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ARTIFICIAL INCUBATION OF THE HORNED GUAN *OREOPHASIS DERBIANUS* (AVES: CRACIDAE) EGGS

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RESUMEN

La incubación artificial es una técnica favorable en los programas de reproducción de especies amenazadas o en peligro de extinción. Sin embargo, la implementación de un método para monitorear el proceso de incubación es de suma importancia. Los parámetros físicos importantes que influyen en el éxito de la eclosión del huevo y que requieren ser contralados son la temperatura y la humedad relativa de acuerdo a las condiciones de la región. Como una alternativa a la incubación natural del pavón (*Oreophasis derbianus*) utilizamos la técnica de incubación artificial por medio de incubadoras. Dos métodos fueron utilizados para monitorear el proceso de incubación de los huevos: pérdida de peso y densidad, considerando una pérdida final del 15 % del peso fresco de los huevos. La nidada fue de dos huevos, puestos a intervalos de dos días. En un período de 4 años (1997-2000) cinco hembras pusieron un total de 30 huevos, cuatro en 1997, seis en 1998, seis en 1999 y 14 en el año 2000; los huevos fueron puestos en los meses de marzo, abril, mayo y junio. Tres huevos fueron puestos desde la percha y se rompieron, los cuales no son considerados en los análisis. En promedio los huevos pesaron 161.04 ± 9.87 g (n = 24) y midieron 85.57 ± 3.16 mm x 57.52 ± 1.31 mm (n = 15). La densidad y volumen promedios de los huevos fueron 1.08 ± 0.036 g-cm^3 y 142.40 ± 10.46 g-cm^3 (n = 15), respectivamente. De 24 huevos, 15 huevos fueron fértiles (62.5 %), de los cuales lograron nacer siete polluelos, equivalente a un 46.6% de eclosión. El proceso de eclosión en dos huevos duró de 14 a 19 horas, respectivamente. Los huevos fueron incubados con parámetros de temperatura de 37.5°C y de 53-56 % de humedad relativa (28.8-30.1°C), con un periodo de incubación de 33 a 35 días. En ocho casos, los embriones alcanzaron desarrollo completo, pero probablemente debido a problemas en el control de la humedad la eclosión no fue exitosa. Para nuestro conocimiento, estos son los primeros datos publicados sobre los parámetros de incubación aritificial de huevos de pavón.

Palabras Clave: *Oreophasis derbianus*, pavón, incubación artificial, nidificación en cautiverio, crácidos, conservación *ex-situ.*

ABSTRACT

Artificial incubation is a favorable technique for reproduction programs of threatened or endangered species. However, implementing a method for monitoring the incubation process is highly important. Temperature and humidity are key physical parameters for egg incubation leading to successful hatching. We used artificial incubation as an alternative to natural incubation of the Horned Guan *Oreophasis derbianus.* Weight and density loss were used to monitor the incubation process of the eggs, considering a final weight and density loss of 15%. Before incubation, the eggs weighed an average of 161.04 ± 9.87 g (n = 24) and measured 85.57 ± 3.16 mm x 57.52 ± 1.31 mm (n = 15). Density and volume averages of eggs were 1.08 ± 0.036 g-cm3 (n = 15) and 142.40 ± 10.46 g-cm3 (n = 15), respectively. Of 24 eggs, 15 (62.5 %) were fertile, and seven resulted in successful hatching (46.6 % hatch). The pip to hatch time in two eggs was 14 y 19 hours, respectively. The eggs were incubated at a temperature of 37.5°C (dry bulb) and

a relative humidity of 53-56% (28.8-30.1°C) (wet bulb) throughout the 33-35 day incubation period. In eight cases, the embryos reached full development but failed to hatch successfully, likely due to improper humidity levels. This is the first published record of Horned Guan artificial incubation parameters and its monitoring process.
Key Words: *Oreophasis derbianus*, Horned Guan, artificial incubation, captive breeding, cracidae, *ex-situ* conservation.

INTRODUCTION

Artificial incubation of wild bird eggs may be an important part of those management and conservation programs, involving rare, threatened or endangered bird species (Kuehler *et al*.1994, Kuehler *et al*. 1996). This incubation type can be used to maximize the potential number of chicks produced in a breeding season by removing the first clutch from the nest to stimulate the female to lay additional clutches (Kuehler *et al*. 2000, Olsen & Clubb 1997). Artificial incubation is also applied when the parents do not naturally incubate in captivity, or when extreme environmental conditions or illnesses are presented (Snelling 1972, Burnham 1983, Kuehler *et al*. 1993). The main benefit to artificial incubation is captive management of birds to increase the number of individuals.

During incubation, all eggs loose weight through water loss due to diffusion of water vapor through the shell (Rahn & Ar 1974, Ar *et al*. 1974). The rate of mass loss is influenced by the geometry, number and structure of pores, and shell thickness. The pore geometry of the shell defines water vapor conductance, as well as the existing water vapor gradient between the inside of the shell and the microenvironment surrounding the egg (Rahn & Ar 1974, Ar *et al*. 1974, Ar & Rahn 1980, Ar 1991).

Temperature and humidity requirements differ among eggs of different bird´s species, and can vary with both the microclimate inside the incubator and the incubation room (Olsen & Clubb 1997). The difference in egg mass, from the time the egg is layed until the chick breaks into the aircell, can be controlled by manipulating the humidity and temperature in the incubator. During natural and artificial incubation, eggs lose approximately 18 and 20% of their initial weight at lay, respectively (Rahn & Ar 1974).

Parameters such as temperature, relative humidity, weight loss and egg density are very well-known in the poultry industry, and for some bird's species of the families Phasianidae, Psittacidae, Anatidae, Struthionidae, Rheidae, Spheniscidae, Drepanidinae, Corvidae, Turdidae (Harvey 1993, Hoyt 1976, Kuehler *et al*. 1994, 1996, 2000, 2001), but they are not very well-known for most avian species, including certain members of the family Cracidae, such as the Horned Guan. Some authors suggest, a temperature of 37.6 °C and 55% relative humidity for incubating cracid eggs in captivity (Todd *et al*.1992, Estudillo-López 1997). The percentage of weight loss during successful artificial incubation depends upon the species; for example in Curassows of the genus *Crax* and *Pauxi*, weight loss ranges from 10-22% (Todd *et al*. 1992).

The Horned Guan is a member of the Cracidae family and it is limited to few isolated mountains ranges in Mexico and Guatemala. Horned Guan clutches consisted of two eggs, with a two day interval between eggs laid. In a four year period (1997-2000), five captive females laid a total of 30 eggs, four in 1997, six in both 1998 and 1999, and 14 in 2000. Eggs were laid during the months of March, April, May and June. Three of the eggs were laid from the perch and broken, and were not included in analyses.

Horned Guan is critically endangered species and an immediate conservation priority due to habitat destruction and hunting pressure is commonable (Brooks & Strahl 2000). In these critical cases, artificial incubation can be used as a conservation strategy to increase individuals in captivity for reintroduction to bolster wild populations.

In the present work, we provide results from a artificial incubation program of Horned Guan eggs in Monterrey, Nuevo León, Mexico, during 1997-2000. Lacking any information available on artificially incubated Horned Guan eggs, this study provides detailed descriptions of successful and unsuccessful hatchings. Successful techniques for hatching are essential elements in captive breeding endangered birds. However, during the initial phases of protocol development for species such as Cracids, in the absence of published information on successful artificial incubation parameters, initial attempts to hatch Horned Guan´s eggs are considered experimental. This is the first published record of Horned Guan artificial incubation parameters and its monitoring process.

METHODS

Breeding stock

The eggs were collected from captive individuals (3 males and 5 females) in the facilities of Fundación ARA, A. C[1]., located to the southeast of Monterrey, Nuevo León, Mexico. The breeding stocks of guans were kept in planted aviaries of soil substrate measuring 12 x 3.5 x 4 m. The aviaries contained an abundance of herbaceous bushes and climbing's plants, as well as perches of different diameter and length, which were distributed the length and width of each enclosure. Nesting opportunities were provided by mounting three artificial nests (2 to 2.5 m high): two nests (wood boxes, 40 x 40 x 10 cm) located in protected corners of the aviary and one rounded basket concealed by vegetation. Each nest was covered with dry grasses. Each aviary had its own irrigation system with misters housed in the roof.

Egg processing and incubation

Most of the eggs were removed from nests when laid. On seven occasions eggs were laid on the ground or from the perch. Surgeon's gloves were worn to handle eggs and eggs were transported to the incubation room in a small box protected with cotton. In the incubation room the eggs were weighed in a Acculab V-333 scale, measured

[1-] Due to Fundación ARA, A. C. facilities closing, the Horned Guan founder group and its descendants were moved to Africam Safari Zoo facilities located in Puebla, México, on October 17, 2000.

with a Mitutoyo digital vernier callipers, and disinfected with an 1% solution of iodine in water at a temperature of 28-30°C, before placing them in the incubator (Olsen & Clubb 1997). Eggs with small crack were repaired using white nail polish. Three eggs were laid from the perch and broke, and therefore, were neither placed in the incubator nor considered in the analysis.

All eggs were set in a forced-air incubator (Humidaire model 21; Humidaire Incubator Co., New Madison, OH, U.S.A.) and were artificially incubated and hatched following protocols and parameters previously used for other Cracids: 37.5°C (dry bulb) and 53.7-56.3% relative humidity (wet bulb) (Anderson & Robbins 1979, Todd *et al*. 1992, Estudillo-López 1997, Harvey 1993, Woodard *et al*. 1993). We took into account the natural incubation period for wild Horned Guans, which has been estimated at 35-36 days (González-García 1995). Most of the eggs were set on their sides and automatically turned 24 times/day at 60 min intervals during the entire incubation process, except two or three days before shell pipped, where upon the turning was suspended (Anderson & Robbins 1979, Harvey 1993).

Egg development

Egg fertility defined as by evidence of embryonic development was determined during the first week of incubation by inspecting blood vessels in each egg with a commercial egg "candler". Infertility was again assessed halfway through the incubation period if no evidence of embryonic development or blood vessels was established. Infertile eggs were extracted from the incubator, and the shells were retained for the collection in Fundación ARA, A.C.

Two methods were used *a priori* and *a posteriori* for monitoring the incubation of the eggs. In most eggs, application of both methods was a priori, except in the number 5 egg in which the analysis was *a posteriori*. The first method was weight loss, determined from the first day of incubation until the chick pipped into the aircell. Expected weight loss was calculated assuming a final loss of 15% of the laid egg weight, divided by the period of incubation (33-35 days), and multiplied by the interval of time monitoring the weight loss (every two days) (Harvey 1993, Woodard *et al*. 1993, Olsen & Clubb 1997). The second method used was the density loss, which was obtained by dividing the laid mass of the egg by its volume. The volume of the egg was obtained by the equation: $V = 0.51\ LW^2$; where L = length, W = width (Hoyt 1976, Grant 1982, Harvey 1993).

Monitoring density loss is similar to monitoring weight loss, and has the advantage of predicting the humidity the egg will need at the beginning of incubation (Paganelli *et al*. 1974, Anderson & Robbins 1979, Harvey 1993, Woodard *et al*. 1993). As a general rule, a thick-shelled egg or one of higher density will need lower humidity, and a thin-shelled egg or one of lower density will be incubated with higher humidity (Harvey 1993).

Analysis

We used ANOVAs to determine differences in egg weight and size among the samples obtained in captivity, and a Tukey test was used for multiple comparison of the corresponding mean. To compare measurements of eggs of wild and of captive origin we used the non-parametric Mann-Whitney test due to the small size of the sample (Zar 1984).

Statistical analyses were carried out using SigmaStat for Windows version 2.0 (Systat Software Inc.). All values are reported as mean ± standard deviation and statistical signifance was set at $P < 0.05$.

RESULTS

Weight, size and density of eggs

The average clutch size in captivity was 1.87 ± 0.34 eggs, which is the range per clutch, although females laid one to four eggs during the annual reproductive period. The Fundación Ara´s Horned Guans laid a total of 30 eggs between March-June. Eggs weighed 161.80 ± 9.51 g (n = 24), and mass differed between years ($F_{3,20} = 6.344$ P =0.003). The eggs of 1999 and 2000 were heavier and were less variable than eggs of 1997 (Tukey: P <0.05). The mean egg length from captive birds was 85.57 ± 3.16 mm (n = 15), versus 83.94 ± 1.13 mm (n = 6) in wild eggs (González-García 1995, Méndez 2000), with no significant differences between the samples (U = 21, $n_1 = 15$, $n_2 = 6$, P = 0.06). The mean egg width from captive birds was 57.52 ± 1.31 mm (n = 15), versus 58.46 ± 1.02 (n = 6) in wild eggs (González-García 1995, Méndez 2000), with no significant differences between the samples (U = 25, $n_1 = 15$, $n_2 = 6$, P = 0.12). The eggs obtained from captive birds did not differ in length ($F_{2,12} = 0.751$, P = 0.493) or width ($F_{2,12} = 1.645$, P = 0.234) between years. The mean egg density obtained from captive birds was 1.08 ± 0.036 g-cm^3 (n = 15), and mean volume was 142.40 ± 10.46 g-cm^3 (n = 15). The shells of fertile and infertile eggs had a mean dry weight of 18.9 ± 3.16 g.

Fertility, weight and density loss

Of 24 eggs, 15 were fertile (62.5%), and 9 were infertile (37.5%). Three were laid from the perch and broken, and therefore were not included in analyses. However, at least one egg laid from the perch could have been fertile, since it came from the same clutch of a fertile egg. Although, no in all cases, both eggs laid were fertile. Fertility rate was higher during the last two years of the study (Table 1).

Table 1

Summary of the number, viability, weight and size of captive Horned Guan eggs (1997-2000).

Year	# egg laid	# fertile eggs	# eggs hatched	Egg weight (g)	Egg length (cm)	Egg width (cm)
1997	3	1	0	147.4 ± 13.2	84.4 ± 2.9	56.3 ± 1.4
1998	6	2	1	159.1 ± 6.7	84.9 ± 4.3	57.9 ± 1.1
1999	6	6	2	162.4 ± 5.6	86.8 ± 1.7	57.7 ± 1.2
2000	9	6	4	168.0 ± 6.2	ND	ND

In 1997 the Horned Guans laid three eggs on ground, including one fertile (Table 1). The fertility in 1997 was 33.3%, but none of the eggs hatched (Table 1). The fertile egg of 1997 was incubated for nearly one month, but the embryo died early probably due to contamination by fungus through a fracture in the shell. The embryo length was 26.38 mm and probably two embryonic growth weeks old. In 1998, a total of six eggs were laid, of which two were fertile (33.3% fertility). One of the two fertile eggs hatched (Table 1). In 1999 six eggs were laid, with a substantial increase in fertility (100%), of which two hatched (33.3% hatching rate) (Table 1). From 1999 to 2000 fertility decreased from 100 to 70%. In 2000, six eggs of 9 were fertile, and hatching rate increased (Table 1). Additionally unpaired females laid four eggs. One of the females hatched at Fundación ARA, A. C., laid her first egg at nearly two years of age. Various females laid infertile eggs and the infertility in eggs varied among females and in a same clutch.

Artificial incubation of eggs

Fertile eggs with similar weight showed contrasting incubation requirements, with incubation periods ranging from 33 to 35 days. Incubation parameters of these eggs are shown in Table 2. The observed weight loss from egg no. 5 moved substantially away from the expected (Fig. 1a). The weight loss should be 24.03 g to the end of incubation and not almost 30.6 g (19% of its initial weight). On average this egg lost 1.61 ± 0.45 g every other days, resulting in a final mass that is two grams away from expected (Fig. 1a).

Table 2

Mean of laying weight, incubation period, incubation temperature, relative humidity, total weight loss observed, initial and final density observed of Horned Guan's eggs.

Fertile eggs	n	Mean weight fresh eggs(g)	Mean incubation period	Mean incubation temperature °C	Mean % Relative Humidity	Mean total weight loss observed (g)	Mean initial density (g/cm3)	Mean final density (g/cm3)
Hatched	7	164.1 ± 5.9	33.4 ± 1.2	37.4 ± 0.30	53.8 ± 1.3	24.6 ± 1.6	1.11 ± 0.31	0.95 ± 0.26
Failed	8	161.3 ± 13	33.4 ± 1.6	37.47 ± 0.08	54.4 ± 1.2	22.2 ± 5.5	1.08 ± 0.36	0.94 ± 0.49

After 37 days of incubation a necropsy was performed on this egg. The chick developed and grew to hatch size, however the yolk sac was not fully withdrawn into the abdominal cavity. It is probable that by using low humidity (56.5% relative humidity), the egg underwent an excessive decrease of weight, which can generate a quick rate of development, dehydration, and consequently the embryo did not have enough lubrication to break the shell. The embryo becomes stuck to the internal membranes of the egg, causing death before pipping the aircell (Anderson & Robbins 1979, Olsen & Clubb 1997). *A posteriori* analysis of 15% weight loss showed that this egg should lose less weight (Fig. 1a). At the beginning with 58.9% of relative humidity (30.2°C), the egg weight loss nearly matched the expected weight loss, however, mass loss tended to deviate from expected later in incubation (Fig. 1a). The analysis *a posteriori* of the egg density loss, showed a similar behavior to weight loss. This loss stayed below the expected density indicating that the applied relative humidity was too low, resulting in a higher loss of density. The initial density of the fresh egg was also considered a low value, which suggests a higher value of relative humidity should have been used to counteract the initial loss of density to fit the curves (Harvey 1993). The contrary seems to have occurred among other eggs in which hatching was not successful. Measured densities suggested that humidity levels were too high (Fig. 1b,c,d).

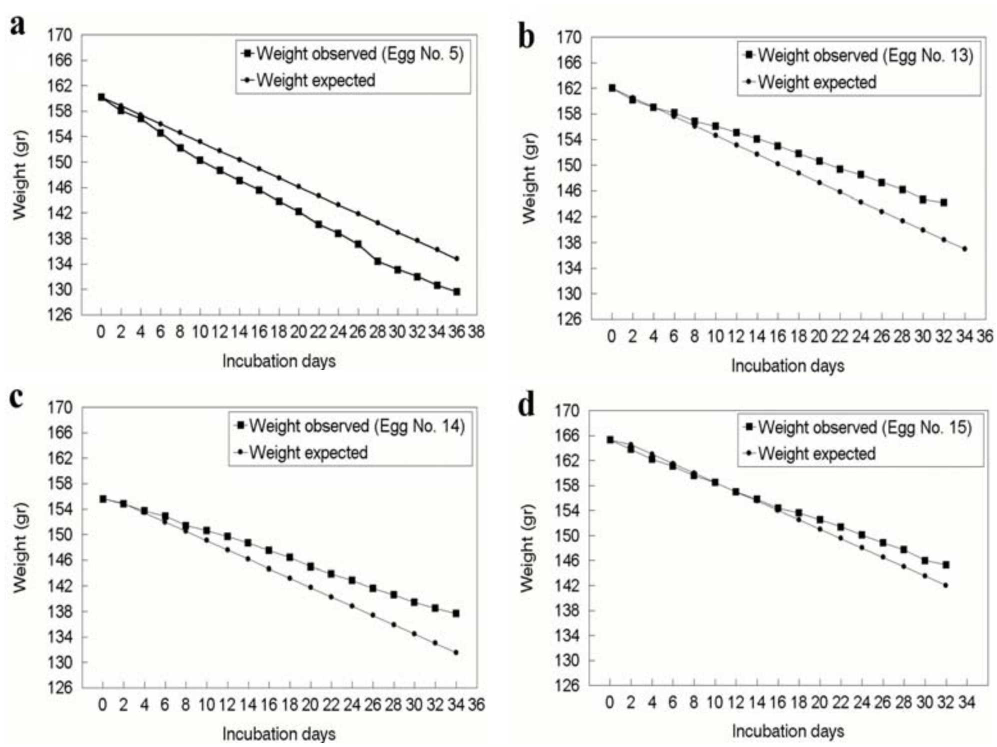

Figure 1

Weight loss in four viable Horned Guan eggs. Note that in a it was necessary to apply higher values of humidity, and in b, c, and d it was necessary to apply lower levels so that the observed curves fit the expected ones.

In contrast to the egg no. 5, the weight and density loss and humidity of the seven hatched eggs (Fig. 2 and 3) were monitored *a priori* in 1998-1999. Data on incubation, temperature and relative humidity are indicated in the Table 2. Total weight loss at the end of the process was of 23.2 to 24.2 g (14.52-15.07 %), very similar to total weight loss expected. On average weight loss was of 1.30 to 1.39 g which is very similar to the expected weight loss. The estimated weight loss was of 1.40 to 1.44 g with intervals of two days, and of 0.70-0.72 g daily. The weight loss observed of each eggs was adjusted to fit the expected curve, by manipulating the relative humidity (Fig. 2a-2c). Higher weight loss ocurred at the beginning and toward the end of incubation (Fig. 3).

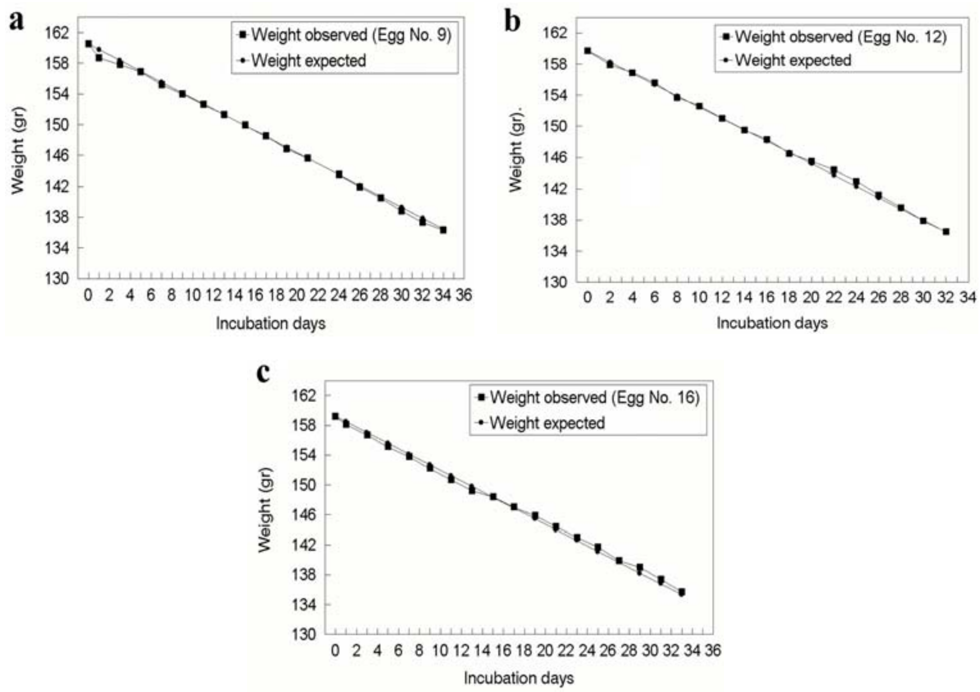

Figure 2

Observed weight loss in three viable Horned Guan eggs (1998-1999). Note the almost perfect fit between the expected weight loss and the observed loss.

Hatching began at 33-35 days of incubation at which time the humidity was increased to 58.9% (30.2°C) as suggested (Anderson & Robbins 1979, Grant 1982, Harvey 1993). Hatching, from aircell entry to emergence from shell, in two eggs lasted 14-19 hours, respectively. Two chicks had hatch weights of 101.8 and 94.5 g respectively.

Density loss presented a very similar pattern to weight loss. On average density loss was 1.01 to 1.08 g/cm^3, similar to the estimated density for 33-35 days of incubation (Table 2). After hatch, chicks remained in the incubator for 48 hours at the reduced humidity of 55 % (28.2°C wet bulb). At 48 hr chicks were transferred to a wooden box 61 x 91 x 61 cm with a 60 watt light bulb at 32° C. They received food at 48 hours post-hatch, consisting of a granulated balanced food (Roudybush), fruits of the season (papaya, melon, apple, lettuce), wild fruits of Anacua (*Ehretia elliptica*: Boraginaceae). The chicks were fed with the help of a puppet mimicking the head of adult Horned Guan.

In the year 2000, with four successful eggs (Table 1), the percentage weight loss observed was 14.8 ± 0.68 % (range 14.2-15.6%). Overall the eggs lost 24.8 ± 1.46 g on average (range 22.7-25.8 g), which is very near the expected weight loss (25.1 ± 0.9; Fig. 3). The incubation period ranged from 33 to 35 days. Although temperature stayed constant (37.5°C), the state of humidity varied within certain limits during the incubation period, with one exception (Fig. 3).

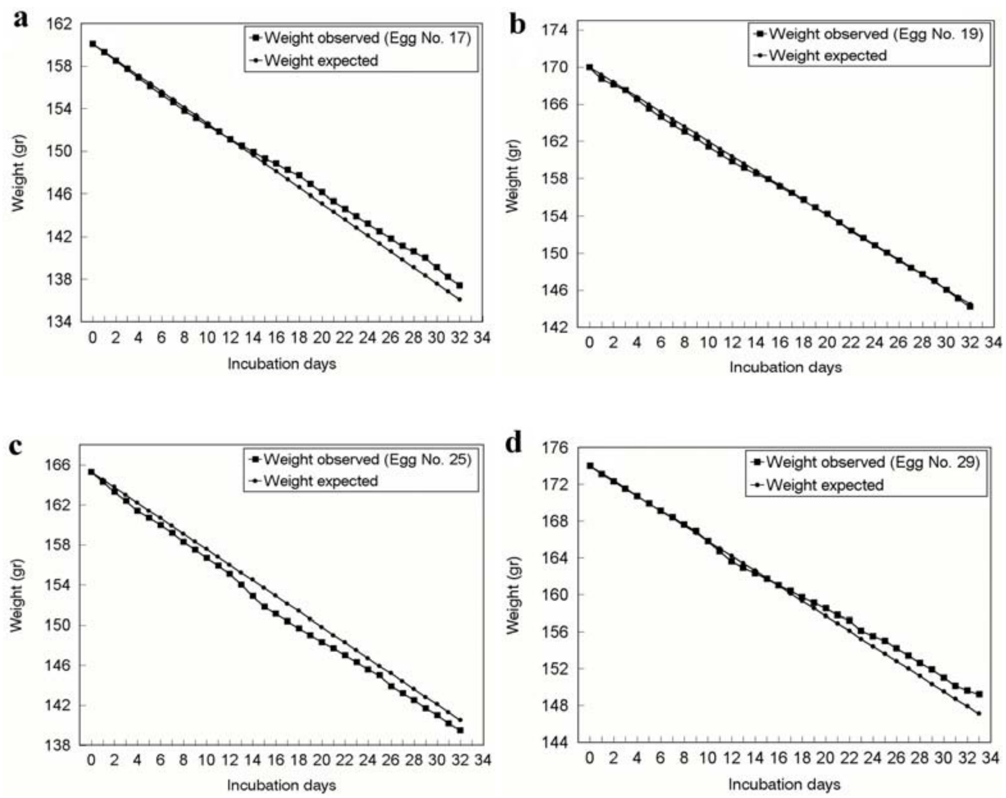

Figure 3

Observed weight loss in four viable Horned Guan eggs (2000). Note that contrary to Fig. 2, the fit between expected and observed curves was not so precise, get successful hatching was achieved.

DISCUSSION

The two-egg clutch, two day laying interval, and egg size were all similar to those of wild guans (González-García 1995). Apparently age, time in captivity and first lay does not influence egg size in Horned Guans, as in other captive species whose first eggs are generally smaller (Anderson & Robbins 1979). However, some differences in the weights of these eggs was observed. The first year eggs (1997) were of lower weight than those laid during the last two years (1999-2000). This increase in weight is a regular phenomenon in captive birds, and is probably related to age and diet (Anderson & Robbins 1979) or maybe the birds got used to the environments after the first year.

The period of incubation in captivity ranged from 33-35 days, similar to the estimate of 35-36 days for wild Horned Guans (González-García 1995) and the allometric incubation equation of Rahn & Ar (1974).

The important physical parameters to consider during incubation are temperature, humidity and air flow, since evaporation rate depends upon this factors (Anderson & Robbins 1979, Olsen & Clubb 1997). Temperature control is critical, especially during the first one-third of the incubation period (Olsen & Clubb 1997, Deeming & Ferguson 1991). The temperature with the highest percentage of successful hatching in other species is 37.5°C (White & Kinney 1974, Anderson & Robbins 1979) and this is also an acceptable temperature for incubating Horned Guan eggs. Although some temperature variation was present from the beginning of incubation; for example in egg number five, there was no evidence that temperature variation was a critical factor in embryo development, when the temperature difference did not exceed 0.5°C. This is consistent with previous studies showing that temperature elevations exceeding 1.0 °C can have negative effects on the embryo, mainly in the early stages of incubation, when the embryo is more suceptible to trauma (Anderson & Robbins 1979, Olsen & Clubb 1997, Deeming & Ferguson 1991).

Horned Guan eggs had differences in weight and density, due to different incubation patterns. Weight loss followed a sigmoidal curve, similar to other birds species (Anderson & Robbins 1979). Similar patterns of weight loss rates have been reported in other birds species, where higher loss is experienced at the beginning and toward the end of the incubation period (Anderson & Robbins 1979, Olsen & Clubb 1997).

The differences in the percentages of fertility and hatching between the first two and the last two years are possibly a reflection of management or increased maturity of the birds. Fertile eggs that did not hatch may have been the result of various deficiencies inhibiting embryonic development, such as genetics or other factors (Burnham 1983, Harvey 1993, Kuehler *et al*. 2000).

A fertile egg will not always hatch, and viability will depend on several factors, including age of parents, egg quality, the egg´s microclimate, and parental or artificial regulation of egg temperature before and during incubation, and egg turning (Harvey 1993, Anderson.& Robbins 1979). Higher mortality in damaged or faulty eggs occurs towards the end of the incubation process (Anderson & Robbins 1979). Of the

Fundación Ara Horned Guans, six embryos died at the end of incubation, and at least two died in the early stages of incubation. Although fertility generally increased throughout the study, the hatch rate did not.

Although temperature is an important factor, humidity is also critical. For example, of the eggs that failed to hatch successfully, humidity should have been higher for egg number five, and lower in the others (Fig. 1). In most of the cases the eggs experienced excess humidity, causing embryo deaths. Higher humidity levels during incubation result in eggs with smaller air cells, and soft embryos with increased incidence of poor umbilical seal and exposed yolk sacks (Olsen & Clubb 1997). Corresponding necropsies indicated excessive liquid inside the egg, broken air cell and embryos developed with liquid in the coelomic cavity, windpipe, lungs and oral cavity, and edema in the neck. Likewise, in one case a yolk sac with 50% absorption resulted in hemorrhaged and ruptured blood vessels, and in two cases the yolk sac was wrapped around the leg. During the final one-third of its incubation period, the embryo swallows the amniotic fluid and the remains of the albumen are absorbed. If both fluids are excessive the embryo can drown. Such problems are caused by inadequate humidity control (Anderson & Robbins 1979, Harvey 1993). Hatching rate will be higher if humidity stays within proper limits (Anderson & Robbins 1979); in Horned Guans the appropriate relative humidity seems to be 53-56% (28.8-30.1 °C). Harvey (1993) has previously shown that control of humidity and temperature is critical during the first third of incubation. Otherwise it is very difficult to change the rate of weight loss of the eggs, and in consequence the embryos can experience early or late death, either from an excess or lack of humidity, as probably happened in 1998 and 1999 (Fig. 1).

Low relative humidity permits weight and density to be kept within expected limits. A large egg will lose more weight per day than a small egg of the same species (Harvey 1993). In a sample of seven fertile eggs, six had density values between 1.08-1.11 g/cm3, while only one egg had a density of 1.16 g/cm3. For example, egg number five and nine were of similar fresh weight (160.2 g and 160.5 g., respectively), but the density values were different. Egg number five had a density of 1.08 g/cm^3, and egg number nine had a density of 1.16 g/cm^3, suggesting to use different levels of humidity. Eggs with lower density require higher levels of humidity (Anderson & Robbins 1979; Harvey 1993). For example, the initial density of one fresh egg (1.16 g/cm^3) was considered a high value, which suggested the use of a low value of relative humidity (Harvey 1993) (Table 2). In contrast, initial density of the other fresh eggs (1.09 to 1.10 g/cm^3) was considered low, which suggested the use of high values of relative humidity (Harvey 1993). When the eggs were slightly outside of the expected weight loss, it was necessary to adjust the humidity (Fig. 2 and 3).

Horned Guan eggs should lose at least 15% of their weight and/or initial density for successful hatching. The observed and expected curves do not necessarily have to coincide, but should stay within certain limits (13-17%), especially during first one-third of the incubation. A tolerance of around ± 2-3% of weight and/or density loss toward the end of the incubation process is acceptable for a successful hatching. An *a posteriori* analysis of the percentage of egg weight loss using the allometric equation of Rahn &

Ar (1974), (considering the mean weight of the same) indicated Horned Guan eggs should lose 14.68% of their initial weight at the end of the incubation process.

This is the first published data of Horned Guan artificial incubation parameters. Both techniques, weight and density loss, proved to be effective to monitor and guide successful hatching. The parameters of temperature and humidity assigned are appropriate for the incubation of Horned Guan eggs, but not optimum. It is advisable to begin with a relative humidity of 55% and a temperature of 37.5°C, and to monitor the pattern of weight and density loss. If the observed weight loss is below the expected loss curve it is necessary to increase the humidity; if the converse pattern arises it is necessary to decrease the humidity. We do not try natural incubation in captive conditions because we want to persuade to females to lay a repeat clutch and finally as a management insurance policy to avoid break eggs either by accident or intentionally. Under natural conditions perhaps the percentage of hatchability is 100 % (n = four nest with two eggs each). Experimental artificial incubation has produced suggestions to improve captive breeding of *O. derbianus*. Implementating a method for monitoring the incubation process is very important. Both techniques, weight and density loss, proved to be satisfactory to monitor and guide successful hatching of the Horned Guan. However density loss could be a better alternative because this technique takes the size of the egg into account, and may be able to predict what humidity needed before incubation starts. Horned Guan eggs should lose 15% of their weight and/or initial density for successful hatching. A temperature of 37.5°C and relative humidity of 53-56% (28.8-30.1°C) appears to be appropiate for incubating Horned Guan eggs. The brood, laying interval and the size of the eggs were similar to situations in the wild. However, this study did find differences in the weight of these eggs, which is probably related to age and diet. These results suggest ways to improve the breeding success of the Horned Guan. These methods can be applied as a starting point for other captive cracid reproduction programs.

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