood, AIC_c is the Akaike's information criterion corrected for small samples, ΔAIC_c is the difference between the model's AIC_c value and the minimum AIC_c for the whole set of 190 competing models, and w_i is the Akaike weight for a model. 15 models within 6 AIC_c units from the top model are listed, in ascending order of ΔAIC_c. Additionally, listed is the top model (#16) among those which contained treatment instead of swelling among candidate predictors. For each model, no. = model number, swelling = PHA-induced skin swelling, treatment = feeding treatment, species = Mew Gull or Black-headed Gull, egg = egg position within laying sequence, sex = sex of the chick (0, female; 1, male). All models had the binomial error structure, with the identity of brood entered as a random factor.

No.	Model (fixed parameters)	k	LL	ΔAIC_c [a]	w_i
1	Swelling + egg + sex + species + egg:swelling + egg:species + sex:species	12	−292.632	0.00	0.200
2	Swelling + egg + sex + species + egg:species + sex:species	10	−294.851	0.31	0.171
3	Swelling + egg + sex + species + egg:swelling + egg:species + swelling:species + sex:species	13	−292.236	1.28	0.105
4	Swelling + egg + sex + species + egg:species + swelling:species +sex:species	11	−294.600	1.87	0.078
5	Swelling + egg + sex + species + egg:swelling + egg:species + swelling:sex + sex:species	13	−292.628	2.06	0.071
6	Swelling + egg + sex + species + egg:species + swelling:sex + sex:species	11	−294.755	2.18	0.067
7	Swelling + egg + sex + species + egg:swelling + egg:species + swelling:sex + swelling:species + sex:species	14	−292.234	3.35	0.037
8	Swelling + egg + sex + species + egg:swelling +egg:sex + egg:species + sex:species	14	−292.353	3.59	0.033
9	Swelling + egg + sex + species + egg:sex + egg:species + sex: species	12	−294.444	3.62	0.033
10	Swelling + egg + sex + species + egg:species + swelling:sex + swelling:species + sex:species	12	−294.511	3.76	0.031
11	Swelling + egg + sex + species + egg:swelling + egg:sex + egg:species + swelling:species + sex:species	15	−291.940	4.85	0.018
12	Swelling + egg + sex + species + egg:sex + egg:species + swelling:species + sex:species	13	−294.183	5.17	0.015
13	Swelling + egg + sex + species + egg:swelling + sex:species	10	−297.345	5.30	0.014
14	Swelling + egg + sex + species + egg:sex + egg:species + swelling:sex + sex:species	13	−294.329	5.47	0.013
15	Swelling + egg + sex + species + egg:swelling + egg:sex + egg:species + swelling:sex + sex:species	15	−292.348	5.66	0.012
16	Treatment + egg + sex + species + egg:species + sex:species	11	−380.553	173.78	<0.001

[a] The lowest AIC_c was 609.7.

females reduces body mass and protein reserves by only 4–8% (Różycki 2014, Wiśniewska 2014, D. Bukaciński and M. Bukacińska personal communication) it was sufficient, as in the Lesser Black-backed Gulls, for the hatchling sex ratio to be significantly different from 50:50 (Figure 1; see also Monaghan et al. 1998).

The differences in sex ratios along the treatment-induced female condition gradient were slightly less extreme in first clutches than in replacement clutches, both in MGs and BHGs. Experimental MG females in worsened condition lowered the proportion of sons to 32% in first clutches (this study) and to 27% in replacement clutches (Bukaciński et al. 2020b). For BHG, the figures were 44% and 41%, respectively. This pattern is in agreement with the reproductive cost hypothesis of sex allocation (Myers 1978, Cockburn et al. 2002), coupled with the expectation that while laying the first clutch adult females are less stressed and are less likely to manipulate their brood sex

ratio than during replacement clutches (Bukaciński et al. 2020a, 2020b). On the other hand, females MG receiving supplemental feeding increased the proportion of sons to 60% in first clutches and to 69% in replacement clutches (Bukaciński et al. 2020b), while supplementary fed BHGs had 51% sons in first clutches and 56% sons in replacement clutches (Bukaciński et al. 2020b). Increasing proportion of sons by good condition parents is compatible with Trivers–Willard hypothesis (Trivers and Willard 1973), given the strong inter-male competition for territories in gulls (Pierotti 1981, Southern 1981, Butler and Janes-Butler 1983), promoting large and good condition males (but see Merkling et al. 2015).

However, supplemented females invested more in sons in replacement clutches than in first clutches when they are presumably in worse condition. It should be noted, however, that the described patterns refer to sex ratio at hatching (equivalent to primary sex ratio in our study)
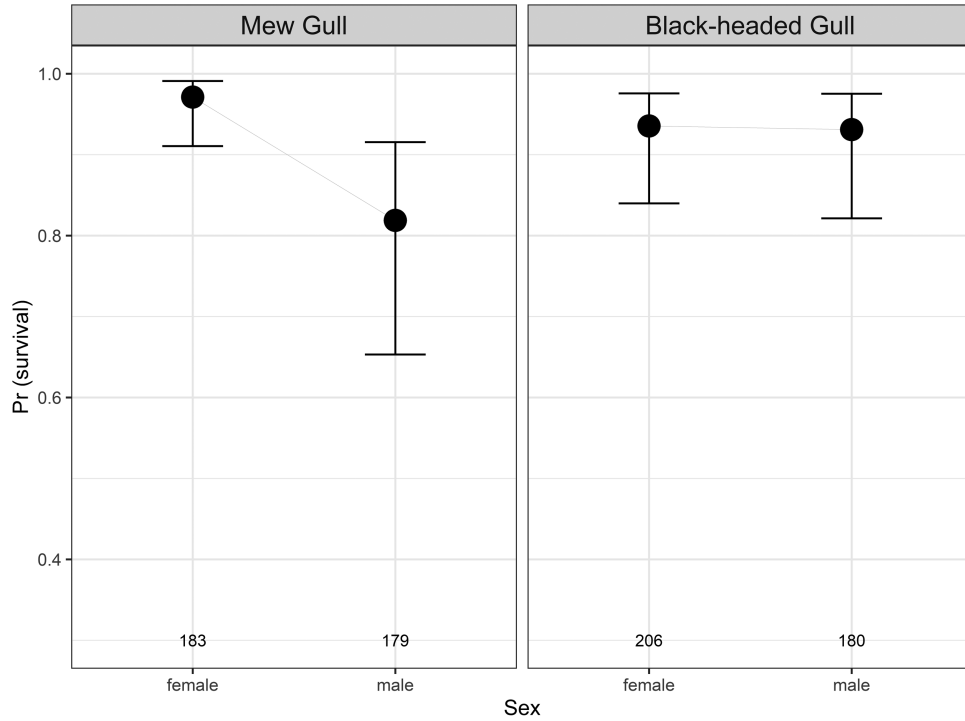
FIGURE 4. Probability of a chick surviving until the fifth day post-hatching in relation to the sex of the chick and species (panels). Modeled means ± 95% confidence intervals from the second-best model selected (Table 3) are shown, based on the values obtained from the *effects* package. Figures within panels denote sample sizes.
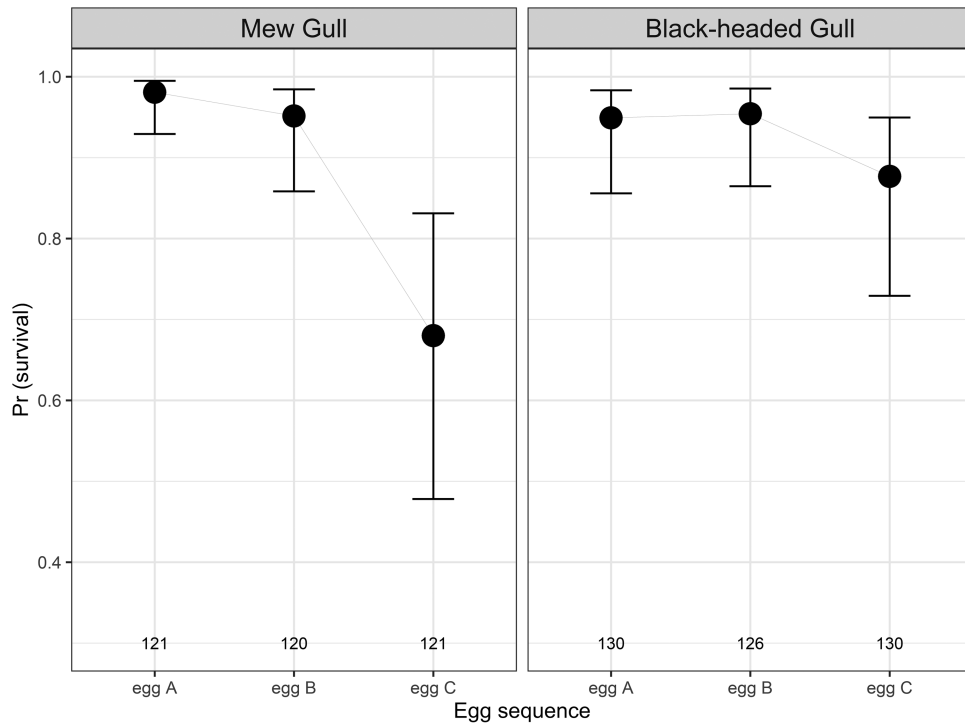

FIGURE 5. Probability of a chick surviving until the fifth day post-hatching in relation to egg position in the laying sequence (egg A = first laid egg, egg B = second laid, egg C = third laid) and species (panels). Modeled means ± 95% confidence intervals from the second-best model selected (Table 3) are shown, based on the values obtained from *effects* package. Figures within panels denote sample sizes.
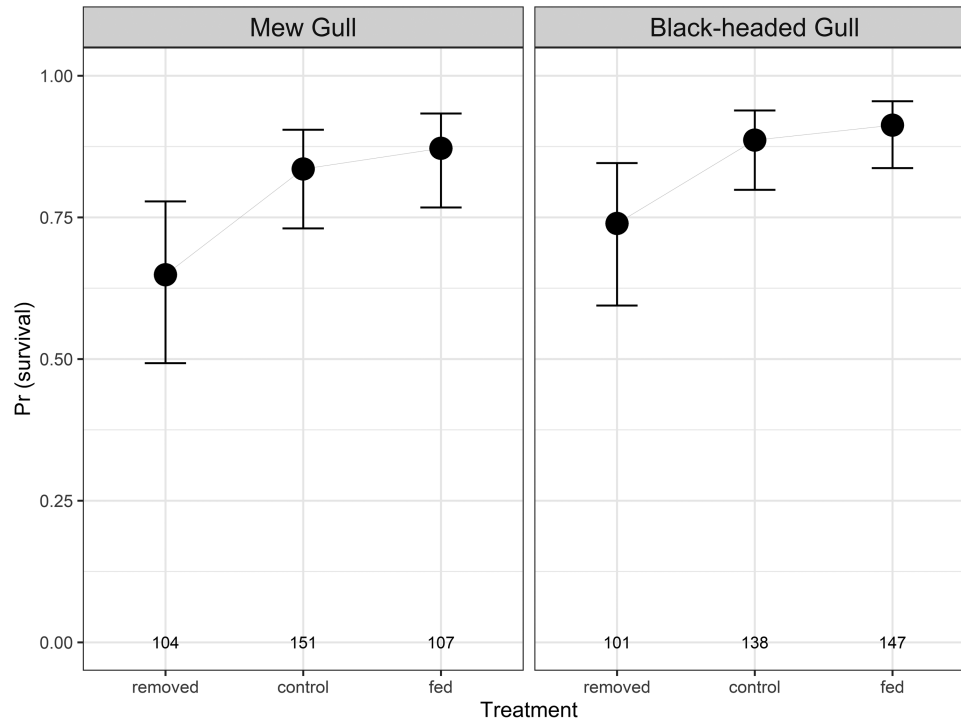
FIGURE 6. Probability of a chick surviving until the fifth day post-hatching in relation to experimental treatment and species (panels). Shown are modeled means ± 95% confidence intervals from the best model selected among those including treatment instead of immune response (model 16 from Table 3; see text), based on the values obtained from *effects* package. Figures within panels denote sample sizes.

TABLE 4. Summary of model selection results for GLMMs explaining sex ratio at fifth day post-hatching (probability of chick being male), recorded in first clutches of 2 gull species. For each model, k is the number of estimated parameters, LL is the model log-likelihood, AIC_c is the Akaike's information criterion corrected for small samples, ΔAIC_c is the difference between the model's AIC_c value and the minimum AIC_c for the whole set of 18 competing models, and w_i is the Akaike weight for a model. Only models within 6 AIC_c units from the top model are listed, in ascending order of ΔAIC_c. For each model, no. = model number, treatment = feeding treatment, species = Mew Gull or Black-headed Gull, egg = egg position within laying sequence, sex = sex of the chick (0, female; 1, male). All models had the binomial error structure, with the identity of brood entered as a random factor.

No.	Model (fixed parameters)	k	LL	ΔAIC_c [a]	w_i
1	Treatment	4	−448.167	0.00	0.175
2	Treatment + species + treatment:species	7	−445.352	0.48	0.137
3	Treatment + egg	6	−446.612	0.96	0.108
4	Treatment + species + egg + treatment:egg + treatment:species	13	−439.501	1.17	0.097
5	Treatment + egg + treatement:egg	10	−442.696	1.34	0.090
6	Treatment + species + egg + treatment:species	9	−443.802	1.49	0.083
7	Treatment + species	5	−447.982	1.66	0.076
8	Treatment + species + egg	7	−446.437	2.65	0.046
9	Treatment + species + egg + treatment:egg	11	−442.493	3.00	0.039
10	Treatment + species + egg + egg:species + treatment:egg + treatment:species	15	−438.437	3.23	0.035
11	Treatment + species + egg + egg:species + treatment:species	11	−442.706	3.43	0.032
12	[Null]	2	−452.327	4.28	0.021
13	Treatment + species + egg + egg:species	9	−445.427	4.74	0.016
14	Egg	4	−450.707	5.08	0.014
15	Treatment + species + egg + egg:species + treatment:egg	13	−441.527	5.23	0.013
16	Species	3	−452.089	5.82	0.010

[a]The lowest AIC_c was 904.4.

which was later modified by the selective mortality of chicks, mostly those last-hatched, during their first days of life. Mortality of chicks from eggs C was much larger in replacement clutches (52%, both species; Bukaciński et al. 2020b) than in first clutches (32% MG, 12% BHG; this study). Thus, gulls are apparently able to adjust the brood sex ratio to prevailing conditions, including their own brood-rearing ability (presumably linked to condition; Lorentsen 1996, Nager et al. 1999, Verboven et al. 2003, this study). In our sample, however, the sex ratio at hatching was unrelated to the egg position in the laying sequence. Increased mortality of chicks from eggs C did not translate into changes to hatching sex ratio during subsequent days of peak chick mortality.

Similar brood sex ratio skews were observed for the first clutches of Lesser Black-backed Gulls, Yellow-legged Gulls (*Larus cachinnans*), and Common Terns (*Sterna hirundo*; Nager et al. 1999, 2000a, 2000b, Alonso-Alvarez and Velando 2003, Benito et al. 2013). Despite the fact that in the early-chick period size differences between chicks of different sexes were smaller than in adult birds (Dejtrowski 1993, Różycki 2014, D. Bukaciński and M. Bukacińska personal communication), the prevalence of dominating sex in a treatment group in MG broods was still greater than in BHG broods (Figure 1). This may indicate that sexual size dimorphism may be more important in shaping the sex ratio skew in this species than in BHG.

Chick Mortality and PHA-Induced Immunological Response in the Early-Chick Period

The role of sex in determining the likelihood of a chick's survival is still debated. If sex does have a significant role, then important questions remain regarding the mechanistic basis for this differential mortality rate and the role of parents in this process (Clutton-Brock 1991, Bukaciński et al. 1998, González-Solís et al. 2005, Ležalova et al. 2005, Kim and Monaghan 2006, Benito et al. 2013, Spelt and Pichegru 2016). Previous studies have indicated that the factors affecting chick mortality may differ not only between species, but also in different populations of the same species (Spaans et al. 1994, Bukaciński and Bukacińska 1995, 2003, Thyen and Becker 2006, Becker and Ludwigs 2011). In the BHG and MG colonies on the islands of the Vistula River's middle course, where parental investment in reproduction was greater almost every year than in other populations of this species (Bukaciński and Bukacińska 2003, 2015a, 2015b), poor condition of parents increased mortality rate in an early-chick period. Similar relationships have been found for Herring Gulls, Lesser Black-backed Gulls, and Common Terns (Bukacińska et al. 1996, Bukaciński et al. 1998, Benito et al. 2013). Notably, despite nonrandom early chick mortality, the sex ratio on day 5 post-hatching remained as skewed as at hatching. This suggests that even if early chick mortality is shaped by differential parental effort, parents apparently

stick to sex allocation determined during egg production. It is also worth noting that the chick mortality rate in broods of MGs was 10–15% higher than in BHG broods (depending on parental condition, see Supplementary Material Figure S3). This could be related to the fact that MG chicks are generally larger (Różycki 2014, Wiśniewska 2014, D. Bukaciński and M. Bukacińska personal communication), and thus potentially may be more sensitive to decreased parental care. Moreover, these between-species differences were driven mainly by selective mortality of male MG chicks, the largest of newly hatched chicks in the sample, reinforcing the idea that size was the primary reason for mortality differences.

In BHG broods, the mortality rate of chicks hatched from the last laid eggs was only slightly (and insignificantly) higher than for the hatchlings from eggs A and B and was not related to the sex of the chicks. This was different in the MG broods, where, regardless of the parental condition, sons experienced significantly higher mortality than daughters, as did the chicks from C eggs, in contrast to those from eggs A and B (Supplementary Material Figure S5). These differences can have serious consequences, especially since in populations of BHGs and MGs, as in many other gull species, offspring mortality during the first few days after hatching is usually the highest and has the largest effect on the annual breeding success of the parents (Lundberg and Väisänen 1979, Hahn 1981, Bukaciński and Bukacińska 1995, Bukacińska et al. 1996, Bukaciński et al. 1998, Buczyński 2000, Różycki 2014, Bukaciński and Bukacińska 2015a, 2015b). The mortality rate of MG chicks from the last laid eggs was twice as high as chicks from the first or second laid egg. This means that brood reduction in the first few days of a chicks' life may be crucial in shaping sex ratio skew at the fledgling stage in this species and much more important in this respect than in BHGs. Our results indicate that the chick mortality rate in subsequent weeks of life is significantly lower and increasingly less related to the egg sequence (Bukacińska 1999, Buczyński 2000). In addition, sex ratio skew among fledglings is affected to a greater degree by the sex of the offspring from eggs A and B at hatching than it is by the sex of the chick from egg C. The strength of this effect increases with decreasing parental condition or with more demanding feeding conditions at the breeding grounds, when parents as a consequence will provide less care (Bukaciński et al. 1998, 2020a, 2020b). Although not included in the most parsimonious models, an interaction between the mortality rate of chicks from successively laid eggs and experimental treatment suggested that a decrease in parental condition was associated with a higher mortality rate in chicks from eggs C, but not for hatchlings from eggs B or (especially) A (Supplementary Material Figure S6). In other words, chicks from egg C are disproportionately affected by the effects of female condition on chick mortality, while

egg A and egg B chicks are buffered to some extent from the parental condition effects. The Carranza (2004) and Carranza and Polo (2012) models show that the relationship between parental resources and brood sex ratio can be more complex than often thought. In the case of species that produce several offspring in one breeding attempt (as in MGs and BHGs), parental resources must be divided among siblings. In such cases, the proportion of resources obtained by each of the descendants will depend not only on the total resources provided by parents, but also on the number and sex of siblings and interactions among them (Carranza 2004, Kalmbach et al. 2005, Uller 2006). Reviews of brood sex ratios indicate that parental condition more often affects the sex of offspring within a specific position in the brood rather than the sex ratio of the entire brood (Pike and Petrie 2003, Alonso-Alvarez 2006).

Immunity (the capability of the organism to resist harmful microorganisms) is one of the basic factors on which the health of the organism depends. We found a strong positive relationship between the parental condition and immunological response of chicks shortly after hatching in both gull species. Maintaining an efficient and effective immune system is costly for birds in terms of energy and has an impact on their reproduction, including the physiological condition of chicks (Lochmiller and Deerenberg 2000). The positive effect of the parental condition of and/or a high-protein diet (in our research—fish) on the PHA-induced immune response of chicks has previously been found, among others, in Northern Bobwhites (*Colinus virginianus*), Great Tits (*Parus major*), and Yellow-legged Gull (Lochmiller et al. 1993, Hõrak et al. 1999, Alonso-Alvarez and Tella 2001).

Explanations for a higher mortality rate of sons compared to daughters in the rearing period have been sought for many years (Clutton-Brock et al. 1985, Griffiths 1992, Emlen 1997). Until the mid-1990s, the opinion prevailed that the higher mortality rate of male chicks (in species in which they are larger sex) was a consequence of greater nutritional requirements and greater sensitivity to hunger in adverse circumstances (poor parental condition, worse food availability, adverse weather conditions, etc.) related to larger body size of this sex (*size hypothesis*; Clutton-Brock et al. 1985, Clutton-Brock 1991, Anderson et al. 1993, Nager et al. 1999, 2000a, 2000b, Heg et al. 2000, Korpimäki et al. 2000, Weimerskirch et al. 2000, Magrath et al. 2007). Later, however, it was suggested that factors independent of size, such as sex hormones, may decide about increased mortality of sons (*male phenotype hypothesis*, Fargallo et al. 2002, Müller et al. 2003). It was pointed out that androgens (mainly testosterone) may weaken the immune system, leading to increased mortality of males (Schwabl 1993, Saino et al. 1995, Nolan et al. 1998, Fargallo et al. 2002). Buchanan et al. (2001)

also found that androgen activity resulted in the higher energy expenditure, and therefore also higher nutritional requirements for the sons of House Sparrows (*Passer domesticus*), although they did not differ in size from their sisters. However, our results from the Vistula colonies of BHGs and MGs do not correspond well with the predictions of the *male phenotype hypothesis*. In our study species, we find greater support for the *size hypothesis*, with higher rates of the observed chick mortality in the larger species (MG) and greater differences in chick mortality between sexes in the species with a greater sexual size dimorphism (MG, lower survival of sons compared to daughters). The results of the PHA-induced immunity responses in the BHG and MG chicks provide little support for the role of sex hormones in producing differential mortality rates among the sexes, which aligns with the predictions of the *size hypothesis*.

In summary, the results obtained in the Vistula gull colonies indicate that body size can have a significant influence on the greater sensitivity of sons in these MG populations, which is consistent with predictions of *size hypothesis* (Clutton-Brock et al. 1985, Clutton-Brock 1991, Krijgsveld et al. 1998). Of course, we cannot exclude that the effect of testosterone could have strengthened (e.g., by increasing more than it was only due to differences in body size, differences in nutritional requirements between brothers and sisters) the effect of body size on the MG mortality rates of different sex in the early-chick stage.

Finally, we would like to draw attention to one intriguing result that is difficult for us to interpret clearly. The level of immune response in all BHG chicks hatched in the removed group or those from eggs C, including males and females and those that survived and died in the early period, was higher on average than in MG offspring (Figures 2 and 3). Speculating, continuous contact with significantly more individuals (BHG breed in larger and denser colonies than MG) may increase the risk of transmitting pathogens (Alexander 1974, Patterson and Ruckstuhl 2013). Living in much larger colonies and in higher densities results in more frequent aggressive neighborly interactions than occurs in the MG colonies (Bukacińska and Bukaciński 1994, Bukaciński 1998, Bukaciński and Bukacińska 2003, 2015a, 2015b). A higher baseline level of immunity in the BHG chicks could therefore be an adaptation to life under a greater risk of contracting pathogens and permanent stress during the breeding season. Surely, further, dedicated studies are needed to confirm these results and identify possible drivers behind the observed pattern.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Ornithology* online.

ACKNOWLEDGMENTS

Ethics statement: We captured and marked Mew Gulls under bird ringing license no. 27/2014, issued by Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences, based on the decision of the General Director for Environmental Protection (DZP-WG.6401.03.73.2014. km). All methods meet ethical guidelines for the use of wild birds in scientific research stipulated by Polish law. The temporary exchange of Mew Gull and Black-headed Gull eggs with wooden dummies, the artificial incubation of eggs in incubators, the injection of a phytohemagglutinin solution in chicks, and collection of blood samples and sampling from dead chicks were made under licenses of the Local Board of Ethics in Warsaw (no. 75/2010 and 76/2010) and Regional Director for Environmental Protection in Warsaw (RDOŚ-14-WPN-I-EB-6631-278/10) and in Lublin (WPN.6402.13.20111.TB).

Authors contributions: D.B. formulated the idea, D.B. and M.B. performed fieldwork, M.B. performed laboratory analyses, P.C. and D.B. performed statistical analyses and made the figures and tables, and D.B. and P.C. wrote the original manuscript. All authors contributed to editing the manuscript.

Data depository: Analyses reported in this article can be reproduced using the data provided by Bukaciński et al. (2021).

Conflicts of interest statement: There are no conflicts to declare.

LITERATURE CITED

Alexander, R. D. (1974). The evolution of social behavior. The Annual Review of Ecology, Evolution, and Systematics 5:325–383.

Alonso-Alvarez, C. (2006). Manipulation of primary sex-ratio: An updated review. Avian and Poultry Reviews 17:1–20.

Alonso-Alvarez, C., and J. L. Tella (2001). Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. Canadian Journal of Zoology 79:101–105.

Alonso-Alvarez, C., and A. Velando (2003). Female body condition and brood sex ratio in Yellow-legged Gulls Larus cachinnans. Ibis 145:220–226.

Anderson, D. J., J. Reeve, J. E. M. Gomez, W. W. Weathers, S. Hutson, H. W. Cunningham, and D. M. Bird (1993). Sexual size dimorphism and food requirements of nestling birds. Canadian Journal of Zoology 71:2541–2545.

Arctander, P. (1988). Comparative studies on avian DNA restriction fragment length polymorphism analysis: Convenient procedures based on blood samples from live birds. Journal für Ornithologie 129:205–216.

Arnold, T. W., D. W. Howerter, J. H. Devries, B. L. Joynt, R. B. Emery, and M. G. Anderson (2002). Continuous laying and clutch-size limitation in Mallards. The Auk 119:261–266.

Azen, R., D. V. Budescu, and B. Reiser (2001). Criticality of predictors in multiple regression. The British Journal of Mathematical and Statistical Psychology 54:201–225.

Barth, E. K. (1967). Egg dimensions and laying dates of Larus marinus, L. cachinnans, L. fuscus, L. canus. Norwegian Journal of Zoology 15:5–34.

Bartoń, K. (2019). MuMIn: Multi-Model Inference. R package version 1.43.6. https://CRAN.R-project.org/package=MuMIn

Bates, D., M. Maechler, B. Bolker, and S. Walker (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Becker, P. H., and J.-D. Ludwigs (2011). Common Tern Sterna hirundo. In Birds of Western Palearctic Interactive 2.03. BirdGuides Ltd and Oxford University Press, Oxford, UK.

Becker, P. H., and M. Wink (2003). Influences of sex, sex composition of brood and hatching order on mass growth in Common Terns Sterna hirundo. Behavioral Ecology and Sociobiology 54:136–146.

Benito, M. M., H. Schielzeth, J. González-Solís, and P. H. Becker (2013). Sex ratio adjustments in Common Terns: Influence of mate condition and maternal experience. Journal of Avian Biology 44:179–188.

Blanco, G., J. Martínez-Padilla, D. Serrano, J. A. Dávila, and J. Viñuela (2003). Mass provisioning to different-sex eggs within the laying sequence: Consequences for adjustment of reproductive effort in a sexually dimorphic bird. Journal of Animal Ecology 72:831–838.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. Stevens, and J. S. White (2009). Generalized linear mixed models: A practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135.

Bolton, M., D. Houston, and P. Monaghan (1992). Nutritional constraints on egg formation in the Lesser Black-backed Gull: An experimental study. Journal of Animal Ecology 61:521–532.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378–400.

Buchanan, K. L., M. R. Evans, A. R. Goldsmith, D. M. Bryant, and L. V. Rowe (2001). Testosterone influences basal metabolic rate in male House Sparrows: A new cost of dominance signaling? Proceedings of the Royal Society of London, Series B: Biological Sciences 268:1337–1344.

Buczyński, A. (2000). Straty jaj w koloniach mewy pospolitej (Larus canus) na Wiśle - znaczenie trzeciego jaja w lęgu (in Polish). MSc thesis, Warsaw University, Warsaw, Poland.

Bukacińska, M. (1999). Wpływ opieki rodzicielskiej na sukces reprodukcyjny u mewy pospolitej Larus canus (in Polish). PhD dissertation, Institute of Ecology, PAN, Dziekanów Leśny, Poland.

Bukacińska, M., and D. Bukaciński (1994). Seasonal and diurnal changes in aggression and territory size in the Black-headed Gull (*Larus ridibundus*) on the islands of the middle reaches of the Vistula river. Ethology 97:329–339.

Bukacińska, M., D. Bukaciński, and A. L. Spaans (1996). Attendance and diet in relation to breeding success in Herring Gulls (*Larus argentatus*). The Auk 113:300–309.

Bukaciński, D. (1998). Adaptacyjne znaczenie terytorializmu u mewy pospolitej *Larus canus*. PhD dissertation (in Polish), Instytut Ekologii PAN, Dziekanów Leśny, Poland.

Bukaciński, D. (2015). Strategia Czynnej Ochrony Zagrożonej Awifauny Wysp Środkowej Wisły: Podręcznik Najlepszych Praktyk (in Polish). OTOP, Marki, Poland.

Bukaciński, D., and M. Bukacińska (1994). Czynniki wpływające na zmiany liczebności i rozmieszczenie mew, rybitw i sieweczek na Wiśle środkowej (in Polish). Notatki Ornitologiczne 35:79–97.

Bukaciński, D., and M. Bukacińska (1995). The factors limiting breeding success in the Black-headed Gull (*Larus ridibundus*) in different habitat types on the middle course of the Vistula River, Poland. Archive für Hydrobiologie 101, Large Rivers 9:221–228.

Bukaciński, D., and M. Bukacińska (2000). The impact of mass outbreaks of black flies (Simuliidae) on the parental behaviour and breeding output of colonial Common Gulls (*Larus canus*). Annales Zoologici Fennici 37:43–49.

Bukaciński, D., and M. Bukacińska (2003). *Larus canus* Common Gull. In Birds of Western Palearctic Update 5 (D. Parkin, Editor). Oxford University Press, Oxford, UK. pp. 13–47.

Bukaciński, D., and M. Bukacińska (2008). Threatened bird species of the middle Vistula River islands: Status, necessity for protection and proposed activities. In Theoretical and Applied Aspects of Modern Ecology (J. Uchmański, Editor). UKSW, Warsaw, Poland. pp. 219–239.

Bukaciński, D., and M. Bukacińska (2015a). Kluczowe Gatunki Ptaków Siewkowych na Środkowej Wiśle: Biologia, Ekologia, Ochrona i Występowanie. T.1. Mewa Siwa *Larus canus* (in Polish). Monografia. STOP, Warszawa, Poland.

Bukaciński, D., and M. Bukacińska (2015b). Kluczowe Gatunki Ptaków Siewkowych na Środkowej Wiśle: Biologia, Ekologia, Ochrona i Występowanie. T.2. Śmieszka *Chroicocephalus ridibundus* (in Polish). Monografia. STOP, Warszawa, Poland.

Bukaciński, D., M. Bukacińska, and A. Buczyński (2018). Threats and the active protection of birds in a riverbed: Postulates for the strategy of the preservation of the middle Vistula River avifauna. Studia Ecologiae et Bioethicae 16:5–30.

Bukaciński, D., M. Bukacińska, and A. Buczyński (2020a). Offspring sex ratio in the first and replacement clutches of the Mew Gulls (*Larus canus*): Breeding tactics in the riverine population of a sexually size-dimorphic bird. Waterbirds. 43:174–185.

Bukaciński, D., M. Bukacińska, and P. Chylarecki (2020b). Effect of food availability on offspring sex ratios in replacement clutches of Mew Gulls (*Larus canus*) and Black-headed Gulls (*Chroicocephalus ridibundus*) in a highly unstable environment of the Vistula River. Journal of Ornithology 161:829–847.

Bukaciński, D., M. Bukacińska, and P. Chylarecki (2021). Data from: Manipulating parental condition affects brood sex ratio, immunocompetence and early chick mortality in two gull species differing in sexual size dimorphism. Ornithology 138:1–21. doi:10.5061/dryad.6q573n5z3

Bukaciński, D., M. Bukacińska, and A. L. Spaans (1998). Experimental evidence for the relationship between food supply, parental effort and chick survival in the Lesser Black-backed Gulls *Larus fuscus*. Ibis 140:422–430.

Burnham, K. P., and D. R. Anderson (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd edition. Springer-Verlag, New York, NY, USA.

Butler, R. G., and S. Janes-Butler (1983). Sexual differences in the behaviour of adult Great Black-backed Gulls (*Larus marinus*) during the pre- and post-hatching periods. The Auk 100:63–75.

Cameron, E. Z., W. L. Linklater, K. J. Stafford, and C. J. Veltman (1999). Birth sex ratios relate to mare condition at conception in Kaimanawa horses. Behavioral Ecology 10:472–475.

Cameron-MacMillan, M. L., C. J. Walsh, S. I. Wilhelm, and A. E. Storey (2007). Male chicks are more costly to rear than females in a monogamous seabird, the Common Murre. Behavioral Ecology 18:81–85.

Cantarero, A., A. Pilastro, and M. Griggio (2018). Nestling sex ratio is associated with both male and female attractiveness in Rock Sparrows. Journal of Avian Biology 49:1–9.

Carranza, J. (2004). Sex allocation within broods: The intrabrood-sharing out hypothesis. Behavioral Ecology 15:223–232.

Carranza, J., and V. Polo (2012). Is there an expected relationship between parental expenditure and sex ratio of litters or broods? Animal Behaviour 84:67–76.

Cassinello, J., and M. Gomendio (1996). Adaptive variation in litter size and sex ratio at birth in a sexually dimorphic ungulate. Proceedings of the Royal Society of London, Series B: Biological Sciences 263:1461–1466.

Clutton-Brock, T. H. (1991). The Evolution of Parental Care. Princeton University Press, Princeton, NJ, USA.

Clutton-Brock, T. H., S. D. Albon, and F. E. Guiness (1985). Parental investment and sex differences in juvenile mortality in birds and mammals. Nature 313:131–133.

Clutton-Brock, T. H., S. D. Albon, and F. E. Guinness (1986). Great expectations: Dominance, breeding success and offspring sex ratios in red deer. Animal Behaviour 34:460–471.

Cockburn, A., S. Legge, and M. Double (2002). Sex ratios in birds and mammals: Can the hypotheses be disentangled. In Sex Ratios: Concepts and Research Methods (I. C. W. Hardy, Editor). Cambridge University Press, Cambridge, UK. pp. 266–286.

Cook, M. I., and P. Monaghan (2004). Sex differences in embryo development periods and effects on avian hatching patterns. Behavioral Ecology 15:205–209.

Dejtrowski, R. (1993). Rozwój postembrionalny piskląt mewy pospolitej (*Larus canus* L. 1758) w zależności od kolejności klucia i siedliska (in Polish). MSc thesis, Warsaw University, Warsaw, Poland.

Douhard, M. (2017). Offspring sex ratio in mammals and the Trivers-Willard hypothesis: In pursuit of unambiguous evidence. BioEssays 39:700043.

Eising, C. M., C. Eikenaar, H. Schwabl, and T. G. Groothuis (2001). Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: Consequences for chick development. Proceedings of the Royal Society B: Biological Sciences 268:839–846.

Emlen, S. T. (1997). When mothers prefer daughters over sons. Trends in Ecology & Evolution 12:291–292.

Fargallo, J. A., T. Laaksonen, V. Pöyri, and E. Körpimaki (2002). Inter-sexual differences in the immune response of Eurasian Kestrel nestlings under food shortage. Ecology Letters 5:95–101.

Folstad, I., and A. J. Karter (1992). Parasites, bright males and the immunocompetence handicap. The American Naturalist 139:603–622.

Foo, Y. Z., S. Nakagawa, G. Rhodes, and L. W. Simmons (2017). The effects of sex hormones on immune function: a meta-analysis. Biological Reviews of the Cambridge Philosophical Society 92:551–571.

Fox, J. (2003). Effect displays in R for generalised linear models. Journal of Statistical Software 8:1–27.

Fridolfsson, A. K., and H. Ellegren (1999). A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116–121.

Galipaud, M., M. A. F. Gillingham, and F.-X. Dechaume-Moncharmont (2017). A farewell to the sum of Akaike weights: The benefits of alternative metrics for variable importance estimations in model selection. Methods in Ecology and Evolution 8:42–49.

González-Solís, J., P. H. Becker, H. Wendeln, and M. Wink (2005). Hatching sex ratio and sex specific chick mortality in Common Terns *Sterna hirundo*. Journal for Ornithology 146:235–243.

Griffiths, R. (1992). Sex-biased mortality in the Lesser Black-backed Gull *Larus fuscus* during the nestling stage. Ibis 134:237–244.

Griffiths, R., M. C. Double, K. Orr, and R. J. Dawson (1998). A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Grueber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson (2011). Multimodel inference in ecology and evolution: Challenges and solutions. Journal of Evolutionary Biology 24:699–711.

Hahn, D. C. (1981). Asynchronous hatching in the Laughing Gull: Cutting losses and reducing rivalry. Animal Behaviour 29:421–427.

Heaney, V., and P. A. Monaghan (1995). Within-clutch trade-off between egg production and rearing in birds. Proceedings of the Royal Society of London, Series B: Biological Sciences 261:361–365.

Heg, D., N. J. Dingemanse, C. M. Lessells, and A. C. Mateman (2000). Parental correlates of offspring sex ratio in Eurasian Oystercatchers. The Auk 117:980–986.

Hewison, A. J. M., and J.-M. Gaillard (1999). Successful sons or advantaged investment in ungulates. Trends in Ecology & Evolution 14:229–234.

Hõrak, P., L. Tegelmann, I. Ots, and A. P. Møller (1999). Immune function and survival of great tit nestlings in relation to growth conditions. Oecologia 121:316–322.

Ilichev, V. D., and V. A. Zubakin (Editors) (1988). Pticy SSSR, volume 4 (in Russian). Chaikovyie. Nauka, USSR, Moskva, Russia.

Kalmbach, E., R. W. Furness, and R. Griffiths (2005). Sex-biased environmental sensitivity: Natural and experimental evidence from a bird species with larger females. Behavioral Ecology 16:442–449.

Kilpi, M., L. Hillström, and K. A. I. Lindström (1996). Egg-size variation and reproductive success in the Herring Gull *Larus argentatus*: Adaptive or constrained size of the last egg? Ibis 138:212–217.

Kim, S -Y., and P. Monaghan (2006). Sex of the first hatched chick influences survival of the brood in the Herring Gull (*Larus argentatus*). Journal of Zoology 270:116–121.

Komdeur, J. (2012). Sex allocation. In Evolution of Parental Care (N. J. Royle, P. T. Smiseth, and M. Kolliker, Editors). Oxford University Press, Oxford, UK. pp. 171–188.

Korpimäki, E., C. A. May, D. T. Parkin, J. H. Wetton, and J. Wiehn (2000). Environmental- and parental condition-related variation in sex ratio of Kestrel broods. Journal of Avian Biology 31:128–134.

Krackow, S. (1995). Potential mechanisms for sex ratio adjustment in mammals and birds. Biological Reviews of the Cambridge Philosophical Society 70:225–241.

Krijgsveld, K. L., C. Dijkstra, G. H. Visser, and S. Daan (1998). Energy requirements for growth in relation to sexual size dimorphism in marsh harrier *Circus aeruginosus* nestlings. Physiological Zoology 71:693–702.

Ležalova, R., E. Tkadlec, M. Obornik, J. Šimek, and M. Honza (2005). Should males come first? The relationship between offspring hatching order and sex in the Black-headed Gull *Larus ridibundus*. Journal of Avian Biology 36:478–483.

Li, X. Y., and H. Kokko (2019). Sex-biased dispersal: A review of the theory. Biological Reviews of the Cambridge Philosophical Society 94:721–736.

Lochmiller, L. R., and C. Deerenberg (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos 88:87–98.

Lochmiller, R. L., M. R. Vestey, and J. C. Boren (1993). Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. The Auk 110:503–510.

Lorentsen, S. H. (1996). Regulation of food provisioning in the Antarctic Petrel *Thalassoica antarctica*. Journal of Animal Ecology 65:381–388.

Lundberg, C.-A., and R. A. Väisänen (1979). Selective correlation of egg size with chick mortality in the Black-headed Gull (*Larus ridibundus*). The Condor 81:146–156.

Magrath, M. J., E. van Lieshout, I. Pen, G. H. Visser, and J. Komdeur (2007). Estimating expenditure on male and female offspring in a sexually size-dimorphic bird: A comparison of different methods. The Journal of Animal Ecology 76:1169–1180.

Martin, L. B. II, P. Han, J. Lewittes, J. R. Kuhlman, K. C. Klasing, and M. Wikelski (2006). Phytohemagglutinin-induced skin swelling in birds: Histological support for a classic immunoecological technique. Functional Ecology 20:290–299.

McDonald, P. G., P. D. Olsen, and A. Cockburn (2005). Sex allocation and nestling survival in a dimorphic raptor: Does size matter? Behavioral Ecology 16:922–930.

Merkling, T., S. Leclaire, E. Danchin, E. Lhuillier, R. H. Wagner, J. White, S. A. Hatch, and P. Blanchard (2012). Food availability and offspring sex in a monogamous seabird: Insights from an experimental approach. Behavioral Ecology 23:751–758.

Merkling, T., J. Welcker, A. J. M. Hewison, S. A. Hatch, A. S. Kitaysky, J. R. Speakman, E. Danchin, and P. Blanchard (2015). Identifying the selective pressures underlying offspring sex-ratio adjustments: A case study in a wild seabird. Behavioral Ecology 26:916–925.

Monaghan, P., R. G. Nager, and D. C. Houston (1998). The price of eggs: Increased investment in egg production reduces the offspring rearing capacity of parents. Proceedings of the Royal Society of London, Series B: Biological Sciences 265:1731–1735.

Müller, W., C. Dijkstra, and T. G. G. Groothuis (2003). Inter-sexual differences in T-cell-mediated immunity of Black-headed Gull chicks (*Larus ridibundus*) depend on the hatching order. Behavioral Ecology and Sociobiology 55:80–86.

Müller, M. S., Y. Roelofs, K. E. Erikstad, and T. G. Groothuis (2012). Maternal androgens increase sibling aggression, dominance, and competitive ability in the siblicidal black-legged kittiwake (*Rissa tridactyla*). PLoS One 7:e47763.

Myers, J. H. (1978). Sex ratio adjustment under food stress: Maximization of quality or numbers of offspring? The American Naturalist 112:381–388.

Nager, R. G., P. Monaghan, R. Griffiths, D. C. Houston, and R. Dawson (1999). Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Sciences of the United States of America 96:570–573.

Nager, R. G., P. Monaghan, R. Griffiths, D. C. Houston, and R. Dawson (2000b). Within-clutch trade-offs between the number and quality of eggs: Experimental manipulations in gulls. Ecology 81:1339–1350.

Nager, R. G., P. Monaghan, D. C. Houston, and M. Genovart (2000a). Parental condition, brood sex ratio and differential young survival: An experimental study in gulls (*Larus fuscus*). Behavioral Ecology and Sociobiology 48:452–457.

Nishiumi, I. (1998). Brood sex ratio is dependent on female mating status in polygynous Great Reed Warbler. Behavioral Ecology and Sociobiology 44:9–14.

Nolan, P. M., G. E. Hill, and A. M. Stoehr (1998). Sex, size, and plumage redness predict House Finch survival in an epidemic. Proceedings of the Royal Society of London, Series B: Biological Sciences 265:961–965.

Ostrowska, J. (1995). Ekologia żerowania mew na Wiśle w okresie lęgowym, ze szczególnym uwzględnieniem mewy pospolitej (*Larus canus*) (in Polish). MSc thesis, Uniwersytet Warszawski, Warszawa, Poland.

Patterson, J. E., and K. E. Ruckstuhl (2013). Parasite infection and host group size: A meta-analytical review. Parasitology 140:803–813.

Pierotti, R. (1981). Male and female parental roles in the Western Gull under different environmental conditions. The Auk 98:532–549.

Pike, T. W., and M. Petrie (2003). Potential mechanisms of avian sex manipulation. Biological Reviews of the Cambridge Philosophical Society 78:553–574.

R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Richards, S. A. (2008). Dealing with overdispersed count data in applied ecology. Journal of Applied Ecology 45:218–227.

Richards, S. A., M. J. Whittingham, and P. A. Stephens (2011). Model selection and model averaging in behavioural ecology: The utility of the IT-AIC framework. Behavioral Ecology and Sociobiology 65:77–89.

Riechert, J., O. Chastel, and P. H. Becker (2013). Mothers under stress? Hatching sex ratio in relation to maternal baseline corticosterone in the common tern (*Sterna hirundo*). Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology 199:799–805.

Różycki, A. Ł. (2014). Fenologia rozrodu i produkcja jaj mew: uwarunkowania i konsekwencje w warunkach środkowej Wisły (in Polish). PhD dissertation, Uniwersytet w Białymstoku, Białystok, Poland.

Rubolini, D., M. Romano, K. J. Navara, F. Karadas, R. Ambrosini, M. Caprioli, and N. Saino (2011). Maternal effects mediated by egg quality in the Yellow-legged Gull *Larus michahellis* in relation to laying order and embryo sex. Frontiers in Zoology 8:24.

Rutkowska, J., and A. V. Badyaev (2008). Meiotic drive and sex determination: Molecular and cytological mechanisms of sex ratio adjustment in birds. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1675–1686.

Saino, N., A. P. Møller, and A. M. Bolzern (1995). Testosterone effects on the immune system and parasite infestations in the Barn Swallow (*Hirundo rustica*): An experimental test of the immunocompetence hypothesis. Behavioral Ecology 6:397–404.

Schindler, S., J. M. Gaillard, A. Grüning, P. Neuhaus, L. W. Traill, S. Tuljapurkar, and T. Coulson (2015). Sex-specific demography and generalization of the Trivers-Willard theory. Nature 526:249–252.

Schwabl, H. (1993). Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Sciences of the United States of America 90:11446–11450.

Seutin, G., B. N. White, and P. T. Boag (1991). Preservation of avian blood and tissue samples for DNA analysis. Canadian Journal of Zoology 69:82–90.

Smits, J. E., G. R. Bortolotti, and J. L. Tella (1999). Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. Functional Ecology 13:567–572.

Southern, L. K. (1981). Sex-related differences in territorial aggression by Ring-billed Gulls. The Auk 98:117–129.

Spaans, A. L., M. Bukacińska, D. Bukaciński, and N. D. van Swelm (1994). The relationship between food supply, reproductive parameters and population dynamics in Dutch Lesser Black-backed Gulls *Larus fuscus*. IBN research report 94/9, IBN-DLO Wageningen, The Netherlands.

Spelt, A., and L. Pichegru (2016). Sex allocation and sex-specific parental investment in an endangered seabird. Ibis 159:272–284.

Stoffel, M. A., S. Nakagawa, and H. Schielzeth (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods in Ecology and Evolution 8:1639–1644.

Tagirov, M., and J. Rutkowska (2013). Chimeric embryos—potential mechanism of avian offspring sex manipulation. Behavioral Ecology 24:802–805.

Thyen, S., and P. H. Becker (2006). Effects of individual life-history traits and weather on reproductive output of Black-headed Gulls *Larus ridibundus* breeding in the Wadden Sea, 1991–1997. Bird Study 53:132–141.

Torres, R., and H. Drummond (1999). Does large size make daughters of the Blue-footed Booby more expensive than sons? Journal of Animal Ecology 68:1133–1141.

Townsend, H. M., T. J. Maness, and D. J. Anderson (2007). Offspring growth and parental care in sexually dimorphic Nazca Boobies (*Sula granti*). Canadian Journal of Zoology 85:686–694.

Trivers, R. L., and D. E. Willard (1973). Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92.

Tveraa, T., B.-E. Saether, R. Aanes, and K. E. Erikstad (1998). Regulation of food provisioning in the Antarctic Petrel: The importance of parental body condition and chick body mass. Journal of Animal Ecology 67:699–704.

Uller, T. (2006). Sex-specific sibling interactions and offspring fitness in vertebrates: Patterns and implications for maternal sex ratios. Biological Reviews of the Cambridge Philosophical Society 81:207–217.

Velando, A. (2002). Experimental manipulation of maternal effort produces differential effects in sons and daughters: Implications for adaptive sex ratios in the Blue-footed Booby. Behavioral Ecology 13:443–449.

Veller, C., D. Haig, and M. A. Nowak (2016). The Trivers–Willard hypothesis: Sex ratio or investment? Proceedings of the Royal Society of London, Series B: Biological Sciences 283:20160126.

Verboven, N., P. Monaghan, D. M. Evans, H. Schwabl, N. Evans, C. Whitelaw, and R. G. Nager (2003). Maternal condition, yolk androgens and offspring performance: A supplemental feeding experiment in the lesser black-backed gull (*Larus fuscus*). Proceedings. Biological Sciences 270:2223–2232.

Weimerskirch, H., C. Barbaud, and P. Lys (2000). Sex differences in parental investment and chick growth in Wandering Albatrosses: Fitness consequences. Ecology 81:309–318.

West, S. A. (2009). Sex Allocation. Princeton University Press, Princeton, NJ, USA.

Wiśniewska, M. (2014). Wpływ wielkości i kondycji matek na inwestycje w lęg u mew na środkowej Wiśle (in Polish). MSc thesis, Uniwersytet Kardynała Stefana Wyszyńskiego, Warszawa, Poland.