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HAND REARING OF THE HOODED GREBE (*Podiceps gallardoi*) AND THE SILVERY GREBE (*Podiceps occipitalis*) AS A FUNDAMENTAL RECOVERY STRATEGY FOR A CRITICALLY ENDANGERED SPECIES

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ABSTRACT: Habitat loss, climate change, and invasive alien species have led the critically endangered Hooded Grebe (*Podiceps gallardoi*) to decline by 80% of its population. The Silvery Grebe (*Podiceps occipitalis,* least concern), is a closely related species that can be used as a biological proxy. As a complement to the *in situ* conservation actions developed over the past decade to protect the Hooded Grebe, we proposed hand rearing as part of the conservation strategy, given that in 97.4% of clutches, the nesting pairs abandon the second egg. Given the limited information regarding captive rearing of grebes, we evaluated variables that could affect hatching success and chick survival, including incubation stage at collection, transportation duration, temperature-humidity incubation protocol, daily egg-weight loss, and care (feeding and swimming) frequency. We collected and artificially incubated eggs from Hooded Grebes and Silvery Grebes for four breeding seasons. We achieved a 73% hatching success rate. The longest Hooded Grebe chick survival was 144 hours, and we successfully raised a Silvery Grebe fledgling, which was released into the wild after 67 days. Transportation duration, daily egg-weight loss, and incubation protocol did not affect hatching success. Eggs collected in the later stages of incubation had a higher hatching rate. We observed a higher survival time associated with the high-temperature and humidity incubation protocol. Chicks hatched from eggs with a daily egg-weight loss of more than 1% showed higher survival. This was also observed in chicks with more assiduous care. We have defined an appropriate incubation protocol for the Hooded Grebe. In the future, we will focus on determining the appropriate frequency of care and diet. The low reproductive success of the Hooded Grebe in the last years urges the need for actions aimed at increasing annual population recruitment.

KEYWORDS: *captive breeding, ex situ management, Hooded Grebe, Podiceps gallardoi, Podiceps occipitalis, reproductive biology, Silvery Grebe*

The grebes (Podicipediformes) are one of the taxonomically of birds of greatest conservation concern, indeed three of the 23 species have been declared extinct in the last three decades, and five others are currently threatened (BirdLife International 2023). Furthermore, habitat alteration, pollution, the introduction of non-native species, and climate change can cause the rapid decline of a species (Winkler et

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al. 2020). The Hooded Grebe (*Podiceps gallardoi*) is an endemic breeding bird of Santa Cruz Province, Argentina, where it breeds in highland lakes on basaltic plateaus in the western part of the province. During winter, Hooded Grebes migrate to the Atlantic Coast estuaries of the Coyle, Gallegos, and Chico-Santa Cruz rivers (Imberti et al. 2020). In the early 1980s, the Hooded Grebe population was estimated to be 3000-5000 individuals (Fjeldså 1986, Beltrán et al. 1992). Counts conducted between 2010 and 2011 showed that in 25 years the population suffered an 80% decrease and an increase in its threats (invasive alien species and loss of reproductive sites due to climate change), with only c. 800 individuals left (Roesler et al. 2012). Consequently, the species was categorized globally as Critically Endangered in 2012 (BirdLife International 2023). In Argentina, it is also considered critically endangered (MAyDS y AA 2017). The causes of decline include predation of adults and juveniles by the invasive American Mink (*Neovison vison*), the destruction of colonies by the Kelp Gull (*Larus dominicanus*), and competition with Rainbow Trout (*Oncorhynchus mykiss*), which has been introduced in some lakes (Roesler et al. 2012, Lancelotti et al. 2017, Fasola & Roesler 2018). In addition, climate-related factors, such as the decrease in precipitation during winter (snowfall) and an increase in wind speed, cause the lakes to dry up (Lancelotti et al. 2020), affecting the viability of reproductive colonies.

The Silvery Grebe (*Podiceps occipitalis*) is evaluated globally as Least Concern because this species has an extremely large range and a large population size. However, the overall population trend is decreasing, and the trend of some of its populations is unknown (BirdLife International 2023). In Argentina, it is considered not threatened (MayDS y AA 2017). This is a closely related species with similar life history to the Hooded Grebe and inhabits the same environments in austral Patagonia (Fjeldså 1983). For this reason, the threats facing the species could be similar. In addition, Hooded and Silvery Grebes frequently form mixed colonies, so any information generated from one species could help to understand the other.

Ex situ management has been used to benefit the conservation efforts of threatened species. Species extinctions have been prevented, and for an increasing number of species, conservation restorations or introductions have followed periods of *ex situ* management (IUCN/SSC 2014). Artificial incubation of eggs removed from the wild, hand rearing of chicks, and the subsequent return of birds into their native habitat have been incorporated into the overall recovery strategy for endangered species such as the Mauritius Kestrel (*Falco punctatus*, Jones et al. 1995), the Mauritius Fody (*Foudia rubra*, Cristinacce et al. 2008), the California Condor (*Gymnogyps californianus*, Kuehler & Whitman 1988), and the Hawaiian Crow (*Corvus hawaiiensis*, Kuehler et al. 1994). Nevertheless, *ex situ* breeding experiences in grebes are anecdotal. Between 1924 and 1933, the German biologist Oskar Heinroth attempted several times to breed individuals of Great-crested Grebe (*Podiceps cristatus*) but was never able to get the chicks to survive beyond four days (Hick 1966). Following his example, Hick (1966) artificially incubated eggs of the same species in 1962 and obtained chicks that also died on the fourth day of life. However, in 1963, he managed to breed two individuals until fledgling, whose survival age is not specified. Also working with *Podiceps cristatus*, Kop (1972) collected and artificially incubated five eggs from the field, all five hatched and two individuals lived 11 days, and three individuals lived 61, 72, and 73 days. Herman artificially incubated three *Aechmophorus occidentalis* eggs in 1974 and collected three chicks in the field. The six chicks were satisfactorily reared until at least four months of age, two were released at the age of 14-15 months (MacVean 1988). Ratti successfully reared seven *Aechmophorus clarkii* from eggs collected in the field to 9-12 weeks of age in 1977 (MacVean 1988). Nuechterlein (1981) incubated two abandoned eggs of the Hooded Grebe after the hatching of the first chick and managed to hatch one of them, which survived up to the age of four days. In 1986, within the framework of a plan to conserve the currently extinct *Podilymbus gigas*, MacVean (1988) successfully reared five individuals of *Podilymbus podiceps* from 20 eggs collected in the field and artificially incubated them, obtaining chicks that were two, 20, and 60 days old, and 14-16 months old. In this last case, the strategy appeared to have extremely high potential, however, unfortunately, it was not continued, and the species became extinct (Hunter 1988). Some of these articles do not provide a detailed description of the work methodology. Others are highly detailed, but they are not suitable for working with species from habitats quite different from those of the Hooded Grebe. In any case, they provide the basis for creating experimental work protocols for other species of grebes.

Since 2011, the 'Hooded Grebe Project' (Aves Argentinas / BirdLife International) has been performing *in situ* efforts to conserve the Hooded Grebe population in the short term (Roesler et al. 2018). This project includes actions to protect the colonies by the 'colony guardians' (i.e., specialized technicians who camp at the colonies to watch and protect the nest from predators; see Roesler et al. 2016). In addition, there is a component of invasive alien species control aimed at preventing depredation of adults, maximizing seasonal recruitment, and restoring reproductive sites (Roesler et al. 2018). These efforts have been successful in halting the population decline; however, due to the low reproductive success of recent seasons, the population is declining again (Roesler et al. 2025).

As a complement to *in situ* actions, we proposed a long term strategy of hand rearing individuals from 'ecologically lost' eggs (i.e., eggs that do not hatch in the wild). This is possible because of the unique life tactic of the Hooded Grebe compared to other grebes (Fjeldså 1986), as 97.4% of breeding pairs raise only one chick and abandon the second egg after the first chick hatches (Roesler 2016). Hand rearing the second egg and releasing individuals may duplicate the recruitment per season. Furthermore, several colonies are destroyed by strong winds, so recovering the eggs before this happens could help mitigate their effect. In addition, given the precarious state of other grebe species, the development of *ex situ* breeding techniques might be helpful in future population recovery efforts.

Our goal is to generate theoretical and practical knowledge for the development of a hand rearing program for the Hooded Grebe, aiming to increase the number of young that survive until migration (i.e., enhance season recruitment) by recovering ecologically lost eggs. Due to the low reproductive success of the species in recent years, we used the Silvery Grebe as a proxy when there was a lack of Hooded Grebe eggs. Our research objectives were to develop a method for obtaining chicks from eggs collected on the lakes, encompassing egg collection, transportation, incubation, and hatching. Additionally, to create a protocol for hand rearing the chicks until they are fledglings, including environmental, management, and feeding variables.

To evaluate the effect on hatching, we compared the incubation period, considering the eggs' collection time, transportation duration between lakes and the hand rearing facilities, five incubation protocols (varying by temperature and humidity), and daily egg-weight loss during artificial incubation. To assess the factors influencing chick survival time, we analyzed the five incubation protocols, variations in daily egg-weight loss during incubation, food options, and the frequency of chick feeding and swimming periods. Here, we present results from initial trials in rearing the critically endangered Hooded Grebe and Silvery Grebe, along with an evaluation of variables affecting hatching success and chick survival for both species.

METHODS

Study Area

Between 2015 and 2020, we worked in the breeding area of the Hooded Grebe in western Santa Cruz Province, Patagonia, Argentina, particularly on the plateaus of Lake Buenos Aires and Lake Strobel (Fig. 1). We were able to work in four breeding seasons (December–April), given that breeding colonies were not present in some years. The first rearing facility was located at Estancia Laguna Verde Fishing Lodge (48°30'S, 71°14'W), in the central area of the Strobel Lake Plateau, where we were situated in a container with a large thermal range. We reared grebes there during three seasons (2015–2016, 2016–2017, 2017–2018). In our last season (2019–2020), we developed a fully equipped hand rearing station at Juan Mazar Barnett Biological Station (JMBBS - Aves Argentinas; 47°14'S, 71°11'W). This place was conditioned with thermal isolation on the roof and walls, which allowed ambient temperature to be stable between 15-20°C. This was a significant improvement to the operation of the incubators. We collected eggs of Hooded Grebe and Silvery Grebe in five lakes: Rodríguez 9, Rodríguez 19, LA4 (Buenos Aires Lake Plateau, Fig. 1B), and Ocho and Nueve (Strobel Lake Plateau, Fig. 1C).

Collection and transportation of eggs

As part of the Hooded Grebe Project, we monitored the lakes where colonies may form every breeding season, looking for nests. When we locate a colony, a 'colony guardian' stays at the site to protect and record the date the eggs are laid (i.e., when eggs are visible on the nest), marking the start of incubation. For hand rearing, we tried to collect eggs after more than 15 days of natural incubation, but if there's a risk of losing the eggs due to a windstorm, we gather them earlier.

To collect the eggs, we used an inflatable boat with oars for two people (Roesler 2016). One person rowed while the other took the eggs from the nest (Fig. 2A). We collected one of the two eggs from each nest. Approaching the colony was always against the wind and never took more than 10 min. All colonies were monitored before and after collection to detect any potential adverse effects of our intervention, and colony reproductive success did not vary. We transported the eggs in thermally insulated boxes with a heat source (hot-water bottles at 55-60°C), which were isolated from the eggs with cotton. With this range of water temperature, we kept the internal temperature of the box between 25 and 30°C throughout the trip. Eggs were then classified according to the transportation time (from the lake to the breeding center) and the incubation stage at the time of collection. We defined three groups based on transportation duration: <1 h, 1–3 h, and >3 h. We also defined three groups of incubation stages (1–7 days, 8–14 days, 15–21 days) based on the typical incubation period of the Hooded Grebe (20–21 days; Roesler 2016), and we assumed the same period for the Silvery Grebe. We based the assignment of laying dates in some cases on information from direct observation obtained by 'colony guardians', when the colony was found before the eggs were laid. In others, this was estimated by the dates of the lakes' monitoring (i.e., the lake was visited and there was no colony, and subsequently, during monitoring, a colony was found) and the number of days of artificial incubation of the eggs until hatching. In this case, we consider the laying date to be 21 days before the hatching day, although transport and artificial incubation may lengthen the hatching time.

Artificial egg incubation

Once at the rearing facilities, we marked the eggs with an identification number using a pencil on the shell, weighed them, and placed them horizontally in an incubator (Fig. 2B). After 12 hours, we candled the eggs to check their viability and measured them (length and width). We waited 12 h until then to let the eggs stabilize after the transport. If any non-viable egg is found, it is discarded, but this was not the case.

We tested five temperature/humidity protocols applied to the different egg batches obtained per season. Due to the variability in the number of nests between seasons, it was impossible to maintain a balanced treatment. We started with a standard incubation protocol used in domestic poultry species, which works well in many nondomestic species (Gage & Duerr 2007): high temperature-high humidity (37.1-37.5°C/65-70%; n = 12) in the season 2015/2016. Due to the low survival time of Hooded Grebe chicks, we hypothesized that a colder incubation temperature could increase incubation time and thus produce stronger chicks. Therefore, in the next season

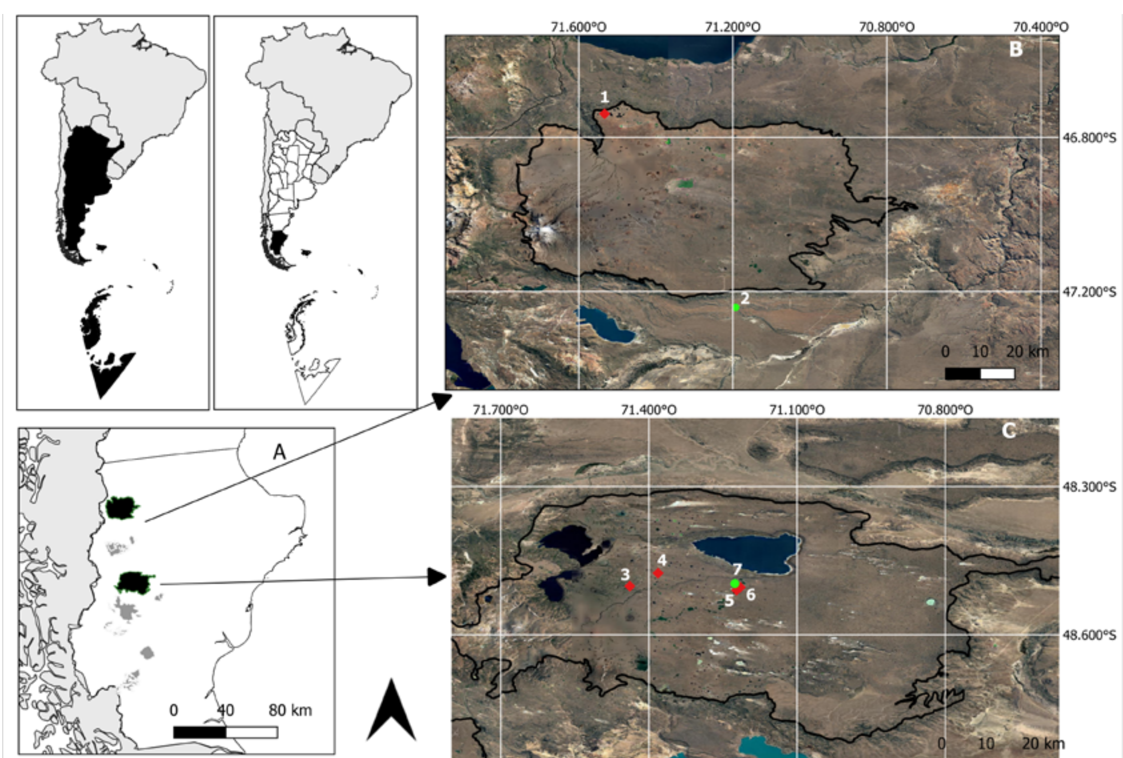

Figure 1. Study area in Santa Cruz Province, Argentina: A) Study area showing the important plateaus: in black, plateaus covered in this study; in grey, other important plateaus for the Hooded Grebe (*Podiceps gallardoi*). B) On Buenos Aires Lake Plateau, location of the lakes (red diamonds) where we collected eggs and *ex situ* breeding sites (green dots): 1 = LA4, 2 = Juan Mazar Barnett Biological Station. C) On Strobel Lake Plateau, location of the lakes (red diamonds) where we collected eggs and *ex situ* breeding sites (green dots): 3 = Rodríguez 19, 4 = Rodríguez 9, 5 = Nueve, 6 = Ocho, 7 = Laguna Verde.

(2016/2017) we tried two protocols simultaneously: A low temperature-high humidity (34.5-34.8°C/65-70%; n = 7) and a middle temperature-high humidity (36.5-36.8°C/65-70%; n = 4), but the chicks hatched immature and weak (i.e., the chicks hatched with their navels open, their back down incomplete, and their eyes closed). Then, due to the arid environment the Hooded Grebe inhabits, we wanted to try decreasing the humidity. So, also during the season 2016/2017, we used a low temperature-low humidity protocol (34.5-34.8°C/35-40%; n = 4) with poor results. Finally, in the season 2017/2018, we tried a high temperature-low humidity protocol (37.1-37.5°C/35-40%; n = 12). Because, subjectively, we obtained the strongest chicks in the first attempt, we decided to return to the high temperature-high humidity protocol in the 2019/2020 season (n = 5). We had technical problems with the middle temperature-high humidity protocol. A hatcher (incubator at a lower temperature) broke, and the eggs suffered an abrupt drop in temperature (almost 2°C).

Daily, we weighed the eggs (scale accuracy 0.01g) to calculate the daily weight loss (Fig. 2C). We defined three intervals of egg-weight loss: interval 1 (0.6–0.9%), interval 2 (1.0–1.3%), and interval 3 (1.4–1.7%). Every three days, we candled the eggs to see embryo development. Over the last 2–3 days, once the eggs were internally pipped, we transferred them to a hatcher. The hatcher was always 0.5°C colder than the incubator in each protocol.

Chick rearing

After hatching, each chick remained in the hatcher for two hours to dry its down before being moved to a breeding box (i.e., a container with a bottom heat source). This was at 36-37°C, like the temperature of a grebes back (Roesler 2016). Daily, the chicks were weighed (0.01 g), fed (Fig. 2D), and placed in a pool (Fig. 2E) to drink and defecate.

We created four day- and night-care scenarios, based on the interval between meals, and one without night feeding, applied according to the number of chicks obtained per season. The difference between treatments was in the frequency of nocturnal feeding. The chicks were fed every hour during the day (06:00-22:00 h) and every one, two, or four hours during the night (scenario one, two, and four hours, respectively). In addition, we used one treatment which did not include night feeding at all (scenario none). Since grebes need to be in the water to defecate and exercise (Gage & Duerr 2007), feeding was always accompanied by a swimming session. In the 'none scenario', the chicks passed through the pool once during the night to stimulate defecation following MacVean (1988). We also created the first scenarios following MacVean's work (1988). Then, the protocols were adapted according to the results or perceptions obtained across the

Figure 2. Working methodology used for Hooded Grebe (*Podiceps gallardoi*) and the Silvery Grebe (*P. occipitalis*): A) Egg collection, B) Incubation, C) Egg weighing, D) Hand feeding of chicks, E) Swimming session, F) Independent feeding of chicks in larger pools.

seasons. In the first two weeks, chicks were fed with tweezers. Then, chicks began to fish for live prey (amphipods) in the pool, which is consistent with their normal feeding behavior. During the first week, chicks were placed in water only long enough to stimulate defecation (15–30 s). After the first week, the time spent in water was gradually lengthened. Chicks were retired from the pool when they showed signs of stress (i.e., vocalizations, restlessness). After four weeks, the only chick alive spent all day in the water and was only retired from the pool at night. The pool was supplemented with live prey (amphipods) collected in the lakes. After seven weeks, following the first plumage transition, it was always in the pool. In the first week, we used a small plastic tub (40 cm x 25 cm x 10 cm). In the second week, we used a 70 cm x 40 cm x 20 cm plastic tub. After three weeks, we placed the chick in a larger pool (1.30 m x 0.95 m x 0.40 m, Fig. 2F). The water used to fill the pools came from local rivers through the ranches' canal system. As it is a gregarious species, we placed mirrors in the pool and breeding box to simulate the presence of other individuals. To define the minimum age of release, we considered it to be older than a month, as juveniles typically become independent of their parents at approximately that age (Roesler 2016). We also thought that it had completed the first plumage transition, which occurs between six and seven weeks of life. We used three food options applied according to the chicks obtained and the food available per season: hypoallergenic cat food and trout (2015/2016), amphipods collected in the lakes (2015/2016-2016/2017-2017/2018-2019/2020), and bloodworms rehydrated with water (2019/2020). We also offered feathers to all the nestlings (Storer 1961, Roesler 2016). We performed a necropsy on all the dead chicks to find out the cause of death.

Data analysis

We considered the response variables hatching success (i.e., whether the egg hatched) and survival time (in hours) of the chicks up to 15 days of life (360 h) or until death. We consider survival through 15 days because, at this age, the chicks are less dependent on external care (e.g., they forage for food and stay longer in the water). To evaluate the effects of incubation stage at collection, transport duration, and incubation protocol on hatching success, we ran generalized linear models with a binomial distribution and a logit link function. We tested univariate models with the three predictor variables, as well as the interaction between natural incubation days and transfer time. For this analysis, we used only Hooded Grebe eggs. Model selection was performed using the corrected Akaike information criterion (AICc). Assumptions were checked using the *DHARMa* statistical package (Harting 2024). To compare daily weight loss between hatched and unhatched eggs, we performed a Mann-Whitney U test. We evaluated the effects of incubation protocol, daily egg weight loss, and chick care frequency on early chick survival time using Kaplan-Meier survival analysis and used the log-rank test for between-group comparisons. We compared the survival of chicks with night feeding versus those without night feeding using a Mann-Whitney U test. We considered chicks that received only one-night feeding within the group without night feeding because we think that four hours is a very long interval, as observed in lakes (Roesler 2016). All analyses were performed in R 4.1.0 (R Core Team 2021).

RESULTS

We collected a total of 45 eggs in nine removal events, comprising 42 Hooded Grebe eggs and three Silvery Grebe eggs. One Hooded Grebe egg broke during measurement and was discarded. We did not detect unviable eggs by candle. Mean Hooded Grebe egg length was 4.58 ± 0.18 cm (range = 4.20–5.10 cm; n = 41); mean Hooded Grebe egg width was 3.08 ± 0.07 cm (range = 2.90–3.20 cm; n = 41). Mean Silvery Grebe egg length was 4.33 ± 0.32 cm (range = 4.10–4.70 cm; n = 3); mean Silvery Grebe egg width was 2.90 ± 0.10 cm (range = 2.80–3.00 cm; n = 3).

Hatching success

Of the 44 eggs, seven (Hooded Grebe) were collected during the first incubation period (1-7 days), 17 (Hooded Grebe) were collected during the second incubation period (8-14 days), and 15 (12 Hooded Grebe / 3 Silvery Grebe) were collected during the third incubation period (15-21 days). Five eggs (Hooded Grebe) could not be classified because they did not hatch therefore, we could not backdate the laying day (no evidence from observations of the colony). Mean egg weight for Hooded Grebe for the first incubation period was 23.70 ± 1.80 g (range = 20.41–26.44 g; n = 7), for the second incubation period, 21.98 ± 1.54 g (range = 18.87–24.42 g; n = 17) and for the third incubation period, 21.40 ± 1.51 g (range = 19.24–24.37 g; n = 12). Mean egg weight for Silvery Grebe, all of which were obtained in the third incubation period, was 18.26 ± 1.77 g (range = 17.00–20.28 g; n= 3). The hatching rate for all eggs was 73% (32/44) – 57% (4/7) for eggs collected in the first incubation third, 82% (14/17) in the second, and 93% (14/15) in the third (Fig. 3).

We documented transport time ranging from 16 minutes to nine hours. To analyze the effect of the incubation protocol on hatching, we discarded the eggs incubated with the middle temperature-high humidity (n = 4). The allocation of eggs among the other four protocols was as follows: seven eggs (low temperature-high humidity), four eggs (low temperature-low humidity), 17 eggs (high temperature-high humidity), and 12 eggs (high temperature-low humidity).

Using AICc for small sample sizes for model selection to assess the effect of variables on hatching success, the univariate model with the predictor variable of days of natural incubation at the time of collection (numerical) was selected as the best model (relative weight = 0.69, Table 1). However, no significant differences were detected (Z = 0.688, $p < 0.491$).

Mean daily egg-weight loss during incubation was 1.05 ± 0.35 % (range = 0.31–1.61%; n = 44). We did not detect significant differences in weight loss for hatched versus unhatched eggs (U = 266, df = 1, p = 0.673).

Survival in the initial stage

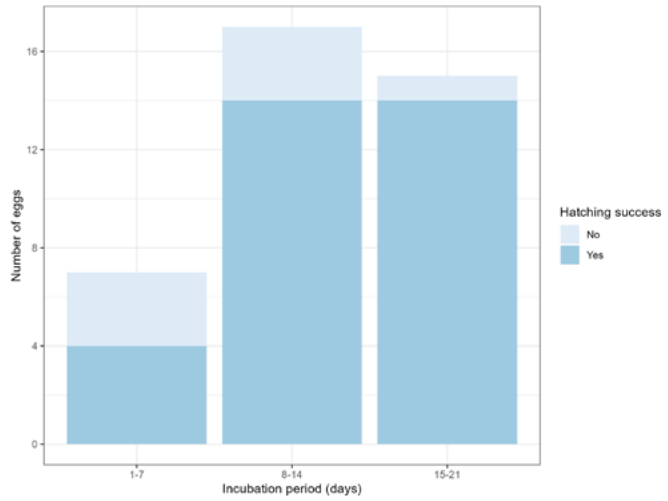

Figure 3. Hatching success of Hooded Grebe (*Podiceps gallardoi*) and Silvery Grebe (*P. occipitalis*) eggs as a function of the incubation period during which the egg was collected.

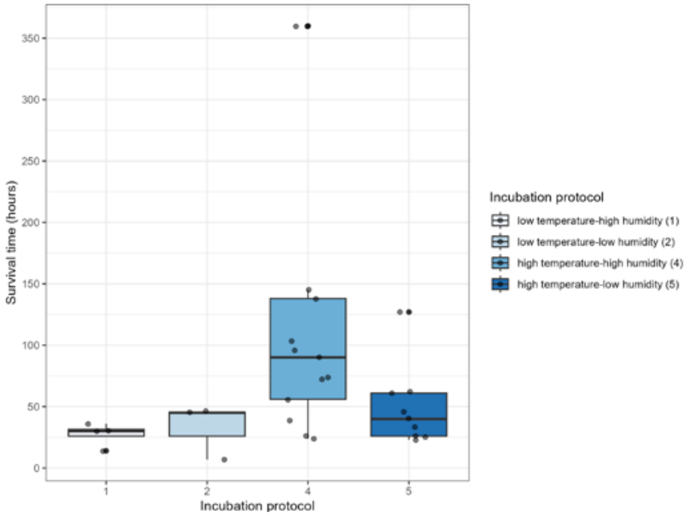

Figure 4. Graph made from raw data showing survival time of Hooded Grebe (*Podiceps gallardoi*) and Silvery Grebe (*P. occipitalis*) chicks according to the incubation protocol.

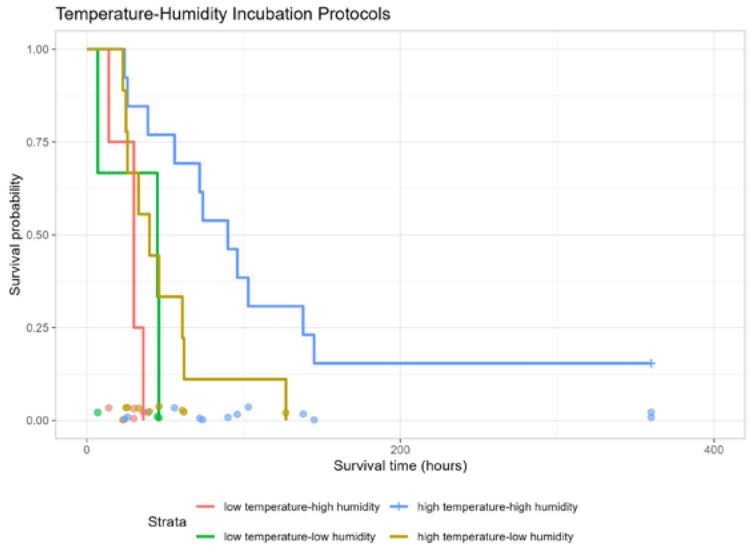

Figure 5. Kaplan-Meier survival curves comparing the survival probability of Hooded (*Podiceps gallardoi*) and Silvery (*P. occipitalis*) Grebes chicks hatched from eggs incubated with different temperature and humidity protocols. The censoring lines represent chicks that did not die before 15 days of age.

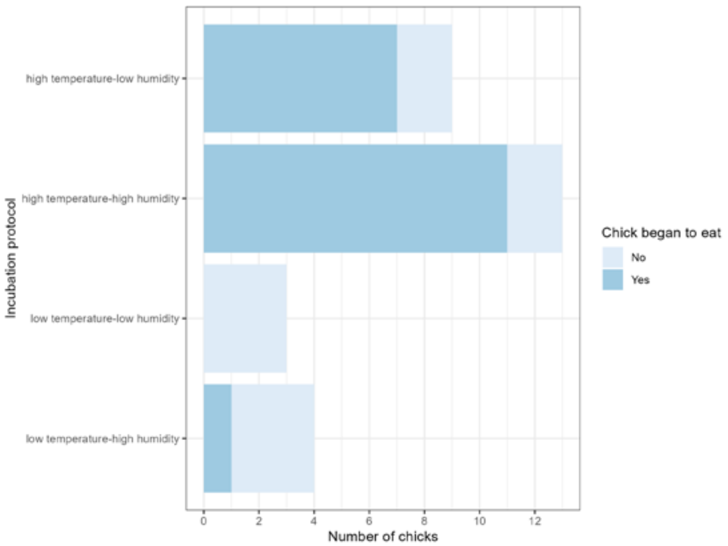

Figure 6. Proportion of Hooded Grebe (*Podiceps gallardoi*) and Silvery Grebe (*P. occipitalis*) chicks that never began to eat according to different temperature and humidity protocols.

Table 1. Multiple model selection analysis (AICc) values of the effect of incubation stage at collection time, transport duration, and incubation protocol on hatching success, including global and null models. Variables: Days_col: days of natural incubation at the time of collection, Transp_dur: duration of transport of eggs from the lakes to the rearing facilities, Incubation: incubation protocol.

Intercep.	Days_col	Transp_dur	Incubation	Days_transp	df	logLik	AICc	Delta	Weight
0.549	0.068				2	-19.081	42.5	0	0.689
3.636	-0.177	-0.008		0.001	4	-17.432	44.2	1.73	0.291
1.115		-0.002			2	-23.295	50.9	8.42	0.010
0.882					1	-24.786	51.7	9.16	0.007
0.288			+		4	-22.153	53.6	11.04	0.003

Thirty-two of 44 eggs hatched (i.e., 73%; 29 Hooded Grebe, 3 Silvery Grebe). The mean hatch weight for Hooded Grebe was 14.91 ± 0.85 g (range = 12.96–16.29 g; n = 29) and 12.28 ± 1.40 g (range = 11.33–13.88 g; n = 3) for Silvery Grebe. Mean survival time was 54.90 ± 35.94 h (range = 7.00–145.00 h; n = 29) for Hooded Grebe and 274.33 ± 148.38 h (range = 103.00–360.00 h; n = 3) for Silvery Grebe. Two Silvery Grebe individuals lived for more than 15 days; one died at day ~15 (362 h), and another was successfully released after 67 days (1608 h).

Mean survival time according to incubation protocol was 27.50 ± 9.43 h (range = 14.00–36.00 h; n = 4) for the low temperature-high humidity protocol; 32.67 ± 22.23 h (range = 7.00–46.00 h; n = 3) for the low temperature-low humidity protocol; 121.77 ± 112.18 h (range = 24.00–360.00 h; n = 13) for high temperature-high humidity protocol; and 49.22 ± 32.64 h (range = 23.00–127.00 h; n = 9) for the high temperature-low humidity protocol (Fig. 4). Kaplan-Meier survival analysis determined that there were statistically significant differences in survival between the different protocols (χ^2 = 14.3, df = 3, p = 0.003; Fig. 5). *Post hoc* comparisons using Bonferroni correction detected higher survival of chicks incubated with the high temperature-high humidity protocol compared to the low temperature-high humidity protocol (χ^2 = 9.3, df = 1, p = 0.013). Most chicks reared under the low temperature-high humidity (75%) and the low temperature-low humidity (100%) incubation protocols never began to eat, while only a few chicks in the high temperature-high humidity (15%) and high temperature-low humidity (22%) incubation protocols never began to eat (Fig. 6).

Mean survival time according to daily egg-weight loss was 35.70 ± 21.13 h (range = 07.00–84.00 h; n = 10) for interval 1, 103.53 ± 110.26 h (range = 24.00–360.00 h; n = 15) for interval 2 and 72.14 ± 39.16 h (range = 23.00–145.00 h; n = 7) for interval 3 (Fig. 7). The Kaplan-Meier survival analysis determined that there were statistically significant differences in survival between groups (χ^2 = 8.9, df = 2, p = 0.012; Fig. 8). *Post hoc* comparisons using the Bonferroni correction detected a lower survival of chicks from group 1 compared to group 2 (χ^2 = 6.5, df = 1, p = 0.032).

Mean survival time according to chick care frequency was: 208.00 ± 214.96 h (range = 56.00–360.00 h; n = 2) for scenario one h; 105.00 ± 43.95 h (range = 53.00–145.00 h; n = 5) for scenario two h; 53.50 ± 20.49 h (range = 40.00–84.00 h; n = 4) for scenario four h; and 89.73 ± 93.69 h (range = 23.00–360.00 h; n = 11) for scenario none (Fig. 9). There were no significant differences in recorded survival times among the different night care scenarios (H = 3.76, df = 3, p = 0.288). In the comparison between chicks with or without night feeding, mean survival time was 134.43 ± 107.31 h (range = 53.00–360.00 h; n = 7) in the night feeding group and 80.07 ± 81.46 h (range = 23.00–360.00 h; n = 15) in the group without night feeding. We did not detect significant differences in survival time between both groups (p = 0.21).

After conducting necropsies, we suspect that most of the nestlings died from multiple organ failure due to compression damage caused by the ‘bloat’ (i.e., accumulation of gas in the stomach). One of the chicks had its stomach pierced by a feather, and one, which died at 362 hours, was due to gizzard impaction.

DISCUSSION

Working on the conservation of endangered species often limits the number of case studies, making it impossible to develop balanced experimental designs. This makes it difficult to arrive at accurate statistical conclusions. However, the results of this work show clear trends that suggest the right direction to follow in the search for an *ex situ* breeding protocol for the Hooded Grebe.

Although we did not detect significant differences, we found that the hatching rate in eggs collected at advanced stages was notably higher than that of those gathered at early stages (93% vs. 57%). This agrees with the literature, which states that some bird species reared in captivity have a higher hatching success *ex situ* if they have a natural incubation period (MacVean 1988, Gage & Duerr 2007). This suggests that collecting eggs at a later stage of incubation would be preferable, but due to the low probability of success for some Hooded Grebe colonies (e.g., colonies exposed to strong windstorms, lakes with low water levels, and high evaporation rates), spending more time on the lake increases the probability of losing both eggs. For the time being, we have achieved the hatching success of eggs incubated in the wild for only five days. Finally, it’s worth noting that our egg collections did not influence the reproductive success of any of the manipulated colonies (i.e., the parents raised one chick per nest).

An extremely valuable result for the future is that the transport method we used proved to be efficient. There were no differences in hatching success between longer and shorter duration trips, which increases the potential range of lakes from which to collect eggs.

During incubation, loss of about 10-20% of the egg's initial mass is essential for maintaining the egg's relative water content and forming the air cell (Ar & Rahn 1980, Davis et al. 1984). Eggs that lose too much or too little water have a decreased probability of hatching (Hoyt 1979, Davis et al. 1988) and can affect the viability of the chicks (Gage & Duerr 2007). We did not detect significant differences in mass losses between hatched and unhatched eggs; however, we observed that chicks hatched from eggs with daily mass losses below 1% had lower survival rates. This finding suggests that Hooded Grebe eggs could lose 21% or more of the initial mass.

An important variable for the proper development of the chicks is the incubation parameters (temperature and humidity), as they influence embryonic growth and the body condition of chicks (Barri 2008, DuRant et al. 2010). We did not find differences in hatching success between proposed incubation protocols with high and low temperatures and humidities. However, significant differences in chick survival were observed in relation to the incubation protocol. Chicks hatched using a high-temperature and high-humidity protocol had higher survival rates compared to those incubated at a low temperature and high humidity. Nevertheless, the most important thing to highlight is that none of the chicks that hatched from eggs incubated at low temperatures began to feed.

Although we did not obtain conclusive results, we observed a tendency for higher survival in chicks that were fed more frequently. Grebes are susceptible to the accumulation of gas in the stomach, usually called 'bloat', with the consequent compression of organs that leads to the death of the individual due to multiple organ failure (Gage & Duerr 2007). This ailment was the cause of death for the majority of chicks in this study. These results suggest the need for nearly constant feeding to maintain gastrointestinal motility (Barrett et al. 2010) and prevent gas accumulation (Gabarain 2021). This extraordinarily high and constant feeding rate is consistent with behavioral observations of individuals in the wild (Roesler 2016).

Nutritional requirements for grebes have not been established yet. As precocial birds, they need to be fed live prey, such as small insects, to stimulate foraging behavior (Padilla 2014). Hooded Grebe chick diet, estimated from the isotopic signature of two individuals found dead at a lake, is composed of a mixture of food items, 78% of which consists of chironomid larvae, and the remaining 22% was made up of a combination of amphipods, copepods, and *Daphnia* sp. (Lancelotti 2009). In addition, it has been observed that parents frequently give feathers to the chicks

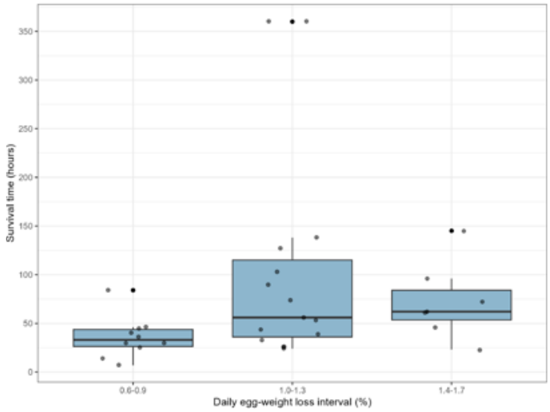

Figure 7. Graph made from raw data showing survival time of Hooded Grebe (*Podiceps gallardoi*) and Silvery Grebe (*P. occipitalis*) chicks according to the daily egg-weight loss interval.

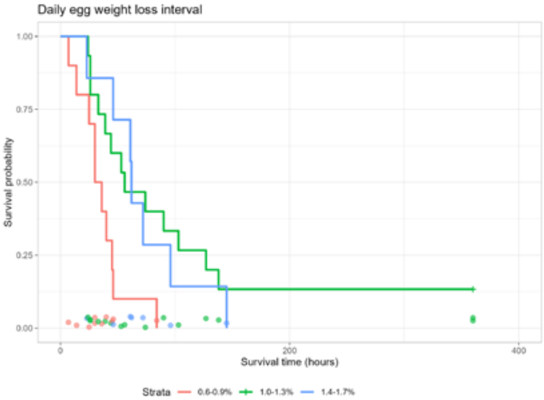

Figure 8. Kaplan-Meier survival curves comparing the survival probability of Hooded (*Podiceps gallardoi*) and Silvery (*P. occipitalis*) Grebes chicks hatched from eggs with different egg-weight loss. The censoring lines represent chicks that did not die before 15 days of age.

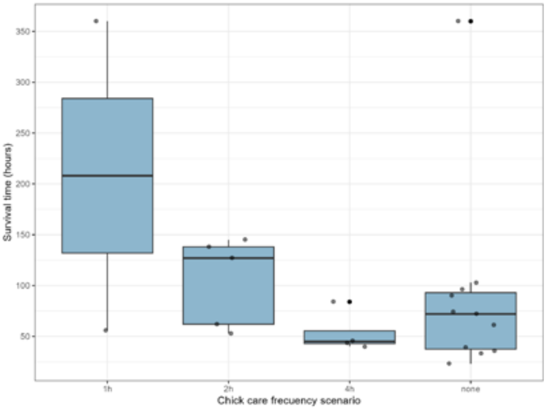

Figure 9. Graph made from raw data showing survival time of Hooded Grebe (*Podiceps gallardoi*) and Silvery Grebe (*P. occipitalis*) chicks according to the chick care frequency scenario.

soon after hatching (Roesler 2016), a behavior also observed in all the grebe species of the genus *Podiceps* (Fjeldså 2005). In this study, the administration of different foods was not standardized and was mainly based on the resources available in the lakes. For this reason, we excluded this variable from the analyses.

In summary, we describe the first *ex situ* hand rearing trials of Hooded and Silvery Grebe, where we successfully reared and released a single Silvery Grebe. At the time of release, the individual was already exhibiting natural behaviors typical of the species (i.e., fishing, diving, grooming). We released it into a lake used for fishing large trout, which ensures that it provides food for grebes. Additionally, this lake is often used by some grebes as a wintering site. On the release day, there were more than 700 Silvery Grebes in the lake. We marked the individual with an orange ring on the right leg, but unfortunately, we have not seen it again. The large number of Silvery Grebes on the plateaus makes locating and identifying specific individuals difficult. Currently, we are collaborating with universities and other partners on the development of a suitable transmitter for grebes. However, it is a challenging task the transmitter needs to weigh only a couple of grams, with a battery life prepared to support extended periods in cold, fresh, and saltwater, among other things.

Although the results are preliminary, mainly due to the low number of events analyzed, we observed tendencies in the different variables considered that could indicate how future experiments should be designed. It should be noted that when working with a species with a reduced population (less than 400 breeding pairs) and with extreme variability in reproductive attempts and success amongst seasons (seasons without colony formation), it is not possible to guarantee an adequate number of cases for carrying out sophisticated analyses (especially in terms of statistical treatment). It is also very ambitious to propose a static experimental design. It is valid to consider conducting some experimental tests with the Silvery Grebe, which can help refine procedures and methods. However, this may have its limitations due to the degree of specialization of the Hooded Grebe to the plateau environment; the information collected with the Silvery Grebe may not be directly applicable to the Hooded Grebe. Considering the critical situation of the Hooded Grebe, any conservation action requires extreme adaptability. Urgent need for results (in the short term) requires that the protocols be adapted not only based on statistically significant results but also on empirical information and even anecdotal data. This urgency makes it even more challenging to obtain large sample sizes. It would be extremely useful to have a quantity of data that would allow multifactorial analysis to evaluate how the different variables, currently analyzed independently, interact to affect both hatching success and chick survival. Understanding the effects of these interactions may help maximize the survival success of this imperiled species.

Next steps and management

So far, we have learned how to retrieve eggs from nests and resolve the logistics of safely transporting them to breeding facilities, but it is important to determine the minimum natural incubation time required to proceed safely with artificial incubation, given the possibility of recovering all of the eggs from colonies at risk of destruction at any time from laying. We have made progress in the incubation process, resulting in the hatching of chicks with a reasonable degree of maturity (Gabarain 2021). However, the safe range of egg mass loss for the species is still to be determined, which is essential for the hatching of healthy chicks (Gage & Duerr 2007). The next step is to prevent the two leading causes of death in chicks: starvation and bloat. Chicks may require some form of early stimulation to encourage them to start eating. Additionally, more assiduous feeding (perhaps every 30 min) could prevent gas buildup by stimulating gastrointestinal motility. Finally, finding a highly digestible, balanced feed that reduces bloating and facilitates feeding logistics is essential.

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