

The relationship between nest-site characteristics, parental investment, brood sex ratio, and sex-specific growth in the little egret

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Abstract

Background

Sex allocation theory predicts that parents may adjust offspring sex ratios in response to environmental conditions and the expected fitness consequences of producing male or female offspring. However, empirical evidence for facultative sex-ratio adjustment in wild birds remains inconsistent. We investigated whether nest-site characteristics and brood size were associated with brood sex ratio and whether brood sex composition was related to nestling body mass in the little egret (*Egretta garzetta*).

Methods

The study was conducted in a single little egret breeding colony in the Caspian coastal wetlands of northern Iran during the 2023 breeding season. Nest-site characteristics were used as proxies for local habitat conditions, while brood size was considered an indicator of parental investment. Nestlings aged 7 days were molecularly sexed, and analyses were restricted to complete broods in which all chicks successfully hatched. Generalized linear models were used to evaluate associations between brood sex ratio and nest-site and reproductive variables, with model selection based on Akaike's information criterion (AIC).

Results

None of the measured nest-site characteristics or reproductive variables showed a significant association with brood sex ratio. Similarly, brood sex composition was not significantly associated with nestling body mass. The results therefore provided no evidence of facultative adjustment of brood sex ratio in relation to the measured environmental or reproductive variables within the study colony and breeding season.

Conclusions

Our findings suggest that, under the conditions examined, brood sex ratio in little egrets may be relatively insensitive to the measured variation in nest-site characteristics and brood size. However, because the study was conducted at a single colony during one breeding season, these results should be regarded as exploratory. Multi-colony and multi-year studies incorporating broader measures of habitat quality, parental condition, food availability, and subsequent offspring survival are needed to determine the generality of sex-allocation patterns in this species.

Background

In avian evolutionary biology, the brood sex ratio is defined as the proportion of male offspring relative to the total number produced within a single reproductive attempt. This concept is traditionally rooted in the seminal work of Fisher (1930), who postulated that natural selection should favor equal investment in both sexes, leading to a population-wide sex ratio of 1:1. Fisher's reasoning was based on the premise that if one sex were less costly to produce, parents would shift their investment toward that sex; this would eventually diminish its fitness value until equilibrium is restored [1, 2]. However, in many bird species, the brood sex ratio is frequently unequal, suggesting that the direction and magnitude of these skews represent adaptive parental decisions rather than random variation [3]. As the heterogametic sex (ZW) in birds, females possess a unique physiological potential for maternal control over the brood sex ratio, facilitating adjustments either before ovulation or during post-hatching periods [4, 5]. Consequently, sex allocation is understood as a strategic process, greater investment in one sex over the other to maximize long-term reproductive success [2, 6].

The determination of the brood sex ratio in birds is influenced by a complex interplay of environmental, genetic, and physiological factors. Building on Fisher's fundamental reasoning, the sex allocation hypothesis—most notably the Trivers-Willard model (1973)—suggests that females should bias their primary or secondary brood sex ratio toward the sex with the greatest potential for fitness returns under specific local conditions [7, 8]. One of the key factors influencing sex allocation is habitat characteristics. In this context, structural nest-site variables are frequently used as proxies [9, 10]. In colonial species, specific structural attributes—such as nest height or placement—reflect the degree of protection from terrestrial predators and environmental stressors, thereby serving as indicators of the safety available to the breeding pair [11, 12]. For instance, the attractive mate hypothesis posits that females mating with high-quality males in superior territories should allocate more resources to sons to ensure they inherit those advantageous traits [13, 14]. Nest-site selection further influences this dynamic by determining the level of protection and microclimatic stability, which directly impacts the resources available for provisioning [9]. Accordingly, nest-site characteristics and breeding timing correlate with sex allocation biases, as parents in superior sites are better equipped to support the growth of larger, more demanding male nestlings [15].

In addition to habitat quality, brood size serves as a critical proxy for parental investment, as larger broods often signal the capacity of high-quality parents to support the more energetically demanding sex. For example, in mew gulls (*Larus canus*), parents in superior condition who are capable of maintaining larger broods tend to overproduce larger, more costly male offspring [15]. Conversely, in species such as the European shag (*Phalacrocorax aristotelis*), restricted investment in specific breeding contexts can lead to female-biased ratios to ensure offspring survival [16]. These adjustments optimize parental fitness by aligning offspring sex with available energy reserves and habitat quality [9].

In birds, however, it is essential to distinguish between this initial sex allocation and subsequent sex-specific survival or growth. Distinct from the allocation phase, the post-hatching period introduces sex-specific growth dynamics. Larger broods often intensify sibling competition, where the more physically robust sex may outcompete smaller siblings; this leads to sex-biased mortality if parental condition is

insufficient [17]. In aquatic species with sexual size dimorphism, such as many gulls and herons, males are often larger and grow faster, making them more vulnerable to starvation during periods of food scarcity [18, 19]. Consequently, while the initial allocation may be biased by habitat quality, the final brood composition is frequently a result of sex-specific survival rates [20].

The study of these relationships is particularly significant in colonial species like the little egret (*Egretta garzetta*). As colonial breeders, little egrets are highly sensitive to the ecological health of their foraging and nesting habitats, making them useful indicators for assessing the environmental status of aquatic regions [21]. Despite the global importance of the Ardeidae family, no prior studies have evaluated the factors determining the brood sex ratio in this specific region. The present research provides an exploratory analysis of these dynamics within a single colony during one breeding season in the Caspian coastal wetlands—a critical site for numerous migratory species. Given the exploratory nature of this study, the findings serve as a preliminary benchmark for understanding sex allocation and growth patterns in this geographic context.

Based on the theoretical frameworks of adaptive sex allocation and the specific ecological constraints of the study colony, several research hypotheses are proposed. Regarding sex allocation, we first hypothesize that the brood sex ratio will be skewed toward males in nests with superior structural parameters (proxies for high-quality nest-sites), as these conditions allow parents to meet the higher energetic costs of male offspring. Second, we predict that initial sex allocation in nests with larger brood sizes will be skewed toward males, reflecting a higher investment strategy by high-quality parents. Regarding sex-specific growth, our third hypothesis posits that nestlings with higher body mass will more likely be male, as robust physiological condition is a prerequisite for the successful development of the larger sex in dimorphic colonial birds. These predictions align with the Trivers-Willard model, suggesting that when local conditions are favorable, parental investment and subsequent offspring success will shift toward the sex that benefit most from additional resources [22].

Methods

Study area

The investigation took place at the Zaghmarz Forest and Rangeland Research Station, located in North Iran. Positioned approximately one kilometer from the Caspian Sea's southern coastline, the site lies at 36°50'35" N and 53°18'15" E within Mazandaran Province, at an elevation of 20 meters below sea level. This station, bordered to the west by the Lapoo-Zaghmarz and Miyankaleh international wetlands, has featured 3×3 meter grid plantings of *Pinus brutia*, *Pinus longifolia*, and *Pinus pinea* since 1997 [23]. The protected status and enclosure of the area, combined with its wetland proximity, establish it as a secure nesting environment for Ardeidae species.

Selection and monitoring of nests and broods

Fieldwork spanned from April 1 to July 31, 2023, during which we monitored a single little egret colony nesting in trees. After an initial survey of the entire colony, we selected and numbered a random subset of nests for observation. To ensure independent sampling, we monitored one randomly chosen nest per tree [24]. Physical inspections occurred every 3–5 days throughout the incubation and chick-rearing periods. We conducted these visits only during favorable weather to avoid biasing results and to protect the birds from predation or early fledging. To minimize researcher impact, we limited nest fate determinations to a maximum of three visits per attempt, prioritizing distant observation during the sensitive incubation stage. To obtain an accurate assessment of primary sex allocation, we analyzed only those nests where every egg successfully hatched. This approach was designed to eliminate the potential for sex-specific embryo mortality to skew the secondary sex ratio [25]. By excluding clutches with unhatched eggs, we avoided the interpretative errors associated with unsexed individuals [26]. While this focused our study on fully successful broods, it provided a definitive census of hatched offspring for each unit, which might otherwise be obscured by differential survival during the early stages. Brood size was defined as the count of surviving nestlings present [27].

Evaluation of nest-site characteristics and chick body mass

We recorded several habitat quality parameters for each study nest, including diameter, height above ground, distance to the nearest neighbor, and the total number of nests on the supporting tree. We also measured the trunk circumference at breast height (CBH) and noted the nest's location within the tree (main trunk vs. proximal or distal branches), as well as the host tree species, primarily *P. longifolia* and *P. pinea* [28, 29]. Morphometric data, specifically body mass, were collected for each chick on 7 days after hatching. To account for the statistical non-independence of nestmates, we calculated the mean body mass for each brood and used these nest-level averages as the primary independent variables in our analyses [30]. Sex ratio data were derived from blood samples taken during the morphometric assessment. Thus, our findings reflect the sex distribution among surviving chicks at the time of sampling. To prevent stress and abandonment, trained handlers processed each chick for less than five minutes before returning them to the nest.

Collection of blood samples

Following morphometric measurement, we collected blood samples using a rapid, minimally invasive protocol (under 5 minutes). We used a 26–30 gauge needle to puncture the brachial vein beneath the wing. Approximately 10 microliters of blood (representing $\leq 1\%$ of body weight) were gathered via capillary tubes and preserved in microtubes containing 96% ethanol. Samples were refrigerated until DNA extraction and sexing procedures were initiated [31–33].

DNA isolation procedures

We utilized the salt-based method for DNA isolation [34]. First, the ethanol used for preservation was removed, and 10–15 microliters of the remaining blood-ethanol mixture from the bottom of the tube were transferred to a fresh microtube. This sample was heated at 55°C to evaporate residual ethanol, which could otherwise inhibit extraction. Digestion was achieved by adding 30 µl of Proteinase K, 410 µl of extraction buffer (Tris-HCl, NaCl, EDTA, and deionized water), and 80 µl of 10% SDS. This mixture was incubated in a water bath at 37–55°C for 12–24 hours. After digestion, the samples were vortexed for 30 seconds and centrifuged at 13,000 rpm for 5 minutes. The resulting supernatant was mixed with 180 µl of NaCl solution, vortexed again, and centrifuged. We then transferred the supernatant to a sterile tube and added 420 µl of chilled (-21°C) isopropanol to precipitate the DNA. After gentle inversion and centrifugation, the supernatant was discarded, and the DNA pellet was washed twice with 80% ethanol. Following a final vacuum-drying step to remove all alcohol, the DNA was dissolved in 20–50 µl of ddH₂O at 37°C and stored at -20°C [34].

PCR amplification and molecular sexing

Sex was determined using polymerase chain reaction (PCR) amplification of DNA segments followed by agarose gel electrophoresis. We employed P2 and P8 primers [35] to distinguish sexes. The PCR protocol involved initial denaturation at 94°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds (denaturation), 52°C for 45 seconds (annealing), and 72°C for 45 seconds (extension). A final extension was performed at 72°C for 5 minutes, with a hold at 12°C. Products were visualized on a 3% agarose gel under UV light after staining. Males were identified by a single DNA band, whereas females exhibited two bands [36, 37]. The brood sex ratio was defined as the proportion of males relative to the total number of chicks. Any ambiguous results were re-tested. To validate the accuracy, we performed repeat PCRs on a random 10% subset ($n = 10$), which consistently confirmed the initial sex assignments [38].

Statistical analyses

The relationship between predictive factors and the little egret brood sex ratio was evaluated using generalized linear models (GLMs) with binomial error distributions. The response variable was specified using the $cb \in d$ function (number of males, number of females) within the *glm* function. Predictive variables included nest diameter, distance to the nearest nest, nest height above ground level, number of nests on the host tree, circumference of the trunk at breast height (CBH) of the host tree, nest position on the host tree (main trunk, proximal branches, and distal branches), and nesting host tree type (specifically *P. longifolia* and *P. pinea*), brood size, and mean nestling mass. We employed automated stepwise selection via the *step* function to identify the most parsimonious model, using the Akaike Information Criterion (AIC) as the selection metric (Table 1). The best-fit model was designated as the one with the lowest AIC value (*AIC* function). We performed model diagnostics to verify the suitability of the binomial distribution and to check for overdispersion. Model fit was assessed using McFadden's Pseudo-R²; although values between 0.2 and 0.4 typically indicate high explanatory power, our values remained low across all models (Table 1). Because the stepwise procedure resulted in a null model

(intercept only) as the most supported fit, we also reported the full model statistics for comparative interpretation. Results were interpreted using predicted least-squares means $\pm$ standard errors (calculated via the *emmeans* or *lsmeans* functions) and visualized with relevance plots featuring shaded error bands generated by the *polygon* function. All computations were executed in R (version 4.4.2) using the *stats* package [39].

Table 1

Results of the model selection—including the full, intermediate, and null generalized linear models (binomial distribution)—examining the relationship between nesting site parameters, brood size, and nestling body mass with the brood sex ratio of little egret. AIC: Akaike Information Criterion; Δ AIC: difference in AIC relative to the best model; K: number of parameters; McFadden's Pseudo- R^2 : goodness-of-fit measure for the selected models.

Model	Formula (predictors)	K	AIC	Delta AIC	McFadden's Pseudo- R^2
Full Model	Number of nests per nest tree + Circumference of the trunk at breast height + Nest diameter + Nest height above the ground + Nearest neighbor nest distance + Nesting host tree type + Nest position + Brood size + Nestlings' body mass	10	73.11	17.33	0
Intermediate	Number of nests per nest tree + Circumference of the trunk at breast height + Nest height above the ground + Nearest neighbor nest distance + Nesting host tree type + Nest position + Brood size + Nestlings' body mass	9	69.17	13.39	0.049
	Number of nests per nest tree + Circumference of the trunk at breast height + Nest height above the ground + Nearest neighbor nest distance + Nesting host tree type + Brood size + Nestlings' body mass	8	67.2	11.42	0.048
	Number of nests per nest tree + Circumference of the trunk at breast height + Nearest neighbor nest distance + Nesting host tree type + Brood size + Nestlings' body mass	7	65.23	9.45	0.047
	Number of nests per nest tree + Circumference of the trunk at breast height + Nearest neighbor nest distance + Nesting host tree type + Nestlings' body mass	6	63.35	7.58	0.045
	Number of nests per nest tree + Nearest neighbor nest distance + Nesting host tree type + Nestlings' body mass	5	61.46	5.68	0.043
	Nearest neighbor nest distance + Nesting host tree type + Nestlings' body mass	4	59.49	3.71	0.043

Model	Formula (predictors)	K	AIC	Delta AIC	McFadden's Pseudo-R ²
	Nearest neighbor nest distance + Nestlings' body mass	3	58.05	2.27	0.032
	Nestlings' body mass	2	56.62	0.84	0.022
Null Model	-	1	55.78	0	0.050

Results

The mean and standard deviation of the measured parameters for nest-site selection, brood size, nestling body condition, and brood sex ratio of little egret are presented in Table 2.

Table 2
Descriptive statistics of nesting site parameters, brood size, nestling body mass, and brood sex ratio of little egret.

Factors	Parameters	Mean ± SD
Nesting site (n = 30)	Nest diameter (cm)	34 ± 6.65
	Nearest neighbor nest distance (cm)	64.57 ± 23.54
	Nest height above the ground (m)	7.69 ± 0.89
	Number of nests per tree	11.83 ± 6.96
	Circumference of the trunk at breast height (cm)	74.87 ± 10.48
Brood (n = 81)	Brood size	2.77 ± 0.73
	Nestling body mass (kg)	0.21 ± 0.08
Brood sex ratio (n = 81)	Number of nestling males	2 ± 2.05
	Number of nestling females	0.79 ± 1.81
	Proportion of males (number of males / brood size)	0.75 ± 0.19

The results of the initial model examining the relationships between nest parameters—including nest diameter, distance to the nearest nest, nest height above ground level, number of nests on the host tree, circumference of the trunk at breast height (CBH) of the host tree, nest position on the host tree (main trunk, proximal branches, and distal branches), and nesting host tree type—as well as reproductive parameters (brood size and nestling body mass) and the brood sex ratio of little egrets are summarized in Table 3. None of the aforementioned parameters showed a significant relationship with the brood sex ratio of little egrets (Table 3. Figure 1a-i).

Table 3

Full generalized linear models with binomial distribution examining the relationships of nesting site parameters, brood size, and nestling body condition with the brood sex ratio of little egret.

Intercept	Estimate	Standard error	Z-value	P-value
	1.168	4.034	0.290	0.772
Nest diameter	0.003	0.045	0.063	0.950
Nearest neighbor nest distance	0.010	0.014	0.734	0.463
Nest height above the ground	-0.058	0.378	-0.152	0.879
Number of nests per nest tree	0.017	0.071	0.247	0.805
Circumference of the trunk at breast height	-0.013	0.034	-0.386	0.700
Nest position, Distal trunk	0.160	0.818	0.195	0.845
Nest position, Main trunk	-0.065	0.772	-0.084	0.933
Nesting host tree type, <i>Pinus pinea</i>	-0.273	0.617	-0.443	0.658
Brood size	-0.087	0.394	-0.221	0.825
Nestlings' body mass	3.234	4.436	0.729	0.466

Discussion

The results of this exploratory study indicate a compelling lack of relationship between a wide range of nest-site characteristics, body mass, and brood size parameters and the brood sex ratio of little egrets in the studied colony during a single breeding season. This finding is significant in the field of evolutionary biology, as it challenges several classical models of sex allocation and suggests that, within this specific ecological context, the brood sex ratio is maintained at a stable 1:1 equilibrium, unaffected by the immediate socio-ecological or physical environment. These findings are largely consistent with other studies in the Ardeidae family, suggesting that a lack of sex-ratio bias may be a broader trend within this group under certain conditions.

Sex allocation hypothesis: nest-site characteristics and parental investment

To interpret the lack of biased sex allocation, one must first consider Fisher's Principle, which posits that natural selection favors a 1:1 sex ratio when the costs of producing and rearing males and females are equal [1, 2]. In many birds within the order Pelecaniformes, sexual dimorphism at the nestling stage is minimal. Although adult little egrets exhibit subtle size differences, if the energetic investment required to raise a male chick does not significantly exceed that for a female, there is no evolutionary pressure for parents to bias the primary sex ratio. The absence of a relationship between brood size—a common

proxy for total parental investment—and sex ratio supports this interpretation [15]. This mirrors results found in the black-crowned night heron (*Nycticorax nycticorax*), where research similarly showed that brood size had no significant impact on the brood sex ratio [40].

The reliance on structural nest-site variables as proxies for nest-site quality is a common approach in colonial waterbird research, based on the assumption that parameters like nest height and position reflect protection from terrestrial predators and microclimatic stability [11, 41]. While these assumptions require further validation within the Caspian wetland system, they provide an exploratory framework for testing the Trivers-Willard hypothesis (1973). This hypothesis suggests that parents in superior condition or possessing better resources (proxied here by superior nest placement) should bias investment toward the sex that benefits more from such an advantage.

However, the lack of relationship between nest position and brood sex ratio suggests that in the little egret, the benefits of producing high-quality males may not outweigh the costs of sex-ratio manipulation. Indeed, in the great cormorant (*Phalacrocorax carbo*), nest-site location did not result in skewed ratios despite influencing overall success [42], and research on the black-crowned night heron also shows that nest height has no significant impact on sex allocation [40]. The little egret's status as a generalist forager in unpredictable wetland environments likely favors a conservative 1:1 strategy, perhaps due to the unpredictable nature of foraging environments where water levels and resource availability fluctuate daily. Committing to a biased ratio at the egg-laying stage would be risky if environmental conditions deteriorate before fledging [22].

Furthermore, parameters linked to Local Resource Competition (LRC) or Local Resource Enhancement (LRE), such as nest density and distance to the nearest neighbor, showed no effect [43]. This suggests that dispersal patterns and competitive threats from offspring in this colony are likely balanced between the sexes, removing the incentive for density-dependent sex-ratio adjustments.

Sex-specific survival and growth after hatching

Distinct from the initial allocation, the results also suggest a lack of sex-specific growth or survival dynamics in the studied population. In species where one sex is significantly larger and more expensive to produce, a relationship between brood condition and sex ratio is common due to sex-biased mortality [18]. The null relationship with nestling body mass implies that little egrets in this colony do not experience the significant sex-specific growth patterns or competitive hierarchies that would necessitate a skewed secondary sex ratio.

However, deviations in other species highlight the role of resource predictability and dimorphism. For example, in the Eurasian spoonbill (*Platalea leucorodia*), biases are often linked to hatching sequence and maternal condition [44]. This contrasts with the little egret but aligns with species like the grey heron (*Ardea cinerea*), where fierce sibling competition often leads to biased outcomes [19]. By maintaining a 1:1 ratio across varying conditions, little egrets may minimize the risk of total brood failure that could occur if multiple high-cost chicks were competing in a single nest under resource scarcity.

Conclusions and applications

This study concludes that the brood sex ratio of little egrets in the studied colony is not significantly influenced by nesting and reproductive parameters, suggesting a stable 1:1 equilibrium. Given that these results are based on an exploratory analysis of a single colony over one breeding season, they support the conclusion that little egrets do not exhibit facultative sex-ratio adjustment or sex-specific growth biases under these specific conditions. Theoretically, this indicates that the costs and benefits of producing male versus female offspring remain balanced, and reproductive strategies may be more heavily governed by mechanisms like asynchronous hatching rather than sex-specific allocation.

From a practical perspective, these preliminary findings suggest that conservationists can simplify recruitment models by assuming a constant sex ratio across varying nest-site characteristics. Management efforts should focus on broader ecological priorities, such as preserving high-quality foraging habitats like wetlands and rice paddies, which drive overall juvenile survival. While structural nest-site characteristics did not drive sex skews in this instance, future research should expand the scope to multiple colonies and seasons to determine the broader applicability of these findings and explore alternative drivers such as maternal endocrine-mediated influences.

Declarations

Ethics approval and consent to participate

All experimental protocols and procedures involving birds in this study were reviewed and approved by the Institutional Animal Ethics Committee of Sari Agricultural Sciences and Natural Resources University, Iran (Approval No. SMANS, 2023), and were conducted in accordance with the approved protocol and relevant institutional and national guidelines and regulations governing the ethical treatment and use of animals in research.

Consent for publication

This manuscript does not contain any person's data, and further consent for publication is not required/applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

Negin Soleimani: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Conceptualization. Seyed Mehdi Amininasab: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Zarbakht Ansari Pirsarai: Visualization, Methodology, Investigation, Supervision, Funding acquisition, Data curation. Seyedeh Farahnaz Vesali: Project administration, Methodology, Investigation, Conceptualization.

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Data Availability

Data will be made available on request.

References

1. Fisher RA. The Genetical Theory of Natural Selection. Clarendon; 1930.
2. Charnov EL. The theory of sex allocation. Princeton: Princeton University Press; 1982.
3. Szász E, Kiss D, Rosivall B. Sex ratio adjustment in birds. *Ornis Hung*. 2012;20:26–36.
4. Kilner R. Primary and secondary sex ratio manipulation by zebra finches. *Anim Behav*. 1998;56:155–64.
5. Rutkowska J, Badyaev AV. Meiotic drive and sex determination: molecular and cytological mechanisms of sex ratio adjustment in birds. *Philos Trans R Soc Lond B Biol Sci*. 2008;363(1497):1675–86.
6. West S. Sex allocation. Princeton (NJ): Princeton University Press; 2009.
7. Hamilton WD. Extraordinary sex ratios. *Science*. 1967;156:477–88.
8. Trivers RL, Willard DE. Natural selection of parental ability to vary the sex ratio of offspring. *Science*. 1973;179(4068):90–2.
9. Jaatinen K, Öst M, Gienapp P, Merilä J. Facultative sex allocation and sex-specific offspring survival in Barrow's Goldeneyes. *Ethology*. 2013;119(2):146–55.
10. Minias P, Wojczulanis-Jakubas K, Kaczmarek K. Offspring sex ratio varies according to nest location within a colony of Great Cormorants. *Auk*. 2014;131:388–95.

11. Suorsa P, Helle H, Huhta E, Jäntti A, Nikula A, Hakkarainen H. Forest fragmentation is associated with primary brood sex-ratio in the treecreeper (*Certhia familiaris*). *Proc R Soc Lond*. 2003;270:2215–22.
12. Bell S, Owens I, Lord A. Quality of breeding territory mediates the influence of paternal quality on sex ratio bias in a free-living bird population. *Behav Ecol*. 2013;25(2):352–8.
13. Burley N. Sex ratio manipulation and selection for attractiveness. *Science*. 1981;221(4483):721–2.
14. Yamaguchi N, Kawano KK, Eguchi K, Yahara T. Facultative sex-ratio adjustment in response to male tarsus length in the varied tit *Parus varius*. *Ibis*. 2004;146:108–13.
15. Bukaciński D, Bukacińska M, Chylarecki P. Manipulating parental condition affects brood sex ratio, immunocompetence, and early chick mortality in two gull species differing in sexual size dimorphism. *Ornithology*. 2021;138(2):ukab007.
16. Velando A, Graves J, Ortega-Ruano J. Sex ratio in relation to timing of breeding, and laying sequence in a dimorphic seabird. *Ibis*. 2002;144:9–16.
17. Dijkstra C, Daan S, Pen I. Fledgling sex ratios in relation to brood size in size-dimorphic altricial birds. *Behav Ecol*. 1999;9(3):287–96.
18. Nager RG, Monaghan P, Houston DC. The cost of egg production: Increased egg production reduces future fitness in gulls. *J Avian Biol*. 2001;32:159–66.
19. Alonso-Alvarez C, Velando A. Female body condition and brood sex ratio in Yellow-legged Gulls *Larus cachinnans*. *Ibis*. 2003;145(2):220–6.
20. Kilgour DAV, Linkous CR, Pierson TW, Guindre-Parker S. Sex ratios and the city: Secondary offspring sex ratios, parental corticosterone, and parental body condition in an urban-adapted bird. *Front Ecol Evol*. 2022;10:894583.
21. Rubio I, Martinez-Madrid M, Méndez-Fernández L, Galarza A, Rodriguez P. Heavy metal concentration in feathers of Little Egret (*Egretta garzetta*) nestlings in three coastal breeding colonies in Spain. *Ecotoxicology*. 2016;25:30–40.
22. McGowan A, Sharp S, Simeoni M, Hatchwell B. Competing for position in the communal roosts of Long-tailed Tits. *Anim Behav*. 2006;72:1035–43.
23. Khorankeh S, Sardabi H, Espahbodi K. Investigation on growth and performance of three exotic softwood species on eastern Caspian Sea littoral of Iran (case study: Zaghemarz Experimental Station, Mazandaran). *IJFPR*. 2014;21:542–56.
24. Carrasco L, Mashiko M, Toquenaga Y. Application of random forest algorithm for studying habitat selection of colonial herons and egrets in human-influenced landscapes. *Ecol Res*. 2014;9(3):483–91.
25. Benito MM, Gonzalez-Solis J. Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds. *J Evol Biol*. 2007;20:1522–30.
26. Dyrce A, Sauer-Gürth H, Tkadlec E, Wink M. Offspring sex ratio variation in relation to brood size and mortality in a promiscuous species: The Aquatic Warbler *Acrocephalus paludicola*. *Ibis*. 2004;146:269–80.

27. Xirouchakis SM, Botsidou P, Baxevas K, Andreou G, Tsaparis D. Brood sex ratio variation in a colonial raptor, the Eleonora's falcon, *Falco eleonora*. Anim Behav. 2023;195:93–106.
28. Bennetts RE, Fasola M, Hafner H, Kayser Y. Influence of environmental and density-dependent factors on reproduction of Little Egrets. Auk. 2000;117(3):634–9.
29. Mohammedi A, Kouidri M, Mahmoudi A, Ababou A. Ecological and biological behavior of nesting colony of *Bubulcus ibis* in North-Western Algeria. Leban Sci J. 2020;21(1):001–11.
30. Luny E. The interactive effects of incubation temperature and organic contaminants on shorebird embryo development. 2019; Doctoral dissertation. University of Saskatchewan.
31. Poláková R, Schnitzer J, Vinkler M, Bryja J, Munclinger P, Albrecht T. Effect of extra-pair paternity and parental quality on brood sex ratio in the scarlet rosefinch *Carpodacus erythrinus*. Folia Zool. 2012;61(3–4):225–32.
32. Ágh N, Pipoly I, Szabó K, Vincze E, Bókony V, Seress G, Liker A. Does offspring sex ratio differ between urban and forest populations of great tits (*Parus major*)? Biol Futura. 2020;71(1–2):99–108.
33. Rubalcaba JG, Polo V. Density dependence of clutch size and offspring sex ratio in starling colonies. J Avian Biol. 2022; e02993.
34. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 1997;25(22):4692–3.
35. Griffiths R, Double MC, Orr K, Dawson RJ. A DNA test to sex most birds. Mol Ecol. 1998;7(8):1071–5.
36. Whittingham LA, Dunn PO, Nooke JK. Maternal influences on brood sex ratios: an experimental study in tree swallows. Proc Biol Sci. 2005;272(1574):1775–80.
37. Que P, Székely T, Wang P, Lu Q, Lei W, Liu Y, Zhang Z. Offspring sex ratio is unrelated to parental quality and time of breeding in a multiple-breeding shorebird. J Ornithol. 2019;160:443–52.
38. Fridolfsson AK, Ellegren H. A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol. 1999;30(1):116–21.
39. R development core team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2024; Accessed July 14, 2024. <https://www.r-project.org/>.
40. Vesali SF, Varasteh Moradi H, Amininasab SM, Pirsaraei ZA. The Relationship of Nest-Site Selection Parameters, Timing of Breeding, Brood Size, and Nestling Body Condition With Brood Sex Ratio in the Black-Crowned Night Heron *Nycticorax nycticorax*. Ecol Evol. 2026;16(6):e73737.
41. Lessells CM, Oddie KR, Mateman AC. Parental behaviour is unrelated to experimentally manipulated great tit brood sex ratio. Anim Behav. 1998;56:385–93.
42. Janiszewski T, Minias P, Lesner B, Kaczmarek K. Age effects on reproductive success, nest-site location, and offspring condition in the Great Cormorant *Phalacrocorax carbo sinensis*. J Ornithol. 2017;158(1):193–202.
43. Clark AB. Sex ratio and local resource competition in a prosimian primate. Science. 1978;201(4351):163–5.

Figures

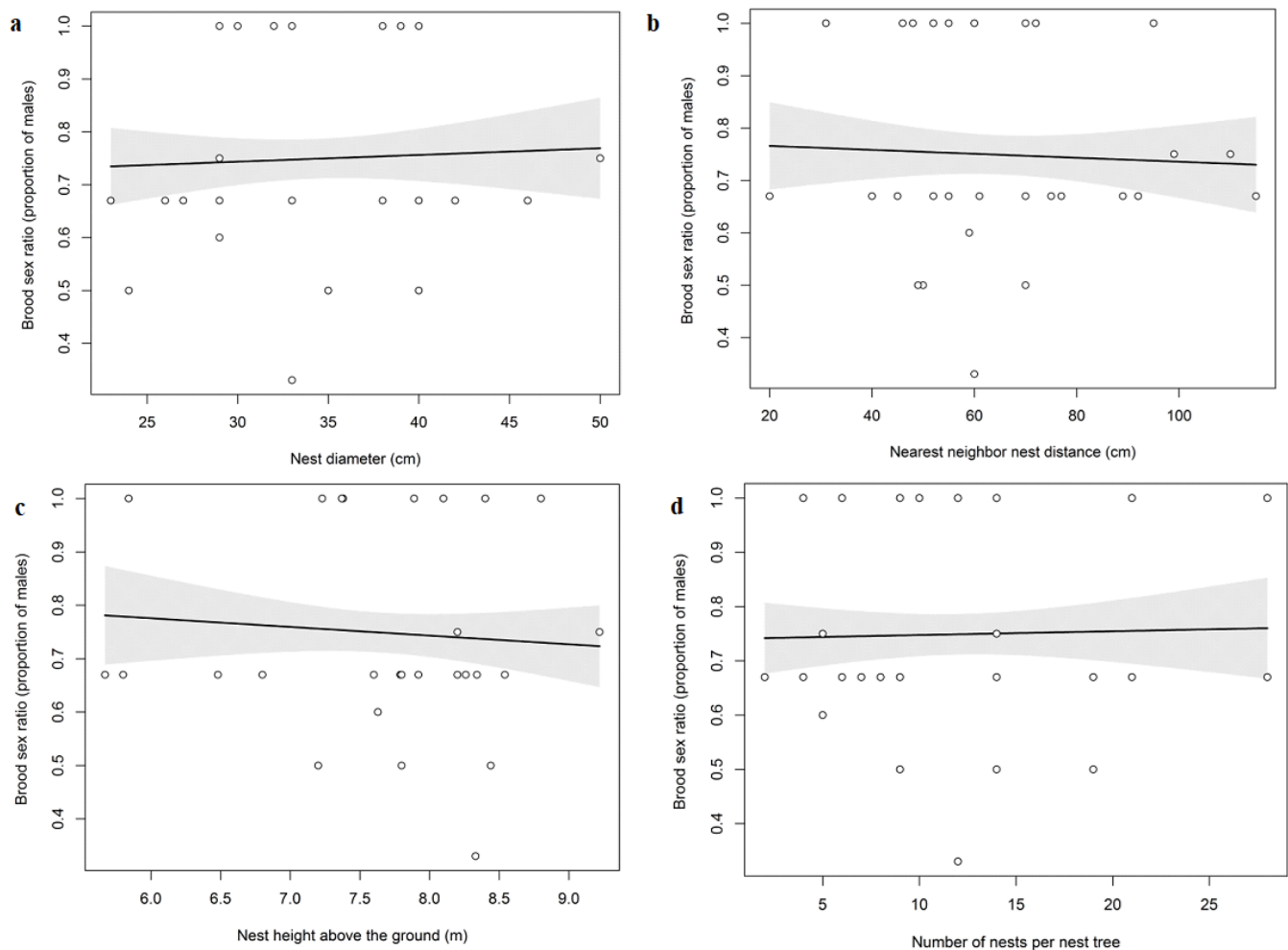


Figure 1

Fig. 1 Predicted relationships between a) Nest diameter, b) Nearest neighbor nest distance, c) Nest height above the ground, d) Number of nests per nest tree, e) Circumference of the trunk at breast height, f) Nest position, g) Nesting host tree type, h) Brood size, and i) Nestlings' body mass with the brood sex ratio of little egret. Lines represent least-squares means, and gray shaded bands indicate the standard error (SE).

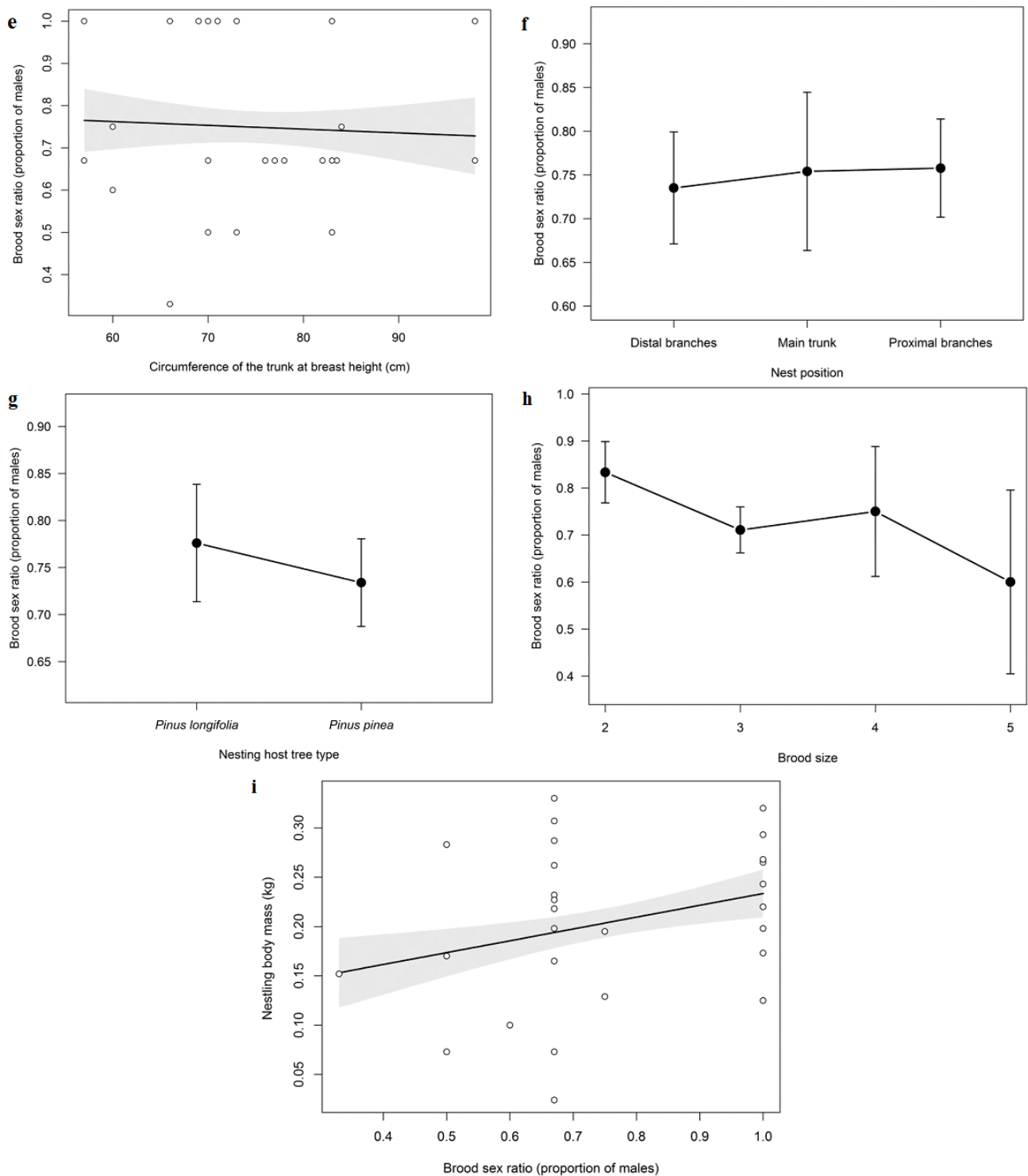


Figure 2

Fig. 1 (continued) Predicted relationships between a) Nest diameter, b) Nearest neighbor nest distance, c) Nest height above the ground, d) Number of nests per nest tree, e) Circumference of the trunk at breast height, f) Nest position, g) Nesting host tree type, h) Brood size, and i) Nestlings' body mass with the brood sex ratio of little egret. Lines represent least-squares means, and gray shaded bands indicate the standard error (SE).

