RESEARCH ARTICLE

Field observations can accurately detect interspecific brood parasitism in *Aythya valisineria* (Canvasback) nests

Michael K. Johnson,[1]* Caitlin P. Wells,[1] Philip Lavretsky,[2] Vergie M. Musni,[2] Christopher A. Nicolai,[3] Frank C. Rohwer,[3] and David N. Koons[1]

[1] Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, Colorado, USA
[2] Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas, USA
[3] Delta Waterfowl Foundation, Bismarck, North Dakota, USA
* Corresponding author: mk.johnson@colostate.edu

ABSTRACT

Validating the accuracy of protocols and field observations is important to maintain consistent data collection and inference amidst changing observers or study sites. To aid inferences on interspecific brood parasitism, we validated field observations of egg identification to the species level against mitochondrial DNA sequencing for nests where interspecific brood parasitism was suspected. Field observations of parasitic *Aythya americana* (Redhead), and host *Aythya valisineria* (Canvasback) eggs proved highly accurate (Aame_ID = 0.99 ± 0.05; Aval_ID = 0.99 ± 0.04) even among observers with different levels of experience ($Obs_{1yr} = 0.99 \pm 0.03$; $Obs_{3yr} = 0.97 \pm 0.02$). Hatched eggshell membranes contained the best tissue samples for successful sequencing compared to eggshell membranes earlier in development ($Pr_{seq\_succ} = 0.84 \pm 0.08$ for 0–9 days incubation, 0.63 ± 0.12 for 10–24 days incubation, and 0.88 ± 0.04 for hatched membranes), while DNA concentration following extraction was not an important predictor of sequencing success. Despite obtaining sufficient DNA concentrations, we observed 15% failure rate among samples, with 69% and 31% of the 29 failed samples being due to either a lack of quality DNA or non-target sequencing, respectively, suggesting failure was likely due to bacterial growth from exposure after eggs were abandoned or preyed upon. Our investigation showcases the utility of non-invasive molecular techniques to validate field protocols and rejects egg identification as a major source of uncertainty when trying to account for interspecific brood parasitism in parasitized *A. valisineria* nests.

Keywords: DNA sequencing, egg tissues, eggshell membrane, field methodology, non-invasive sampling, Redhead Duck

How to Cite

Johnson, M. K., C. P. Wells, P. Lavretsky, V. M. Musni, C. A. Nicolai, F. C. Rohwer, and D. N. Koons (2026). Field observations can accurately detect interspecific brood parasitism in *Aythya valisineria* (Canvasback) nests. Ornithological Applications 128:duaf000.

LAY SUMMARY

- Some female ducks lay their eggs in the nests of other species, a behavior called interspecific brood parasitism.
- Parasitic eggs can often be identified by physical characteristics, like color or texture, but the validity of this methodology is uncertain.
- We used DNA from *Aythya americana* (Redhead) and *Aythya valisineria* (Canvasback) duck egg membranes and found that field observations correctly identified eggs to the species level in parasitized canvasback nests 97% of the time.
- Intermittent validation tests are important to maintain consistency among studies that vary across space and time.

Le parasitisme de couvée interspécifique peut être détecté avec fiabilité par les observations de terrain dans les nids d'*Aythya valisineria* (Fuligule à dos blanc).

RÉSUMÉ

Pour garantir la cohérence de la collecte et de l'interprétation des données malgré la variation des observateurs ou des sites d'études, il est important de valider l'exactitude des protocoles et des observations de terrain. Afin de faciliter les déductions sur le parasitisme de couvée interspécifique, nous avons validé les observations de terrain d'identification des œufs au niveau de l'espèce en les comparant avec les résultats de séquençage d'ADN mitochondrial pour des nids présentant une suspicion de parasitisme de couvée interspécifique. Les observations de terrain des œufs parasites d'*Aythya americana* (Fuligule à tête rouge) et de l'hôte *Aythya valisineria* (Fuligule à dos blanc) se sont révélées extrêmement fiables ($Aame\_ID$ = 0.99 ± 0.05; $Aval\_ID$ = 0.99 ± 0.04), même avec des observateurs de niveaux d'expérience différents (Obs_{1an} = 0.99 ± 0.03; Obs_{3ans} = 0.97 ± 0.02). Les membranes de coquilles d'œufs collectées après éclosion contenaient les meilleurs échantillons de tissus pour la réussite du séquençage, comparées aux membranes de coquilles d'œufs collectées plus tôt au cours du développement ($Pr_{séq\_réussi}$ = 0.84 ± 0.08 à 0-9 jours d'incubation, 0.63 ± 0.12 à 10-24 jours d'incubation, et 0.88 ±0.04 pour les membranes de coquilles écloses), tandis que la concentration d'ADN après extraction n'a pas été un factor important dans la réussite du séquençage. Malgré l'obtention de concentrations d'ADN suffisantes, nous avons observé un taux d'échec de 15% de nos échantillons. Les pourcentages respectifs de 69% et de 31% des 29 échantillons dont le séquençage a échoué étaient dus à un manque d'ADN de qualité et à un séquençage non ciblé, ce qui suggère que l'échec était probablement dû à une croissance bactérienne provenant d'une exposition ayant eu lieu après un abandon ou une prédation des œufs. Notre étude démontre l'utilité des techniques moléculaires non invasives pour valider les protocoles de terrain et réfute l'identification des œufs comme source majeure d'incertitude lorsque l'on cherche à quantifier le parasitisme de couvée interspécifique dans les nids parasités de *A. valisineria.*

Mots clés : séquençage de l'ADN, tissus ovulaires, membrane de la coquille d'œuf, méthodologie de terrain, échantillonnage non invasif, canard à tête rouge

INTRODUCTION

Brood parasitism is an alternative reproductive strategy where eggs are parasitically laid in the nests of other individuals (Yom-Tov 1980, 2001). Identifying both the presence and extent of brood parasitism is important to understanding drivers of population dynamics for species exhibiting this breeding strategy, and for species subjected to the behavior (e.g., interspecific brood parasitism; de Valpine and Eadie 2008). Abnormally large clutch sizes have historically been used to document brood parasitism in the field; however, relying on the number of eggs in a nest can be prone to error because eggs may be removed, displaced, or damaged following a parasitic event. Alternatively, egg characteristics may be used to differentiate host from parasite eggs but are rarely consistent enough to be conclusive given the extraordinary variation in morphology, pigmentation, and patterns, both across and within species of birds (Romanoff 1949, Hauber 2020). Egg characteristics have even been shaped by brood parasitism itself, as is the case with egg mimicry where a parasitic species' egg closely resembles the egg of a host species to avoid discovery and potential egg rejection (Brooke and Davies 1988, Kilner 2006, Honza et al. 2014). More commonly, similarities between the parasitic species' egg and the host's egg occur due to convergent evolution because of shared diet, environment, or predation pressures (Harrison 1968, Mason and Rothstein 1987). Nonetheless, parasitic eggs laid by heterospecifics are not identical to those of the host. Visual and morphological characters may be reliable for distinguishing and enumerating interspecific brood parasitism but have not been properly validated for many host-parasite relationships.

There exists a strong host–parasite relationship between *Aythya americana* (Redhead) and *Aythya valisineria* (Canvasback), two North American waterfowl species. It is common for *A. americana* to lay eggs in *A. valisineria* nests. These two species share nesting habitat preferences and the duration of their incubation period is similar, creating an opportunity for *A. americana* to increase their fecundity by parasitizing *A. valisineria* nests (Baldassarre 2014, Sorenson 1991, 1997, Péron and Koons 2012). Conversely, *A. valisineria* fecundity may be negatively impacted by *A. americana* parasitism when scuffles between the host and parasitic females non-discriminately displace eggs from the overwater nest causing the eggs to die (Sorenson 1997). After multiple parasitic events at a single nest, the number of host eggs is typically reduced and parasitized nests are commonly a mixture of *A. americana* and *A. valisineria* eggs (Erickson 1948, Weller 1959, Bouffard 1983, Stoudt 1982, Sorenson 1997). Following a successful hatch, *A. americana* ducklings are then reared alongside *A. valisineria* ducklings by the attending female.

Methods to quantify the number of host eggs in *A. valisineria* nests exposed to interspecific parasitism have traditionally relied on visual observations to distinguish parasitic *A. americana* eggs within *A. valisineria* nests (Figure 1). Differentiating eggs between the two species is described to be straightforward; *A. valisineria* eggs appear olive green in color, somewhat ovate in shape, and with a coarse outer texture. *Aythya americana* egg color is beige or cream-colored, with a shape that is typically rounder than long, and a smooth, nearly glossy eggshell texture (Baldassarre 2014). In some cases, however, *A. valisineria* and *A. americana* eggs appear similar, introducing opportunities for eggs to be misidentified afield. Advancements in molecular techniques have proven to be especially useful for identification of eggs to species and even to individual parent levels (Martin-Gálvez et al. 2011, Šulc et al. 2022) and can be applied to validate field methods where visual cues are used to discern egg species. Our primary goal was to measure the accuracy of field observations discerning parasitic *A. americana* eggs in *A. valisineria* nests. If accurate, field observations could be used to quantify, e.g., the host (*A. valisineria*) clutch size at hatch (CSH, the quantity of host species' egg per nest immediately before transition to the offspring stage of a life cycle) among studies spanning large spatial and temporal scales, which is an important component of reproductive success at the population level (Cooch et al. 2001). To do this, we used non-invasive molecular techniques to identify eggs to the species level and compared those results with egg identification by observers in the field where visual and morphological characteristics were used to guide their determinations.

METHODS

Sample Collection

During the 2022 nesting season, *A. valisineria* nests were located via foot searching emergent wetland vegetation, primarily *Typha* spp. (cattail) and *Scirpus* spp.(bulrush), as part of a larger multi-year study assessing the impacts of predator management on the nest survival of overwater nests encompassing 388.5 km^2 near Minnedosa, Manitoba, Canada. This landscape is characterized as Prairie Parkland habitat with gently rolling topography, interspersed stands of *Populus* spp. (aspen) and *Quercus* spp. (oak) trees, and many semi-permanent and permanent wetlands that attract high densities of overwater nesting ducks. The Minnedosa area has been especially important to study overwater-nesting ducks and has shaped our understanding of *A. valisineria* and *A. americana* breeding ecology including interactions between these two species (Weller 1959, Stoudt 1982, Sorenson 1991, 1997, Anderson et al. 1997). Daily searches for overwater nests were conducted over a 6-week period during the primary nesting season from mid-May to the end of June in 2022.

Observers were given detailed descriptions on the color, texture, and morphology of *A. valisineria* and *A. americana* eggs, and experienced observers also provided in-person training to first-year observers at located nests during the beginning of the nest-searching season. Once an *A. valisineria* nest was discovered, the eggs were visually identified to species (*A. valisineria* or *A. americana*) by observers if interspecific brood parasitism was suspected (e.g., noticeable differences in egg characteristics, location of displaced eggs lost during parasitic scuffles) and all eggs were uniquely marked using permanent markers (see Figure 2). Nests were monitored weekly and eggshell membrane tissue samples were collected after nest termination if fragment markings could be definitively matched to an egg's ID. We collected samples as they were encountered to ensure we would meet the maximum sample size (200) allowed by Canadian Wildlife Service permitting, noting that samples could only be collected from inactive nests (e.g., abandoned, preyed upon, or hatched). In addition to field-recorded egg species, we also noted the experience level of the individual identifying eggs (e.g., first year, second year, 3+ years) as we suspected accuracy may be influenced by observer experience. We estimated embryonic development of each egg via candling (Weller 1956) as eggs early in development (i.e., incubation) were suspected to be more challenging to sequence using only mitochondrial DNA (Bush et al. 2005). Egg membrane samples were individually labeled and preserved at –20°C until DNA extraction.

DNA Extraction

Sample tissues were transported to and extracted at Colorado State University, Fort Collins, Colorado, ~14 months after collection. We chose the part of an eggshell membrane most likely to yield suitable DNA concentrations by searching for collapsed blood vessels or other substantial tissues. To begin the lysing process, samples were approximately 1.5 × 1.5 cm in size to begin but were cut into smaller pieces using a sterile razor to accelerate lysing. Genomic extractions were completed using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) with the following modifications from Bush et al. (2005) to enhance lysis of membrane tissue: we doubled the amount of proteinase K (to 40 µL) and increased the amount of buffer animal tissue lysis (ATL) (to 400 µL). While we initially followed the recommended incubation time (12–48 hr) and temperature (55°C) as outlined in Bush et al. (2005), our optimum incubation times ranged from 16 to 72 hr at 55°C depending on sample type. DNA concentrations were quantified using high-sensitivity chemistry in a Qubit Fluorometer (dsDNA HS Assay Kit, Life Technologies, Carlsbad, CA) to ensure samples contained DNA for sequencing and for inclusion as a predictor variable for analysis of sequencing success. DNA concentrations ranged from 0.05 ng mL^{-1} to 25.0 ng mL^{-1} across membrane samples. DNA quality across samples was also visualized on a 1% agarose gel.

Mitochondrial DNA PCR and Sanger Sequencing

The mitochondrial DNA (mtDNA) control region has a relatively high mutation rate within the hyper-variable region that ensures sufficient polymorphisms to demarcate between even closely related species (Nabholz et al. 2009). Here, we used primers L78 and H774 to amplify ~625 base pairs (bp) of the mtDNA control region (Sorenson and Fleischer 1996, Sorenson et al. 1999), following polymerase chain reaction (PCR) concentrations and thermocycler conditions described in Lavretsky et al. (2015). PCR products were visualized via agarose electrophoresis and then purified using ExoSAP-IT (ThermoFisher). Clean PCR products were then sent for Sanger sequencing using the L78 primer on a 3130XL Genetic Analyzer at the University of Texas at El Paso, Border Biomedical Research Center's Genomic Analysis Core Facility. Sequences were aligned and edited using Sequencher DNA Sequence Analysis Software (version 4.8, Gene Codes), and all unique sequences were subsequently BLASTed against the GenBank repository to confirm species identity (accession numbers PX520515-PX520780). Confirmed species were then compared with species identified via visual observations afield to quantify accuracy of field assignment of species to each egg in the study sample.

Statistical Analysis

We used Bayesian binomial logistic regression models in JAGS (Plummer 2003), cast from the R software (R Core Team 2021), to examine factors that may influence the ability of observers to accurately identify eggs, and separately, to examine tissue sample characteristics that may influence successful PCR sequencing. For the egg species validation model, we divided observer experience into 3 categorical levels: first year, second year, and 3+ years field experience, using the most experienced group as the reference group because of skewed sample sizes. Additionally, we used a binary variable to demarcate the true identify of each egg to test for differences in validation between the two species. The response variable was treated as a binary outcome where a correctly identified egg was represented by "1" and a misidentified egg by "0". For the sequencing success model, we divided development stages into 3 categorical age classes representing notable development benchmarks: 0–9 days incubation, 10–24 days incubation, and hatched, using hatched eggs as the reference group because of skewed sample sizes between groups. These 3 developmental age classes may also represent constraints regarding parts collection protocols. DNA concentration was treated as a continuous covariate and the response variable was treated as a binary outcome variable where a successfully sequenced sampled was represented by "1", and unsuccessful by "0". In all cases, prior distributions were specified using a normal distribution with a mean of 0 and variance of 100 (0, 0.01). We assessed convergence of our Bayesian models using traceplots in the *MCMCvis* package as well as $\hat{R}$ scores, concluding convergence was met when $\hat{R}<1.01$. We made inference on the relationship and strength of covariates within each model, respectively, by evaluating the mean value and 95% Bayesian credible interval (BCI) for each regression coefficient. Bayesian credible intervals describe the proportion of the posterior distribution falling within 95% of the probability mass. To derive predicted probabilities for both models, we back-transformed model coefficient estimates by applying the inverse logit function to the predicted log-odds from our model results.

RESULTS

We collected 200 tissue samples from eggs visually identified as *A. valisineria* (57%, *n* = 115) or *A. americana* (43%, *n* = 85) in the field. First, despite having non-zero DNA concentrations, 20 of the 29 failed samples did not have any visible DNA when gel checked, and thus, were not carried forward to PCR amplification. Of the 180 samples carried forward through PCR amplification and Sangar sequencing, 9 (5%) failed due to non-target sequencing. The remaining 171 samples were successfully amplified and sequenced returning a haplotype network that recovered both *A. valisineria* (*n* = 97) and *A. americana* (*n* = 74) haplogroups (Supplementary Material Figure S1). Comparisons between field and molecular assessment of eggs to the species level for the 171 samples that successfully amplified and sequenced resulted in 97% similarity assignments, including no difference in derived validation success between parasitic *A. americana* eggs and *A. valisineria* eggs (Aame_ID = 0.99, SD = 0.05; Aval_ID_= 0.99, SD = 0.04). Among the eggs that were misidentified, 2 were *A. valisineria* eggs misidentified as *A. americana*, and 4 were *A. americana* misidentified as *A. valisineria*. The difference between the most experienced (3-yr experience) and second-year observers was nearly 0 ($\beta_{obs\_2yr} = -0.001$, 95% BCI = –1.630 to 1.730); however, the difference between the most experienced and least experienced group (i.e., first-year observers) was large ($\beta_{obs\_1yr} = 7.310$, 95% BCI = –1.279 to 21.332), but only equated to 0.03 difference in identification probability (i.e., 0.96 vs. 0.99).

DNA concentration measured via fluorometry was not a predictor of successful mtDNA sequencing as the estimated coefficient was near 0, and 95% BCI widely overlapped 0 ($\beta_{conc} = 0.014$, SD = 0.023, 95% BCI = –0.030 to 0.059). MtDNA sequencing success (i.e., Pr_{seq_succ}) varied with development stage of the egg: 0.84 (SD = 0.09) for eggs 0–9 days incubation, 0.63 (SD = 0.12) for eggs 10–24 days incubation, and 0.88 (SD = 0.04) for hatched membranes (Figure 3).

DISCUSSION

Despite similarities in egg characteristics, visual observations afield accurately differentiated *A. valisineria* and *A. americana* eggs in parasitized *A. valisineria* nests. Of the few misidentified eggs, twice as many eggs were misidentified as *A. valisineria* suggesting some *A. americana* eggs were indiscernible from *A. valisineria* eggs to the naked eye, but the low frequency of overall misclassification (3%) does not deem field protocols unreliable. To the contrary, our molecular determinations validate the ability of trained field observers to distinguish between *A. americana* and *A. valisineria* eggs. In our tests, the interspecific variation in egg characteristics was enough to visually index clutch compositions and suggests historical, unvalidated counts of CSH is sufficient for measuring and comparing the productivity of *A. valisineria* nests at population levels. We recognize that our results are limited only to *A. americana* and *A. valisineria* eggs; yet parasitic *A. americana* readily lay eggs in the nests of other overwater nesting ducks including *A. affinis* (Lesser Scaup), *A. collaris* (Ring-necked Duck), and *Oxyura* *jamaicensis* (Ruddy Duck) (Baldassarre 2014). Egg characteristics between *A. americana* and *A. valisineria* are most similar; therefore, field determination of egg species by observers should also be highly accurate in nests of other overwater-nesting ducks.

Numerous external factors limited our ability to hire a perfectly stratified field crew based on prior observation experience for the purposes of this field experiment, which created skewed sample sizes among observer groups and contributed to some issues of estimate uncertainty (e.g., β_{obs_1yr}; Table 1). Admittedly, we regret not collecting detailed morphometric and pigment data for eggs, which would have potentially helped explain the small number of misclassifications we observed and future research could add these traits to the repertoire of those used to distinguish eggs between species and potentially achieve 100% accuracy. DNA concentration was not related to sequencing success; however, development stage was supported as an explanatory variable. Hatched membranes led to the highest sequencing success probability at 88%, likely because hatched membranes often included developed tissues for extractions (e.g., dried blood vessels). Early-development and hatched membrane samples were 20–25% more likely to be successfully sequenced than mid-development eggs (10–24 days incubation) and this difference may hinge on DNA quality (Figure 3). Field protocols to collect egg membrane samples at mid-development required nests that had been abandoned for >1 week, which allowed time for decay and mtDNA breakdown to occur. We suspect samples that failed to be sequenced but yielded suitable DNA concentrations are likely to contain non-target DNA, as humid conditions in an overwater nest quickly promote the growth of bacteria. Thus, to ensure high-quality DNA, we recommend collecting membrane samples as soon as possible following nest desertion, sampling embryonic tissue instead of membrane tissue from abandoned eggs (Morland et al. 2023) and reducing the duration that samples are stored before extractions begin. Moreover, sampling of eggshells instead of membranes can result in potentially higher yield and less degraded DNA (Oskam et al. 2010, but see Trimbos et al. 2009). Finally, while the circular nature of mitochondrial DNA results in a slower rate of degradation than linear nuclear chromosomes (Foran 2006), failure was driven by a lack of quality DNA or non-target amplification; and thus, amplifying a shorter fragment that still includes sufficient differences can increase PCR success for samples with degraded DNA (Broquet et al. 2007).

To limit in-the-field variability between observers, we recommend identifying vision impairments (e.g., color blindness) that could influence one's ability to discern subtleties in pigmentation and affect identifications of eggs to the species level if unaccounted for. Marking eggs during nest visits is also an important consideration when trying to quantify CSH as well as change in the number of host and parasitic eggs present in a nest throughout incubation, and we found unique egg markings are necessary if samples are to be collected for genetic testing (Figure 2). We have no evidence suggesting that even vibrantly marked eggs were noticed or discriminated against by incubating females in our study; however, marking eggs of species capable of egg discrimination should be approached with caution to avoid egg rejection. This methodology is best suited for species with precocial young that desert the nest after hatch and leave samples behind but is less suited for altricial birds where nest visits immediately after hatch may negatively influence chick survival or do not ensure collection of egg remains (Grant et al. 2005).

The importance of validating field protocols to ensure reliable and accurate data cannot be overstated and is especially important when data may be collected over broad temporal or spatial scales. Eggshell membrane tissues serve as a suitable alternative for non-invasive DNA sampling in waterfowl and we encourage continued efforts to develop innovative and non-invasive techniques that improve our understanding of ecological systems as well as offer ethical and accessibility advantages over many traditional methods (Orlans 1988, Pauli et al. 2010). Potential egg misidentification is a minor source of error when indexing host CSH of parasitized *A. valisineria* nests, and refinement of the molecular methods could be further applied to assess individual host clutch size after laying that could otherwise be confounded with intraspecific brood parasitism, and the field identification of eggs to species level along with egg marking would be accurate for measuring dynamic change in host egg numbers throughout incubation that could otherwise be confounded by interspecific brood parasitism, displacement of eggs from parasitic scuffles, and partial predation. Molecular techniques provide a wide variety of opportunities to inform our understanding of brood parasitism interactions, from validating field methods (Andersson and Åhlund 2001, Sorenson and Payne 2002, Grønstøl et al. 2006, Lavretsky et al. 2023) to investigating more nuanced features like extra-pair copulations, relatedness among parasitic females, and how parasitism shapes local population structures (Harvey et al. 2021, Lavretsky et al. 2023, Fukunaga at el. 2026).

Supplementary material

Supplementary material is available at *Ornithological Applications* online.

Acknowledgements

We would like to thank the field assistants who aided in identifying and collecting eggshell samples: Troy Blackwell, Craig Casto, Zack Peck, Grant Rhodes, Hannah Sabatier, and Camryn Vestby. We would like to thank the reviewers. And we would also like to thank Cassandre Venumière-Lefebvre for the French translation of the abstract.

Funding statement

Delta Waterfowl Foundation provided the funding to support field work, materials for extractions, mtDNA sequencing and species identification, and supported MKJ. DNK and MKJ were additionally supported by the James C. Kennedy Endowment for Wetland and Waterfowl Conservation at Colorado State University.

Ethics statement

Eggshell parts collection was approved by permit #22-MB-SC004 granted by the Canadian Wildlife Service and extractions approved via Colorado State University IACUC protocol #3334.

Conflict of interest statement

The authors declare no conflict of interest.

Author contributions

Conceived the idea - FCR conceptualized the idea with a simple question: “Are you sure?”. Experimental design and drafting of manuscript - MKJ and DNK developed a proposal for funding and permit acquisition. MKJ drafted the manuscript, and all co-authors contributed towards editing the manuscript. Data collection/analysis – MKJ completed field collections, statistical analysis, and lab extractions under the mentorship of CPW at Colorado State University. PL and VM conducted sequencing procedures at University of Texas at El Paso, Border Biomedical Research Center’s Genomic Analysis Core Facility.

Funding support - CAN aided in allocating funds to cover field operations and lab work.

Data availability

Analyses reported in this article can be reproduced using the data provided by Johnson et al. (2026).

LITERATURE CITED

Andersson, M., and M. Åhlund (2001). Protein fingerprinting: A new technique reveals extensive conspecific brood parasitism. *Ecology* 82:1433–1442.

Baldassarre, G. (2014). *Ducks, Geese and Swans of North America*. John Hopkins University Press, Baltimore, MD, USA.

Bouffard, S. H. (1983). Redhead egg parasitism of Canvasback nests. *Journal of Wildlife Management* 47:213–216.

Brooke, M. de L., and N. B. Davies (1988). Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* 335:630–632.

Broquet, T., N. Ménard, and E. Petit (2007). Non-invasive population genetics: A review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conservation Genetics* 8:249–260.

Bush, K. L., M. D. Vinsky, C. L. Aldridge, and C. A. Paszkowski (2005). A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of Greater Sage-Grouse (*Centrocercus uropihasianus). Conservation genetics* 6:867–870.

Cooch, E., R. F. Rockwell, and S. Brault (2001). Retrospective analysis of demographic responses to environmental change: A Lesser Snow Goose example. *Ecological Monographs* 71:377–400.

de Valpine, P., and J. M. Eadie (2008). Conspecific brood parasitism and population dynamics. *The American Naturalist* 172:547–562.

Erickson, R. C. (1948). Life history and ecology of the Canvas-back, *Nyroca valisineria* (Wilson), in south-eastern Oregon. Ph.D. dissertation, Iowa State College, Ames, IA, USA.

Foran, D. R. (2006). Relative degradation of nuclear and mitochondrial DNA: An experimental approach. *Journal of Forensic Sciences* 51:766–770.

Fukunaga, K., C. P. Wells, and P. Lavretsky (2026). Determining sex ratios and mitochondrial haplotypes of Hawaiʻi's endemic and introduced ducks. *Journal of Ornithology* 167:211–222

Grant, T. A., T. L. Shaffer, E. M. Madden, and P. J. Pietz (2005). Time-specific variation in passerine nest survival: New insights into old questions. *The Auk* 122:661–672.

Grønstøl, G., D. Blomqvist, and R. H. Wagner (2006). The importance of genetic evidence for identifying intra-specific brood parasitism. *Journal of Avian Biology* 37:197–199.

Harvey, K., P. Lavretsky, J. Foth, and C. K. Williams (2021). Nest parasitism, promiscuity, and relatedness among Wood Ducks. *PLoS One* 16:e0257105.

Harrison, C. J. O. (1968). Egg mimicry in British Cuckoos. *Bird Study* 15:22–28.

Hauber, M. E. (2020). *The Book of Eggs: A Life-Size Guide to the Eggs of Six Hundred of the World's Bird Species*. University of Chicago Press, Chicago, IL, USA.

Honza, M., M. Šulc, V. Jelínek, M. Požgayová, and P. Procházka (2014). Brood parasites lay eggs matching the appearance of host clutches. *Proceedings of the Royal Society Bulletin: Biological Sciences* 281:20132665.

Johnson, M. K., C. P. Wells, P. Lavretsky, V. M. Musni, C. A. Nicolai, F. C. Rohwer, and D. N. Koons (2026). Data from: Field observations can accurately detect interspecific brood parasitism in *Aythya valisineria* (Canvasback) nests. Ornithological Applications 128:duag000. https://doi.org/10.5061/dryad.r4xgxd2t4 [Dataset].

Kilner, R. M. (2006). The evolution of egg colour and patterning in birds. *Biological Reviews* 81:383-406.

Lavretsky P, J. M. Dacosta, B. E. Hernández-Baños, A. Engilis Jr, M. D. Sorenson, and J. L. Peters (2015). Speciation genomics and a role for the Z chromosome in the early stages of divergence between Mexican Ducks and Mallards. *Molecular Ecology* 24:5364–5378.

Lavretsky, P., A. Hoyt, V. M. Musni, D. Howell, and C. K. Williams (2023). Frequency and types of alternative breeding strategies employed by nesting American Black Ducks in North Carolina. *PLoS One* 18:e0278905.

Martín-Gálvez, D., J. M. Peralta-Sánchez, D. A. Dawson, A. M. Martín-Platero, M. Martínez-Bueno, T. Burke, and J. J. Soler (2011). DNA sampling from eggshell swabbing is widely applicable in wild bird populations as demonstrated in 23 species. *Molecular Ecology Resources* 11:481–493.

Mason, P., and S. I. Rothstein (1987). Crypsis versus mimicry and the color of Shiny Cowbird eggs. *The American Naturalist* 130:161–316.

Morland, F., S. Patel, A. W. Santure, P. Brekke, and N. Hemmings (2024). Including the invisible fraction in whole population studies: A guide to the genetic sampling of unhatched bird eggs. *Methods in Ecology and Evolution* 15:80–90.

Nabholz, B., S. Glémin, and N. Galtier (2009). The erratic mitochondrial clock: Variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology* 9:1–13.

Orlans, F. B. (1988). *Field Research Guidelines Impact on Animal Care and Use Committees.* Scientists Center for Animal Welfare, Bethesda, MD, USA.

Oskam, C. L., J. Haile, E. McLay, P. Rigby, M. E. Allentoft, M. E. Olsen, C. Bengtsson, G. H. Miller, J. Schwenninger, C. Jacomb, R. Walter, A. Baynes, et al. (2010). Fossil avian eggshell preserves ancient DNA. *Proceedings of the Royal Society Bulletin: Biological Sciences* 277:1991–2000.

Pauli, J. N., J. P. Whiteman, M. D. Riley, and A. D. Middleton (2010). Defining noninvasive approaches for sampling of vertebrates. *Conservation Biology* 24:349–352.

Péron, G., and D. N. Koons (2012). Integrated modeling of communities: Parasitism, competition, and demographic synchrony in sympatric ducks. *Ecology* 93:2456–2464.

Plummer, M. (2003). JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. In *Proceedings of the 3rd International Workshop on Distributed Statistical Computing* (Vol. 124, No. 125.10). pp. 1–10.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Romanoff, A. L., and A. J. Romanoff (1949). *The Avian Egg*. J. Wiley, New York, NY, USA.

Sorenson, M. D. (1991). The functional significance of parasitic egg laying and typical nesting in Redhead ducks: An analysis of individual behavior. *Animal Behavior* 42:771–796.

Sorenson, M. D. (1997). Effects of intra- and interspecific brood parasitism on a precocial host, the Canvasback, *Aythya valisineria*. *Behavioral Ecology* 8:153–161.

Sorenson, M. D., and R. C. Fleischer (1996). Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proceedings of the National Academy of Sciences USA* 93:15239–15243.

Sorenson, M. D., J. C. Ast, D. E. Dimcheff, T. Yuri, and D. P. Mindell (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution* 12:105–114.

Sorenson, M. D., and R. B. Payne (2002). Molecular genetic perspectives on avian brood parasitism. *Integrative and Comparative Biology* 42:388–400.

Stoudt, J. H. (1982). Habitat use and productivity of Canvasbacks in southwestern Manitoba, 1961–1972. Washington D.C. U.S. Fish and Wildlife Service Special Science Report, Wildlife 248.

Šulc, M., A. E. Hughes, J. Troscianko, G. Štětková, P. Procházka, M. Požgayová, and M. Honza (2022). Automatic identification of bird females using egg phenotype. *Zoological Journal of the Linnean Society* 195:33–44.

Trimbos, K. B., J. Broekman, R. Kentie, C. J. Musters, and G. R. de Snoo (2009). Using eggshell membranes as a DNA source for population genetic research. *Journal of Ornithology* 150:915–920.

Weller, M. W. (1956). A simple field candler for waterfowl eggs. *The Journal of Wildlife Management* 20:111–113.

Weller, M. W. (1959). Parasitic egg-laying in the Redhead (*Aythya americana*) and other North American Anatidae. *Ecological Monographs* 29:333–365.

Yom-Tov, Y. (1980). Intraspecific nest parasitism in birds. *Biological Reviews* 55:93–108.

Yom-Tov, Y. (2001). An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis* 143:133–143.

Table 1. Descriptive sample sizes of eggshell membrane and covariate data used in molecular analysis to identify egg species of *A. valisineria* (Canvasback) and *A. americana* (Redhead). “Egg age classes” represents the respective samples size of eggshell membrane tissues grouped by the age of the egg in incubation days determined via candling. “Observer experience” represents the number of years an individual had worked on the project differentiating parasitic *A. americana* eggs from *A. valisineria* eggs, and the sample size collected by observers from each group.

Sample sizes	***Aythya valisineria***	***Aythya americana***	**Total**
Number of nests	55	N/A	55
Number of eggs	115	85	200
Successfully sequenced	99	72	171
Correctly identified	95	70	165
Covariate Data			
Egg age classes			
0–9 days	6	21	27
10–24 days	20	15	35
Hatched	89	49	138
Observer experience			
1 year	2	5	7
2 years	49	34	83
3+ years	64	46	110

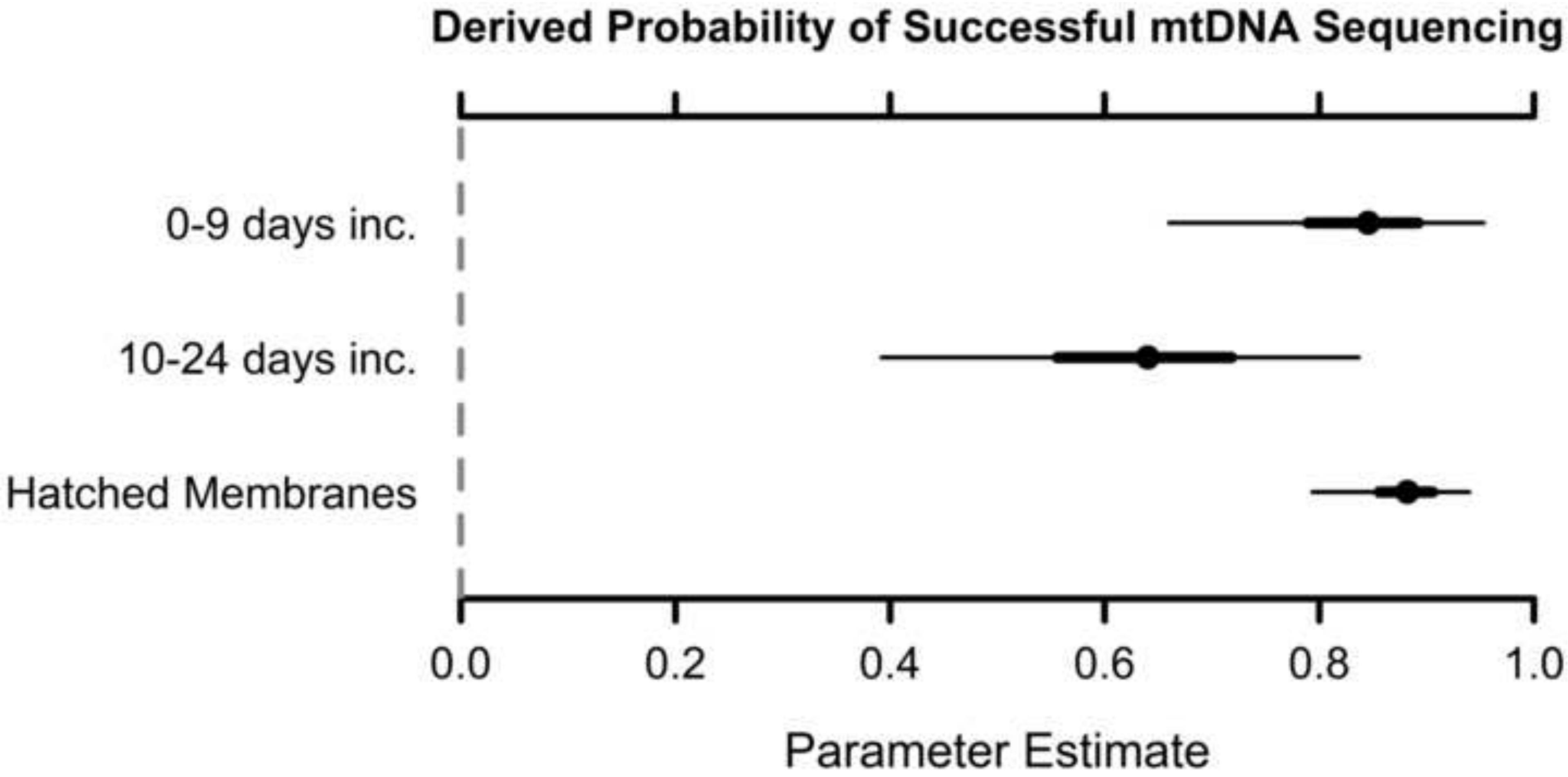
Figure 1. Photo of an *Aythya valisineria* (Canvasback) nest containing both *A. valisinera* and parasitic *A. americana* (Redhead) eggs. The inset contains 3 eggs denoted with “R” that might be classified as *A. americana* eggs based on shape and color (though egg texture could also be considered).

Figure 2. Eggs in parasitized *Aythya valisineria* (Canvasback) nests were marked on ends with an “R” for *Aythya americana* (Redhead), or “C” for *A. valisineria* with black permanent marker. Additionally, we uniquely marked eggs using a combination of permanent marker colors so that no two eggs were colored the same. Often only fragments of eggs are recoverable following hatch or a predation event and this unique coloring system allowed us to match up to 10 individual eggs per nest with initial field observations.

Figure 3. Derived probabilities for mtDNA sequencing success relative to embryonic development stage of *Aythya valisineria* (Canvasback) and *A. americana* (Redhead) egg membrane tissue samples. Groups represent important developmental stages based on incubation duration and could also represent potential constraints for parts collection.

Figure 1

Figure 2


Derived Probability of Successful mtDNA Sequencing
0-9 days inc.
10-24 days inc.
Hatched Membranes
0.0
0.2
0.4
0.6
0.8
1.0
Parameter Estimate