# Breeding Ecology and Related Life-History Traits of the Hoatzin, *Opisthocomus hoazin*, in a Primary Rainforest Habitat

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**Antje Müllner**

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Mitglieder der Promotionskommission:

Vorsitzender: Prof. Dr. Th. Dandekar

1. Gutachter: Prof. Dr. K. Eduard Linsenmair

2. Gutachter: Prof. Dr. Michael Wink

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# 1. Introduction

## 1.1 The hoatzin - an enigmatic bird and “flying cow”

Hoatzins (*Opisthocomus hoazin*) are bizarre birds that live in the Amazon and Orinoco river basin in South America. They are pheasant sized, long tailed, and have a colourful and crested head. As a result of this unusual appearance they are called *Cigana* (“Gypsy”) in Brazil. Hoatzins are locally common along small watercourses and oxbow lakes where they live in small year-round territories (Strahl, 1988; del-Hoyo et al., 1996). Despite their considerable size hoatzins are not hunted in most parts of their distribution range. This is likely to be due to reports that their meat is unpalatable and their nickname “Stinky Turkey” reflects this attribute. Nonetheless, the further destruction of riverine habitat might also affect hoatzins in the long-run and in fact Dias (1995) reports declining numbers on the Marajo Island in Brazil.

Hoatzins are exiting birds – for several reasons:

- Hoatzins belong to the few extant bird species (3%) that heavily rely on foliage in their diet (Morton, 1978). Furthermore, they are the only known birds in the world that have a ruminant-like digestion and the discovery of this unusual habit gave them the privilege of decorating the front cover of the magazine “Science” in 1989 (Vol. 245; Grajal et al., 1989). The hoatzin’s leafy diet is cracked by the help of anaerobe bacteria and ciliate protozoa living in their foregut (Domínguez-Bello, Lovera et al., 1993) and by this means the birds are even able to digest cellulose (Grajal, 1995*a*). Protozoa have not been found in the crop of other birds and their numbers resemble numbers from the rumen of Ruminantia (Domínguez-Bello, Lovera et al., 1993; Domínguez-Bello, Ruiz et al., 1993). There are also similarities in the lysozymes of hoatzins and cattle, demonstrating the digestive adaptations in this birds and in foregut fermenting mammals as a case of a very remarkable convergence (Kornegay et al., 1994; Ruiz et al., 1994; Kornegay, 1996). Since this digestive mode has further implications on the bird’s biology the name “flying cow” describes the hoatzin quite appropriately.

Leaves are low in nutrients and high in bulk, and thus herbivorous animals must consume large quantities in order to satisfy their energy requirements and at the same time they have to provide sufficient storage room. Fermenting chambers loose efficiency with decreasing size, whereas energy demands increase disproportionally in smaller mammals and birds. These counteracting constraints put a limit on body size with respect to fibre digesting endotherms. However, the hoatzin does not follow this allometric model (Grajal, 1995*b*; McWilliams, 1999). Actually it would be much too small for its high digestive efficiency (average body size 700-800g). Two factors might explain this discrepancy. First, the gut and its plant content may account for 20% of the hoatzin's body weight. Not surprisingly, the non-migrating hoatzin is a weak flyer. Second, hoatzins have very long digesta passage rates (25-46 hr for solid markers depending on particle size; Grajal and Parra, 1995). This contrasts with other folivorous birds in which fast passage rates maximise the rate of assimilation from cell content at the expense of little cell wall digestion (Lopez-Calleja and Bozinovic, 2000). The long retention times in the fermentation chamber have strong implications on the activity pattern of this species: Hoatzins exhibit a sedentary life-style and are found sitting in a tree most of the day, accommodating the large crop by a thickened skin callus on the tip of the sternum (see Appendix 1). Domínguez-Bello et al. (1994) and Grajal (1995*a*) suggested that this prolonged diet processing in the gut by microbial action detoxifies secondary compounds. It is known that leaves commonly contain secondary metabolites which may reach toxic levels in the diet of herbivores. Jones et al. (2000) demonstrated that hoatzins cannot handle high tannin contents and in fact avoid highly tanniniferous leaves.

- The classification of this unusual bird has perplexed taxonomists since its description as *Phasanius hoazin* (Müller, 1776) more than 200 years ago. Traditionally classified among the fowl-like birds (Galliformes), some authors have allied it with the Turacos (Musophagidae), the Pigeons (Columbidae), the Rails (Rallidae) or the Cracids (Cracidae) (review in Sibley (1973) and in Marceliano (1995)). Modern studies have favoured a relationship of the hoatzin to the Cuculiformes (De Queiroz and Good, 1988; Hedges et al., 1995; Hughes, 1996). Sibley and Ahlquist (1973; 1990) even placed the hoatzin within the Cuculidae, with a strong link to the Neotropical Anis and the Guira Cuckoo (Crotophaginae). Recent analyses based on genetic, osteological, and behavioural evidence indicate that the hoatzin is most closely related to the Turacos (Musophagidae), a small family of arboreal, frugivorous birds which live in sub-Saharan Africa (Hughes and Baker, 1999; Hughes, 2000). However, the latest genetic study could neither support a relationship to the Turacos and Cuculiformes nor to the Galliformes but found a weak link to Doves (Columbiformes) (Sorenson et al., 2003). Wisely, the Handbook of the Birds of the World places the hoatzin as the only member of the family in the monotypic order Opisthocomiformes (del-Hoyo et al., 1996). The search continues…

Despite the debate over which extant bird clade the hoatzin is most closely related to, it is generally accepted that the modern hoatzin lineage represents a relatively ancient divergence from other groups of birds (Avise et al., 1994), presumably having evolved from ancestors in Gondwanaland (Hughes, 2000). Only one fossil modern Hoatzin has been found to date: *Hoazinoides magdalenae* has been found in the Western Andes of Colombia, being part of the La Venta fauna from the upper Miocene ca. 18 million years ago (Miller, 1953). A bird fossil from southern Argentina (*Onychopteryx simpsoni*), thought to represent another hoatzin family, provides evidence that Opisthocomiformes already existed in the early Eocene approximately 55 million years ago and that they had a much wider distribution than they do today (Cracraft, 1971).

- Linked to this phylogenetic uncertainty hoatzins still live with the fame of being a prehistoric bird. Hoatzin chicks possess two wing claws at each wrist joint and the species has been linked to the prehistoric *Archaeopteryx* which lived in the upper Jurassic 138 million years ago. Emílio Goeldi, a recognized researcher of Amazonian Brazil stated that the hoatzin is “living evidence of the transition between reptiles and birds” (Goeldi, 1898). Many tourist guides in the Neotropics still present the hoatzin as a living fossil. However, wing claws are in fact known in a variety of bird species, e.g. in some Turacos (Stephan, 1992). It is most likely that the wing claws are a secondary adaptation closely linked with the aquatic environment of the species and the very peculiar predator avoidance behaviour of the chicks: When frightened the young hoatzin drops into the water and either dives or swims into the vegetation and hides (see Appendix 2). Later it climbs another tree in an inconspicuous manner using its wing claws (Grimmer, 1962; Strahl, 1988).

Young hoatzins also use the wing claws while climbing in the nesting tree before fledging. Normally the claws are lost in the adult stage.

- Hoatzins exhibit a complex social organisation: First of all, they belong to the few bird species (3%) that are classified as co-operative breeders (Brown, 1987). According to observations by Strahl (1988) in Venezuela approximately 55% of hoatzin breeding units are composed of a single pair together with offspring from previous years. Units with helpers have a better annual breeding success than single pairs, probably due to a faster growth of the chicks (Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990). Sons mainly act as helpers-at-the-nest whereas daughters appear to be the dispersing sex (Strahl, 1985; 1988). Secondly, joint-female-nesting has also been observed in this species, thus several females may lay their eggs in one common nest (Strahl, 1988). Furthermore, one record indicates communal nesting (Torres, 1987).

## 1.2 Aims and structure of the study

Despite the fact that there are numerous articles concerning the phylogeny and the digestive peculiarities of the hoatzin, little has been published regarding its nesting ecology and breeding performance. What is known about hoatzin reproduction to date relies almost exclusively on populations in the savannah of Venezuela (Ramo and Busto, 1984; Strahl, 1988; Strahl and Schmitz, 1990; Domínguez-Bello et al., 1994) and the social organisation has only been investigated in one of these populations (Strahl, 1988; Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990). Savannahs and their gallery forests are situated at the limit of the species' distribution and differ from Amazonian rainforests in many abiotic and biotic factors. A comparative approach should reveal the potential consequences of these differences on the hoatzin's breeding performance and thus allow a better understanding of the species' life-history.

Here, I provide the first detailed and comprehensive data set on the nesting ecology of the hoatzin from a rainforest habitat. I studied a population of approximately 700 individuals in the Cuyabeno Wildlife Reserve in Amazonian Ecuador, particularly focussing on breeding effort and success as well as on the social organisation of this bird. My overall aim was to collect urgently needed information on this species from another geographical region and habitat. Furthermore, I examined the hoatzin reproductive traits in the context of tropical avian life-histories: Do the typical traits known for passerines (low clutch size, Murray, 1985; Skutch, 1985; low annual reproductive success, Skutch, 1966; Fogden, 1972; Robinson et al., 2000, and longevity Fogden, 1972; Francis et al., 1999) also occur in a non-passerine bird from an isolated lineage? Since the study area is target of increasing rainforest tourism I also had a close look at the interactions between tourists and breeding hoatzins. I started a thorough investigation of this topic because I had become aware of the worrying effects of the so-called eco-tourism.

The thesis is divided into two major parts that are each composed of several chapters. The first part deals with the nesting biology and social organisation of hoatzins, while the second part focuses on the interactions of breeding hoatzins and their chicks with rainforest tourists. Each chapter has its own introduction to the specific topic and can be read independently.

First of all I examined the timing of reproduction and general breeding patterns, e.g. brood size and breeding success (chapter 2.1). Then I determined *intra*- and *inter*-clutch egg size variation, looked at its adaptive significance and investigated the consequences of hatching asynchrony (chapter 2.2). Hoatzins are known to breed co-operatively in Venezuela, and the composition of breeding units and the potential effects of differing sizes on reproductive output are presented in chapter 2.3. The assumed sex-bias in helping behaviour of the hoatzin raises the interesting question of adaptive off-spring sex ratios, deviating from equality. The offspring sex ratio had to be determined using molecular methods because male and female hoatzins look alike. Chapter 2.4 elaborates on this topic. The occurrence as well as the potential benefit of joint-female nesting is examined in chapter 2.5 using DNA fingerprints to identify full sibling broods from broods with more than one parent.

The second part of this thesis deals with the problems that hoatzins are confronted with in face of the current rainforest tourism in the Cuyabeno Wildlife Reserve. An overview of the impacts of tourism on animals is given in chapter 3.1, followed by a comprehensive investigation into impacts of tourist exposure on the behaviour of adult and young hoatzins, on chick survival and on the hormonal stress response of chicks (chapter 3.2). Chapter 3.3 summarises the chances and risks of rainforest tourism with respect to the conservation of biodiversity, using the Cuyabeno Wildlife Reserve in Ecuador and its hoatzin population as a case study.

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# 2. Breeding effort of the hoatzin in an Ecuadorian rainforest

## 2.1 Nesting patterns and breeding success of the neotropical hoatzin *Opisthocomus hoazin*

Antje Müllner and K. Eduard Linsenmair

### Abstract

Hypotheses on systematic latitudinal variations in avian life histories are based on a huge data set for temperate birds whereas, even basic information is lacking for many tropical birds. This holds especially true for non-passerine species. We investigated the nesting activities of the neotropical hoatzin *Opisthocomus hoazin* in a primary rainforest in Amazonian Ecuador. At our study site hoatzins live and breed in the inundated forest which surrounds lakes and channels. Hoatzin's breeding attempts were closely linked to the rainy seasons and thus clumped within a few months annually. Normally hoatzins nested only once a year. Re-nesting was rare and only occurred after a failure during incubation. Nests were built in the most common trees and shrubs of the habitat. The number of eggs in a single hoatzin nest ranged from one to seven, but 51% of all clutches contained only two eggs. The mean incubation time was 32 days. Calculated on a per-nest basis the mean success for all years was 35% for hatching and 50% for fledging, resulting in an overall nesting success of 18%. The major cause of nesting failure was predation, with birds and snakes being the most frequent predators, whereas mammals did not contribute considerably to the predation rate. The small time window of breeding activities seems to be the result of strong constraints for successful reproduction due to a better nest protection and the abundance of high quality food during high water levels. The taxonomically very isolated hoatzin exhibits life-history traits similar to those of tropical passerines. This indicates that effective and general environmental selection pressures may be involved in shaping these traits.

## Introduction

Tropical birds are known to show some fundamental differences in their life histories compared to temperate birds. Typical characteristics for birds in tropical regions seem to be a low clutch size (Murray, 1985; Skutch, 1985), a low annual reproductive success (Skutch, 1966; Fogden, 1972; Robinson et al., 2000), and longevity (Fogden, 1972; Francis et al., 1999). These traits are all suggested to be inter-related . There are several hypotheses regarding how latitudinal variation in climate and food availability (Lack, 1947; Ricklefs, 1980) or predation pressure (Skutch, 1945; Skutch, 1985) influence avian life histories. However, despite recently developed models that quantify the effects of temperature and body mass on metabolic rate and on development rate and that claim to be applicable to all organisms (Gillooly et al., 2001; Gillooly et al., 2002), a satisfactory framework for understanding tropical birds' peculiarities is still lacking. Ricklefs and Wikelski (2002) pointed out that physiological mechanisms might considerably constrain the adaptive responses to different environments and thus have to be taken into account. Whereas many data on temperate bird life histories exist, data on tropical birds are rare because the number of individuals is often low, reproduction is much more difficult to observe, and because in general much less research has been done. The few existing studies focus on passerine birds (Kulesza, 1990; Ricklefs, 1997; Magrath et al., 2000; Padmanabhan and Yom-Tov, 2000), while even basic information is lacking for other taxa (but see Vehrencamp et al., 1988; Sandercock et al., 2000).

Here we provide a quantitative description of the nesting patterns and the breeding success of a hoatzin population (*Opisthocomus hoazin*) in a primary rainforest in Ecuador. The pheasant-sized hoatzin is the only member of the family Opisthocomidae and only lives in forests in the Amazon and Orinoco river basin of South America. The taxonomic position of this species still remains unclear; a former consensus about a relationship to the Cuculiformes (De Queiroz and Good, 1988; Hedges et al., 1995; Hughes and Baker, 1999) has recently been questioned by Sorenson et al. (2003). Although hoatzins are locally common in riparian habitats, information on their natural history published so far relies almost exclusively on observations from the gallery forest in the savannahs ("llanos") of Venezuela (Ramo and Busto, 1984; Strahl, 1988; Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990; Domínguez-Bello et al., 1994). The aim of our study was to investigate whether the reproductive traits known and believed to be typical for tropical passerines also apply to the non-passerine hoatzin.

Furthermore, our data may help to identify factors shaping these traits by comparing our findings from a rainforest habitat with the data from the savannah habitat. Finally, the information presented in this article complements the knowledge about this relatively unknown species.

## Methods

### *Study site and species*

Fieldwork was conducted from 1995 to 1998 and in 2000 in the Cuyabeno Lakes ("Lagunas de Cuyabeno"), a system of several black water lakes, small channels, flooded forest, and *terra firme* forest in Amazonian Ecuador (0°02' N, 76°9' W and 0°03' S, 76°14' W). The area of the Cuyabeno Lakes covers about 10 x 10 km and is intersected by the equator. Average rainfall per year for the region amounts to 3494 ± 473 mm (mean 1981-2000; min. 2505 mm, max. 4668 mm; all rainfall data from the Dirección de Aviación Civil at the airport in Lago Agrio, about 100 km away from the Cuyabeno Lakes). Rainfall is seasonal and in general the wettest months are from April to June and from October to November (Fig. 1A), but events such as the El Niño in 1995 and the following La Niña strongly influence the Amazonian lowlands too: in 1996 heavy rains already began in January, with the highest precipitation ever measured for a single month between 1981 and 2000. Because the water level of the lakes and the flooded forest is totally dependent on the rainfall in the region, it directly follows the precipitation pattern (Fig. 1B). In average years the flooded forest is dry between December and March and for a shorter period in September.

Stretches of flooded low-density vegetation surround the lakes and creeks, composed of only a few species of trees and shrubs (see figures in Appendix 1). Tree height ranges from five to ten meters and water level may rise up to four meters. This lagoon-forest is the habitat of a population of approximately 700 adult hoatzins (*Opisthocomus hoazin)* (A. M., own census data). Hoatzins are co-operative breeders and may live as single pairs or in family groups of up to 7-8 adults (Strahl, 1988; Müllner and Linsenmair in prep., see chapter 2.3). During the wet seasons every breeding unit defended its all-purpose territory, whereas in dry months the hoatzins formed flocks of up to 50 birds (A. M., unpubl. data). Hoatzins are obligate folivores and their daily food intake was composed of the leaves of the most common plants of the inundated forest (A. M., unpubl. data). More details on the study site and its faunal composition are described elsewhere (Müllner and Pfrommer, 2001; Müllner et al., 2004, see chapter 3.2).

### *Nest controls*

We regularly searched for hoatzin nests from June to December 1995, from April to December in 1996, 1997, and 1998, and in May 2000. Because hoatzins live at the forest edge along rivers and lakes and always build their nests on branches overhanging the water, their nests are relatively easy to locate, although sometimes they are well hidden in dense vegetation. All nest monitoring was conducted by the same observer (A. M.) using a paddled dugout canoe. Once a nest was located, we noted its position on a self-made map, but did not place a marker flag. For every nest site, the height above water level was estimated and the plant species determined. The aquatic environment sometimes made access to nests difficult, but had the advantage that we did not create trails that might allow nest predators to follow us. We only approached nests closely at the initial control to determine the nest content or to inspect the nest when no activity was observed. Eggs were marked individually with a felt pen and their length (L) and maximal breadth (B) were measured to the nearest mm using a sliding calliper. Their volumes were estimated by means of the formula $0.51LB^2$ (Hoyt, 1979). Afterwards we checked nest sites one to three times a week, generally using binoculars from a distance of 15 to 25 meters. In 1998 egg laying dates were estimated by the egg floating method (Dunn et al., 1979; Alberico, 1995), if we had not been able to directly observe laying.

### *Data analysis*

In order to avoid pseudoreplication and seasonal effects we only included the first annual breeding attempt of a hoatzin pair in all general analyses. The few cases of second breeding attempts were analyzed separately. Clutch sizes are presented as means ± SD. However, frequency distribution might be a more appropriate way of reflecting the clutch size of a population because clutch size is a discrete parameter and its distribution is often skewed. Thus, the proportion of clutch sizes is also given. We combined the clutches of all years because the distribution of clutch sizes did not differ between the years ($G = 3.98$, $df = 9$, $p = 0.91$, categories 4-7 eggs and years 1995 and 2000 were pooled to meet the test assumptions).

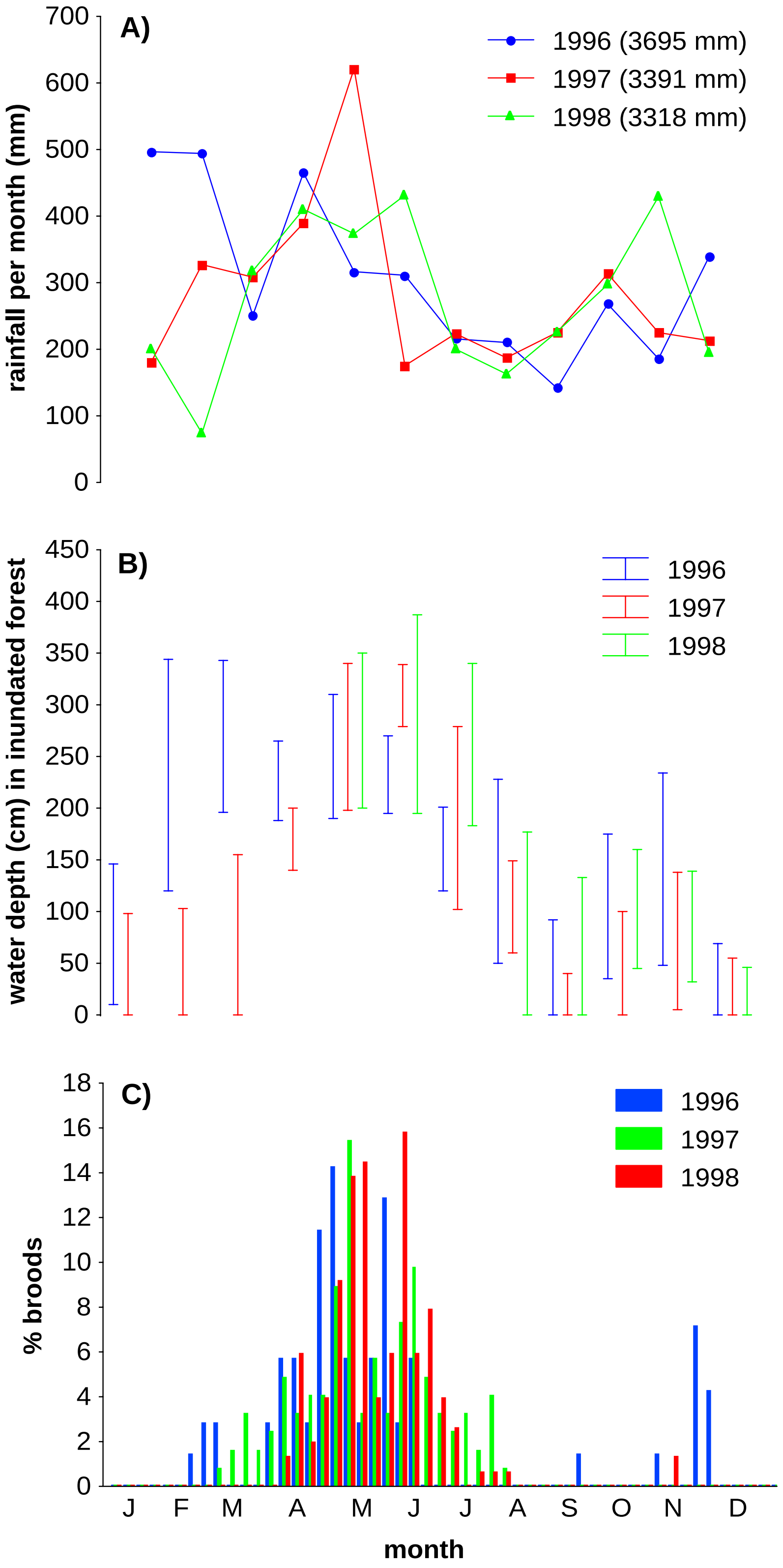

**Figure 1**. Monthly rainfall at the airport of Lago Agrio in the north-eastern lowlands of Ecuador (A), monthly range of water depth in the inundated forest of the Cuyabeno Lakes (B) and the weekly proportions of initiated hoatzin clutches (C) in 1996, 1997 and 1998 (sample sizes were *n* = 70 broods in 1996, *n* = 123 in 1997, and *n* = 145 in 1998). Rainfall data were obtained from the Dirección de Aviación Civil, annual total is given in parentheses.

Breeding success was calculated as the percentage of successful nests, a measure that allowed us to compare our data with results from other sites. We divided the nesting cycle into the incubation period and the brooding period until fledging, and nests were considered successful after the incubation period when they had produced at least one hatchling and after the brooding period when at least one fledgling was produced. We only included nests that contained eggs not older than a week or nestlings with a maximum age of five days in the analysis to prevent a bias towards successful nests. Otherwise, nests found later during the incubation or nestling period, which hence had already survived for quite a while, would have led to an overestimation of success.

## Results

### *Nest parameters*

Major nest plants were *Genipa americana* (Rubiacea) and *Macrolobium acaciifolium* (Leguminosae), which hosted 39% and 23%, respectively, of nests (*n* = 693). Additionally, *Macrolobium acaciifolium* represents the most important food source in the hoatzin habitat (see Appendix 1). The remainder of the nest sites were composed of *Licania* spec. (15%, Chrysobalanaceae), several species of *Ficus* (10%, Moraceae), and *Pithecellobium longifolium* (7%, Leguminosae). Less than 7% of nests were situated in *Bactris* spec. (Arecaceae), *Coussapoa trinerva* (Moraceae), *Inga* spec. (Leguminosae) and a few others. This selection of nest sites reflects the overall vegetation composition and does not reveal any preference for particular plant species.

Nests were situated at a height of about 4-6 meters above ground. Because the maximum water depth in the inundated forest was 4 m (see Fig. 1B) nests could be very near the water surface at the high peak of the rainy season. Nests were mostly simple platform nests with a diameter of 25 to 30 cm. The sticks were often put together in a very loose manner. Sometimes the stump of a *Macrolobium* or *Ficus* tree was used as a natural nest cup.

### *Clutch size, egg characteristics and incubation time*

The number of eggs we found in a single hoatzin nest ranged from one to seven. Mean clutch size was 2.4 ± 1.1 SD in 1995 (*n* = 16), 2.3 ± 1.0 SD in 1996 (*n* = 56), 2.5 ± 1.3 SD in 1997 (*n* = 79), 2.4 ± 1.0 SD in 1998 (*n* = 120), and 2.3 ± 1.3 SD in 2000 (*n* = 20), with an overall mean for all nests of 2.4 eggs ± 1.1 SD. The frequency distribution in Figure 2 shows that approximately half of the clutches contained two eggs (50.9%), whereas clutches of three eggs were found in 19.6% of nests and 14.4% of nests contained only a single egg. Only 15.1% of clutches contained four or more eggs. Consequently, the frequency distribution was significantly different from a binomial distribution (G = 345.0, df = 6, $p < 0.00001$).

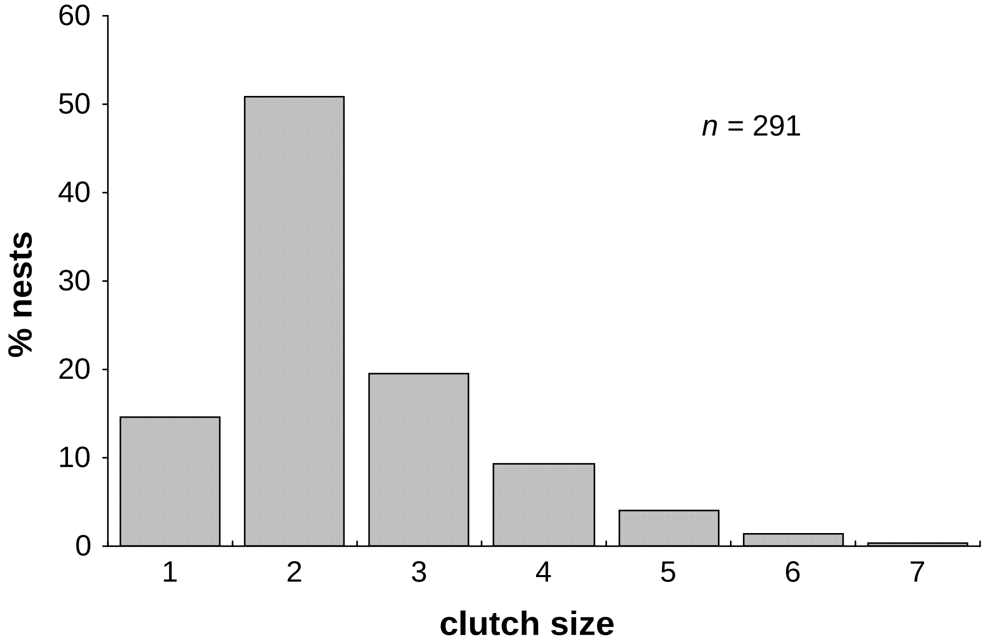

**Figure 2.** Frequency distribution of hoatzin clutch sizes in the Cuyabeno Lakes. Only first breeding attempts per season were considered. Data from 1995 – 2000 were pooled.

We measured a total of 522 hoatzin eggs from 230 first annual breeding attempts. Mean ± SD of length and breadth were 46.4mm ± 1.84 (range 41.0–52.5mm) and 34.0mm ± 1.13 (range 30.5-37.0mm), respectively, resulting in an average egg volume of 27.4 $cm^3$ ± 2.33 (range 21.0-34.5$cm^3$). The mean weight of freshly laid eggs was 29.7g ± 1.6 ($n$ = 28). There was a strong but non-significant trend for egg volumes to differ between the years (ANOVA, $F_{3,518} = 5.38$, $p = 0.052$). This was due to a difference between 1997 with the lowest mean egg volume and 1998 with the biggest mean volume (Scheffé post hoc-test: $p = 0.07$).

The incubation of the clutch started with the first egg laid. Normally one hoatzin remained sitting until another hoatzin arrived to replace it, so that the eggs were never uncovered. Mean incubation time was 32 days ± 1.51 SD per egg ($n$ = 20 clutch means), ranging from as much as 28 to 34 days for a single egg. This variation was positively correlated to clutch size, i.e. eggs in bigger clutches were incubated for a longer period before hatching (Spearman rank correlation, $r_s = 0.55$, $p = 0.01$; see Appendix 3).

### *Breeding success and nesting failure*

Hatching success of hoatzin clutches ranged from 33.3 to 36.2% (Table 1) but did not differ significantly between the years investigated ($G = 0.19$, df = 2, $p = 0.91$). The mean annual rate was 35.0%. Fledging success ranged from 44.4% to 61.5%, but the difference was also not significant ($G = 3.08$, df = 2, $p = 0.21$). Mean annual success during the fledging period was 50.3%, resulting in an overall nesting success of 17.6%.

**Table 1.** Hatching and fledging success of hoatzin breeding attempts in the Cuyabeno Lakes in Amazonian Ecuador. Success was calculated as the proportion of nests with at least one hatchling or one fledgling, respectively.

| year | % hatching success | *n* | % fledging success | *n* | % total nesting success |
|---|---|---|---|---|---|
| 1996 | 36.17 | 47 | 44.44 | 27 | 16.08 |
| 1997 | 32.39 | 71 | 61.54 | 39 | 19.93 |
| 1998 | 33.33 | 96 | 44.93 | 69 | 14.98 |

During the incubation period nesting failure was mainly due to the disappearance of the entire clutch, while the nest construction remained intact. Although, there were some joint-nesting nests where egg removal occurred (Müllner et al. in prep., see chapter 2.5). Nests were either destroyed or missing in only a few cases, and only 8% of nest failures (*n* = 142) were clearly linked to severe climate: After a heavy thunderstorm in 1998 three nests with eggs were found in the water, still entwined in vegetation. In 1997 and 1998 several nests were flooded because the water level had risen more than a meter in one week. We conclude that all clutches which vanished completely from otherwise intact nests were predated.

If not disturbed hoatzin chicks normally stayed in their nest for between two and three weeks before starting to climb in the nest tree. Fledging occurred at 45-55 days but young birds stayed in their natal group and still begged for food for a further few months. Nestlings often disappeared from the nest without any evidence of what had taken them. However, in contrast to the egg-stage, nests with young suffered also partial brood losses. Although 85.6% of clutches contained more than a single egg, one fledged young was the most common result of a successful hoatzin breeding attempt (75%, *n* = 89, years 1995-1998). Only in 24% of successful broods were two fledglings found and in all of the study years we only once observed a breeding unit with three fledglings.

Due to the aquatic environment, hoatzin nest sites were well protected from non-flying mammals. The most obvious potential predators were diurnal birds and - at a much lower detection rate - snakes. We saw Black Caracaras *Daptrius ater* mobbing hoatzins that were incubating or brooding, and eggs (2 incidences) or nestlings (1 incidence) had vanished the next day. Toucans (*Ramphastos* and *Pteroglossus*) were often seen in hoatzin breeding territories, once at an empty nest that had contained eggs two days before. Small raptors, e.g. Forest Falcons *Micrastur* spec. were also regularly detected. Interestingly, in the language of the native Siona Indians the name of the fishing buzzard *Busarellus nigricollis*, which was resident at some lakes, means "feed on hoatzins" (Tomás Criollo, pers. comm.). Although, this bird only seems to be a potential threat to fairly young hoatzins. However, bigger raptors such as the Ornate Hawk-Eagle *Spizaetus ornatus* and the Great Black Hawk *Buteogallus urubitinga* were observed attacking adult hoatzins, provoking panic and intense alarm calls. Both species probably would take juveniles as well.

Snakes were not often noted in the inundated forest, although most species are good swimmers. Most frequently we detected the Anaconda *Eunectes murinus* which certainly feeds on hoatzin nestlings: Once we saw a 1.5 m long Anaconda with a full stomach resting directly beside an empty hoatzin nest, which had contained two ten-days-old chicks a few hours before. Another time we detected a much smaller Anaconda strangling a Greater Ani *Crotophaga major*, which is somewhat bigger than a young hoatzin, to death. The Amazon Tree Boa *Corallus hortulans* and the Tiger Rat Snake *Spilotes pullatus* (Colubridae) were occasionally noted and they are also potential predators of nestlings. We never observed hoatzins actively defending their broods by attacking a predator or an approaching human. Instead the attending individual eventually fled from the nest to a nearby tree, protesting with its typical hissy vocalization. Then the entire group joined in and participated in this concert.

### *Timing of reproduction and temporal variation of breeding success*

The breeding activities of the hoatzins in the Cuyabeno Lakes were closely linked to the onset of the rainy seasons in all three years (Fig. 1C). Pooling all observed breeding events, egg laying occurred from February to July and from September to November but always peaked in the three months April, May and June: 76% (1996), 79% (1997) and 93% (1998) of all annual breeding attempts started in this period. Within this time window May was a further peak already containing more than a third of all initiated clutches (37%, 36% and 39% of all clutches in 1996, 1997 and 1998, respectively). We observed only very few breeding attempts in the short rainy season in fall. These were most common in 1996, when several breeding units did not breed at all in spring, probably due to the La Niña anomaly.

There was a strong trend for clutches laid late in the season to be smaller than clutches laid at the beginning and during the course of the season both in spring and in fall (Fig. 3, U-test, March to June and October versus July and November, Z = 1.91, *p* = 0.056).

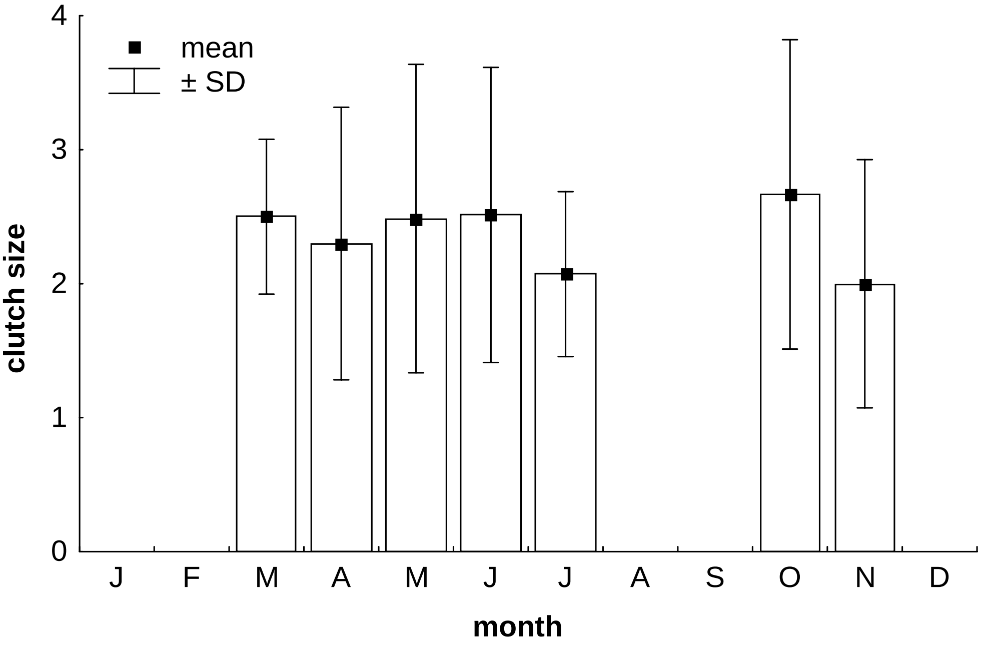

**Figure 3**. Mean clutch size ± SD of hoatzin broods during the breeding season. Data were pooled from 1996-1998 (*n* = 70 for 1996, *n* = 123 for 1997 and *n* = 145 for 1998). Only first annual breeding attempts were considered.

Hatching success decreased significantly during the breeding season (Fig. 4, G = 4.58, df = 1, *p* = 0.032, months 3-6 vs. 7-11). This pattern could not be confirmed for fledging success (Fisher's exact, *p* = 0.28; months 3-4 *vs.* 5-7), but the small sample size at the end of the season has to be considered. Breeding attempts that had started in August or later never successfully produced any fledglings.

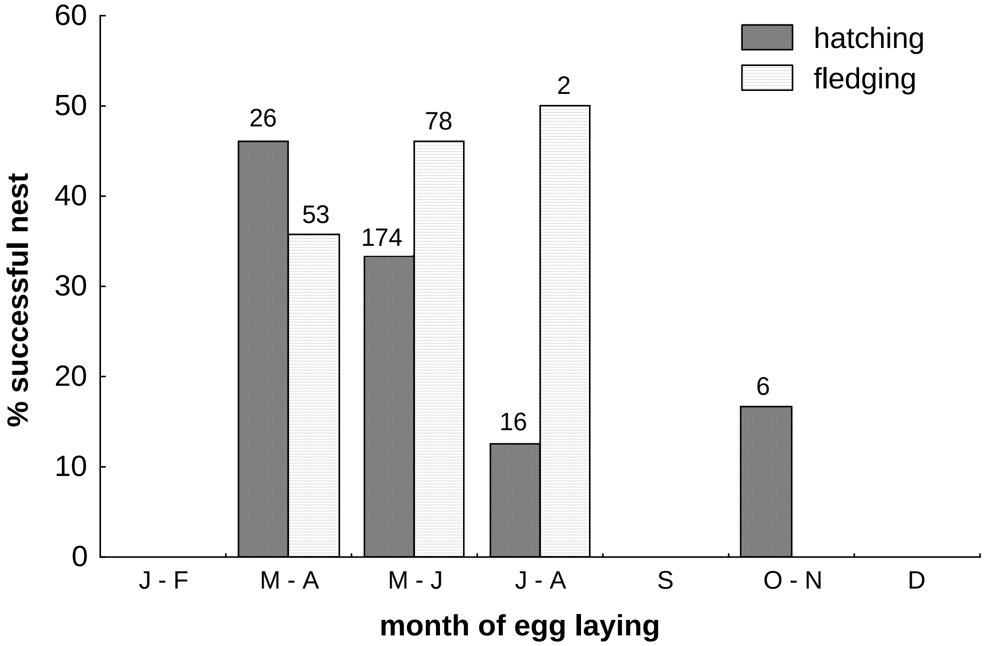

**Figure 4.** Hatching and fledging success of hoatzin nesting attempts in the course of the breeding season. Data from 1997 and 1998 were pooled; sample sizes are given above the bars.

### *Renesting*

We normally observed only one nesting attempt per hoatzin breeding unit per year. Renesting was rare and only occurred after an early nesting failure, and never after a successful breeding cycle. Proportions of first and seconds attempts from all annual attempts were 93.9% and 6.1% in 1996 (*n* = 82), 93.8% and 6.2% in 1997 (*n* = 130). In 1998 we observed two hoatzin units who tried a third attempt in the short rainy season of the fall and the proportions were 89.0% for the first, 9.8 % for the second and 1.2% for the third attempt (*n* = 163). Renesting hoatzins often used the same platform nest for the consecutive attempt but sometimes a new nest was built in the same tree or a nearby tree inside the territory boundaries. The mean time lapse in weeks ± SD between the loss of the first clutch and the laying of the replacement clutch was 4.5 ± 2.38 in 1996 (*n* = 4), 5.1 ± 1.81 in 1997 (*n* = 8) and 5.1 ± 2.08 in 1998 (*n* = 16). There was no association between replacement interval and the size of the first clutch ($r_P$ = -0.09, *p* = 0.68).

Furthermore, the clutch size of second broods did not differ from those of first broods. Instead clutches produced by the same breeding unit were strongly positively correlated in their size between the first and second breeding attempts (Spearman rank correlation, $r_s = 0.88$, $p < 0.05$, $n = 24$). Likewise, egg volumes of replacement clutches were similar to those of first clutches, both with respect to their means as well as to their variances (means: 27.3 $cm^3$ ± 2.32; t = 0.25, $p = 0.81$; variances: F = 1.01, $p = 1.0$; $n = 55$ eggs from 21 second breeding attempts). The recorded inter-annual difference in egg volumes (see above) also seemed to apply to the replacement clutches, but the sample size was too small in 1996 and 1997 to analyze this assumption statistically. Hatching success of second breeding attempts was similar to the success of first attempts (35.5% and 33.6%, respectively). Although fledging success was much lower in second than in first attempts (16.7% and 49.6%, respectively) the difference was not statistically significant (Fisher's exact, $p = 0.43$).

## Discussion

### ***Clutch size, egg size and incubation time of the hoatzin***

51% of all hoatzin clutches in the Cuyabeno Lakes contained two eggs. From further analyses we know that the maximum clutch laid by a single female was only three eggs whereas bigger clutches were the result of joint-female nesting (Müllner et al. in prep., chapter 2.5). Consequently, the percentage of two-egg clutches even raises to 60% when only those clutches are considered where the eggs were laid by a single female. This predominance of two-egg clutches is confirmed in other hoatzin breeding sites situated more to the North (Venezuela, 8°34' N, 67°35' W, Strahl, 1988; 9°, Domínguez-Bello et al., 1994) and to the South (Peru, 11°51' S, 71°19' W, Torres, 1987). This finding indicates that there is no substantial regional variation in egg number laid by a single female within the tropical zone. Furthermore it supports the observation that two-egg clutches are typical for the tropics (Skutch, 1966; Skutch, 1985).

The most important factors which are considered to explain the small tropical clutches are high annual nest predation rates together with a longevity of adults (Skutch, 1985; Murray, 1985). Assuming that egg production is costly (Monaghan et al., 1998; Nager et al., 2001) and that also birds living in a "rich" tropical environment are energetically constrained, the low investment in a single breeding attempt is strongly influenced by the high probability of loosing this brood. A high adult survival in contrast allows several breeding attempts over many consecutive years. Hoatzins are thought to live for at least ten years with an annual mortality of approx. 10% (Strahl and Schmitz, 1990). Two female hoatzins living in the Bronx Zoo in New York, USA, were at least 14 years old (J. Rowden, pers. comm.). With a mean annual nesting success of approx. 18%, a hoatzin pair would need about 5 to 6 years of breeding to raise at least one fledging and about twice that to replace itself. According to Strahl (1988) hoatzins do not start breeding normally before their third year of life. Thus a life span of 13 years would be the minimum required to maintain the population in the area. Although this is a very rough calculation it demonstrates that the existing data are conclusive in a tropical life-history framework. High adult survivorship is also reported in other tropical bird species (Fogden, 1972; Ricklefs, 1997; Francis et al., 1999). However, the reasons for this fact remain unresolved (Ricklefs and Wikelski, 2002).

Although two-egg clutches were predominant at all hoatzin breeding sites, the mean clutch size of 2.4 eggs found at our study site is larger than reported from Venezuela with 2.2 and 2.06 eggs in different reports (Strahl, 1988; Domínguez-Bello, 1994). This difference is mainly due to a much higher percentage of joint-nesting broods, i.e. clutches with more than three eggs, in Cuyabeno (15.5%) than in Venezuela (4.6% and 1% in two different studies). Furthermore, the number of seven eggs in a single nest is the highest number reported so far for a hoatzin breeding attempt. The topic of single- versus joint-female nesting is discussed in detail in Müllner et al. in prep. (chapter 2.5).

Egg shape was broader in Cuyabeno than in Strahl’s study site (mean length and breadth were 4.64cm and 3.40cm, respectively, in Cuyabeno, and 4.67cm and 3.31cm, respectively, in Venezuela (Strahl, 1988)) resulting in a larger mean egg size (27.4cm$^3$ in Cuyabeno compared to only 26.1cm$^3$ in Venezuela). Data from Schönwetter (1967) lay in-between but the origin of the eggs he measured is not known. Likewise, the mean incubation time for hoatzin eggs was longer in Cuyabeno than found by Strahl (32 days in Cuyabeno compared to 30-31 days, Strahl, 1988) but was similar to another place in the Llanos of Venezuela (Domínguez-Bello, 1994). Considering the mean fresh weight of a hoatzin egg of only 29.7g, the incubation time should actually be a week shorter, according to the calculations of Rahn et al. (1975). It is interesting that Ricklefs (1992) attributed long incubation times and thus long embryonic development found in many tropical birds to a low parasitic load and an apparent good immune response which in turn may enhance survival.

### *Breeding success and nesting failure*

The overall nesting success of hoatzins in the Cuyabeno Lakes ranged between 15% and 20% per year which is less than has been reported from other regions (22–27%, Strahl and Schmitz, 1990; Ramo and Busto, 1984). These authors did not give separate data for hatching and fledging success, but Domínguez-Bello et al. (1994) reported a hatching success of 27%, which in contrast is lower than the 32-36% observed in Cuyabeno. All three cited studies were made in Gallery forests of the Llanos of Venezuela, whereas the Cuyabeno Lakes lay within a huge area of relatively pristine rain forest, where many environmental factors may contribute to a relatively low overall success. However, some of the Cuyabeno Lakes suffer high tourism loads during the summer months, i.e. just at the time when brooding of young takes place. Tourism certainly negatively affects fledging success (Müllner et al., 2004), but even when only undisturbed sites were considered nesting failure was still high in absolute terms (77-82%).

Although some authors doubt the general phenomenon of low tropical nesting success (Oniki, 1979; Gibbs, 1991) the majority of studies support this pattern (Skutch, 1966; Ricklefs, 1969; Fogden, 1972; Robinson et al., 2000). Perhaps the reason for the contrasting results is the fact that there is considerable annual and inter-specific variation in the nesting success of tropical birds (Robinson et al., 2000). Very intense predation is seen as the major factor leading to the sometimes extremely high rates of nesting failure in the tropics (Skutch, 1966; Ricklefs, 1969). This is caused by predator faunas which are more diverse and abundant in tropical than in temperate forests. We have only little direct but conclusive evidence that most nesting failures in the Cuyabeno Lakes were due to predation. Our observations suggest that small raptors, toucans and snakes were the main predators of hoatzin broods. Raptors and especially toucans are both recognized by other authors to play an important role in stealing eggs and nestlings (Skutch, 1966; Ricklefs, 1969), a fact which is reconfirmed by the Siona Indians of Cuyabeno (Aurora Payaguaje, pers. comm.). The composition of hoatzin nest predators is consistent with a report of Robinson and Robinson (2001) on several bird species (no hoatzins) from mainland Panama. The authors witnessed 14 nest predation events of which 8 were by birds and 4 by snakes (altogether 86%). A high influence of snakes as nest predators for tropical forest birds is suggested by Skutch (1966) and Loiselle and Hoppes (1983). Their findings together with our findings complement the results of Roper and Goldstein (1997), who found nocturnal mammals to be the most important nest predators in a Costa Rican rainforest. Of course, we have to consider the fact that most hoatzin nests were protected against forest-dwelling mammals through the aquatic environment, explaining the minor role of mammal predators. Nevertheless, the overall rate of nest failure was still high.

Whilst during incubation the entire clutch fell victim to predation, during the fledging period a partial brood loss occurred. There is no evidence to indicate that starvation was a major cause of the disappearance of young hoatzins and we consider predation to be the reason for the loss of single chicks too, a conclusion that is consistent with the observations of Strahl (1985). One reason for the survival of a hoatzin young from a nest that has been attacked is the very specialized escape behaviour: from their first days of life young hoatzins are able and willing to leave the nest and to jump into the water when menaced. They can dive more than 10 meters and eventually will leave the water in an inconspicuous manner. Their famous wing claws help them when climbing another tree (see Appendix 2). We never observed them coming back to the nest. The adults find and brood them at the new place, but mortality is higher for these individuals compared to undisturbed individuals (Müllner et al., 2004, chapter 3.2). However, this strategy at least increases the chance that an attacked individual might escape successfully.

### *Timing and reproduction effort*

Approximately 80-90% of all annual breeding attempts were initiated in a three month period of which 50% were clumped in a single month. Thus hoatzins in the Cuyabeno area, living directly at the equator, were breeding as seasonally as temperate zone birds do. In all study years their nesting activities were closely linked to the beginning of the rainy season which is consistent with observations from Peru (Torres, 1987), Venezuela (Strahl, 1988) and British Guyana (Grimmer, 1962). But in contrast to the other sites we found two clearly distinct nesting seasons in the Cuyabeno area, although the first one from April to June is clearly the major one. The second breeding season is most probably explained by the bimodal precipitation pattern in Cuyabeno and indicates that rainfall or associated changes in water level or daily temperature are important triggers of reproductive behaviour. Together with endogenous rhythms these factors may act as proximate timing cues to regulate the physiological level, such as for example the release of reproductive hormones. A link between the beginning of the wet season and gonadal growth is known for other rainforest birds (Wikelski et al., 1999). In temperate zone birds proximate control processes involve effects of increasing day length in spring, which triggers the secretion of gonadotropin. Hau et al. (1998) showed that passerine birds at 9° N in Panama could also measure slight changes in tropical photoperiod. In the Cuyabeno Lakes, however, this change should be at its minimum because of the equatorial position of the area.

Although tropical habitats are exposed to much slighter seasonal changes in environmental conditions than temperate zones, many tropical birds have specific breeding seasons. Seasonal differences in food availability and in the degree of nest predation are suggested as ultimate factors that limit breeding in the tropics to certain parts of the year (Fogden, 1972; Young, 1994; Komdeur, 1996). The hoatzin seems to be an additional, non-passerine example, where the following causes might be applicable: First of all, the high water level, a consequence of the wet season, serves as a shield which protects nest sites against mammal predators. Mammals are known to heavily influence the mortality of hoatzin eggs and nestlings in the Gallery forest of Venezuela (Strahl, 1988) when access to nest sites is possible. In the Cuyabeno Lakes, broods late in the season, which experienced the beginning of the dry season, were immediately lost by predation from one day to the next when nest sites fell dry (A. M., unpubl. data). Once a banded juvenile, already able to fly short distances but resting at night in the just recently dry-ran territory was caught at night by an ocelot which left its footprints, the uneaten gut and the individual’s colour bands. Secondly, the water level in the inundated forests also strongly influences the phenology of the plants there. Hoatzins are obligate folivores and prefer fresh leaves and buds where N-content is high (A. M., unpubl. data) while tannin content is probably low. Jones et al. (2000) showed that hoatzins cannot handle high tannin content in their diet and their gut analyses indicate that hoatzins avoid highly tanninferous leaves and consequently are supposed be selective in their leafy diet. The high water level in the first part of the rainy season regularly leads to leaf fall of many inundated trees and shrubs. The flushing of new leaves coincides with the beginning of the feeding period of the young.

The relatively small time window of favourable ecological breeding conditions might also be the reason for the extremely low number of renesting attempts. Replacement clutches were also absent in the rainforest of Peru (Torres, 1987) but were very common in Venezuela from where Strahl (1988) also reported several successful breeding attempts within a season. In Cuyabeno, nesting late in the season tremendously increases the probability of experiencing a dry period and thus suffering higher predation. Thus renesting should be started as soon as possible. A mean interval of 4-5 weeks between the loss of the first clutch and the laying of the replacement clutch seems quite long and not appropriate to secure an optimal chance of successful reproduction. Physiological or energetical constraints might account for the long pause and serve as an additional explanation for the regular single nesting attempt per year.

However, this conclusion is not consistent with the small clutch sizes which actually are presumed to enhance the possibility of laying replacement clutches quickly. In addition, the hoatzin’s fresh egg weight of 29.7 g is much lower than the expected 46.5 g (Rahn et al., 1975) for a bird of this body size (mean 775 g, $n = 3$, range 695-859 g). Depending on whether the number of eggs laid was one, two or three, the clutch weight made up only about 4%, 8% or 12%, respectively, of the female’s body mass. These findings indicate that hoatzin females do in fact both invest only a small amount of energy in one breeding attempt, and exhibit only a low number of breeding attempts. The underlying causes for this paradox might be the unique feeding habits and physiology of these birds: the hoatzin is not only one of the few obligate folivorous birds but is also the only known foregut fermenter in the avian world (Grajal et al., 1989) and supposed to be particularly energy limited (Grajal and Strahl, 1991; Bosque et al., 1999). The forest flooded by black water at the Cuyabeno Lakes represents an extremely nutrition-poor environment and in combination with a high predation pressure this might force hoatzins towards another resource allocation than in the Venezuelan savannah. Of course, a reproduction pattern in such a “slow lane” can only evolve together with a high adult survival and hence the potential of many breeding attempts during an individual's lifetime.

Apart from the strong timing of reproduction there was an additional effect of decline in clutch size towards the end of the breeding season. This effect is also known in temperate birds, especially in species which breed once a year (Meijer et al., 1990), and adaptive and non-adaptive reasons are discussed (Winkler and Allen, 1996). The time-saving hypothesis of Birkhead and Nettleship (1982) suggests that decreasing clutch sizes help to speed up incubating and brooding times and thus help to compensate late-season conditions that lower the survival prospects of late hatchlings. Indeed we found incubation time positively associated with the number of eggs in the clutch. But nonetheless we have no evidence that a smaller clutch enhanced the chance of successful breeding. The observed decrease might simply be a consequence of variation in the capacity of the female to produce eggs. For example, young females may need more time for the production of egg yolk and therefore may lay later and also lay fewer eggs (Hipfner et al., 1997).

In conclusion, our findings on the breeding biology of the hoatzin in a tropical rainforest habitat give further support to the existence of typical life history characteristics for tropical birds, such as small clutch size and low annual reproductive success. To date these traits were almost exclusively reported from passerine birds and we are able to expand the validity of this pattern to a non-passerine bird. The isolated taxonomic position of the hoatzin and its very peculiar habits and needs indicate very broad and general environmental selection pressures in the low latitudes.

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## 2.2 Effects of hatching rank and egg size variation in the neotropical hoatzin *Opisthocomus hoazin*

Antje Müllner and K. Eduard Linsenmair

### Abstract

Hatching asynchrony results from incubation before the clutch is completed. It creates size hierarchies in nestlings which normally reflect the order in which the eggs were laid and which strongly influence survival. Egg size variation in turn is often seen as an adaptive trait, which may either strengthen or mitigate the consequences of hatching asynchrony. We examined growth rates and fledging success of chicks as well as egg size variation with respect to laying and hatching sequence in the neotropical hoatzin *Opisthocomus hoazin*. At our study site in a primary rainforest in Ecuador, hoatzins laid 1-3 eggs and began incubation as soon as the first egg had been laid. The laying interval was 1-2 days and chicks hatched in regular succession according to the order in which they had been laid. Fledged hoatzins were composed of 75% first-hatched (A) chicks and 25% second-hatched (B) chicks; we only found a fledged third-hatched chick once, indicating that the female operated a brood reduction strategy by means of hatching asynchrony. However, the growth rates of A and B chicks did not differ during the nestling or the fledging stage, suggesting that no differential feeding took place. Therefore, starvation is unlikely to account for the higher mortality of junior siblings, but we cannot offer another satisfactory explanation for the different survival prospects. In multi-egg clutches the last eggs to be laid were smaller than the first and second eggs and had a lower hatchability. Thus intra-clutch variation in egg volume seems to enhance the size hierarchies of chicks and gives further support to a brood reduction strategy. However, physiological or resource constraints may also have enforced a decreasing investment in egg production, and moreover may explain an apparent trade-off between clutch and egg size. Predation was high during the egg stage and early incubation might be selected for in order to protect the clutch better. Thus, hatching asynchrony may have to be viewed as a non-adaptive and costly consequence of an enemy-induced incubation pattern.

## Introduction

Many birds lay more eggs than they successfully raise and than life history theory predicts (Roff, 1992; Stearns, 1992). This apparent wasting of resources has puzzled avian biologists for a long time. According to the brood reduction hypothesis parents adjust their brood size *post hoc* with respect to the actual development of the decisive environmental conditions (Lack, 1947; Lack, 1954). If, for example, food is scarce after hatching one or more nestlings are sacrificed to enhance the survival prospects of the others.

Lack (1947; 1954) considered hatching asynchrony to be the main proximate mechanism of adaptively down-sizing an actual brood under adverse environmental conditions. Hatching asynchrony of eggs results from starting to incubate a clutch before laying has been completed and many species of altricial and semialtricial birds exhibit this behavioural pattern (Clark and Wilson, 1981; Magrath, 1990; Stoleson and Beissinger, 1995). As a consequence of hatching asynchrony a brood is composed of nestlings of different ages and sizes. Within this size hierarchy the last nestling(s) may starve to death or be killed by older and larger siblings. Lack (1947; 1954) suggested that hatching asynchrony was adaptive because it facilitated the reduction of brood size. By hatching their brood asynchronously, parents confer advantages to first-hatched offspring and handicap others from later-laid eggs. Assuming a brood reduction strategy, first-laid eggs should receive the biggest quantity of resources whereas the last egg(s) should receive less. The last eggs would be skipped when environmental conditions are bad or average and would produce fledglings only under extraordinarily good conditions.

However, hatching asynchrony might have to be interpreted, in some cases at least, as a side-effect or even a cost of other selection pressures (Clark and Wilson, 1981; Magrath, 1990; Stoleson and Beissinger, 1995). Then, parents should compensate the disadvantage of hatching later, and the brood survival hypothesis predicts the last egg(s) of a clutch should be bigger than the clutch mean. This would reduce size hierarchies and there is evidence for this strategy in some species (Slagsvold et al., 1984; Cichón, 1997). Based on the controversial adaptive and non-adaptive explanations of hatching asynchrony, Slagsvold et al. (1984) considered patterns of intra-clutch egg size variation as adaptive breeding strategies in themselves by modifying hatching hierarchies.

Of course, a general model to explain all egg laying patterns and breeding modes of birds would be most desirable. However, the wide variety of avian life-histories makes it unlikely that such a universal explanation will ever be found. In particular, breeding patterns of tropical birds may be shaped by other factors than those of temperate birds, from which most hypotheses were derived (see chapter 2.1). But information on tropical species is scarce and more data are necessary in order to enable the development of a comprehensive and consistent framework. In this article we examine the consequences of egg laying sequence on hatching rank and on nestling mortality in the neotropical hoatzin *Opisthocomus hoazin*. We also ask whether intra-clutch egg size variation strengthens or rather mitigates a possible effect of hatching asynchrony. Whereas observations on hoatzin clutch size are consistent at different study sites (1-3 eggs for single breeding females) contradictory data exist about when incubation begins: Grimmer (1962) and Domínguez-Bello et al. (1994) reported that incubation started when the first egg had been laid, but Strahl (1988) observed that incubation did not start before the second egg had been laid. Therefore, another objective of this study was to verify the incubation and hatching pattern for a population of this relatively unknown bird species living in an undisturbed lowland rainforest habitat.

## Methods

### *Study site and species*

We studied hoatzins from 1995 to 1998 and in 2000 in the Cuyabeno Lakes (“Lagunas de Cuyabeno”) in Amazonian Ecuador (0°02’ N, 76°9’ W and 0°03’ S, 76°14’ W). The area is a mosaic of primary rainforest with *terra firme* forest, several black water lakes and channels and large parts of flooded forest during the rainy season. Rainfall averaged 3300mm per year in a seasonal, bimodal pattern (April to July and October to November). The average annual air temperature was 26-27° Celsius with monthly minimum and maximum values of 19°C and 33°C, respectively.

The hoatzin (*Opisthocomus hoazin*) is a pheasant-sized bird that inhabits the riparian lowlands of the Amazon and Orinoco basin (del-Hoyo et al., 1996). It is related to the Cuculiformes but its exact taxonomical position remains unclear (Hedges et al., 1995; Hughes and Baker, 1999; Sorenson et al., 2003). Hoatzins mainly feed on leaves and buds which are digested with the help of foregut fermentation (Grajal et al., 1989). The sexes look alike and may live as single breeding pairs or together with offspring from previous years, together defending an all-purpose territory (Strahl, 1988). Breeding is linked to the onset of the rainy seasons and a simple open-cup nest is built on branches overhanging the water. Mean clutch size is two eggs, representing 51% of all broods and even 60% of clutches laid by single females. Joint-female-nesting occurs in about 15% of breeding attempts and may result in clutches of up to 7 eggs (Müllner et al. in prep., see chapter 2.5). At our study site incubation time is about 32 days and chicks fledge at an age of 45-55 days. Breeding success is 18% and only one brood per year is raised. Renesting is very rare and occurs only after early nesting failure (A. M. and K. E. L., in prep., see chapter 2.1). Both sexes incubate and brood the young hoatzins, but the offspring from previous years also help their parents with these duties (Strahl, 1985). Chicks are fed plant material which has been pre-digested and regurgitated by adults until they are at least two months old.

All monitoring of breeding activities was carried out using a dugout canoe. In general we checked the habitat one to three times a week in order to search for active nests and to control the fate of nests. All nest checks were made in the morning between the hours of 6:00am and 11:00am, depending on the nest’s position along the monitoring route.

### *Measuring egg volume and egg laying sequence*

A hoatzin was considered incubating when it was found sitting on at least one egg and the egg(s) felt warm. The length (L) and maximal breadth (B) of eggs discovered for the first time were measured using a sliding calliper to the nearest mm. The eggs were marked individually with a felt pen and their volume was estimated by means of the formula $0.51LB^2$ (Hoyt, 1979). In 1996 and 1997 it was only possible to observe the successive laying of a complete clutch in a few cases. In 1998 we increased visitations to a daily rate at some nests in order to record egg-laying order more precisely. Additionally, in 1998 and 2000 we used the egg-floating method to determine the laying sequence (Dunn et al., 1979; Alberico, 1995). Since gas is produced in the eggs during development the grade of submersion and the angle of an egg floating in water indicates the time that has passed since the egg was laid. We calibrated our estimation curve with eggs of known laying date and the method exactly reflected the relative age of eggs and was reliable in absolute terms for 1-2 days when used during the first two weeks of incubation (A. M. unpubl. data).

### *Analysis of egg size variation*

The analysis of egg size variation is restricted to samples from 1998 and 2000 because the laying order of eggs could only be determined in these years. In order to avoid pseudoreplication by repeatedly sampling the same breeding pair we only included first annual breeding attempts. We only considered clutches with a maximum of three eggs because only those can be assumed to come from a single female (Müllner et al. in prep., see chapter 2.5). We employed paired t-test (two eggs) or repeated measurement ANOVA (three eggs) to determine egg size variation *within* the clutch. A possible association *between* clutch size and egg size was evaluated by calculating the mean egg volumes for every clutch and comparing different clutch sizes using a t-test.

### *Determination of hatching order and further development of chicks*

In 1998 we approached nests containing chicks and weighed them using a Pesola spring balance to the nearest gram.  We also marked them with expandable plastic bands on the left or right leg (two chicks) or on both legs (three chicks). Non-hatched eggs found in the nest were collected and opened to determine whether any embryonic development had occurred. When hatching order could not be observed directly it was concluded post hoc for nestlings not older than 7 days according to the development features of downs, known from focal nests visited at a daily rate. Young hoatzins were caught and weighed again at the age of 10-15 days and, if possible, also at the age of 45-55 days when they were colour banded with individual combinations. At other control dates the development of juveniles was observed using binoculars, normally from a distance of approximately 25m to avoid triggering the typical escape behaviour in disturbed juveniles, which involves diving into the water with possibly very negative consequences (Müllner et al., 2004, see chapter 3.2). Undisturbed hoatzins stay in the nest until day 15-20 (nestling stage) and then start climbing in the surrounding vegetation until fledging between days 45-55 (fledging stage). Growth rates during the nestling period were analysed by linear regression for a population wide value with one individual providing one data point. If more than one measurement had been taken for one individual one measurement was selected by chance for use in further analysis in order to avoid pseudoreplication. Individual growth rates until fledging were calculated on the basis of at least two measurements with a time interval of 3-4 weeks.

## Results

### *Laying interval and onset of incubation*

Eggs were laid within an interval of one to two days (*n* = 8 clutches with 19 eggs), but it is necessary to consider that nest controls were always conducted in the morning, and thus no more precise laying times could be observed. Incidental flushing of incubating birds that allowed some unplanned nest inspections suggest that laying did not occur in the afternoon but rather during the night or at dawn.

The interval between the laying of the first and the second egg was 1.3 days ± 0.5 (*n* = 9 intervals) whereas two days always passed between the laying of the second and the third egg (*n* = 3 intervals). Although the sample size was too low to allow further analyses these findings correspond with the estimation of laying date by the egg floating method. Nests were attended from the first egg laid and this first egg was always warm to the touch. We never saw a nest containing any egg/eggs without a bird sitting on them.

### *Egg size and hatchability with respect to laying order*

There was an apparent negative association between laying order and egg size with second and later laid eggs being smaller (Fig. 1). In two-egg clutches the last-laid egg had on average a 3% smaller volume than the first-laid egg (mean difference = 0.7cm$^3$, paired t-test, t = 3.34, df = 48, p = 0.002). In three-egg clutches the pattern was more variable and as a consequence standard deviations were higher. Therefore, the difference between the volume of the last egg and the mean volume of the remainder failed to be significant (mean difference = 0.8cm$^3$, paired t-test, t = 1.79, p = 0.09). Nevertheless, two-egg clutches, which represent 60% of all clutches laid by single females and 51% of all clutches, exhibited a clear size hierarchy in laying order.

Interestingly, we detected an additional effect of egg number on egg volume for clutches of three eggs: mean volumes of the eggs in three-egg clutches were on average 1.3cm$^3$ smaller than the mean egg volumes in clutches with one and two eggs (Fig. 1, t = -2.23, df = 83, *p* = 0.029). Consistent with this pattern the lowest mean egg volume of all clutches was calculated for a three-egg clutch (22.1cm$^3$) and likewise the egg with the lowest volume out of all the hoatzin eggs we measured was found in a three-egg clutch (21.0cm$^3$; *n* = 557, 1996-2000).

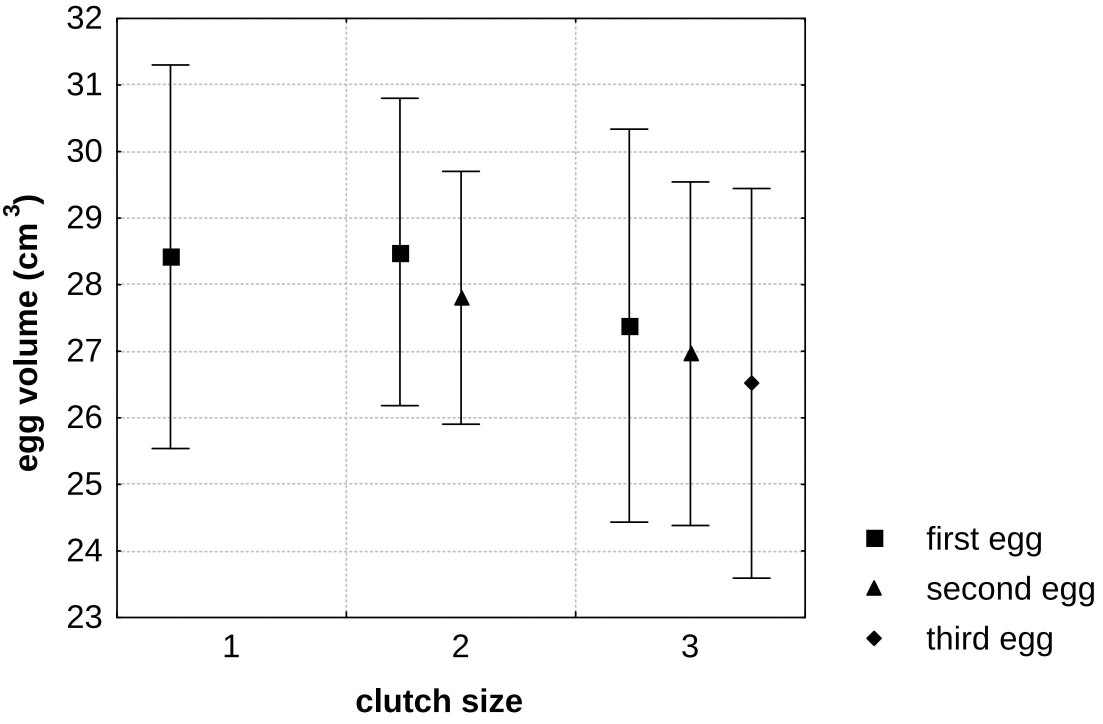

**Figure 1.** Mean egg volume ± SD in relation to the laying order of hoatzin eggs for *n* = 13 clutches with one egg, for *n* = 49 with two eggs, and for *n* = 23 with three eggs. Data from 1998 and 2000 were pooled.

Approximately 17% of eggs that had not been predated did not hatch (*n* = 95 eggs from 39 clutches). They were composed of eggs containing dead embryos (5 embryos from 5 nests; 5.3% of all eggs) and of eggs without development, assumed to be infertile (11 eggs from 10 nests, 11.6% of all eggs). Two of the infertile eggs came from single-egg clutches where they had been incubated for at least one week more than the usual 32 days. In multi-egg clutches hatching failure occurred almost exclusively in second- and third-laid eggs (G = 8.2, df = 1, *p* = 0.004, *n* = 11): The five dead embryos all came from a last-laid egg and the six infertile eggs, from which the laying order was known, were predominantly last-laid eggs. There was no difference in the volumes of hatched and non-hatched eggs of a clutch indicating that in fact laying order played a crucial role in hatchability (paired t-test, t = -1.5, *p* = 0.17).

### *Hatching pattern and growth development of hatchlings*

Eggs always hatched in the order they were laid and hatching intervals were as long as laying intervals, and thus reconfirmed our estimations of laying dates. Accordingly, hatching spans were 1-2 days in two-egg clutches and 3-4 days in three-egg clutches.

However, in one three-egg clutch an interval of three days between the hatching of the second and of the last egg was observed. Mean hatching weight ± 1 SD of chicks was 20.9g ± 2.31 (*n* = 7); no further separation with respect to hatching rank was performed due to the small sample size.

As a consequence of asynchronous hatching, nestmates from a common brood exhibited a size hierarchy, which in some cases persisted until fledging. Growth rates of first-hatched (A) and second-hatched (B) chicks did not differ significantly during the nestling stage both in broods with two-chicks as well as in broods with three chicks (Table 1, Fig. 2 B, C). In the latter, there was also no difference in the growth rates of C chicks compared to those of their senior siblings. However, while growth rates of single chicks were similar to those of chicks with one further sibling, broods with three chicks had much lower growth rates independent of hatching rank (Fig. 2 A, B, C; single chicks *vs.* two chicks: t = 0.73, $p = 0.47$; two chicks *vs.* three chicks: t = 3.18, $p = 0.002$).

Likewise, during the fledging period growth rates were indistinguishable between A and B siblings of a common brood (Fig. 3, paired t-test: t = 0.23, $p = 0.83$). There was also no difference in growth rate between broods bringing only one nestling to fledge and broods with two nestlings (mean ± SD; one chick: 6.6g/day ± 1.85; two chicks: 6.35g/day ± 0.81, t = 0.39, df = 16, $p = 0.7$). These growth rates were similar to those of the nestling stage (see Table 1), indicating that no fundamental change in development rate took place after leaving the nest.

### *Survival of first- and second-hatched chicks*

Normally chicks just vanished between two control dates without any hint as to their fate. We have no evidence to indicate that starvation was a major cause of the disappearance of young hoatzins and dead or severely weakened chicks were only found in the nest in a few cases. Some chicks had small wounds from bites of the vampire bat (*Diphylla caudata*) and had probably suffered blood loss. Despite similar growth rates, the survival of A chicks was much higher than the survival of B chicks. When comparing success on a per-nest basis, in 70% of successful breeding attempts only the first-hatched chick fledged, in 26% of broods both chicks survived until fledging, and in 4% only the second-hatched chick fledged (*n* = 27). This distribution was highly significantly different from a uniform distribution (G = 19.99, $p = 0.00005$). Calculated on the basis of individuals, 76.5% of all fledglings from which hatching order and fate were known were A chicks whereas 23.5% were B chicks (G = 9.89, $p = 0.002$, $n = 34$). We only once observed a brood with three fledglings.

**Table 1.** Growth rates of hoatzin chicks during the nestling stage (1-20 days) according to hatching rank and brood size.

| | **A** | **B** | **C** |
|---|---|---|---|
| **Single chick** | | | |
| Weight gain / day (g) | 7.2 | | |
| *n* | 14 | | |
| Linear regression | Y = 16.19 + 7.19 x<br>($r^2 = 0.87$) | | |
| **Two chicks** | | | |
| Weight gain / day (g) | 7.0 | 6.0 | |
| *n* | 31 | 29 | |
| Linear regression | Y = 13.48 + 6.99 x<br>($r^2 = 0.90$) | Y = 14.5 + 5.98 x<br>($r^2 = 0.80$) | |
| slope A *vs.* B | t = 1.34, $p = 0.19$ | | |
| **Three chicks** | | | |
| Weight gain / day (g) | 3.1 | 4.6 | 3.7 |
| *n* | 10 | 10 | 10 |
| Linear regression | Y = 31.0 + 3.01 x<br>($r^2 = 0.54$) | Y = 21.4 + 4.6 x<br>($r^2 = 0.50$) | Y = 20.67+3.67x<br>($r^2 = 0.82$) |
| slope A *vs.* B | t = 0.77, $p = 0.46$ | | |
| slope A *vs.* C | | t = 0.49, $p = 0.63$ | |
| slope B *vs.* C | | | t = 0.57, $p = 0.58$ |

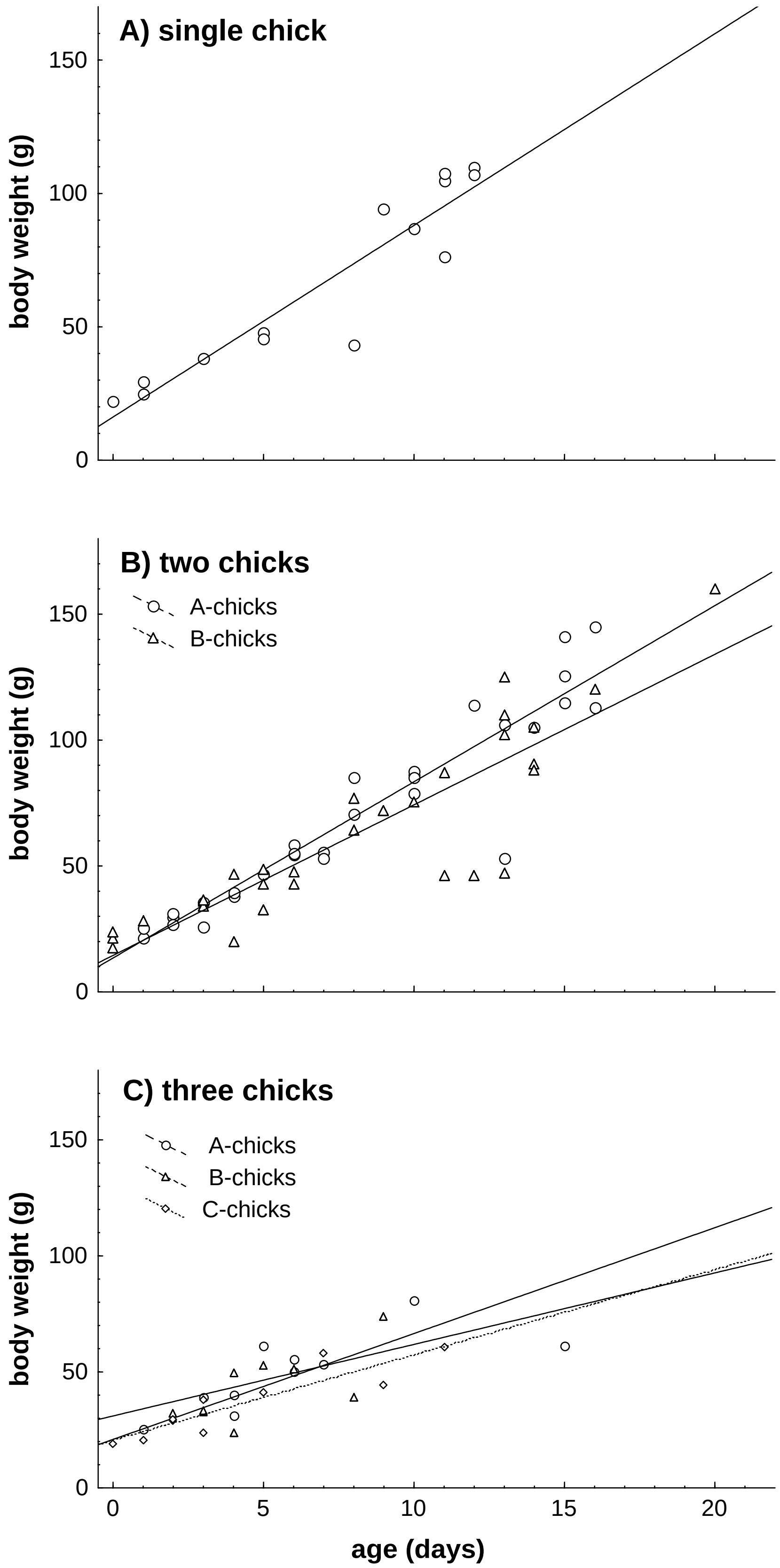

**Figure 2.** Body weight with respect to age of young hoatzins during the nestling stage in broods with A) a single chick, B) two chicks, and C) three chicks, grouped by hatching rank. One individual contributes one data point. Sample size was *n* = 14 for A), *n* = 30 + 31 for B), and *n* = 10 + 10 + 10 for C).

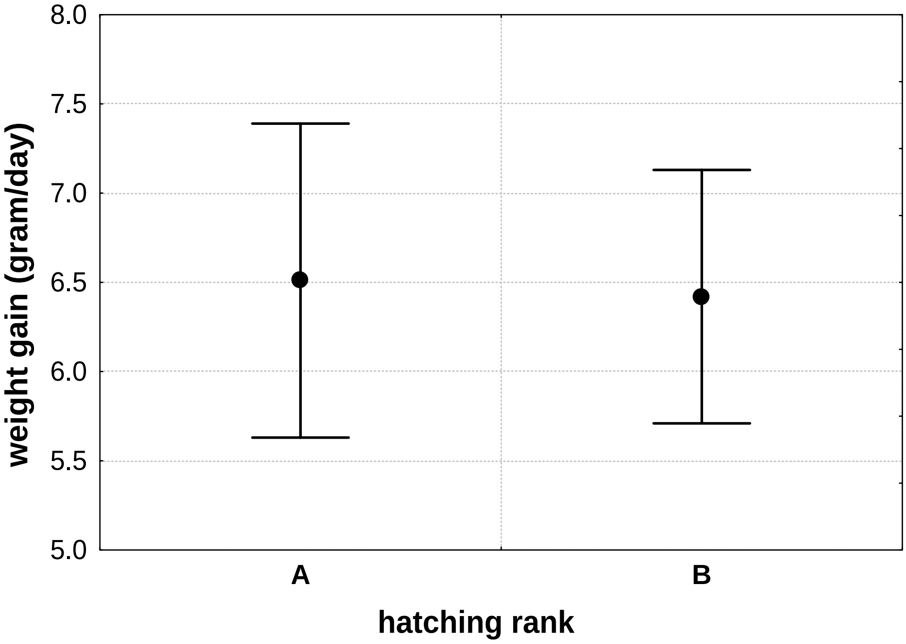

**Figure 3.** Mean growth rates ± SD of 5 hoatzin sibling pairs with respect to hatching rank during the fledging period measured at 7-15 days and at 45-55 days of age, respectively.

## Discussion

### *Onset of incubation and hatching pattern*

In our study area in Ecuador hoatzins started incubation with the first egg laid and successive hatching reflected the laying order and the laying interval. Early incubation in hoatzins has also been reported for populations in British Guyana (Grimmer, 1962) and Venezuela (Domínguez-Bello et al., 1994). Interestingly, hoatzins at another site in Venezuela did not start incubation before the second egg had been laid and chicks of two-egg clutches hatched within one day (Strahl, 1988). Variation in the onset of incubation within a bird population has recently been demonstrated (Bortolotti and Wiebe, 1993; Wiebe et al., 1998; Greiner and Beissinger, 1999) and it is therefore to be expected that variation between different populations also occurs. Furthermore, variation in incubation pattern is a prerequisite for explaining the adaptive significance of hatching asynchrony, because this hypothesis assumes that females can control hatching patterns by altering their incubation behaviour. Unfortunately, the consequence which the more synchronous hatching had on hoatzin chick survival at Strahl’s study site is unknown. However, despite the measured individual variation in asynchrony, both reports by Greiner and Beissinger (1999) and Wiebe et al. (1998) support our finding that eggs hatch in the order they were laid. Bortolotti and Wiebe (1993) associated irregular hatching pattern with small female body size due to age or poor body condition, which might cause problems in covering all eggs during incubation. However, hoatzins should not have problems fully incubating three eggs because of their considerable body size, and in fact no correlation exists between egg number and incubation time ($R_S = 0.34$, $t = 1.27$, $p = 0.23$, $n = 14$; Müllner unpubl. data).

When explaining the presumed adaptiveness of hatching asynchrony most hypotheses or experiments have focused on the nestling stage in order to determine some post-hatch benefit such as increased reproductive success. However, it is important to remember that early incubation may be caused during the laying period by other proximate factors including hormonal constraints (Mead and Morton, 1985), food supply (Wiebe and Bortolotti, 1994), egg viability (Veiga, 1992) or predation pressure (Bollinger et al., 1990). Clark and Wilson (1981) predicted that an equal or greater predation on eggs than on nestlings should select incubation starting with the first egg laid because this minimises the period when the nest contains obvious and unprotected eggs. Therefore, differences in size among nestlings may be a side-effect of selection for early incubation and brood reduction might be a non-adaptive and costly consequence. Indeed, our study population of hoatzins suffers a high predation pressure during the egg stage: 65% of the clutches vanished before hatching whereas 49% of broods failed during the nestling and fledging stage (A. M. and K. E. L. in prep., see chapter 2.1). In addition, the main predators of hoatzin eggs at our site were birds, which locate their prey optically. Hoatzin eggs are whitish and were easy to detect in the open-cup nest when birds were flushed off. It is therefore reasonable to conclude that early incubation might be indispensable for egg protection in this tropical environment where there is certainly a high abundance of predators.

### *Brood size and survival prospects of chicks*

Hatching rank strongly influenced the survival of hoatzin chicks: many more first hatched chicks (A) survived than did last hatched (B or C) chicks. This consequence of hatching asynchrony is well known and originally led to the formulation of several adaptive and non-adaptive hypotheses explaining the apparent wasting of energy (reviews in Clark and Wilson (1981) and Stoleson and Beissinger (1995)). The most frequently cited explanation is that avian parents may create more offspring than they can normally rear as a backup against unpredictable environmental conditions (Lack, 1947; 1954). In our study population over four years on average only 24% of successful hoatzin broods raised two fledglings whereas 75% produced one fledgling (A.M. and K. E. L. in prep., see chapter 2.1). Obviously a chance of 1:4 for a second fledgling was sufficient to warrant the regular investment in a two-egg clutch.

The reasons for the higher mortality of B and C chicks than that of A chicks were not obvious to us. Mortality of all chicks was highest within the first three weeks when the chicks were still in the nest (see survival tables in Müllner et al., 2004, chapter 3.2) and when food demand should not have been as high as during the fledging period. C chicks which hatched later just vanished more often than their older nestmates and we have no evidence that starvation played a role. Similar growth rates to those of their first-hatched nestmates demonstrate that no differential feeding took place either by the parents or by sibling competition. However, we have to consider that our fledgling sample bears a natural bias towards surviving individuals and therefore may be masking a potential disadvantage of later-hatched chicks. Surprisingly, the latter could never catch up the head start of their siblings who were at most two days older and the difference in body weight from that of their senior siblings often persisted until fledging eight weeks later. This indicates maximum growth rates which were not constrained by food provision but by other maturation processes. For instance, the ruminant-like digesting system in the hoatzin, unique for birds, may mean that there are upper limits to the efficiency by which the plant material is fermented and important compounds are extracted (Grajal et al, 1989; Grajal and Parra, 1995). We lack detailed observations on feeding behaviour which would enable the exclusion of the possibility that the parents treated A and B chicks differently. Theoretically, a strict feeding order might imply differences in the degree of predigestion of the fed material or in the rate of inoculation with the necessary bacteria and protozoa (Domínguez-Bello et al., 1993). The latter are suspected to play an important role in the detoxification of plant secondary compounds (Grajal, 1995).

Predation is the most important factor involved in nesting failure at our study site (A. M. and K. E. L., see chapter 2.1) and in the tropics in general (Skutch, 1966; Ricklefs, 1969). However, it is not very likely that predators selected the smaller chick of a brood so precisely so often. Furthermore, we do not have any evidence which hints towards siblicid or infanticide, and there is no anecdote from another site which favours this possibility, but we cannot exclude it. In conclusion, we have no satisfactory explanation regarding how the rank-related mortality acted. Interestingly, Stoleson and Beissinger (1997) report something similar from a neotropical parrotlet (*Forpus passerinus*): rather than starvation, size disparities among nestmates themselves might have led to the death of the smallest chick but the proximate mechanisms remained unclear. The topic of rank-related chick mortality is clearly worth further investigation.

Growth rates were not only similar within a brood but also did not differ between single chicks and chicks sharing parental care with another sibling. This finding indicates that food provision by the adults was sufficient and is consistent with the fact that food did not play a major role in enhancing breeding success by helping offspring (A. M. and K. E. L., see chapter 2.3). Domínguez-Bello et al. (1994) also observed that hoatzin chick development was independent of brood size in Venezuela. However, in our study we found a marked decline in growth rate in broods with three chicks compared to that of broods with one or two chicks indicating that an essential component of food composition or brood care was still inadequate. C chicks vanished more rapidly than their second hatched siblings (8.1 days ± 3.1 versus 15 days ± 6.7, $n = 7$) and after the death of the third chick the development of the remainder of the nestlings seemed comparable to that of two-chick broods, although we lack data on the body mass of these fledglings. It is possible that our data are misleading because three-chick broods are uncommon and hence sample size is low. Nonetheless, it is striking that, in addition, three-egg broods started with lower mean egg sizes than smaller broods and that third-laid eggs were the smallest of all eggs, indicating that the investment per egg was lower. This suggests a fundamental difference between the female's resource allocation in smaller and larger broods.

If the survival probabilities of three chicks in a single brood and in particular of C chicks are so low, why hoatzins do invest in a third egg at all? In the four breeding seasons throughout which we had thoroughly studied reproductive success we only once observed three fledglings within one breeding unit, although 35% of all clutches contained three eggs or even more by joint-female nesting (A. M. and K. E. L., see chapter 2.1). At first glance this suggests that egg production is cheap and in fact data from a savannah habitat in Venezuela support this view: hoatzin breeding units produced up to six replacement clutches within a season (Strahl, 1988) demonstrating that females of this species are in fact able to lay many more eggs than corresponds to the average brood size. However, in Cuyabeno, replacement clutches were rare and females maintained a time gap of 4-5 weeks between clutch loss and renesting (see chapter 2.1). We consider it likely that the very nutrient-poor environment of the Cuyabeno Lakes, flooded by black water, constrains egg-production due to the lack of essential resources such as protein, calcium carbonate or other minerals. The association between clutch and egg sizes (see below) gives further support to this constraint theory although the current clutch size pattern might be genetically maintained and therefore might not be readily changeable (Gwinner et al., 1995). In any case - cheap or costly egg production - the trade-off between investment in brood size and survival prospects of chicks is linked to the fundamental question of how frequently those special conditions would have to come along to make overproduction cost-effective. This parameter is difficult to demonstrate and remains a central challenge when attempting to test the brood reduction hypothesis.

### *Egg size variation and hatchability*

Egg size declined with position in the laying order in the most common clutches with two eggs. This effect also appears to be present in three-egg clutches but the pattern is less clear. Egg size is known to influence egg quality and hatching weight (Reed et al., 1999), and thus first laid eggs have an advantage over last-laid eggs. Instead of counteracting the consequence of hatching asynchrony, the intra-clutch egg size variation accentuated the size hierarchies of nestlings. This variation pattern appears adaptive when seen in the framework of a brood reduction strategy (Slagsvold et al., 1984). However, chick growth rates were independent of egg laying order or hatching rank, therefore it is unlikely that egg size made a considerable contribution to survival. We lack sufficient measures of newly hatched chicks from eggs from different laying positions (i.e. first-laid or last-laid eggs) to assess any minor effects of egg size variation, but our data suggest that the possible slight effect of egg size on survival was later compensated for anyway. This conclusion is supported by Sydeman and Emslie (1992) and Reed et al. (1999) who did not find that egg volume influenced fledging success. Furthermore, Magrath (1992) demonstrated that hatching asynchrony in Blackbirds (Turdus merula) is far more important than egg size in establishing hatching size hierarchies.

Only 83% of non-predated hoatzin eggs hatched which seems a rather low percentage. For example, Koenig (1982) calculated a mean hatchability of 89.7% for non-passerines. Interestingly, he detected a negative association between hatchability and social factors such as shared incubation and cooperative breeding behaviour, and both factors might apply to the hoatzins. In principle, the high rate of hatching failure might be an ultimate cause for an insurance egg (Simmons, 1997; Forbes, 1991) and therefore might explain the laying of a second and a third egg despite the high mortality. However, this suggestion is not consistent with the fact that the hatchability of eggs was related to their laying rank. This finding rather fits to the association between egg size and laying order and demonstrates that the females put less effort into the production of the last egg. Since survival prospects were considerably higher for chicks from first-laid eggs than for chicks from last-laid eggs, the female hoatzin invested less in the last-laid eggs when resources were limited.

Alternatively, physiological constraints might act during fertilisation or formation of second and third eggs. This non-adaptive interpretation also offers an explanation for the measured intra-clutch egg size variation. There is an ongoing debate about the adaptive significance of intra-clutch egg size variation but several studies show that this variation can often be seen as a population wide response to environmental conditions (Järvinen and Ylimaunu, 1986; Jover et al., 1993). Further evidence for an enforced responsiveness instead of adaptiveness of the hoatzin egg size variation *within* clutches is the egg size variation *between* clutches of different sizes: clutches with the maximum number of three eggs had a lower mean egg volume than clutches with one or two eggs. This indicates a trade-off between egg number and egg size due to resource limitation of the egg laying female (Stearns, 1992; Roff, 1992). The cost of egg production might have been underestimated in many species for a long time. Recently it has been well documented in gulls, showing clear impacts on egg quality (Nager et al., 2000), on parental performance (Monaghan et al., 1998), and on future fitness (Nager et al., 2001). In hoatzins, clutch mass is low compared to the adult body mass (4%, 8%, and 12 % for one, two, and three eggs, respectively, A. M. and K. E. L. in prep, see chapter 2.1) and therefore simple energetic restrictions are unlikely in this leaf-eating species whose food normally is superabundant. However, as mentioned above, females might suffer a lack of other essential elements rare in this environment, limiting both size and number of eggs that can be produced.

## Conclusions

Hoatzins apparently initially over-produce family size when compared to the number of successfully raised offspring. Hatching rank plays a crucial role in chick survival and intra-clutch egg size variation enhances the size hierarchies of nestlings instead of counteracting the consequence of hatching asynchrony. This suggests that hoatzins exhibit a brood reduction strategy adopting hatching asynchrony as a mechanism of down sizing. However, egg size variation *within* and *between* clutches as well as an association between hatchability and hatching rank also support a non-adaptive explanation of egg size pattern due to resource limitations. It is likely that early incubation may be selected for because predation during the egg stage is very high. In this case hatching asynchrony is an inescapable consequence of the incubation pattern. Early incubation in turn may offset the cost of asynchronous hatching and thus rank-related chick mortality.

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# 2.3 Effects of unit size on reproductive output in the co-operatively breeding hoatzin *Opisthocomus hoazin*

Antje Müllner and K. Eduard Linsenmair

## Abstract

Hoatzins are non-passerine birds which live in the Amazon and Orinoco river basin. Based on a study in the savannah of Venezuela they are classified as co-operative breeders in which social units may be composed of a breeding pair and offspring from previous years in approximately 55% of breeding attempts. We examined the composition of breeding units and their reproductive output in a hoatzin population at the Cuyabeno Lakes in the lowland rainforest of Ecuador in order to assess the effect of unit size on breeding success. The most common group size was a trio (40%); single pairs and groups with four adults each represented about 20%, and roughly 20% of units had 5-7 unit members. Thus breeding groups (80%) rather than pairs were more common at our study site than in Venezuela. In our opinion, the more stable environment and the higher predation pressure in Cuyabeno account for this geographic difference. Adults other than the breeding pair participated in incubation, feeding the young and territory defence. The survival of broods was significantly higher in groups than in unaided pairs for both the incubation and the fledging period. However, groups did not produce significantly more fledglings per successful nest than single pairs, although their initial brood size was larger. We suggest that helpers played a major role in enhancing reproductive output by improving predator detection and deterrence, although the effects of differences in age and experience of parents and helpers cannot be excluded. Breeding success did not increase further in groups where there were more than three individuals indicating a trade-off between helping and group size.

## Introduction

In most species of birds co-operation among adults to raise young involves only the breeding pair (Lack, 1968), but in at least in 3% of all extant species more than a single pair co-operate to raise young (308 of 9672 bird species; Arnold and Owens, 1998). Most "alloparental" helpers are offspring from previous years staying in their parent's all-purpose territory (Brown, 1987). Despite the inclusive fitness advantages gained by helping siblings (Koenig et al., 1992), the fitness benefit from staying at home and helping is likely to be considerably less than the potential of direct genetic gain from immediate independent breeding if a territory and/or mate could be obtained (Brown, 1987). Thus co-operative behaviour can be seen as a "best-of-a-bad-job" strategy, adopted only when constraints on successful independent breeding exist.

A vast number of authors have tried to identify the selective pressures towards this rare behaviour. Explanations primarily focus on a) why helping offspring delay dispersal from their natal territory rather than dispersing and attempting to breed independently, and b) why they help to raise young that are not their own. Two major schools of thought can be distinguished: While the ecological constraint hypotheses stress the influence of factors preventing dispersal and breeding, e.g. habitat saturation (Emlen, 1982), others focus on the long-term benefits associated with philopatry and group-living, e.g. learning of particular skills or inheriting a high quality territory (Stacey and Ligon, 1987; Hatchwell et al., 1999; Ligon and Stacey, 1991; Koenig et al., 1992). However, no single concept is able to explain all patterns observed in birds (e.g. Cockburn, 1998) and a recent review concludes that the rate of turnover of suitable breeding opportunities plays a key role but that specific life-history traits and ecological constraints act together to influence this turnover (Hatchwell and Komdeur, 2000).

Independent of the ultimate and proximate causes of helping behaviour most explanations are based on the assumption that helping increases the breeding output of the recipients. In fact there is a lot of evidence which supports a positive effect of helpers (Brown et al., 1982; Brown, 1987; Stacey and Koenig, 1990; Mumme, 1992; Komdeur, 1994) but other studies failed to detect such an effect (Leonard et al., 1989; Magrath and Yezerinac, 1997; Shaw and Shewry, 2000; Bednarz, 1987). Komdeur (1994) demonstrated that in the Seychelles Warbler (*Acrocephalus seychellensis*) a potential benefit from helpers depends on the overall size of the breeding unit in combination with territory quality. Sydeman (1989) observed that the participation of helpers in caring for nestlings only enhanced breeding success in some years in Pygmy Nuthatches (*Sitta pygmaea*). Thus the relationship between the presence and number of helpers and the breeding outcome seems to be complex and variable, and depends on several factors that may change in time and space. Emlen (1991) pointed out that many bird and mammal species are in fact facultatively co-operative, and recently Heinsohn and Legge (1999) concluded that co-operative breeding in birds should be seen as a flexible strategy which can be altered according to demographic constraints and opportunities. Therefore we should expect plasticity in the social organisation of a species in different populations and habitat types. Hence, studying the within-species variation in co-operative behaviour is an approach that offers a better and more appropriate view of the influencing factors.

Here we examine a possible association between the size of the breeding unit and reproductive outcome in the hoatzin (*Opisthocomus hoazin*). Hoatzins are non-passerines which live in the Amazon and Orinoco river basin and were classified as co-operative breeders by Strahl (1988) and Strahl and Schmitz (1990). According to their study in Venezuela hoatzins live in territorial social units that are usually composed of a single breeding pair and from none to six non-breeders. These additional adults were largely offspring from previous years helping with incubation, feeding nestlings and fledglings as well as with territorial defence (Strahl, 1988; VanderWerf and Strahl, 1990). At this study site in the savannahs (“llanos”) of Venezuela the presence of helpers increased annual breeding success through both a higher survival of chicks and a higher number of breeding attempts per season (Strahl and Schmitz, 1990).

Although the hoatzin is well known among ornithologists for its still puzzling taxonomic position as well as for the retention of wing claws and swimming ability of the chicks, all published data on the social organisation of this species come from studies on the above mentioned single study population in Venezuela (Strahl, 1985; Strahl, 1988; Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990). Therefore one aim of our research was to examine hoatzin group composition and breeding success in another geographical region and another habitat in order to assess possible variations. In addition, we investigated specifically if and in which way a differing number of alloparental group members would affect the reproductive output of the breeding units.

# Methods

### *Study population and site*

We investigated the breeding ecology of a hoatzin population (*Opisthocomus hoazin*) in a primary rainforest of Amazonian Ecuador from 1996 to 1998 and in 2000. The study site at the Cuyabeno Lakes is a 65km$^2$ area comprising interconnected black water lagoons, flooded and non-flooded forests and *terra firme* forest (0°02' N, 76°9' W and 0°03' S, 76°14' W). The lakes are surrounded by stretches of inundated forest, which are flooded twice a year for 4-5, and 2-3 months (see chapter 2.1). This forest is six to ten meters high and composed of only eight major tree and shrub species. In many areas the density of this "lagoon forest" is low presenting a rather open habitat (see Appendix 1). For a detailed description of the study site see Müllner and Pfrommer (2001) and Müllner et al. (2004).

The total hoatzin population in these flooded forests varied between 700 and 750 individuals living in social units consisting of two to seven adults. These units defended all-purpose territories of approximately 5000–8000m$^2$, the size of which depended on the area of open water surface. These territories had highly traditional boundaries over the years studied and were left only during the dry season when hoatzins formed larger flocks and followed the shrinking watercourses. Nesting activity was closely linked to the beginning of the rainy season and the majority of clutches were laid in May and June. Nests were built on branches overhanging the water and about 70% of all nest sites were island trees surrounded by open water. The most common clutch size was two eggs (51%, $n$ = 291) with an overall range of 1-7 eggs. Only a single brood per season was raised. Second attempts were very rare and only performed after an early failure during the incubation period (A. M. and K.E. L. in prep., see chapter 2.1). Despite larger clutch sizes broods only fledged one (74%) or two young (26%) (A. M. and K.E. L., see chapters 2.1 and 2.2). Social units that are larger than just the breeding pair may contain both non-breeding adults and co-breeders. At our study site joint-female nesting made up to 15.5% of all breeding events (results of DNA-fingerprinting, A. M., K.E. L. and M. Wink, unpublished; see chapter 2.5). This is in contrast to other places where joint-nesting of hoatzins is quite rare (Peru: 5.3%, Torres, 1987; Venezuela: 4.6%, Strahl, 1988).

Hoatzins feed only on foliage and possess a ruminant-like digestion (Grajal et al., 1989). In Cuyabeno their major food trees are several species of Fabaceae, which were among the most common tree species in the flooded forest (A. M. and K.E. L. in prep., see chapter 2.1). Foraging bouts occurred mainly in the early morning and late afternoon hours, during the day the birds normally were perched quietly, probably in order to digest their leafy diet. This unusual feeding habit may explain the sedentary lifestyle of the species. The taxonomic position of the pheasant-sized hoatzin is still unclear. Sorenson et al. (2003) have recently questioned a former classification of the species among the Cuculiformes (Sibley and Ahlquist, 1990; Hughes and Baker, 1999; Hughes, 1996).

### *Data collection*

In this part of the study we searched for hoatzin nests from April to December in 1997 and 1998. All nest monitoring was done with a paddled dugout canoe by the same observer (A. M.). We only approached nests closely at the initial control to determine the nest content or to inspect the nest when no activity was observed. Every nest site was checked one to three times a week to observe hatching or fledging success, generally from a distance of 20 to 40 meters using binoculars. Additionally, at each visit we recorded the number of adult individuals in the breeding unit. While one individual was incubating or brooding the remainder of the group were often perching together, normally within viewing distance of the nesting tree. We defined the maximum number of adults observed in successive counts as the size of the breeding unit. The variation between counts was low because hoatzins stayed in their territory during the entire breeding season. In hoatzins no plumage features indicate breeding or social status and in addition both sexes look alike. Therefore we were unable to distinguish between the resident pair, non-breeding helpers or potential co-breeders and we could only determine the overall size of the social unit.

To verify whether additional adults contributed to the breeding effort we spent a total of 60 hours using a telescope to observe six breeding attempts by four breeding units from observation towers which were constructed on rafts. These focal groups had either three or four members and it was possible to distinguish different individuals by their pattern of wear and emergence of tail feathers.

### *Analysis*

Breeding success was analysed by both the percentage of successful broods, and the number of fledglings per successful brood. We divided the nesting cycle into the incubation period and the fledging period and broods were considered successful when they had produced at least one hatchling after the incubation period or when at least one young had emerged after the fledging period, respectively. The survival rate of nests was calculated according to Mayfield (1961; 1975) and comparisons of nesting success between groups of different sizes were made according to Hensler and Nichols (1981). Data from all groups were pooled, thus a potential effect of group size includes the effect of offspring helpers and that of co-breeders. Because co-breeding might confound or even hinder hoatzin breeding success (A. M., K.E. L. and M. Wink, unpublished, chapter 2.5) we also analysed a subsample that excluded all breeding attempts with a brood size of more than three eggs or nestlings, as these were likely to have been a result of joint-female nesting (A. M., K.E. L. and M. Wink, unpublished, chapter 2.5). To further examine whether brood size, in particular the number of fledglings, was influenced by additional adults in a breeding unit we also analysed the number of eggs, hatchlings and fledglings per brood in breeding units of different sizes. We used Kruskal-Wallis ANOVA for comparisons because the number of unit categories was too small for a correlation analysis. All *p*-levels given are two-tailed.

## Results

### *Composition of breeding units*

Hoatzin breeding units differed in their size, ranging from two adults, i.e. a single pair, to seven adults (Fig. 1). Mean unit size ± SD was 3.5 ± 1.13 in 1997 and 3.3 ± 1.11 in 1998. Single pairs represented approximately 20% of all breeding units. Groups were usually trios (ca. 40%) or composed of four adults (ca. 23%). Frequencies of larger groups were less than 10% each and decreased with increasing group size. The distribution was similar in both years. In our focal groups all adult unit members were seen to incubate eggs, to feed young and to engage in territorial disputes. However, our observation time did not allow quantification of the effort dedicated to brood care by the different individuals.

**Figure 1.** Number of adults in hoatzin breeding units and the relative frequency of unit sizes.

***Size of breeding unit and brood size***

Clutch size increased steadily with group size, and thus clutch sizes were not uniformly distributed among groups of different size (Fig. 2; eggs: H = 86.74, p < 0.00001). The most pronounced difference was observed between groups of 4 and groups of 5-7 members.

***Size of breeding unit and breeding success***

Nests of breeding units with additional adults had a higher daily survival than nests of single pairs both during the egg stage and during the fledging stage (Fig. 3; hatching: Z = - 2.65, *p* = 0.008, *n* = 147; fledging: Z = -2.32, *p* = 0.02, *n* = 102). The magnitude of this effect was similar in all units that were larger than just a pair. Translated into survival rates for the entire hatching or fledging period, unaided pairs had a chance of 0.11 and 0.07 of producing at least one hatchling or fledgling, respectively, while the rates for groups were 0.33 and 0.48, respectively. This result holds also true when a subsample is analysed that excludes potentially confounding co-breeding events (hatching: Z = - 2.59, *p* = 0.01, *n* = 123; fledging: Z = - 2.37, *p* = 0.02, *n* = 91). The mean number of fledglings produced per successful nest, however, always ranged between 1.0 and 1.4 per nest and did not differ significantly among groups (Figure 4; H = 3.83, *p* = 0.44), although the diagram below suggests a slight increase. The number of fledglings did not differ either between unaided and aided pairs when all aided pairs where pooled (MW-U test, Z corr. = -1.22, *p* = 0.22). There was no influence of group size on the number of broods per year because second breeding attempts were not performed if the first attempt was successful.

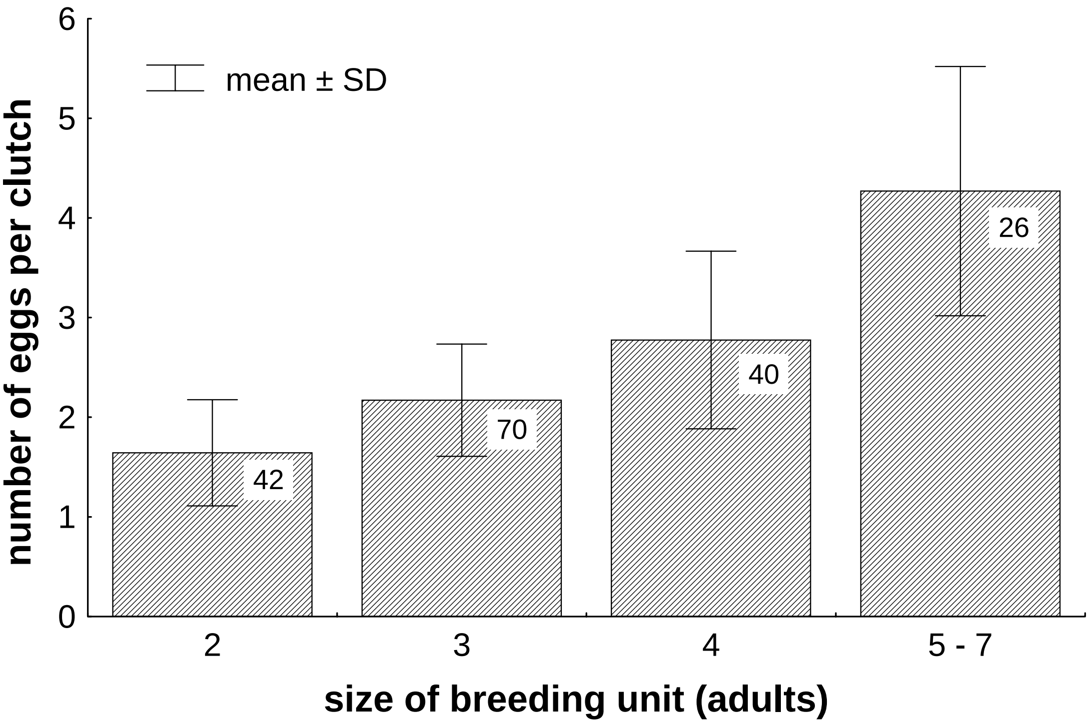

**Figure 2.** Number of eggs per brood ± SD with respect to the number of adults in the hoatzin breeding units. Data from 1997 and 1998 were pooled; sample size is given in the bars.

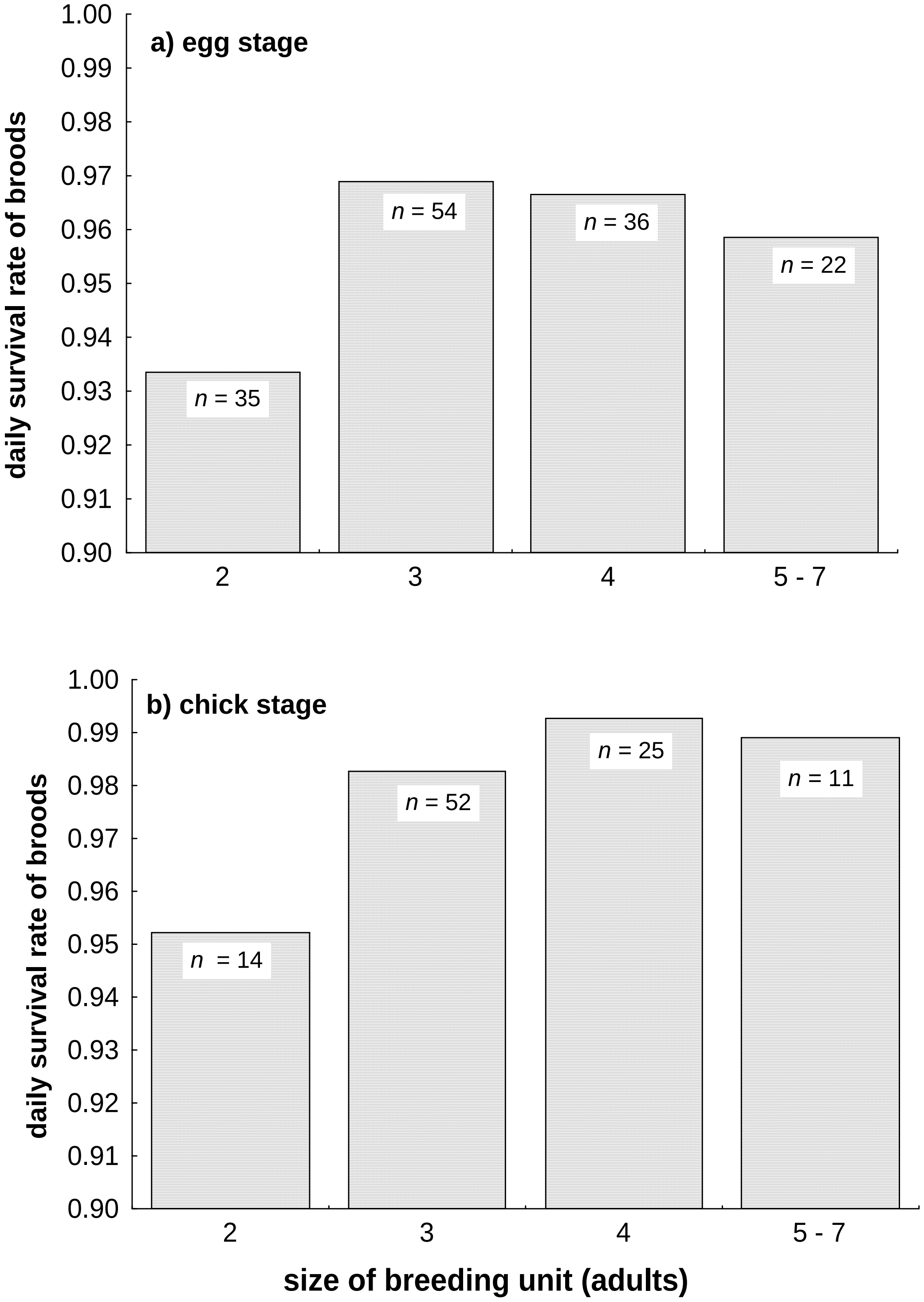

**Figure 3.** Daily survival rate of hoatzin broods during a) the egg stage and b) the chick stage for breeding units of different sizes. In successful nests at least one hatchling or one fledgling survived the egg stage or the chick stage, respectively. Data from 1997 and 1998 are pooled.

**Figure 4.** Number of fledglings per successful brood ± SD with respect to the number of adults in the hoatzin breeding units. Data from 1997 and 1998 are pooled; sample sizes are given in the bars.

## Discussion

### *Composition of breeding units*

In our study population breeding units consisted of between two and seven adults, with single pairs representing about 20% of hoatzin breeding units while alloparental adults were found in 80% of units. Trios were the most common group size (40%). It is likely that the individuals in the groups, apart from the breeding pairs, were offspring from previous years in most cases (Strahl, 1988; observations of colour-banded individuals, A. M.). Although we lack quantitative data on their share of brood care we observed these adults both incubate and feed the young which is consistent with the observations of Strahl (1988) and Strahl and Schmitz (1990). Therefore, it seems justified to call the non-breeding adults “helpers”.

Hoatzins in the Cuyabeno Lakes more commonly lived in large social units with helpers than hoatzins in the savannahs of Venezuela where single pairs made up nearly 45% of breeding units and trios represented 30% of groups (Strahl and Schmitz, 1990). This means that in our study population more young hoatzins delayed dispersal and reproduction. Arnold and Owens (1998; 1999) considered ecological variation to be important in determining exactly which species or population in a predisposed lineage will adopt co-operative breeding. They suggested that co-operative breeding might be linked to the fact that stable environments facilitate sedentary behaviour and the establishment of year-round territories. In fact, the Venezuelan savannahs, although tropical, are intensely seasonal habitats and seem to exhibit a greater range of within-year variation than our study site in the rainforest directly at the equator (Rabenold, 1984; Strahl, 1988; A. M. and K.E. L., unpublished, chapter 2.1).

Apart from the general feature of a year-round territory other factors may have caused delayed dispersal in the hoatzin population at Cuyabeno. Habitat saturation is one factor which is thought to crucially influence whether offspring decide to breed independently or to stay in the natal territory and to help (Emlen, 1982; Komdeur, 1992). Populations at high densities may be co-operative while those at lower densities are either not at all co-operative or only co-operative to a lesser extent. However, hoatzin breeding units were more scattered in the Cuyabeno Lakes than in the Gallery forest (3-10 units/km shoreline in Cuyabeno compared to ca. 20/km in Venezuela (Strahl and Schmitz, 1990)) and space for territories does not seem to be a limiting factor. Therefore, pure habitat saturation is unlikely to account for the difference in group composition.

Komdeur (1992) demonstrated that not only does a shortage of breeding territories influence the decision to stay at home but the food abundance in the territories available also affects this decision. Offspring then face the options of staying in a successful natal home or leaving for an independent but marginal territory. We lack data on the vegetation density and plant inventories for each territory, but leaves, as the hoatzins' exclusive food source, seemed to be superabundant, at least during the breeding season, which coincides with the appearance of new leaves and shoots. In agreement with Strahl (1985) we consider food unlikely to be an important feature of territory quality and we doubt that a pronounced variation in food availability between sites can explain the common formation and persistence of large groups in Cuyabeno. Instead, the presence and abundance of predators and in particular a lack of safe nest sites might cause non-breeding hoatzins to stay in their natal territory: First of all, dispersal may be very costly in the Cuyabeno area, a primary rainforest with the full range of predators. Big raptors were recorded regularly and we saw, for instance, an Ornate Hawk-Eagle (*Spizaetus ornatus*) and a Black Hawk-Eagle (*Buteogallus urubitinga*) attacking adult hoatzins. Harpy Eagles (*Harpia harpyja*), which are known to take large prey, were also present in the area. Secondly, the chance of succeeding when breeding independently may only be small at the Cuyabeno Lakes because nest predation was higher there than in the Venezuelan savannahs (Ramo and Busto, 1984; Strahl, 1988; Domínguez-Bello et al., 1994). Therefore, we consider the more stable environment and the higher predation pressure at our study site to be responsible for geographic differences in the hoatzin social organisation.

### *Clutch size and group size*

Clutch size increased continuously with increasing group size but showed the most pronounced increase from units of 4 to units of 5-7 members. Whereas the high number of eggs in larger groups is almost certainly due to joint-female nesting, i.e. two females laying eggs in the same nest, (A. M. and K.E. L., see chapter 2.5), the increase from single pairs to groups with one or two helpers reflects a true increase in the number of eggs laid by a single female. This suggests that because of the helpers' contributions a considerable release from duties had occurred and thus energy had been saved, which in turn had been invested in the production of more eggs. In addition, the association between group and clutch size could be influenced by the age of the egg-laying females: Females with helping offspring should be older because they have obviously already bred successfully before. Indeed, several reports state that clutch size is linked to the age of the egg-laying female in many altricial birds (e.g. Desrochers and Magrath, 1993; Hipfner, 1997; Reid et al., 2003; but see Moreno et al., 2002; Wiktander et al., 2001).

### *Effect of alloparental unit members on breeding success*

Nests of hoatzin breeding units with at least one additional adult had a higher chance of producing at least one hatchling or fledgling than nests of single pairs. Because alloparental unit members contributed to the breeding effort directly through incubation and chick feeding, and indirectly through territory defence and predator detection it is reasonable to attribute this increased chance of success to their helping. This conclusion is supported for the hoatzin by Strahl (1988) and for other co-operatively breeding species by Brown et al. (1982), Mumme (1992) and Komdeur (1994). According to VanderWerf and Strahl (1990) hoatzin helpers enhanced annual productivity by more frequent feeding visits to the chicks. This rate translated into shorter fledging periods (rather than greater weight at fledging) and consequently reduced chick mortality caused by predation (Strahl and Schmitz, 1990). However, we found that the survival of nests in units with helpers was also enhanced during the incubation period and therefore other contributions in addition to chick feeding must have played an important role as well.

In the tropical Stripe-backed Wren (*Campylorhynchus nuchalis*), for example, better annual productivity was caused by increased predator detection and deterrence through helpers as well as an enhanced defence effort by the territorial male through a release from feeding duties (Rabenold, 1984). Predator detection is presumably better in units with helpers but hoatzins do not effectively defend their nests (Robinson, 1997; own observations), at least not towards larger enemies such as eagles. In contrast to the report from Venezuela we never saw hoatzins in Cuyabeno performing a defence posture at the nest (Strahl, 1988). Instead the incubating bird froze when alerted, apparently trying to camouflage itself, but fled without protest when approached too closely. Chick-brooding birds normally left the nest site earlier and in a more inconspicuous manner than incubating birds (A. M. unpublished data). They left it to their chicks to look after themselves via their peculiar flight behaviour, which involves jumping into the water, diving 5-15 meters away and climbing another tree. This performance offers a real chance of escaping an attack. The adult birds, either incubating or brooding, started scolding nest robbers from a nearby tree (e.g. Caracara (*Daptrius ater*)) after flushing, with other group members then joining in. We suspect a higher vigilance by more group members to elicit a more timely camouflage through earlier freezing of the breeding bird and/or the triggering of faster predator avoidance via an earlier and then perhaps more clandestine flight.

The effect of alloparental helping may be supplemented by an enhanced experience of hoatzin pairs with helpers because those had obviously already been reproductively successful. For example, Fitzpatrick and Woolfenden (1988) showed that both the effect of helpers and experience of the breeding pair independently contributed to reproductive success in the Florida Scrub Jay (*Aphelocoma coerulescens*). This is consistent with our finding that hoatzin females breeding in single pairs laid fewer eggs. Furthermore, we have evidence that experience is helpful in selecting appropriate nest sites because all three nests that were destroyed by flooding through a rapidly increasing water level (from a total of 147 nests) had been constructed by single pairs.

Interestingly, the number of chicks that eventually fledged from successful nests did not differ for groups of different sizes although initial brood size was larger in larger groups. It is possible that we failed to detect such a relationship because of the small sample size of successful unaided pairs; in fact Figure 4 suggests a slight increase. However, a comparison of attributes of all nesting attempts between 1995 and 1998 resulting in one fledgling or two fledglings, respectively, did not show a difference in mean group size either (1 fledgling: 3.6 adults/group, *n* = 48; 2 fledglings: 3.8 adults/per group, *n* = 17; MW-U test Z corr. = -0.144; *p* = 0.89). The lack of a relationship points to a strong influence of predation pressure and further strengthens our conclusion that additional chick feeding was not a major component of the helping effect. Furthermore, our findings are consistent with observations on tropical wrens where feeding by helpers released the parents but did not lead to an overall better feeding of chicks (Rabenold, 1984). Consequently, the number of chicks per successful nest did not increase either. However, in the wrens the support and release of parents by helpers resulted in a higher number of nesting attempts per season and hence a higher productivity per year. Strahl and Schmitz (1990) suggest a similar mechanism for the hoatzin in the Venezuelan savannahs and often observed several successful breeding attempts there. At our study site there was normally only one nesting attempt per season, a fact we have linked to a small time window of favourable breeding conditions due to a bimodal pattern of rain (see chapter 2.1). Although a release through helpers did not result in more frequent nesting attempts per season by the aided breeding pairs we would expect that their life-time reproductive success is higher. Only further long-term data may unequivocally prove the adaptive value of this life-history strategy.

The relationship between number of helpers and breeding success was not a smooth continuously increasing one but showed a sharp non-linear increase between single pairs and aided pairs. Trios therefore seem to constitute a “critical mass” for breeding success and the positive effect did not increase further for groups that were larger than three, neither during the hatching nor during the fledging stage. This pattern suggests a balance between benefits from helping and costs of grouping. For example, Skutch (1961) argued that one of the principal costs of having helpers might be the elevated attraction of predators to the nest site due to increased activity. Larger groups might mean greater vigilance, but extra visits to the nest by helpers might counterbalance this advantage by making nests easier for predators to detect (Martin et al., 2000). In addition, larger groups may also have more complex social interactions and in fact VanderWerf and Strahl (1990) found hoatzin unit size positively correlated with the number of territorial disputes and negatively correlated with nest attendance. Furthermore, helpers might have conflicts with their younger siblings and their willingness to help might decrease in larger groups. In another study we have demonstrated that co-breeding might confound or hinder breeding success (A. M., K.E. L. and M. Wink, see chapter 2.5). However, even when co-breeding events are excluded from the analysis the lack of a positive relationship between unit size and breeding success remains. In contrast to the situation in the insect feeding Seychelles Warbler (Komdeur, 1994), food depletion by extra group members does not seem likely to cause costs because it is reasonable to assume that there was always sufficient food available.

To summarise, our data from the cooperatively breeding hoatzin reconfirm reports on other bird species that social units with alloparental adults were more successful in producing hatchlings and fledglings than single pairs. Our observations justify calling these adults “helpers” and our results strongly suggest that unaided breeding is less productive. The number of fledglings per successful nest was independent of group size and several pieces of evidence suggest that it was not enhanced feeding that accounted for the better nesting success of groups but rather predator detection and avoidance played the crucial role. There was apparently a trade-off between helping and grouping and we consider factors related to group activity and sociality to prevent a further increase in reproductive output in larger groups. In addition, there may be an association between group size and level of experience of the breeding pair. Finally, we revealed a geographical variation in the social system of the hoatzin and argue that predator density and environmental stability were the influencing factors. Our findings support the view that helping behaviour is a flexible trait influenced by ecological circumstances at the population level.

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# 2.4 Offspring sex ratio varies with family size and laying order of eggs in the co-operatively breeding hoatzin *Opisthocomus hoazin*

Antje Müllner, K. Eduard Linsenmair and Michael Wink

## Abstract

In co-operatively breeding birds offspring of one sex usually stay in the natal territory and show helping behaviour. Helping offspring may enhance the parent’s reproductive success and parents that control the sex of their offspring may benefit from producing a bias towards the helping sex. We examined offspring sex ratios in the co-operatively breeding hoatzin *Opisthocomus hoazin* living at the Cuyabeno Lakes in Amazonian Ecuador. We sexed nestlings and unhatched eggs of this sexually monomorphic species using molecular techniques. We found a female-bias at the population level (59%). There was no difference between the sex ratios at egg-laying and at fledging indicating that no differential mortality of the sexes occurred during the fledging stage. This is also supported by similar growth rates in chicks of both sexes. Despite the overall female-bias, single breeding pairs, lacking any helpers, produced only sons from first-laid eggs. In contrast, pairs with at least two helpers produced more female offspring from first-laid eggs than predicted by chance. We did not observe this pattern in later-laid eggs. Hoatzins live in pairs or families and according to a study in Venezuela, sons from previous years predominantly act as helpers-at-the-nest whereas daughters disperse. Although our sample size of single pairs is small our data suggest that hoatzin females adaptively manipulated the sex ratio of their offspring according to their current family size in order to maximise individual fitness.

## Introduction

Although parity seems to prevail in avian sex ratios (Charnov, 1982; Clutton-Brock, 1986), deviation from parity has been reported in several co-operatively breeding bird species (Gowaty and Lennartz, 1985; Emlen, 1986; Ligon and Ligon, 1990; Komdeur, 1996). In these species the offspring from previous broods usually act as helpers-at the-nest, with one sex mainly performing helping behaviour, or at least one sex helping more than the other (Brown, 1987). Parents that control the sex of their offspring may particularly benefit from producing a bias towards the helping sex. Sex allocation theory predicts that natural selection favours a population sex ratio that equalises parental expenditures on sons and daughters (Fisher, 1958). Therefore, sex ratio variations should occur primarily in sexually dimorphic species where a surplus of the cheaper-to-raise sex is expected, which in most cases is the smaller sex. In co-operatively breeding birds each helping offspring repays a part of its rearing costs and thus can be seen as the cheaper sex. This is why the repayment model predicts a helper-biased sex ratio at the population level (Emlen, 1986; Lessells and Avery, 1987; Pen and Weissing, 2000). This model is supported by some empirical data (Gowaty and Lennartz, 1985; Ligon and Ligon, 1990; Arnold et al., 2001), while other investigations failed to find such a connection between helping and sex ratio (Bednarz and Hayden, 1991; Koenig and Dickinson, 1996). It is clear that the repayment model may conflict with local competition between parents and offspring when offspring of one sex compete more with their parents than offspring of the other sex. This can arise when sons and daughters show different levels of philopatry. In this case the parents would benefit from producing an excess of the dispersing sex (e.g. Clark, 1978).

According to Trivers and Willard (1973) the relative fitness benefits through sons and daughters may vary depending on the situation in which the offspring are produced. In such cases, parents should allocate their investment in sons and daughters in such a way as to maximise their own lifetime reproductive success, and skewed sex ratios can be expected. For example, skewed primary sex ratios are reported in relation to parental qualities such as maternal body condition (Wiebe and Bortolotti, 1992; Nager et al., 1999; Whittingham et al., 2002) or paternal attractiveness (Ellegren et al., 1996). Other studies found correlations of the sex ratio to the food supply during the breeding period (Appleby et al., 1997) or to the time of year in which the eggs were laid (Daan et al., 1995). Since the presence and strength of these influencing factors are expected to differ between individual families, a different sex ratio may be adaptive for each parent. Thus, the sex ratio at the family level might show a substantial deviation from the sex ratio at the population level.

All these explanations for skewed sex ratios are not exclusive. Instead the observed sex ratios, irrespective of whether they are biased or unbiased, are the result of a combination of several factors which might act together or which might partly counteract one another (e.g. Lessells et al., 1996; Korpimäki et. al., 2000). For example, laying females of the co-operatively breeding Seychelles Warbler *Acrocephalus seychellensis* adjusted their primary sex ratios both in respect to the number of helpers they already had and to the food abundance in the family's territory (Komdeur, 1996). This resulted in an overall bias towards the non-helping sex.

Data on sex ratios in wild populations of co-operative breeders are still rare and there is an even greater lack of information on the different factors which might influence these sex ratios. Many co-operatively breeding birds live in monogamous pairs and both sexes are the same size and exhibit the same plumage, at least when they are juveniles. These species are suitable objects on which to study the influence of family size on sex ratios because confounding factors are unlikely to play an important role: We do not expect parental quality to have a great influence because the variance in reproductive success is similar in both sexes, in contrast to polygynous species. Additionally, different rearing costs due to sex-specific growth rates or to development of sexual characters should be low or even negligible in monomorphic species. However, the fact that it was necessary to either dissect the birds for sexing or to wait until they performed breeding behaviours hindered previous investigations. Using the new molecular methods which are available today, we were able to sex nestlings and even unhatched embryos (Griffiths et al., 1996; Kahn et al., 1998). In this paper we report patterns of sex ratio variation at egg laying in a population of the co-operatively breeding hoatzin *Opisthocomus hoazin* in the Amazonian rainforest of Ecuador.

Hoatzins are pheasant-sized birds which inhabit riparian forests throughout the Amazon and Orinoco river basin. Until now only one long-term study on a hoatzin population in the savannah ("llanos") of Venezuela provided detailed data about the social system of this bird species (Strahl, 1988; Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990). According to these authors hoatzins live in pairs or family groups of up to eight individuals including the offspring of previous broods. Their long-term observational study showed that sons mainly acted as helpers-at-the-nest: The proportion of males in the helping part of the population was significantly greater than that of females and although both sexes were seen to help, male individuals contributed more to the care of the nestlings and the defence of the territory than females (Strahl 1985; Strahl, 1988; Strahl and Schmitz, 1990). Males were patrilocal whereas most females dispersed in their second year (Strahl and Schmitz, 1990). Since hoatzins are sexually monomorphic, neither adults nor chicks can be sexed by their size or colour. Therefore, Strahl and colleagues were only able to determine the sex of adults after they had witnessed their copulation attempts. For adults which had been colour-banded and observed as juveniles it was possible to retrospectively link the individual sex with the behaviour which that particular animal had exhibited as a juvenile or helper.

In our study area hoatzins lived in single pairs or formed groups of three to seven individuals during breeding time and defended territories in the inundated forest. Hoatzins are obligate folivores and possess a ruminant-like fermentation which is unique in birds (Grajal et al., 1989). Even the chicks are fed exclusively on a pre-digested mash of leaves, regurgitated by the adults. Hoatzin pairs with at least one helper had a significantly enhanced breeding success whereas an increasing number of helpers only slightly increased breeding success (Müllner and Linsenmair unpubl. data, see chapter 2.3). Thus for a single pair one helping offspring would always be of great advantage. In contrast, for pairs that already have a helper the benefit of further helpers would be limited, in particular when considering that one helping son may help in its natal family group for several nesting seasons before breeding on its own. According to the repayment model and to the data from Strahl and Schmitz (1990) we expected a sex ratio skewed towards the helpers, i.e. a bias towards males, on the overall population level. On the family level, however, we expected an individual variation with respect to the size of the social unit. We predicted that families lacking any helper should produce a male-biased sex ratio whereas groups which already have several helpers should produce more of the dispersing females.

Emlen (1997) suggested that female birds could control the sex ratio of their offspring by absorbing eggs. Birds that lay clutches of more than one egg should bias the sex of the first-laid egg and then let the rest of the clutch be determined randomly. If female hoatzins control the sex ratio adaptively, according to Emlen’s hypothesis, the sex bias should be due to a bias of the first egg laid. Therefore, we also analysed sex ratio variation with egg sequence.

## Methods

### *Study site*

We studied a population of approximately 700 hoatzins *Opisthocomus hoazin* in the Cuyabeno Lakes (“Lagunas de Cuyabeno”) in the Amazonian lowlands of Ecuador, South America (0°02’ N, 76°9’ W and 0°03’ S, 76°14’ W) between 1995 and 1998 (details in Müllner and Pfrommer, 2001; Müllner et al., 2004). The area consists of a system of several black water lakes, small rivers, flooded forest and *terra-firme* forest. The hoatzins feed and breed in the stretches of inundated forests which surround the lakes and every pair or family defends its territory. The vegetation in these forests is of low density and dominated by only a few species of trees and shrubs. The leaves of the six most common plants of the inundated forest (*Macrolobium acaciifolium*, *Pithecellobium cf. longifolium, Inga spec*., *Licania spec*., *Coussapoa trinervia*, *Cecropia litoralis*) made up about 80% of the hoatzin’s daily food intake (A. M. unpubl. data).

### *Determination of egg sequence and size of family group*

Hoatzins always build their nests on branches overhanging the water and all observations and measurements were made from a paddled dugout canoe by the same person (A. M.). Nest sites, clutch size, size of breeding group, hatching and fledging success were recorded one to three times a week. Egg size was measured to the nearest mm using a calliper and the volume was calculated using Hoyt’s equation (1979). In total, over all study years, 85% of hoatzin clutches (n = 292) contained one to three eggs with a modal value of two eggs whereas 15% of clutches contained four to seven eggs. These “superclutches” were limited to social units of four or more birds and we have strong evidence that they were the result of joint-nesting of two females in the same social unit (results of DNA fingerprints; Müllner et al. in prep, see chapter 2.5).

In 1998 we increased our rates of nest visitation and the laying order could easily be determined because the hoatzins laid their eggs one to two days apart. All eggs were numbered using a felt pen. Incubation started as soon as the first egg had been laid (Müllner and Linsenmair in prep, see chapter 2.1). In 1998 we determined the egg laying order by the egg-floating method (Dunn. et al., 1979; Alberico, 1995). We calibrated our estimation-curve with eggs for which we knew the laying date and the method was reliable when used during the first two weeks of incubation (A. M. unpubl. data). Incubation lasted for between 29 to 31 days, but was similar in eggs of the same clutch. Eggs always hatched in the order in which they had been laid and consequently a pronounced hatching asynchrony of one to two days existed (see chapter 2.2). When the laying order of eggs was unknown in a clutch of known size and we found the complete brood with chicks that were only a few days old, we determined hatching order by analysing the weight and plumage characteristics of the chicks. Siblings of one nest were marked with an expandable ring on one or both legs, juveniles were colour-banded with individual combinations of plastic rings. The chicks left the nest when they were two or three weeks of age but needed about six weeks more until fledging. In 1998 the survival rates of the first-hatched chicks (= first-laid egg) until fledging were three times higher than that of the second hatched chicks (= second-laid egg) and no third-hatched chick survived until fledging (*n* = 34 eggs; Müllner and Linsenmair unpubl. data, see chapter 2.2).

Group size was defined as the greatest number of adults observed in successive counts during the breeding season on the basis of at least five counts. These counts were very consistent because hoatzins stayed in their territory during the entire breeding season. The most frequent size of 126 different groups which engaged in nesting activities in 1998 was three adults (40.2%), i.e. presumably the breeding pair and one helping offspring. About one fifth of breeding units (22.8%) consisted of a single breeding pair and 37% of units had two or more helpers (Müllner and Linsenmair unpubl. data, see chapter 2.3).

### *Sex determination of young hoatzins*

In 1998 we took blood samples of 122 young hoatzins of which 117 were nestlings between 5 and 14 days after hatching and 5 were fledglings that had not been sampled as nestlings. Approximately 100µl blood were taken from the wing vein and stored in EDTA buffer at ambient temperature until being brought to the laboratory in Heidelberg, Germany, for DNA analysis. Eggs that did not hatch and were still found in the nest on the sample day were opened. Some of them contained a foul liquid and these were probably non-fertilised. In five eggs embryos that had undergone approximately 2–3 weeks of development were found and stored in ethanol. These unhatched eggs were always the last-laid eggs in clutches of two or three eggs, three were males and two were females. Due to a heavy rise in the water level in May 1998 several incubated hoatzin nests were abandoned because of flooding. Two additional eggs with embryos were found in these nests and stored. A tissue sample from the embryos was used for the DNA-analysis. Molecular sexing followed Becker and Wink (2002) using the methods outlined in Kahn et al. (1998) which are based on the detection of the CHD gene on avian sex chromosomes. As in most species, hoatzin males produced one band on the gel and females two (see Appendix 4), presumably reflecting differing intron sizes of the W versus Z chromosomes (Kahn et al., 1998).

### ***Data analysis***

In order to avoid pseudoreplication every breeding pair contributes only one brood to the sample. For most pairs this was the first and sole attempt in the season, we rarely observed re-nesting in the Cuyabeno Lakes. The offspring sample considers 129 different individuals (117 nestlings, 7 embryos and 5 juveniles that were only sampled as fledglings). The nestling subsample includes the 117 nestlings; the fledgling subsample is composed of all 32 nestlings known to have fledged and 5 juveniles that were only sampled as fledglings. We aimed to sample full clutches, but because of nestling mortality before the sample day and capture difficulties, hatchlings were missing in several broods. Since partially sampled broods may have a bias towards first-hatched chicks through the higher mortality of second- and third-hatched chicks, we also calculated the sex ratio for fully sampled clutches separately. We have data for 41 complete broods, containing 95 fertilised and 7 foul eggs which we supposed to be unfertilised.

We tested for deviation from parity using a G-test with William correction. Comparisons between offspring sex ratios of different group sizes were analysed with the two-tailed Fisher’s exact-test. When we made multiple comparisons, we corrected levels of significance ($p < 0.05$) using the sequential Bonferroni-method (Rice, 1989). Differences in volume of male and female eggs were analysed by t-tests. Sample sizes vary among analyses because it was not always possible to obtain data on egg size or sequence and on group size for all individuals or nests.

## Results

### *Population level sex ratio*

The hoatzin offspring sex ratio was 41.1% males to 58.9% females ($G = 4.11$, $p = 0.043$, 129 individuals from 68 broods). This population level female-bias was also present when only complete clutches were considered, with 40.0% males and 60.0% females ($n = 95$; $G = 3.81$, $p = 0.051$) but shortly failed significance. Both the *nestling* as well as the *fledgling* subsample also showed this female bias, but the difference was not significantly different from parity in the *fledgling* sample due to the smaller sampler size (*nestlings*: 41.2% males and 59.8% females, $G = 4.53$, $p = 0.033$, 117 individuals from 62 broods; *fledglings*: 35.1% males and 64.9% females, $G = 3.28$, $p = 0.07$, 37 individuals from 27 broods). Consequently, there was no difference between the *nestling* and the *fledgling* sample ($G = 3.28$, $p = 0.59$). We did not find different sex ratios in different lakes of the study area.

### *Egg volume and weights of male and female offspring*

The volumes of male and female eggs were very similar (mean ± SE, males: 28.0cm$^3$ ± 0.5, $n = 29$; females: 28.44cm$^3$ ± 0.3, $n = 47$; $t = 1.02$, $p = 0.31$). Additionally, nestlings of both sexes had the same growth rate during their development until fledging (Fig. 1). Young hoatzins fledged when they weighed about half as much as an adult, which was 803.0g ± 30 SE ($n = 5$).

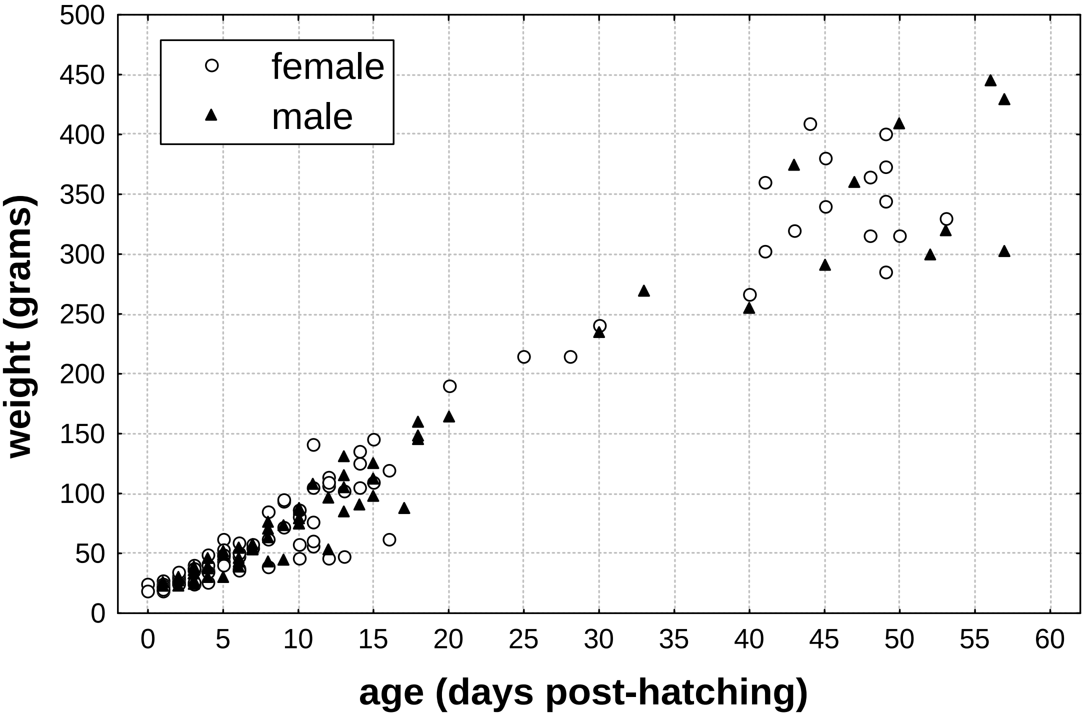

**Figure 1.** Body weight of male and female hoatzins between hatching and fledging at 50-60 days of age. Presented are 97 measurements from 66 females (49 broods) and 74 measurements from 48 males (40 broods).

### *Individual sex ratio with respect to family size and egg sequence*

When we combined all offspring of all broods the percentage of female offspring increased both with an increasing number of adults in the breeding unit and with a higher position in the egg laying sequence (Table 1). However, these associations were not significant when the categories of unit size and laying position were analysed separately (number of adults: G = 5.90, df = 3, *p* = 0.12; egg sequence: G = 4.40, df = 2, *p* = 0.11, categories 3rd and 4th egg pooled). Instead, if we considered the egg sequence in the analysis of different family sizes we detected a striking shift in sex ratios of the first-laid eggs depending on the number of adults in the breeding unit (Fig. 2A): first-laid eggs from single pairs were only males, in pairs with one helper there was an equal percentage of males and females in the first-laid eggs, and first-laid eggs from units of greater size were predominantly females. There was a significant difference in the sex ratio between the offspring of single breeding pairs and the offspring of breeding units with more than one helper (Fisher's exact: group size 2 vs. >= 4, *p* = 0.0036, Bonferroni limit = 0.017; group size 2 *vs.* 3, *p* = 0.03, Bonferroni limit = 0.025; group size 3 vs. >=

4, *p* = 0.17). In contrast, in the later-laid eggs of differently sized groups the sex ratios were almost identical (Fig. 2B).

**Table 1.** Percentage of female offspring in respect to the number of adults in the breeding unit and the laying order of eggs within a clutch. Data are from a population of hoatzins in the Cuyabeno Lakes, Ecuador, in 1998.

| | **% females** | ***n*** |
|---|---|---|
| **number of adults** | | |
| 2 | 30.0 | 10 (6 broods) |
| 3 | 54.7 | 53 (31 broods) |
| 4 | 57.1 | 28 (13 broods) |
| > = 5 | 71.9 | 32 (12 broods) |
| **egg sequence** | | |
| 1st | 52.6 | 59 |
| 2nd | 56.1 | 41 |
| 3rd | 73.3 | 15 |
| 4th* | 75.0 | 4 |

4th - eggs were almost certainly from a second female in the breeding unit.

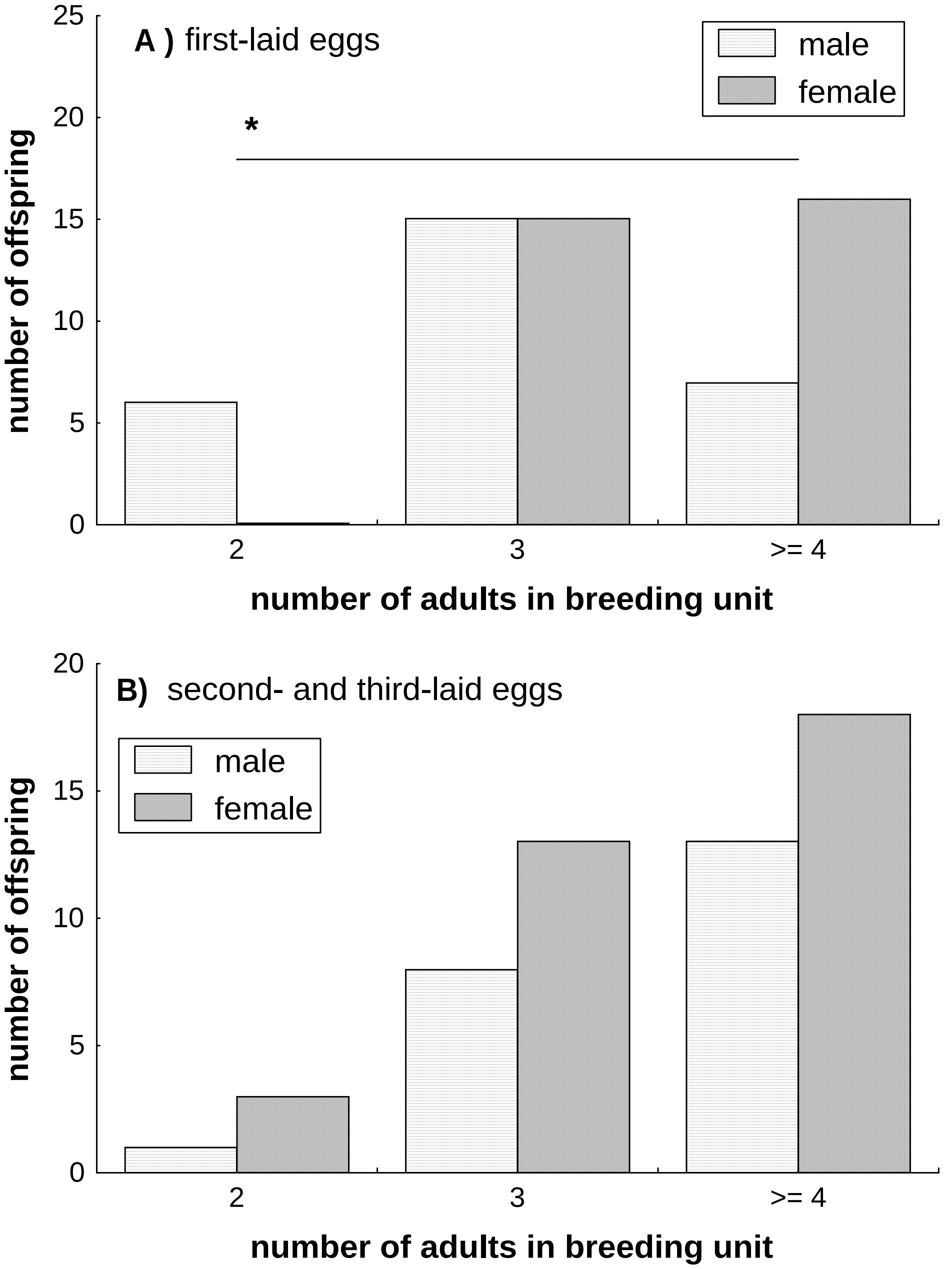

**Figure 2.** Sex ratio of hoatzin offspring from A) first-laid and B) later-laid eggs with respect to the number of adults in the breeding unit. Sample size is *n* = 59 individuals for A) and *n* = 56 for B). In the first-laid eggs the sex ratio differed significantly between single breeding pairs and units with >= 4 members (*).

## Discussion

### *Sex ratio at the population level*

The hoatzin offspring sex ratio was female biased on the population level in 1998 and this bias is consistent with a few nestling samples taken from the same population in 2000 (38.1% males and 61.9% females, respectively; *n* = 21). The rather small magnitude of an overall skew (ca. 10%) is similar to what has been observed in other co-operative bird species (Gowaty and Lennartz, 1985; Ligon and Ligon, 1990). However, small deviations, although biologically relevant, make it difficult to obtain sufficient statistical power without very large sample sizes. This is likely to be the reason why the *fledgling* sample failed to be significantly different from parity. There was no differential mortality of the sexes between hatching and fledging and we don’t have any evidence for differential embryo mortality either. Therefore the sex ratio of our offspring sample almost certainly reflects the sex ratio at egg laying and hence we conclude that the observed skewed sex ratio is caused by a biased egg production.

Volumes of male and female eggs were similar and the growth rates of male and female young hoatzins did not differ. This indicates that there is no difference in the rearing costs of the sexes, at least until fledging. It might be possible that some invisible sex-specific structures demand different quantities of energy during development. However, hoatzins do not reach maturity until they are two or three years old (Strahl and Schmitz, 1990) and therefore we doubt that these structures already cause differential requirements in the nestling stage. Furthermore, the fact that both sexes suffer the same mortality rates during their fledging period shows that hoatzins did not adjust the secondary sex ratio of their broods, for example by differential provision with food (Clotfelter, 1996; Kilner, 1998).

Given that in hoatzins the patriclocal sons contribute much more to helping than daughters (Strahl, 1985; Strahl, 1988; Strahl and Schmitz, 1990) our finding of a female bias in offspring apparently contrasts with Emlen’s repayment model (1986), which predicts an overall bias towards the helping sex in co-operatively breeding birds. However, offspring sex ratio does not necessarily reflect the adult sex ratio. We do not have data about the sex ratio of adults in our study population but Strahl and Schmitz (1990) calculated a percentage of 63% males in the adults for a hoatzin population in Venezuela, i.e. a bias towards the helping sex. It may be possible that females suffer a higher mortality during dispersal than males who mostly stay in or near their natal territory. This could lead to a sex ratio shift resulting in equity or in a male bias at the adult stage.

Assuming that our observed sex ratio would remain unchanged until the adult stage another explanation may apply to the bias. A surplus of dispersing offspring at fledging could also be adaptive if saturation with helpers already existed. This is consistent with the fact that about 80% of the pairs in the Cuyabeno Lakes already had at least one helping offspring living with them. Since males may be helpers for several years and hoatzins are thought to have a low adult mortality (Strahl and Schmitz, 1990) this pattern might not change rapidly. Instead, dispersing females could try to occupy free territories together with males which were formerly helping. In this way the inclusive fitness of the breeding pair would be higher than in the case of producing more helpers which were only of limited use. Hence, producing more females than males could be beneficial for all pairs that already had at least one helper. The success of such a strategy depends on the number of available territories on the regional scale. Indeed, densities of breeding groups in the Cuyabeno Lakes were at the lower range when compared with other regions (Torres, 1987; Strahl and Schmitz, 1990; Domínguez-Bello et al,. 1994), and it might be possible that habitat saturation was not yet reached.

### *Sex ratio at the level of the breeding pair*

We observed a significant association between the sex of first-laid eggs and the number of adults in the breeding unit. In contrast to the population level trend of a female bias in the offspring, single breeding pairs only produced males. In Venezuela male offspring was the helping sex in six out of seven hoatzin groups (86%, Strahl, 1985) and males constituted only 12% of the offspring that dispersed before it was one year old (Strahl and Schmitz, 1990). Assuming that this sex-specific behaviour is similar in our study area, single pairs produced male offspring that would more probably act as helpers-at-the-nest in the coming years than the dispersing females. Pairs living with one offspring produced equal numbers of males and females, and pairs with several offspring produced more females than males. This pattern seems highly adaptive, because single pairs benefit from a helper by enhancing their breeding success (Torres, 1987; Strahl, 1988; Müllner and Linsenmair unpubl. data, see chapter 2.3). Therefore, single pairs should try to manipulate the sex ratio of their clutches towards males. However, A-chicks from first-laid eggs have the highest chance of survival in a brood (75% A-chicks vs. 25% B-chicks) and one helper is sufficient to increase the reproductive success of a breeding pair significantly (Müllner and Linsenmair unpubl. data, see chapter 2.3). Consistently, we only found skewed sex ratios that were related to group size for the first-laid eggs. In contrast, second- and third-laid eggs were constantly female-biased, independent of the size of the family from which they came. This probably explains why differences in sex ratio between different group sizes failed to be significant when all eggs were combined, irrespective of their laying sequence.

The proximate mechanism of sex ratio manipulation in birds is unknown but several suggestions exist (Frank, 1990). Only females have the potential control over the sex of the offspring because they are the heterogametic sex (WZ), whereas males are homogametic (ZZ). Thus, sex chromosomes carried by all sperm are the same (Z) while those in eggs may be Z or W. It is thought that females either control the production or the release of W and Z gametes (e.g. Dijkstra et al., 1990; Ellegren et al., 1996; Lessells et al., 1996), or that they exercise a selective resorption of the “wrong” sex after fertilisation (Emlen, 1997). In order to prevent a time lag during the laying of consecutive eggs due to this manipulation females might be able to wait until the right sex is ovulated before beginning to lay the clutch, the rest of the eggs would then rely on the “Mendelian lottery” (Emlen, 1997). Recently Velando et al. (2002) showed a bias of solely the first-laid eggs in European shags *Phalacrocorax aristotelis*. In our sample the later-laid eggs did not differ significantly from parity either, although they appear slightly female-biased (22 males to 34 females, $G = 2.57$, $p = 0.11$).

Hoatzin pairs with more than one helper did not produce an equal sex ratio but produced more of the dispersing females. According to Strahl and Schmitz (1990) most female offspring leave the natal family group in the second year, whereas males stay. Since territory size increases only slightly with group size (Strahl, 1985; A. M. and K. E. L. unpubl. data, see chapter 2.3), a situation of local resource competition between parents and offspring might be created when more adults live in the same unit. However, food in general did not seem to be limited for the leaf-eating hoatzins in the Cuyabeno area because the birds fed on the most common trees and bushes in their habitat. Although, hoatzins especially prefer the young leaves and buds where the tannin content is probably still low and the flushing of the new leaves coincides with the fledging period, when the young were fed a predigested mash. Therefore, temporal resource depletion might play a role in bigger family groups. The shift in sex ratio according to group size is similar to what has been shown in the Seychelles Warbler in which females skew their single-egg clutch towards the helping sex (females) when their territory is of high enough quality to support more birds, and towards the dispersing sex (males) when their territory is of low quality (Komdeur et al., 1997).

In the hoatzin, another reason might explain the bias towards the dispersing offspring in bigger social units. VanderWerf and Strahl (1990) observed that bigger hoatzin units more frequently engaged in territorial disputes and thus decreased their nest attendance, which included disruption of the feeding of the young. This increased agitation in bigger social units was also observed in the Cuyabeno Lakes by A. M. and again might indicate a limitation of resources. It also suggests difficulties in coordinating the social life in bigger families so that as a consequence the current brood suffers. This might be the reason why we did not find a linearly increasing enhancement of breeding success with respect to group size (Müllner and Linsenmair unpubl. data, see chapter 2.3). We think that an upper limit in family size exists until which it is beneficial for the breeding pair to live with its offspring. When this limit is reached parents should rather release progeny from their territory by producing more dispersing individuals.

However, a non-adaptive explanation for the data is also possible, although not convincing in our case. Krackow (1995) suggested that the segregation of sex chromosomes during meiosis could be influenced by steroid hormones which in turn are known to be influenced by many internal and external factors. Much more endocrinological information would be necessary to support this hypothesis, but according to Krackow a sex ratio bias could be just the passive response to a specific physiological state of the laying female, for example via extraordinarily bad or good environmental conditions. This argument seems primarily applicable to the age of the laying female or to strong differences in food supply. We have no data about the age of the breeding hoatzins in the Cuyabeno Lakes, but it is reasonable to assume that in pairs lacking any helpers the females are still young, or at least have not yet had a successful breeding attempt. If the endocrinological hypothesis was true in the case of the hoatzins in the Cuyabeno Lakes a physiological constraint favouring male eggs in younger females should have an influence on the entire clutch, not only on the first-laid egg. Therefore, we consider it unlikely that individual differences in the age of the mother were responsible for the observed sex bias. The same argument holds true for a potential influence of food abundance.

We conclude, therefore, that the presented adaptive explanations of the observed sex ratio variation on the family level are more convincing than physiological constraints which are to date unknown. Our data indicate that female hoatzins are able to adjust their sex ratio at egg-laying according to the size of their social unit. Because one helping offspring is already sufficient to substantially improve the reproductive success of a pair and because the first-laid egg has the best chance of producing a fledgling the sex bias occurs only in the first egg of a clutch. Both local resource enhancement and resource competition seem to influence the decision of laying female hoatzins. On the population level this resulted in a sex ratio bias towards the non-helping sex, a fact apparently contradicting the repayment model. However, we have to consider that several factors may work simultaneously in which case the repayment model might also be relevant

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# 2.5 Joint-nesting in the neotropical hoatzin *Opisthocomus hoazin* revealed through DNA fingerprinting

Antje Müllner, K. Eduard Linsenmair and Michael Wink

## Abstract

Previous observations of the co-operatively breeding hoatzin suggest that almost all social units are formed by a monogamous pair living with offspring, which may act as helpers-at-the-nest for several years. Thus a single female usually contributes all eggs to the clutch of the breeding unit. Only a small proportion of clutches, all with more than three eggs, were assumed to result from two females laying their eggs in the same nest. We investigated group structure and breeding success of a hoatzin population in the rainforest of Ecuador and collected blood samples from hoatzin nestlings in broods of different sizes for DNA analysis. We found clutches with four to seven eggs in 15.5% of nesting attempts and these were always confined to breeding units with at least four adults. Band sharing comparisons revealed that all nestmates from two-egg clutches and almost all nestlings from three-egg clutches were full siblings. In contrast, chicks from clutches with four or more eggs were not all full siblings, but also either half siblings or aliens, indicating that they were from up to three different parents. Thus, all bigger clutches must have been the result of jointly-nesting females and three eggs seem to be the maximum number laid by a single female. We only observed the disappearance of eggs in joint-clutches, and this occurred at any time during incubation. Breeding success on a per-nest basis did not differ between single- and joint-nests. However, the fledgling to egg ratio was much lower in joint-female nests than in single-female nests with 0.3 fledglings per egg and 0.6 fledglings per egg, respectively. Likewise, when success was calculated for the individual female it was lower in joint-nests than in single nests. This disadvantage is expected to be compensated for by other lifetime benefits.

## Introduction

The majority of birds breed in monogamous pairs which only care for their own nest, eggs and young (Lack, 1968). Thus, with the exception of parasitised hosts, one female contributes all eggs to the clutch. In contrast, joint-nesting is a system of nesting in which two or more females of the same species regularly lay their eggs in the same nest, followed by shared incubation and raising of young (Brown, 1987). Joint-nesting is known to be associated with monogamous and polygamous mating systems (Koenig et al., 1984; Jamieson and Craig, 1987; Koford et al., 1990) and must have evolved independently several times, as it occurs in different avian families (Vehrencamp, 2000).

Brown (1987) and Vehrencamp (2000) listed the bird taxa in which joint-nesting is known, but they did not mention the neotropical hoatzin *Opisthocomus hoazin*. This is probably due to a lack of information, because until now all data about the social organisation of this co-operatively breeding species was based on one single long-term study in the wet savannah (“llanos”) of Venezuela (Strahl, 1985; Strahl, 1988; Strahl and Schmitz, 1990; VanderWerf and Strahl, 1990). In the Venezuelan study population only 4.6% of hoatzin clutches (*n* = 412) were supposed to be the result of two joint-nesting breeding pairs whereas the remainder was thought to be produced by single pairs that were presumably monogamous. In both nesting systems breeding pairs lived together with non-breeding offspring from previous broods and sons mainly acted as helpers-at-the-nest (Strahl, 1988). The supposed joint-clutches always contained four to six eggs whereas the clutches of a single female contained a maximum of three eggs. A frequency of extraordinarily big clutches similar to that found in Venezuela was observed in the rainforest of Peru, but within a much smaller sample size (5.3%; *n* = 18; Torres, 1987). Both investigators relied on behavioural observations of adults in the breeding group and on differences in egg shape and colour within a common clutch in order to make conclusions about joint-nesting. In our study, however, we used band sharing analysis of DNA fingerprints to reveal whether or not hoatzin nestlings were full siblings in clutches with two or more eggs.

According to the previous assumptions we expected full siblings in all broods from clutches with two and three eggs, whereas clutches with more than three eggs were suspected to contain one or more unrelated nestmates. In order to evaluate the reproductive consequences of joint-nesting we also compared the nesting success of supposed single and joint breeders.

## Methods

We investigated breeding ecology and egg laying patterns of the hoatzin *Opisthocomus hoazin* in the Cuyabeno Lakes in Ecuador from 1995 – 1998 and in 2000 (Müllner et al., 2004, Müllner and Linsenmair, unpubl. data). Hoatzins are co-operatively breeding birds which live in the Amazon and Orinoco river basin in South America. At our study site hoatzins live in pairs or in groups of up to seven adults in the flooded forests which surround the black water lakes like a belt. Breeding activities are linked to the rainy season. Eggs were laid within an interval of 1-2 days, but incubation (29-31 days) started with the first egg, resulting in a pronounced hatching asynchrony (A. M. and K. E. L., unpubl. data, see chapter 2.2). Nesting success and group size of breeding units was determined one to three times a week by the same observer (A. M.) using a paddled dugout canoe. The length and breadth of the eggs were measured using a sliding calliper and the eggs were then individually marked with a felt pen. In 1998 we employed the egg-floating method to estimate the laying date and laying order of eggs (Dunn et al., 1979) and took blood samples from nestlings at an age of 7-12 days. About 100µl of blood were collected from the wing vein and stored in EDTA buffer at ambient temperature until they could be brought to Heidelberg for analysis. Isolation of DNA followed the “proteinase K method” as described in Heidrich et al. (1995); plotting procedures followed Wink et al. (1999).

On average we scored 17.04 bands ± 3.34 SD (range 10-24) from 76 individuals and compared the band patterns of DNA profiles between dyads of all mates of a common nest. Seventy-four dyads from 28 nests were analysed and for each nestmate dyad the band-sharing coefficient (BSC) was calculated according to Bruford et al. (1992) as:

$$BSC = \frac{\textit{number of shared bands} \times 2}{\textit{number of all bands in profile of individual } A + \textit{number of all bands in profile of individual } B}$$

The BSC provides an index that ranges from 0 (no bands shared) to 1 (all bands shared). Related individuals share DNA fingerprint bands by common descent. We considered nestmates with a BSC value of more than 0.5 to be full siblings (Wink et al., 1999;

Leisler at al., 2000). Since adult hoatzins are nearly impossible to catch we do not have any genetic samples from the parents. We can confidently identify full siblings, but are unable to determine whether BSC values of less than 0.5 indicate a half sibling (presumably from a different mother but the same father) or a nestmate which is not closely related at all (from a second breeding pair within the same social unit). Nestlings which hatched later always experienced a high mortality, especially in the bigger broods where the time lapse between hatching of the first and the last chick was more pronounced (A. M., unpubl. data). Therefore it was difficult to completely sample clutches with more than three eggs, so we also included broods which were partially sampled. The maximum number of hatched nestmates that we ever found was four.

## Results

### *Frequency of clutches with more than three eggs*

The percentage of clutches with more than three eggs in a given breeding season ranged from 12.5% to 20% (Table 1), resulting in a mean annual frequency of 15.5%. The maximum number of eggs in a single clutch was seven. The frequency of clutch size decreased as clutch size increased (Table 2). Clutches with more than three eggs were confined to breeding units with four or more adults and larger clutches apparently came from bigger groups (Table 2). The number of adults in the breeding units differed significantly between units which produced different clutch sizes (Kruskal-Wallis-test: H = 9.14, $p = 0.006$, clutches with 6 and 7 eggs pooled).

**Table 1.** Percentage of hoatzin clutches with more than three eggs (= supposed joint-clutches) in different breeding seasons. Only the first breeding attempt per social unit within a season was considered in order to avoid pseudoreplication.

| **year** | **clutches with more than three eggs** | ***n*** |
|---|---|---|
| 1995 | 12.5% | 16 |
| 1996 | 12.5% | 56 |
| 1997 | 17.7% | 79 |
| 1998 | 14.9% | 121 |
| 2000 | 20.0% | 20 |

**Table 2.** Frequency distribution of 46 hoatzin clutches with more than three eggs and the corresponding number of adults (mean ± SD) in the breeding group. Data from all years were pooled; only the first breeding attempt per social unit within a season was considered.

| clutch size | frequency | number of adults |
|---|---|---|
| **4** | 60.9% | 4.8 ± 0.65 (*n* = 18) |
| **5** | 26.1% | 5.6 ± 1.13 (*n* = 9) |
| **6** | 10.9% | 6.0 ± 0.82 (*n* = 4) |
| **7** | 2.2% | 7.00 (*n* = 1) |

### *Band sharing analysis of relatedness of nestmates*

We calculated the band-sharing coefficient of all nestmate dyads from clutches with two to six eggs and deduced the composition of nestmates with respect to their relatedness (Table 3). In all broods from clutches with more than three eggs we identified at least one nestmate which was not a full sibling (“alien”), whereas we never detected four full-sibling nestmates. Three of these broods contained one “alien” each, two contained two different sibling pairs and in one we found a sibling pair plus two other nestmates that were not related to each other. Additionally, in an incompletely sampled brood from a four-egg clutch we also detected two nestmates which were not full siblings. In contrast, all nestmates from clutches where there were two eggs were full siblings, as well as most nestmates from three-egg clutches. These results indicate that 1) clutches with more than three eggs were produced by more than one female and 2) the deposition of three eggs is the upper limit for an individual female hoatzin. Consequently, the clutch of seven eggs which we observed must have been the result of at least three joint-nesting females. This is consistent with the fact that we determined three different parental lineages for nestmates of a 5-egg clutch (Table 3).

Although almost all clutches with two and three eggs were produced by a monogamous pair, we found one exception: one brood from a three-egg clutch contained a nestmate with a BSC value that was far below 0.5, strongly suggesting that it was not a full-sibling. This result was retrospectively confirmed by the shape and laying order of the eggs in that clutch. During the control of nest content one egg in the clutch had been noted as being remarkably longer than the two others (length / breadth = 1.42 versus 1.31 and 1.29). Additionally, the conspicuous egg was the last-laid egg of the clutch and the time lag between it and the second-laid egg was one day longer than normal (3 days instead of 1-2 days, see chapter 2.2). Astonishingly, only three adults formed the breeding unit which incubated the clutch and raised the young until they were eaten at the age of 10 days by an anaconda.

**Table 3.** Relatedness of hoatzin nestmates in differently sized clutches. Mean ± SD band sharing coefficient (BSC) and supposed kinship: F = Full sibling (BSC > 0.5), X, Z = half sibling or unrelated nestmate (BSC < 0.5), ? = not sampled.

| clutch size | n | relatedness | BSC of nestmate dyads |
|---|---|---|---|
| **2** | 10 | $F_1$, $F_2$ | $F_1 : F_2 = 0.77 \pm 0.05$; range 0.72-0.87 |
| **3** | 6 | $F_1$, $F_2$, $F_3$ | $F_i : F_{ii} = 0.69 \pm 0.07$; range 0.60-0.76 |
| | 1 | $F_1$, $F_2$, X | $F_1 : F_2 = 0.59$, $F_1 : X = 0.41$, $F_2 : X = 0.11$ |
| **4** | 2 | $F_1$, $F_2$, $F_3$, X | $F_i : F_{ii} = 0.69 \pm 0.12$; range 0.53-0.87<br>$F_i : X = 0.27 \pm 0.12$; range 0.14-0.44 |
| | 1 | $F_1$, $F_2$, $X_1$, $X_2$ | $F_1 : F_2 = 0.82$, $X_1 : X_2 = 0.67$<br>$F_i : X_i = 0.28 \pm 0.08$; range 0.21-0.38 |
| | 1 | F, X, ?, ? | F : X = 0.14 |
| | 3 | $F_1$, $F_2$, ?, ? | $F_1 : F_2 = 0.66 \pm 0.81$; range 0.57-0.82 |
| | 1 | $F_1$, $F_2$, $F_3$, ? | $F_i : F_{ii} = 0.62 \pm 0.4$; range 0.55-0.67 |
| **5** | 1 | $F_1$, $F_2$, $X_1$, $Z_1$, ? | $F_1 : F_2 = 0.69$, X : Z = 0.40<br>$F_i : X = 0.27$, $F_i : Z = 0.31$; range 0.22-0.32 |
| | 1 | $F_1$, $F_2$, $X_1$, $X_2$, ? | $F_1 : F_2 = 0.67$, $X_1 : X_2 = 0.58$<br>$F_i : X_i = 0.19 \pm 0.05$; range 0.15-0.26 |
| **6** | 1 | $F_1$, $F_2$, $F_3$, X, ?, ? | $F_i : F_{ii} = 0.57 \pm 0.01$; range 0.56-0.57<br>$F_i : X = 0.27 \pm 0.15$; range 0.10-0.40 |

### *Breeding success of joint clutches*

We calculated hatching and fledging success as the percentage of nests which had at least one hatchling after the incubation period or at least one fledgling after the fledging period, respectively. The hatching success of joint-clutches was 32.3% ($n = 31$) and fledging success was 52.9% ($n = 9$), resulting in an overall nesting success of 17.1%. These percentages were indistinguishable from the success of clutches with one to three eggs (33.3% for hatching, $n = 183$, Fisher's exact: $p = 1.0$, and 49.6% for fledging, $n = 121$, $p = 1.0$; overall nesting success = 16.5%). Additionally, the mean number of fledglings per successful nest was not statistically different between singly-laid and joint-clutches, although the average clutch size as well as the number of hatchlings per successful nest was higher in joint-clutches (Table 4). Consequently, when the number of fledglings per nest was related to the number of eggs that had been laid in that nest, the effort to success ratio was much lower in joint-clutches than in singly-laid clutches (joint-clutches = 0.30 fledglings / egg, singly-laid clutches = 0.61 fledglings / egg; U-test: Z = 3.93, $p < 0.0001$). Alternatively, the number of fledglings per nest could be divided by the number of contributing females. However, we lack the data regarding whether two or three females were involved in the joint-clutches from which the success had been calculated. Nonetheless, the fledgling to female ratio would be at least 50% lower in joint-nesting females than in singly breeding females. In conclusion, for an individual female, breeding in a joint-nest results in lower annual success than breeding alone.

**Table 4.** Mean number of eggs per nest and number of hatchlings and fledglings per successful nest in broods from singly-laid clutches and from joint-clutches of the hoatzin.

| | **eggs / nest** | **hatchlings / nest** | **fledglings / nest** |
|---|---|---|---|
| **singly-laid clutch** | 2.1 ± 0.62<br>range 1-3, $n = 249$ | 2.02 ± 0.54<br>range 1-3, $n = 89$ | 1.29 ± 0.46<br>range 1-2, $n = 58$ |
| **joint-clutch** | 4.5 ± 0.81<br>range 4-7, $n = 46$ | 3.0 ± 0.71<br>range 1-4, $n = 19$ | 1.33 ± 0.50<br>range 1-2, $n = 9$ |
| U-test | Z = -11.65<br>$p < 0.0001$ | Z = -5.21<br>$p < 0.0001$ | Z = -0.24<br>$p = 0.81$ |

Renesting was generally low in the Cuyabeno Lakes and only occurred after a failure early in incubation, mainly due to predation (A. M. and K. E. L. unpubl. data, see chapter 2.1). In six out of nine second breeding attempts (= 67%) which followed a joint-clutch that had failed, a joint-clutch was produced again. The mean clutch size of these repeated joint-clutches was 4.7 ± 0.82 SD (*n* = 6) and egg number did not differ from that in first attempts.

### *Egg removal*

Predation as well as nest destruction by wind or by rising water levels normally affected the entire clutch. However, in eight joint-clutches we noted that single eggs had disappeared from the nest. This could happen at any time during incubation, even closely before the hatching of chicks. Additionally, we found a cracked egg in two nests and in two clutches with six eggs we observed that eggs had been placed on the periphery of the nest and apparently had not been incubated by the individual which had been flushed. Hoatzins always nest on branches overhanging the water and thus no broken egg shells could be found beneath the nest. We did not observe partial disappearance of eggs in smaller clutches. Instead, foul and unhatched eggs often remained in the nest until the day when we collected blood samples from the nestlings at the age of 7-12 days (see Müllner et al., 2004). There were only two instances where a single egg had been removed from a single nest, almost certainly because we had accidentally cracked them slightly when we picked them out from a tree top nest to make our measurements.

## Discussion

### *Relatedness of hoatzin nestmates*

Hoatzin nestmates were full siblings in all two-egg clutches, i.e. they were the result of a monogamous breeding pair. Apart from one exception, three-egg clutches also contained hatchlings from the same parents. We found no more than three full siblings in a common brood and it is likely that three eggs represent the upper limit for an egg-laying hoatzin female. In contrast, broods from clutches with four, five or six eggs contained a mixture of full siblings, half-siblings or even alien nestlings. These nestmates must have been the offspring of different adults. Considering that a single female can only lay three eggs, joint-female nesting rather than a polyandrous origin of the nestmates is suggested. The observed clutch size of seven eggs and the DNA profiles which revealed four nestmates with three different mothers demonstrate that joint-nesting attempts comprised up to three females.

Our genetic data do not allow the differentiation between half-siblings and nestmates that were not closely related. Therefore, we cannot clearly determine whether joint-nesting in the hoatzin is a result of polygamy of one female, as for example in the Acorn Woodpecker (*Melanerpes formicivorus*) (Koenig et al., 1984), or whether two or more monogamous pairs produced offspring within the same breeding unit (e.g. in the Groove-billed Ani (*Crotophaga sulcirostris*), Vehrencamp, 1978). We consider the latter possibility more likely. First of all, the BSC values were quite low in nestmates that were not full-siblings, indicating that they were not closely related. Secondly, bigger clutches with more than three eggs were always produced by units with at least four adults. In a polygynous system clutches with four or more eggs should theoretically also be found in breeding units with only three adults, comprising a single pair still lacking any helpers and an additional female. Furthermore, the conclusion that these were distinct monogamous pairs is consistent with the behavioural observations of Strahl (1988). Nonetheless, monogamous pairs and polygamous associations are not exclusive and different mating patterns may be realised in different breeding units as e.g. in the Guira cuckoo (*Guira guira*) (Quinn et al., 1994).

Surprisingly, we also found an alien nestmate plus a sibling pair in a brood from a three-egg clutch. We are absolutely certain that this breeding unit only consisted of three adults which were presumably the same three individuals as in the year before. In that year they successfully incubated a two-egg clutch, but suffered fledging failure probably due to tourist activities (Müllner et al., 2004, see chapter 3.2). There are two main scenarios which could explain the alien chick: There might have been a second female in this unit (see above), either mating with the male of this group or with another male from outside the group. Alternatively, a female from an adjacent territory may have laid an egg into that nest, either after mating with a male from the “host”-group (son or father) or with a male from outside this group. Since the BSC value was very low, the third nestmate was probably not a half-sibling, indicating that neither of the males in that breeding unit was the father of this extra-pair-egg. Unfortunately, it is not possible to come to a definite conclusion about this case without genetic information about the breeding adults.

### *Frequency of joint-nesting*

Approximately 16% of hoatzin breeding attempts in the Cuyabeno Lakes were the result of joint-nesting whereas 84% of hoatzin clutches were produced by a singly breeding pair. Hence, monogamous pairs, living singly or with offspring from previous years, seem to be the most common breeding unit. However, the percentage of joint-nesting is much higher than that reported from two other study sites in Peru and Venezuela (Torres, 1987; Strahl, 1988). The high incidence of joint-nesting in the Cuyabeno Lakes is mainly due to hoatzins living at two lakes which are relatively isolated from the rest of the lake system and which lack a corridor to other sites with suitable hoatzin nesting habitat. Hoatzins are weak fliers and predation during dispersal might be high in the rainforest. Thus, females, which represent the dispersing sex in hoatzins (Strahl and Schmitz, 1990), may have a low probability of acquiring a territory with independent breeding opportunities. A limited availability of suitable territories and intense competition for breeding vacancies might support joint-nesting (Koford et al., 1986; Mumme et al., 1988; but see Macedo and Bianchi, 1997). In fact, these two lakes exhibited the highest densities of breeding groups and nests in the entire lake system. Although nest densities in the Cuyabeno Lakes were still lower than in Peru and Venezuela (Torres, 1987; Domínguez-Bello et al., 1994; Strahl and Schmitz, 1990; Müllner et al. unpubl. data), habitat saturation could be a driving force for females or pairs to nest jointly.

Survival increased with unit size in female Anis (*Crotophaga sulcirostris*) due to reduced predation which in turn was due to shared incubation (Vehrencamp, 1978; Vehrencamp et al., 1988). According to the authors survival appears to play a key role in the ecological basis of social nesting. This also might apply for the hoatzin, but the annual survival of breeders seems to be very high (approx. 87%, Strahl and Schmitz, 1990). Another explanation for the high incidence of joint-female nesting in our study population could be a lack of mates for females because the hoatzin offspring sex ratio was female biased (59%; A. M., K. E. L. and M. W., submit., see chapter 2.4). However, this female skew would support polygynous systems rather than the suggested joint-nesting in distinct pairs.

Vehrencamp (2000) proposed that only species where more than 20% of all clutches are joint-nests should be considered as real joint-nesters. This percentage seems somewhat arbitrary and more orientated on the data available at the time than on the need to separate this breeding system from an accidental use of the same nest by several females (e.g. Semel et al., 1988). If joint-nesting is considered to be a response to ecological constraints (Vehrencamp, 1978; Mumme et al., 1988) we would expect that the magnitude of joint-nesting in bird populations varies according to the strength of specific factors. The few studies on joint-nesters which were able to compare several geographically distinct populations of the same species support this theory (Koenig and Stacey, 1990; Jamieson, 1997).

Interestingly, the hoatzin, whose phylogeny is still under discussion, has been linked to the neotropical family Crotophagidae in the past (Sibley and Ahlquist, 1973; Sibley and Ahlquist, 1990). The Crotophagidae consist of three species of anis and the Guira Cuckoo (*Guira guira*), which are all known to be joint-nesters (Davis, 1942; Macedo, 1992; Vehrencamp, 2000). Only recently Hughes and Baker (1999) questioned the relationship of the hoatzin and placed it near the African Turacos, but still within the order Cuculiformes.

### *Breeding success of joint nests*

The percentage of joint-nests with at least one hatchling or one fledgling was indistinguishable from that of nests with a single breeding pair. However, if we consider the greater number of eggs laid in joint-clutches and take into account the fact that joint-clutches actually reflect the breeding attempts of two or more females, the breeding success in relation to the effort made is lower in joint-nests than in nests with a single breeding pair. One reason for this decreasing success is certainly the loss of eggs between laying and hatching. However, there was also a higher discrepancy between the number of hatchlings and fledglings in successful joint-nests than in single nests, indicating increased hatchling mortality. This could be the result of nesting rivalries, but we have no evidence to support this suggestion. Bigger social units engage more heavily in territorial disputes and disregard their breeding duties (VanderWerf and Strahl, 1990; A. M. unpubl. observations). Consistent with this behavioural pattern breeding success did not increase beyond a certain threshold in larger units (Müllner and Linsenmair, in prep., see chapter 2.3). There might be ecological constraints which do not allow more than one or two young per breeding unit to develop successfully and reach fledging. We only once found a group with three fledglings (in all study years). The lower success of joint-nesting units is consistent with data from other joint-nesting species (Mumme et al., 1988; Vehrencamp et al., 1988). In these species the disadvantage of joint-nesting seems to be compensated for by a higher adult survival and/or the later inheritance of the previously shared territory (Vehrencamp, 1978; Mumme, 1988; Koford et al., 1990). Both arguments might also be applicable to the hoatzins in the Cuyabeno Lakes. We lack the necessary data to confirm this theory but we would expect some individual lifetime benefits to be associated with joint-nesting.

### ***Egg removal***

Partial egg disappearance was only recorded in joint-clutches. Predation normally affected the entire clutch during the incubation stage and, therefore, we conclude that hoatzins actively removed eggs from joint-clutches. Hoatzins are territorial and it is unlikely that an individual from a neighbouring group destroyed the eggs. It is most probable that adults from the same breeding unit removed the eggs. This suggestion is supported by anecdotes from the Siona Indians living in the Cuyabeno area. They have regularly observed hoatzins “accidentally” tossing eggs out of the nest into the water and thought that some birds were “bearish” (B. Criollo, pers. comm.). The incidence of egg manipulation might in fact have been higher, but probably stayed undetected because we tried not to flush the incubating birds during our regular controls.

Egg removal indicates a reproductive conflict among adults in the breeding unit. Unfortunately we do not know whether hoatzins are able to identify their own eggs and whether only eggs from other parents were manipulated. Eggs appeared to be quite different in shape and spot patterns in some joint clutches, but a considerable overlap existed. Egg-tossing has also been observed in other joint-nesting species, but most of them were not able to discriminate their own eggs from those of others (Vehrencamp, 1977; Macedo, 1992; Koenig et al., 1995; but see Bertram, 1979, for the ostrich (*Struthio camelus*)). In some species egg destruction stopped when the second female started laying its eggs (Vehrencamp, 1977; Koenig et al., 1995). As a consequence the egg number remained roughly constant throughout incubation. This contrasts with our finding that hoatzin egg removals could occur at any time during incubation. A similar pattern plus infanticide has also been observed in joint-nesting guira cuckoos and was explained by an indiscriminate destructive behaviour due to a pronounced skew in reproductive contributions among group members (Macedo, 1992; Quinn, 1994). This form of “sabotage” from non-breeding members of the group or from the pair which had least contributed might also apply to the egg loss and the egg manipulation in the hoatzin. Further research is necessary in order to evaluate the reproductive skews within joint-nesting breeding units.

To summarise, we have demonstrated that both jointly and singly nesting groups coexist within the same rainforest population of hoatzins. Monogamous pairs produced clutches of up to three eggs whereas bigger clutches were the result of laying by several females, presumably living in distinct pairs within a breeding unit. Jointly-nesting females have significantly lower annual reproductive success than females that nest singly, and we expect other benefits to compensate for this disadvantage.

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# 3. Hoatzins and rainforest tourism

## 3.1 Introduction: Effects of tourism on animal populations

Antje Müllner and Albrecht Pfrommer *

The most important attractions for nature-tourists are free-ranging animals. Bird- and other wildlife observations were stated as the main motivation for visiting Ecuador by the majority of international visitors (i.e. 60%, Boo, 1996). Disturbances and changes of animal populations thus not only contradict the purpose of wildlife conservation areas, but also harm a substantial part of the tourist economy. Despite these consequences, the effects of tourist activity on local animal populations were investigated only sporadically. This lack of investigations is partially due to the common assumption that nature-tourists in fact only take photographs and leave footprints. Furthermore, particularly at the beginning of a disturbance, obvious signs of its effect are scarce. Accumulated effects may become evident only after a habitat has already been changed to a considerable degree. Because of this, studies investigating the effects of even low tourism rates are urgently needed. This will enable the detection of effects as early as possible. Alterations such as trash accumulation or the development of lodgings and roads are the most obvious signs of an increasing impact. Provided that consequent law enforcement is available, these problems can often be abolished or at least controlled.

***“Disturbance”***
*In biological context “disturbance” is commonly used in reference to negative effects on individual organisms or biological systems. However, in common language, the term disturbance may have different meanings. Often it remains uncertain, whether “disturbance” refers to a cause, an effect or the relationship between cause and effect. Likewise, anthropogenic and natural causes are commonly not differentiated. Disturbance is often used to define not only a cause, e.g. a by-passing motorboat, but also its expected negative effect. Within the meaning of this last definition, the term disturbance is also used in statute-books, e.g. in the German Conservation Edict. Generally, conservationists should try to avoid the term and rather differentiate between cause and effect or according to Stock et al. (1994) between “disturbance-stimulus" and “disturbance consequence”.*

Contrary, the existence of partly strong disturbance effects caused by simple tourist presence, noise or approaches is mostly ignored. Some of these disturbance effects and

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their known consequences are listed in Table 1. Further reviews are found in Liddle and Scorgie (1980), Boyle and Samson (1985), Hockin et al. (1992), Ingold et al. (1993) and Keller (1995). Up to now, only few studies were carried out, investigating the long-term effects of tourist activity on animals. In part, this is due to the high costs and difficult realization of such studies. Furthermore, the differentiation between anthropogenic and natural effects is often difficult (Pechmann et al., 1991). Nevertheless, the existence of long-term effects on specific animals has to be acknowledged.

**Table 1**. Documented consequences of tourism, human presence and observational approaches on wildlife.

| **animal reaction** | **immediate consequences** | **long-term consequences** | **species, site, reference** |
|---|---|---|---|
| Increased alertness and unrest | Decreased feeding rates and increased mortality | Population decrease | Plover chicks, Canada (1) |
| | Decreased feeding rates | No data | Flamingo, USA (2)<br>Trumpeter Swan, USA (3)<br>Heron, USA (4)<br>Asian rhinoceros, Nepal (5) |
| | Decreased feeding rates of hatchlings | No data | Marsh Harrier, Spain (6) |
| | No data | No data | Boobies, Galápagos (7) |
| Increased aggression | No data | No data | Seagulls, USA (8) |
| Increased heart beat | Increased energy consumption | No data | Coastal birds, North Sea (9)<br>Long-eared Owl, Germany (10)<br>Penguins, Antarctica (11)<br>Boobies, Galápagos (12) |
| Flight from nests | Increased nest predation, decreased number of hatchings | Population decrease | Great Crested Grebe, Swiss Lakes (13) |
| | | No data | Nesting water-fowl, Patagonia (14) |
| | | No data | Nile crocodile, Uganda (15) |
| | Nest desertion | No data | Beach-nesting birds, Amazonian Peru (16) |
| | No data | No data | Nesting water-fowl, Florida(17) |

| | | | |
|---|---|---|---|
| Flight from feeding and resting sites | Temporal abandonment of sites | None to be expected | Ducks, Nepal (18) |
| | | No data | Water-fowl, USA (19), (20)<br>Macaques, Sulawesi (21)<br>Cheetahs, Kenya (22)<br>Chamois, Alps (23) |
| | Variation of activity time | No data | Waders, Florida (24)<br>Geese, Canada (25)<br>Chamois & marmots, Alps (23)<br>Mammals, Sumatra (26) |
| | Decreased feeding times | No data | Marmots, Alps (23) |
| | Retreat from the area | Lasting expulsion | Mammals, Sumatra (26) |
| | | No data | Geese, England (27)<br>Heron, USA (4) |
| | Desertion of territory | Lasting expulsion | Giant Otter, Peru (28)<br>Park birds, Madrid (29) |
| Micro-injuries caused by turbulences | Increased mortality | Change in community composition | Corals, fish, Caribbean (30) |
| Injuries caused by boats | Mortality rate increased by 10% | No data | Manatee, Florida (31) |
| Uncertain | Loss of nursed young | Local population decrease | Giant Otter, Peru (28) |
| | Increased nestling mortality | No data | Razorbill, Denmark (32) |

References: (1) Flemming et al., 1988; (2) Galicia and Baldassare, 1997; (3) Henson and Grant, 1991; (4) Kaiser and Fritzell, 1984; (5) Lott and McCoy, 1995; (6) Fernández, 1993; (7) Burger and Gochfeld, 1993; (8) Burger, 1981*a*; (9) Hüppop and Hagen, 1990; (10) Berger, 1992; (11) Culik et al., 1990; (12) Jungius and Hirsch, 1979; (13) Keller, 1989, 1992, Fuchs, 1978*a*, 1978*b*, 1982; (14) Yorio and Quintana, 1996; (15) Cott, 1969; (16) Groom, 1991; (17) Rodgers and Smith, 1995; (18) Hulbert, 1990; (19) Klein, 1993; (20) Burger, 1981*b*; (21) Kinnaird and O'Brian, 1996; (22) Gakahu, 1992; (23) Ingold et al., 1993, Ingold et al., 1992, Hamr, 1988; (24) Burger and Gochfeld, 1991; (25) Bélanger and Bedard, 1990; (26) Griffith and van Schaik, 1993; (27) Gill et al., 1996; (28) Schenck and Staib, 1998, Staib and Schenck, 1994; (29) Fernandéz-Juricic, 2000; (30) Hawkins et al., 1999; (31) Shackley, 1992; (32) Lyngs, 1994.

Figure 1 summarises the variety of possible impacts of visitor activities on animals known to date. Depending on species and previous experience the individual animal might adapt to tourists. However, wild animals almost always respond with a change in behaviour, although the reaction might be invisible and only detectable on a physiological level. Often the animals flee the disturbance or try to avoid it spatially or temporally. They may then suffer in other ways, for example via reduced feeding time or interrupted reproductive activities. These disadvantages may be partly compensated for and do not necessarily have to have negative consequences on the population status. If avoidance or compensation is not possible we should expect negative impacts in the long run with a continuum from a slight decline in abundance to local extinction. Furthermore, even if behavioural changes do not lead to severe impacts at the population level, they may have negative consequences on the tourism itself due to a decreased visibility of the animals.

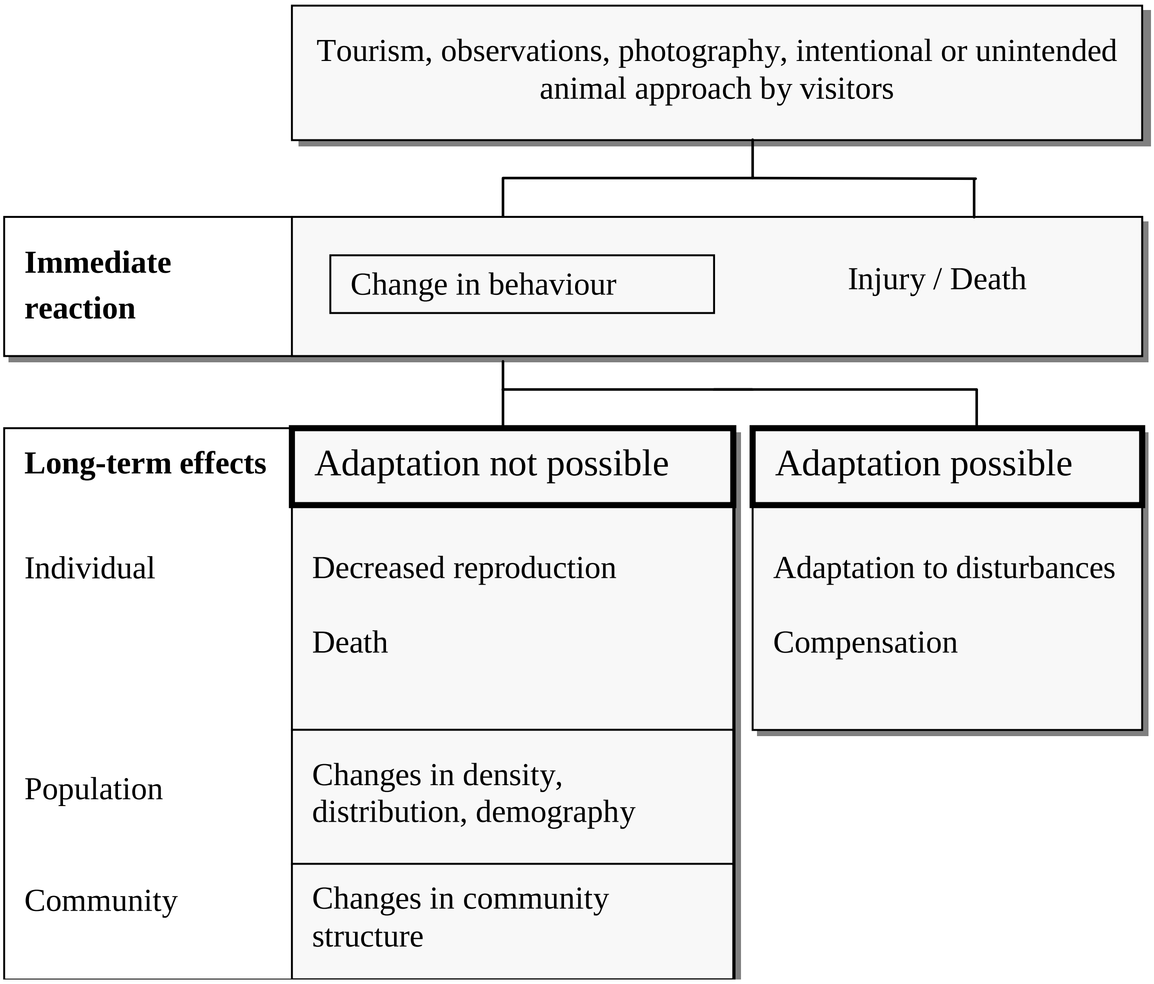

**Figure 1**: Immediate and long-term effects of tourism on animals. Modified after Ingold (1991) and Knight and Gutzwiller (1995).

Ecotourism which aims to be sustainable has to safeguard its natural attractions. This is especially true for tourism in protected areas where all negative impacts on wildlife should be prevented. Only the knowledge between cause and effect of animal responses to tourism allows proper management measures (Sutherland, 1998; Curio, 1996). Therefore, specific investigations about the interaction of tourists and animals should be part of every concept on visitor management. However, only little is known about tourist influences on wildlife in tropical rainforests because tourism is a recent development there and interdependences in these biodiversity-rich ecosystems are extremely complex. Nevertheless, such studies are needed urgently because tourism in tropical protected areas is increasing rapidly.

Investigating the effects of tourist activity on wild animals in a rainforest area researchers are confronted with a number of methodical difficulties. An ideal design for a study would be to compare under parallel control- and disturbance conditions an area before and after a disturbance (Hill et al., 1997; Stewart-Oaten et al., 1986; 1992). A comparison of the obtained data would then allow an evaluation of the disturbance in relation to the observed variations. However, data available on the occurrence of animals before the onset of tourism in the area are mostly too limited to be used as standard of comparison. In many cases rainforest tourism increases rapidly and uncontrolled, and only the observation of first changes bring about a consideration of possible tourism effects. To come as close to the ideal situation as possible, we simultaneously collected data on different parameters in areas used by tourists and areas in which tourist activity was strictly prohibited.

The main questions in this study were whether current tourist activities show detectable effects on the wildlife in the study area and whether serious alterations can be detected for certain species or communities. The obtained data only allow an evaluation of these questions for the actual situation. A projection of the effects on animals if there were greater or fewer visitors is not possible without actually altering visitor numbers in a controlled experiment. However, it is generally neither practical nor ethically acceptable to deliberately produce a disturbance factor. For example, it was not desirable to allow tourists to enter the off-limit area just to enable the scientific investigation of which number of visitors would cause a certain species to vanish.

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## 3.2 Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (Opisthocomus hoazin)

Antje Müllner, K. Eduard Linsenmair and Martin Wikelski *

### Abstract

Ecotourism helps to protect many habitats, but may also have negative impacts on wildlife. We investigated effects of ecotourists on reproductive success of hoatzins (*Opisthocomus hoazin*) and on hormonal status of their chicks in Amazonian rainforest lakes by comparing birds from undisturbed and from tourist-exposed nests. Hatching success was similar in both groups but chick survival was much lower at tourist-exposed nests than at undisturbed nests. This effect was due to an increased mortality of juveniles prior to fledging whereas small nestlings seemed largely unaffected. Juveniles, but not nestlings, living at tourist-exposed sites had a lower body mass and showed a stronger hormonal response to experimental stress compared to individuals at undisturbed sites. These data suggest that juvenile hoatzins were susceptible to tourist-induced stress which in turn may be responsible for the lower survival. In contrast, adult hoatzins that were incubating had apparently habituated to tourist presence because their flush distances at tourist-exposed nests were 50 % lower than at undisturbed sites. Our findings demonstrate that individuals in different life stages show different susceptibility to tourism. We suggest that even just watching animals during breeding can threaten their survival, but a proper scientific management of off-limit zones and area-specific guidelines for wildlife observation could reduce harmful effects.

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## Introduction

Ecotourism is largely perceived to safeguard pristine areas and thereby to contribute to the conservation of the rich tropical biodiversity (Groom et al., 1991*a*; Munn, 1992; Ceballos-Lascuráin, 1996). Revenue from ecotourism operations may compensate local people for the abandonment of other non-sustainable uses (Munn, 1992; Wunder, 1996) and might allow a gentle development of regions where economic alternatives are rare. Because ecotourism is a rapidly growing industry (Giannecchini, 1993) there is an urgent need to assess its impacts and develop rapid ways to avoid potential negative consequences.

Protected rainforests areas are the favoured destinations of many ecotourists in tropical countries. One of the main travel incentives is to experience free-ranging animals in a pristine landscape (Boo, 1990). Whereas effects of tourism on wildlife are well recognised in temperate countries (reviews in Boyle and Samson, 1985; Edington and Edington, 1986; Knight and Gutzwiller, 1995), very little is known whether visitors have any influence on rainforest animals. The rare empirical evidence available indicates that even low numbers of visitors can change activity patterns or expel rainforest animals from potential foraging or breeding sites (Groom, 1991*b*; Griffiths and van Schaik, 1993; Schenck and Staib, 1998; de la Torre et al., 2000). All of these effects can reduce reproductive success and therefore hamper conservation goals of protected areas. At the same time negative impacts on wildlife reduce both the ecotouristic as well as the economic value of the visited area.

Here we concentrate on the Cuyabeno Wildlife Reserve which has become the most important destination for ecotourists in Amazonian Ecuador. Tourism started in the 1980s and developed quickly and largely uncontrolled since 1992. Ecotourists regularly search for close encounters with wildlife and the high visibility of birds make them a major attraction. One target is the hoatzin (*Opisthocomus hoazin*) whose pheasant-like size and colourful plumage with a crest make it conspicuous. Because tourists like to witness on animal’s family life nest sites and young birds are particularly attractive. Reports by tourist guides indicated that hoatzins bred in larger numbers prior to regular tourism than nowadays (Aurora Payaguaje, Galo Sevilla, pers. comm.). In the present paper, we examined the effects of ecotourists on reproductive success of hoatzins in the Cuyabeno Lakes by comparing birds at undisturbed nests in off-limit zones and at tourist-exposed nests. We also measured stress hormones in hoatzin chicks to evaluate the physiological effects of human disturbances. Although field endocrinology is acknowledged as a useful tool in conservation (Wingfield et al., 1995; Schoech and Lipar, 1998), only a few studies have used the hormonal status of vertebrates as an assessment of tourism impacts so far (Fowler, 1999; Romero and Wikelski, 2002).

## Methods

### *Study site and tourism*

The Cuyabeno Reserve is a protected rainforest area of about 6000km$^2$, situated in the Amazonian lowlands of Ecuador. One of its main tourist sites are the Cuyabeno Lakes, a system of rivers and lakes with flooded and unflooded forests (Fig. 1). Providing an extraordinary landscape and rich wildlife, this rather small area (10 x 10 km) attracts ca 4000 tourists per year. Tourists come all year round, but a pronounced high season exists in July and August when about 10 tourist groups with a total of more than 100 people may be based at the same time at the central Laguna Grande. Visitors go on guided jungle walks and explore the lakes and rivers by motorised and paddled canoes, normally of four days duration, to actively seek and approach animals. Most tourist sites are visited every day but boat traffic changes unpredictably: a day with only one boat could be followed by a day with six or ten boats in only two hours. Sites for tourism use and off-limit zones were established in 1996 for the area of the Cuyabeno Lakes (INEFAN-ONISE/OISE, 1995). However, using the argument that wildlife observation is quite harmless, several tour operators tried to suspend the ban on visiting some sites. For more details on the study area and tourism development see Müllner and Pfrommer (2001).

### *Study species*

The hoatzin (*Opisthocomus hoazin*) is distributed patchily throughout the Amazon and Orinoco river basin and is locally common in the Cuyabeno Reserve. It inhabits the stretches of flooded forest which surround the Cuyabeno Lakes, living in pairs or small family groups and defending territories of about 5000-8000m$^2$. Density of groups is 3-7 per km of shore line and lies within the range of another rainforest population of hoatzins in Peru (Torres, 1987). Breeding is closely linked to the beginning of the wet season and starts in April or May. Hoatzins build simple platform nests on branches overhanging the water, and 72% of nest sites in the Cuyabeno Lakes are situated on isolated flooded trees, which are well protected against non-flying and non-swimming predators.

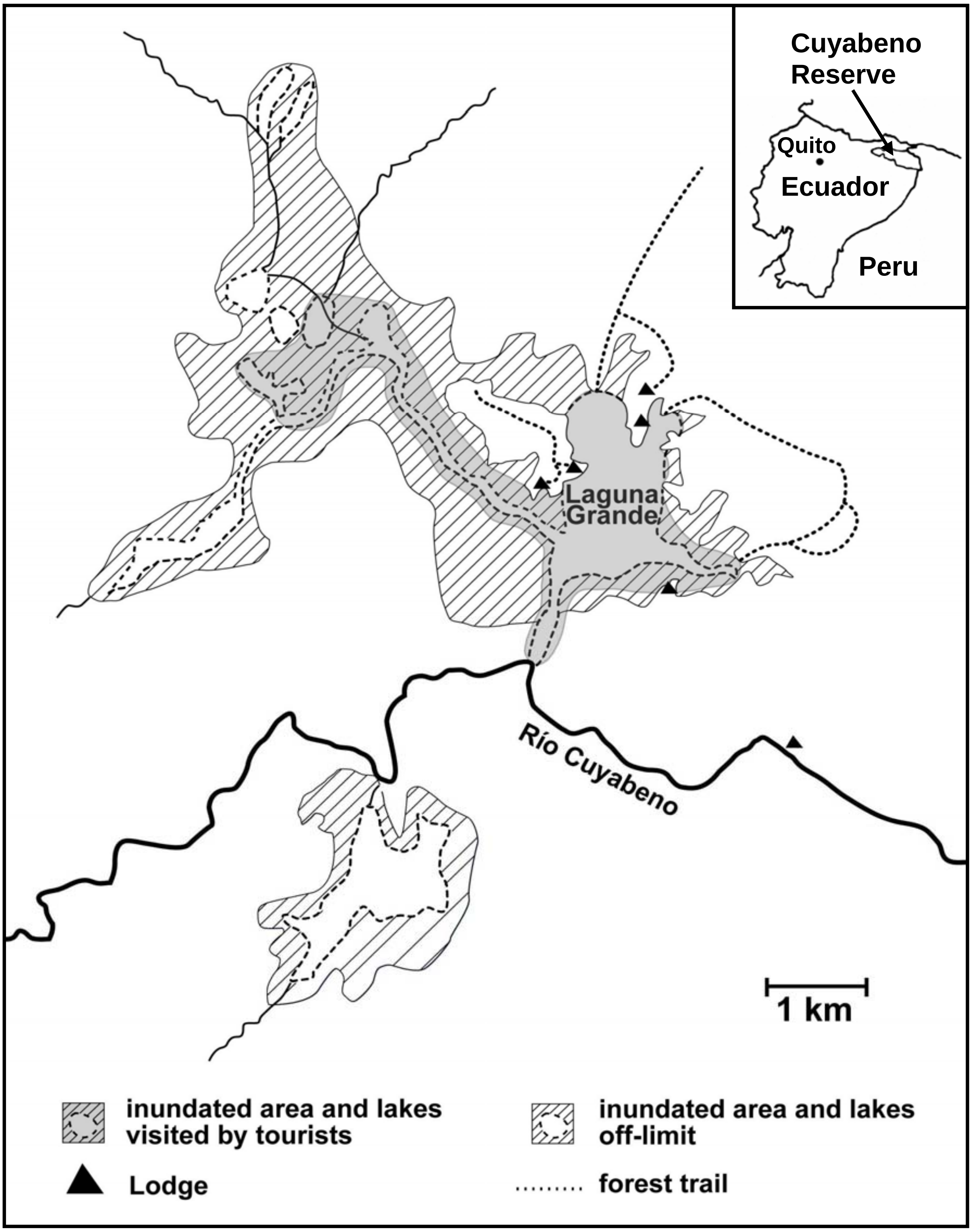


**Figure 1.** The Cuyabeno Lakes in the Amazonian lowlands of Ecuador within the Cuyabeno Wildlife Reserve (0°02’ N, 76°9’ W and 0°03’ S, 76°14’ W). Modified after INEFAN-ONISE/OISE 1995.

The modal clutch size is two eggs and the incubation period lasts 32 days; in 75% of broods only one chick survives until fledging (A. M., unpubl. data). If not disturbed, hatchlings stay in the nest 14-20 days and then begin to climb into the surrounding foliage. First flights occur at seven weeks of age, and individuals reach full flight capabilities at about ten weeks. As an adaptation to life in inundated forests, hoatzin chicks prior to fledging show an extraordinary escape behaviour when approached by a predator: they jump into the water and may dive 5-15m in an attempt to escape. With the help of their wing claws they climb another tree, but do not return to their nest site. Instead, the adults normally find the young and feed them until fledging. The most common predators we observed in the Cuyabeno Lakes preying on eggs or young of the hoatzins were several species of toucans, raptors and snakes, while monkeys or other non-flying mammals were rarely seen. In contrast to reports from other regions (Beebe, 1909; Strahl, 1988) hoatzins in Cuyabeno neither defended the clutch nor their chicks, but always fled from a predator or an approaching observer.

Hoatzins feed only on foliage (Grajal et al., 1989) and were observed to feed on the most common trees and shrubs of their flooded habitat, mainly Leguminosae (A. M., unpubl. data). The chicks were fed a predigested mash that the adults regurgitated from their crops. Overall, food was sufficiently available during the entire breeding season. However, hoatzins prefer the young leaves and buds, whose main annual peak coincides with the feeding of the chicks in July and August.

### ***Data collection and analyses***

#### *Monitoring of nest sites*

The entire area of the Cuyabeno Lakes was checked regularly for hoatzin nests and breeding activities from April to October in 1996, 1997 and 1998. All data were collected by the same observer (A. M.) paddling a dugout canoe. Incubated nests were approached either only once, to inspect the nest content, or a second time to determine the flush distances of adults. To minimise investigator effects the progress of incubation and chick development was observed from a distance of 20-40m by binoculars. Every nest site was checked one to three times a week, depending on accessibility due to water level or fallen logs. In 1998 some nests were subjected to two additional approaches to capture and bleed hoatzin chicks. Nest sites were divided into those exposed to tourism and undisturbed nests situated in backwater and off-limit zones (Fig. 1).

*Reproductive success*

Calculation of nesting success and statistical comparison of undisturbed nests and tourist-exposed nests followed Mayfield (1961) and Hensler and Nichols (1981). We considered breeding at 184 different nest sites in a total area of 580ha of tourist-visited habitat (with ca. 26 km of shoreline) and 660 ha of undisturbed habitat (with ca. 24km of shoreline) in the analyses. Incubation and chick period were analysed separately because tourist activity may influence breeding success in a different manner during different nesting stages. This also allowed us to include nests in the analysis for which only the fate of birds during one of these periods was known. We considered nests successful if at least one chick hatched after the incubation period or if at least one young had fledged after the fledging period, respectively. To determine the most vulnerable time during the fledging period we further employed a life table analysis (SPSS 11.5) for the chicks.

To assure comparable conditions of nest sites with respect to climate, vegetation and tourist load only the first annual breeding attempt of a hoatzin pair was included in the analysis. Neither growth rate nor mortality differed between male and female chicks (results of molecular sexing, A. M., K.E. L. and M. Wink, unpublished) and thus sex was ignored in our analysis. Hoatzins are cooperatively breeding birds and group size is known to influence nesting success (Strahl, 1988). However, group composition was indistinguishable in undisturbed and tourist-exposed territories. In particular, the percentage of single pairs, which are the least successful, was similar in both samples with 19% and 20%, respectively (A. M. and Linsenmair, unpubl. data).

*Hormonal stress response of hoatzin chicks*

In 1998 we measured the plasma concentrations of corticosterone to investigate if young hoatzins were physiologically stressed by tourism activities. We followed the capture stress protocol of Wingfield et al. (1983) which uses capture and handling as a standardised stressor and measures the increase of plasma corticosterone in subsequent blood samples. This method provides information about the sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis and can reveal how an individual might be affected by other stressful stimuli, such as disease or human disturbances (Wingfield et al., 1995). In contrast, basal corticosterone levels only present the bird's current hormonal status.

We sampled hoatzin chicks at an age of 8-18 days when they remain quiet in the nest (nestlings) and as young birds at an age of 40-57 days that had already left the nest but prior to fledging (juveniles). The chicks were caught by hand directly from the nest (all nestlings) or from the branch where they were resting (juveniles). Some chicks jumped into the water when approached and were immediately caught with a fishing net. All stress protocols were carried out between 0900 and 1200 by the same researcher (A. M.). Sample time started (1) when the nesting tree was reached with the canoe or (2) at the moment when the young tried to escape before the canoe had reached the tree. This situation mimics the approach by a tourist group to the nesting site. The first bleeding occurred as soon as possible after capture ("initial sample"). Blood was collected from the wing vein into heparinized capillary tubes, ca. 50µl per sample. Subsequent blood samples were taken at 10, 20, 35 and 70 minutes after the first sample. Between blood sampling events the birds were guarded in cloth bags. After the final bleeding, individuals were weighted with a Pesola spring balance, colour banded, and released at the place where they had been caught. The survival of sampled chicks was within the range of survival of birds that were not sampled.

The blood was stored on ice up to 6h and then centrifuged with a hand centrifuge (3min at 3000rpm). Plasma was extracted with a Hamilton syringe and the volume was measured. The plasma was stored in 0.5ml 100% ethanol (Wikelski et al., 2002) at 4° C for several months and transported to the University of Illinois, where it was kept at -20° C until analysis in 2000. Corticosterone was analysed by radioimmunoassay after extraction of corticosterone with dichloromethane (described in detail in Wikelski et al., 2000). Each sample was assayed in duplicate with a small amount of radiolabelled steroid added to determine recovery. The intra-assay variation was 9%, the inter-assay variation 12.5% and assay sensitivity was at 3ng/ml. Differences in the stress response between tourist- exposed and undisturbed nest sites were compared by repeated-measures ANOVA. In juveniles the variances of the last sample (70min sample) failed to be homogeneously distributed, but the variances were not correlated with the means and the *F*-statistic is known to be quite robust to a violation of this assumption. For several initial bleedings sampling time was delayed. To calculate true basal concentrations we therefore split the initial samples into those taken before and after 3 minutes after capture as 3 minutes is a common threshold for detecting stress-induced corticosterone secretion (M. Romero, unpubl. data).

To further test for associations between age or body weight and stress response, we calculated the maximum level of corticosterone achieved during the restraint, regardless of sample time and used Spearman´s rank-order correlation. No sex differences existed in the stress response or body weights of sampled chicks, thus sex of the young was ignored in the analysis. There were three sibling pairs in each nestling group and two pairs in each of the juvenile groups. Because there was as much variation between siblings as there was between unrelated chicks (from different nests), the stress responses of all siblings were included in the analysis. We tried to sample the same individuals both as nestlings and as juveniles but were not successful in all cases. Thus we included additional juveniles (not sampled as nestlings) in the analysis.

### *Flight behaviour of incubating hoatzins*

We used flight distances of incubating hoatzins as an indicator of the behavioural response towards humans, assuming that the reactions to the investigator's canoe were at least as strong as to a bigger tourist boat with several people inside. In 1996 and 1997 hoatzin nests were approached approximately at a 45° angle to the shore line until the incubating bird was flushed away. The distance between boat and nest was measured with a range-finder to the nearest metre. The bird normally waited in a nearby tree and returned a few minutes later when the observer was outside the critical flight distance. We restricted experimental flushing to the period between week 2 and 4 of incubation (i.e. before the chicks start to hatch) to prevent abandonment of incubated nests and because flight distances might change during the nesting cycle. Every territory was considered only once and flight distances measured in 1996 and 1997 were combined. Comparisons of distances at undisturbed and at tourist-exposed nest sites were made by t-test.

## Results

### ***Breeding success***

A total of 131 undisturbed nests and 83 tourist-visited nests were monitored during incubation of eggs, and 74 and 61 respectively during fledging (Table 1). Nest survival during the incubation period was similar at 23-28% for undisturbed and for tourist-exposed nests over the three years (Table 1). However, chick survival during the fledging period was always higher in undisturbed nests compared to tourist nests. This difference was most pronounced in 1996, also significant in 1998, but supported only by a weak trend in 1997. The survival functions for the chicks (Fig. 2) revealed that the lower survival of tourist-exposed chicks was due to a higher mortality in week 3-6 when the chicks had left the nest.

**Table 1.** Success of hatching and fledging of hoatzins from undisturbed nests (U) and from tourist-exposed nests (T).

| year | exposure (days) | successful nests | unsuccessful nests | daily survival | survival for entire period |
|---|---|---|---|---|---|
| **1996** | | | | | |
| *Incubation* | | | | | |
| U | 339 | 7 | 14 | 0.9582 | 0.26 |
| T | 338 | 10 | 16 | 0.9573 [a] | 0.25 |
| *Fledging* | | | | | |
| U | 399 | 9 | 4 | 0.9900 | 0.57 |
| T | 338 | 3 | 11 | **0.9638** [b] | 0.13 |
| **1997** | | | | | |
| *Incubation* | | | | | |
| U | 822 | 15 | 32 | 0.9611 | 0.28 |
| T | 364 | 8 | 16 | 0.9560 [c] | 0.24 |
| *Fledging* | | | | | |
| U | 892 | 18 | 6 | 0.9933 | 0.69 |
| T | 567 | 8 | 8 | 0.9859 [d] | 0.46 |
| **1998** | | | | | |
| *Incubation* | | | | | |
| U | 968 | 20 | 43 | 0.9556 | 0.23 |
| T | 538 | 12 | 21 | 0.9610 [e] | 0.28 |
| *Fledging* | | | | | |
| U | 1183 | 22 | 15 | 0.9873 | 0.50 |
| T | 734 | 9 | 22 | **0.9700** [f] | 0.19 |

A successful nest produced at least one hatchling after the incubation period (32 days) or one fledgling after the fledging period (55 days), respectively. *p*-levels are two-tailed. Bold print indicates significant results.

*Incubation:* [a]$Z = 0.17$, $p = 0.87$; [c]$Z = 0.40$, $p = 0.35$; [e]$Z = -0.50$, $p = 0.31$;
*Fledging*: [b]$Z = 2.21$, $p = 0.027$; [d]$Z = 1.30$, $p = 0.096$; [f]$Z = 2.44$, $p = 0.01$

### *Hormonal response and body weight of hoatzin chicks*

Hoatzin chicks at both undisturbed and tourist-exposed nests responded to our experimental capture and handling by a rapid increase in secretion of corticosterone. Maximum values were reached at 10 to 20 minutes of restraint and followed by a decrease, but even 70 minutes after capture the concentrations were still higher than initially. Among nestlings, the magnitude and pattern of this response was indistinguishable between undisturbed and tourist nests (Fig. 3A; $F_{1,25} = 0.90$, $p = 0.35$, Table 2). Among juveniles, however, patterns of corticosterone secretion were strikingly different between individuals from undisturbed and from tourist-exposed nests (Fig. 3B, $F_{1,19} = 8.53$, $p = 0.01$). Levels in tourist-exposed juveniles showed a much stronger increase within the first 20 to 30 minutes after capture and stayed at that level for more than one hour, while previously undisturbed juveniles secreted less corticosterone and had already decreased plasma concentrations at the end of the stress protocol. Maximal corticosterone titres of the tourist-exposed juveniles were twice as high as those from birds living at undisturbed sites (Table 2).

Initial corticosterone levels were similar for hoatzin chicks from undisturbed and from tourist-exposed nests, for nestlings and for juveniles (Table 2). Baselines values, only from samples taken within the first three minutes after capture, were at 3.3ng/ml ± 0.73 for the pooled nestlings ($n = 8$) and were similar for juveniles (3.8ng/ml ± 0.81, $n = 6$). These basal values were lower than the initial samples indicating that the latter include chicks that had already rapidly increased their corticosterone secretion after the first three minutes. For the graphic presentation in Fig. 3 the initial samples are therefore divided into those taken within and after the first three minutes. Thus, six sample groups are shown instead of the five considered in the ANOVA.

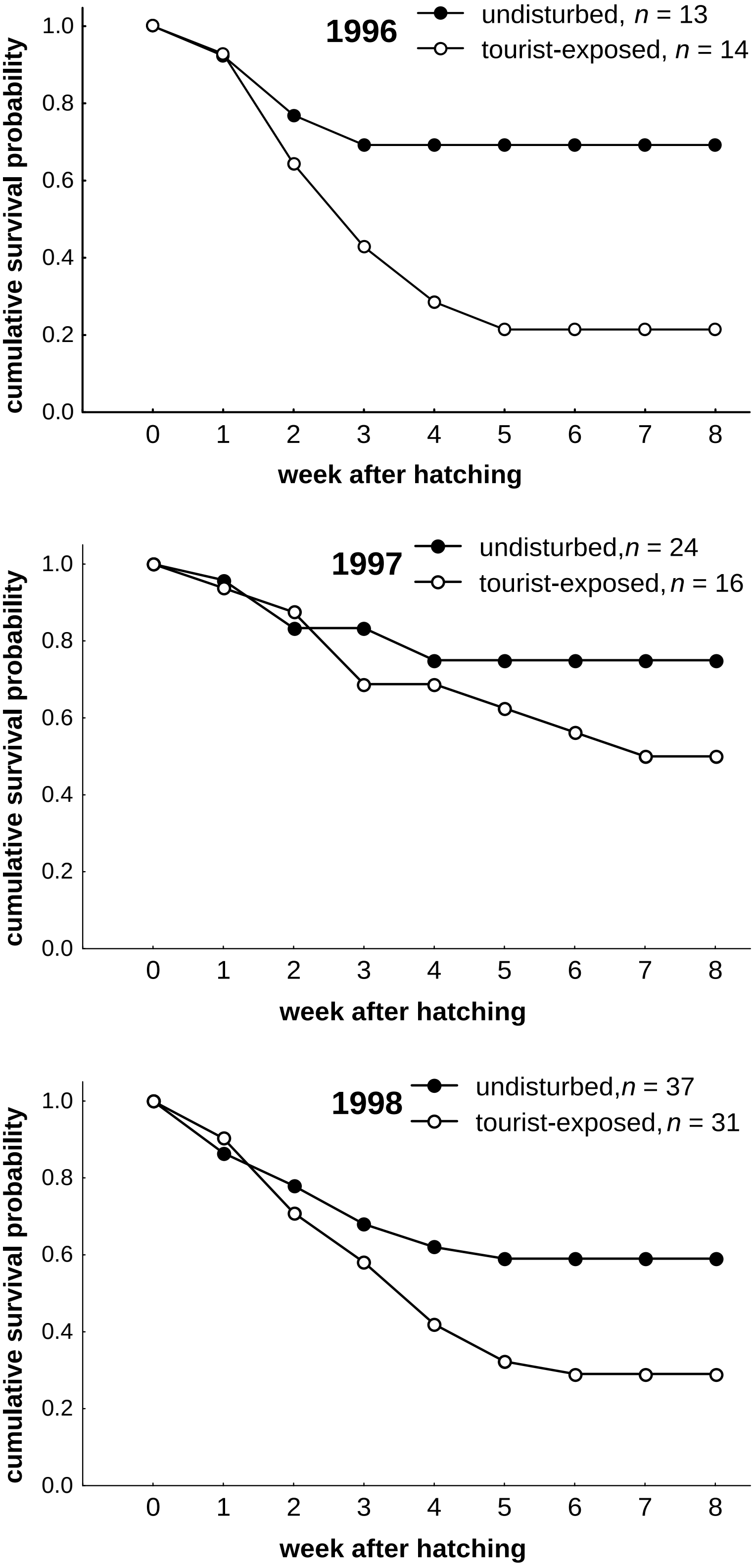
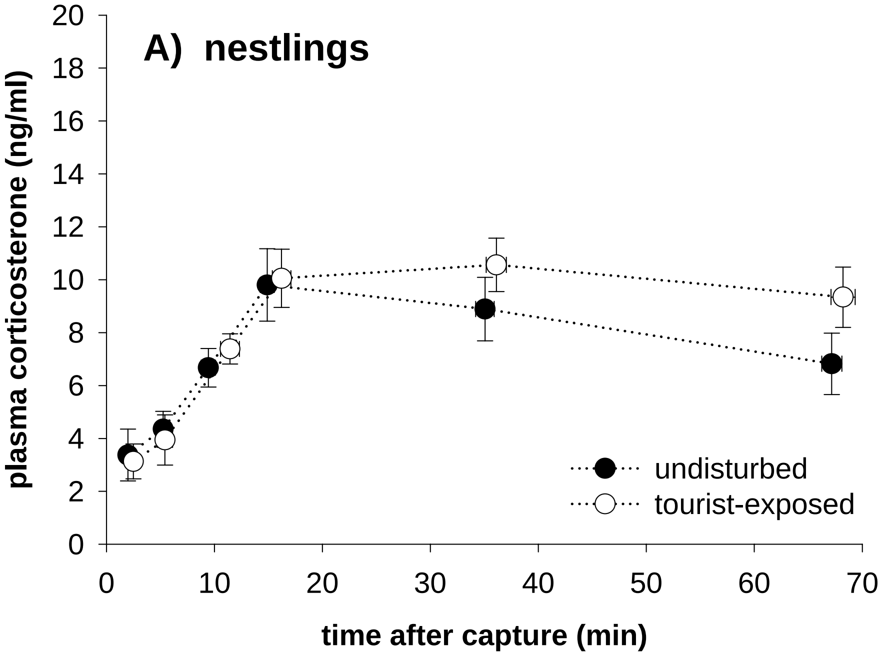


**Figure 2.** Survival function of hoatzin chicks at undisturbed nests and at tourist-exposed nests. Functions differ significantly between undisturbed and tourist-exposed chicks in weeks 3-7 of chick development. Wilcoxon (Gehan) test 1996: 4.47, *p* = 0.03; 1997: 4.34, *p* = 0.04; 1998: 5.19, *p* = 0.02.



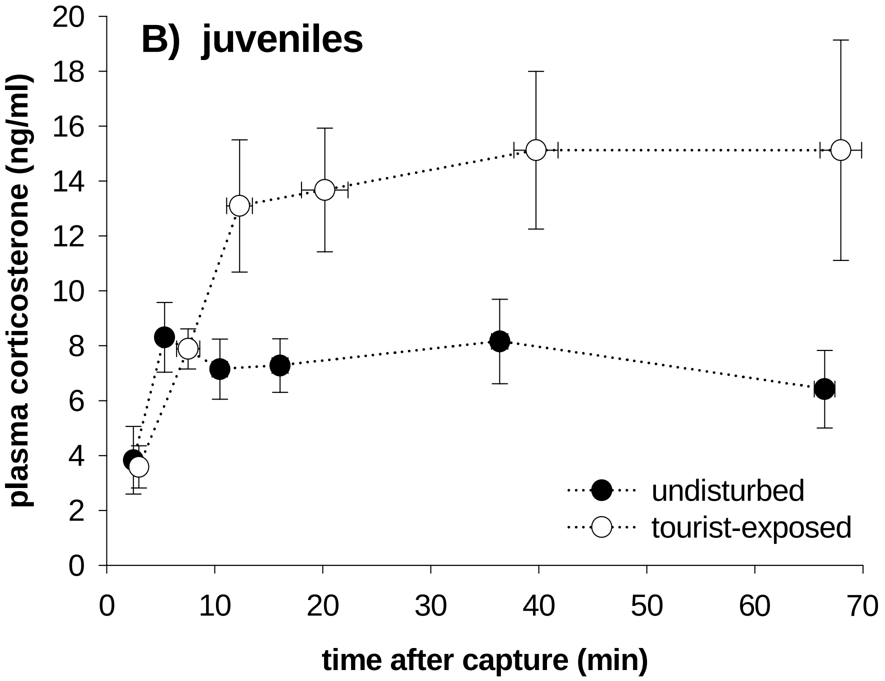


**Figure 3**. Stress-induced corticosterone secretion of hoatzin chicks from undisturbed and from tourist-exposed nests. Each point represents the mean ± SE for the concentration of plasma corticosterone and sampling time for A) *n* = 18 and 9 nestlings from undisturbed and tourist-exposed sites, respectively, and B) *n* = 12 and 9 juveniles from undisturbed and tourist-exposed sites, respectively.

Body weights of nestlings were lower in the undisturbed group than in the tourist-exposed group, but this may have been because there was a suggestion that the sample of undisturbed individuals was slightly younger than the tourist-exposed one (Table 2). We found no correlation of maximum corticosterone values with body weights (undisturbed: $r_s = 0.14$, $p = 0.58$; tourist-exposed: $r_s = 0.35$, $p = 0.35$) or with age for both nestling groups (undisturbed: $r_s = 0.003$, $p = 1.0$; tourist-exposed: $r_s = 0.25$, $p = 0.51$). In contrast, juveniles from undisturbed nests had significantly higher body weights than juveniles from tourist-exposed nests, although the former were not older (Table 2). There was no association between body weight and maximal corticosterone values, either for undisturbed ($r_s = -0.03$, $p = 0.92$) or for tourist-exposed individuals ($r_s = -0.47$, $p = 0.20$). Interestingly, we detected a negative relationship of maximal corticosterone values with age only for tourist-exposed juveniles: older juveniles responded less to acute stress ($r_s = -0.72$, $p = 0.03$) whereas undisturbed juveniles did not show this relation ($r_s = -0.19$, $p = 0.56$).

**Table 2.** Plasma corticosterone concentrations prior and in response to experimental stress and body condition of hoatzin nestlings and juveniles from undisturbed nests and tourist-exposed nests in the Cuyabeno Lakes 1998.

| | initial cort. (ng/ml) | max. cort. (ng/ml) | age (days) | body weight (g) |
|---|---|---|---|---|
| **Nestlings** | | | | |
| Undisturbed, *n* = 18 | 4.02 ± 0.55 | 12.69 ± 1.32 | 11.3 ± 0.6 | 83.6 ± 4.4 |
| Tourist-exposed, *n* = 9 | 3.76 ± 0.75 | 12.02 ± 0.93 | 13.4 ± 1.1 | 119.22 ± 6.8 |
| Significance *p* | 0.78 | 0.74 | 0.08 | 0.0002 |
| **Juveniles** | | | | |
| Undisturbed, *n* = 12 | 6.82 ± 1.11 | 10.55 ± 1.17 | 47.7 ± 1.2 | 371.9 ± 12.9 |
| Tourist-exposed, *n* = 9 | 6.94 ± 0.85 | 21.23 ± 3.73 | 46.1 ± 1.9 | 314.8 ± 17.2 |
| Significance *p* | 0.94 | 0.006 | 0.46 | 0.014 |

Results are given as arithmetic mean ± SE. Comparisons were made by t-test, after verifying the normal distribution of the data by K-S test. Only age and body weight of nestlings were compared by Monte Carlo simulations (100,000 permutations) because data failed to be normally distributed.

### *Flight reactions of incubating hoatzins*

Incubating hoatzins differed strikingly in their flushing distances, depending on whether they inhabited an undisturbed territory or a site with tourism activity (Fig. 4; $t = -5.6$, $p < 0.0001$). Hoatzins at undisturbed nests left their clutches much earlier when approached by a human compared to their conspecifics at tourist-exposed nests.

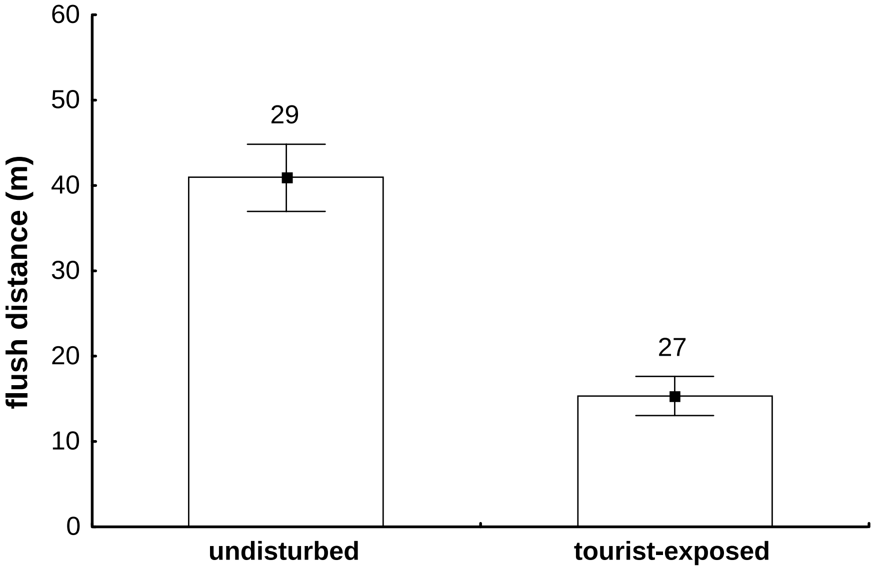


**Figure 4.** Average distances at which incubating hoatzins flushed from the nest in response to an approaching observer. Whiskers represent SE; sample size is indicated above.

**Table 3.** Number of tourist boats and visitors during the breeding season of the hoatzins as well as nest survival during the fledging period in the Cuyabeno Lakes from 1996 to 1998

| year | incubation period (May – June) | | fledging period (July – August) | | |
|---|---|---|---|---|---|
| | boats | visitors | boats | visitors | chick survival |
| 1996 | 80 | 538 | 207 | 1461 | 0.13 |
| 1997 | 53 | 310 | 147 | 938 | 0.46 |
| 1998 | 82 | 470 | 188 | 1298 | 0.19 |

Data are based on the visitor registration at the reserve entrance to the Cuyabeno Lakes by the reserve administration of INEFAN.

## Discussion

### *Hoatzin reproductive success*

Breeding success of hoatzins was higher at undisturbed nests than at tourist-exposed nests. This difference was solely due to an increased mortality of young birds prior to fledging whereas the success of incubation was similar in both groups. The effect fluctuated in its magnitude among years and seems to be associated with the number of visitors and boats during the fledging period (Table 3). We suggest that the direct influence of tourism activities are to blame for the observed higher mortality of hoatzin chicks in tourist-exposed areas and we suggest that the temporal overlap of the tourist high season in July and August with the hoatzin fledging period may be crucial for that impact. Diurnal predators were not more abundant at tourist-exposed territories and there is no evidence that differences in predation pressure could be responsible for the reduced chick survival of only tourist-exposed nests. The finding that nest survival during incubation was the same for all nest sites supports this conclusion.

Negative influences of human activities on development and survival of offspring are also reported for shore breeding and sea birds and have been linked to increased vigilance and reduced feeding rates (Safina and Burger, 1983; Flemming et al., 1988; Hatchwell, 1989). Other studies, however, showed effects of tourism activity on breeding success mainly during the incubation period (e.g. Anderson and Keith, 1980; Pierce and Simons, 1986; Keller, 1989). The fact that hoatzin hatching success was not affected by tourist-exposure might be explained by the apparent habituation of adult incubating hoatzins. The low tourist numbers in May and June may additionally aid in reducing an impact on incubation success.

The survival functions of chicks demonstrate that tourist chicks die more readily during the second part of the fledging period. In this period chicks have already left the nest and are climbing through the foliage. Although juveniles are guarded by their parents most of the time they are also found alone. Especially when the family group has been flushed away juveniles prior to fledging cannot follow and have to rely on their unusual escape behaviour. In fact, we have evidence that juveniles were disturbed more frequently at tourist-exposed sites compared to undisturbed sites: on several occasions we found them resting on another tree than their initial nesting tree. This change of location involves swimming which is only elicited when the individual feels severely threatened.

When only undisturbed sites are considered the overall survival for both the incubation and the fledging period ranged between 0.12 and 0.19 in all study years (survival for entire incubation and the entire fledging period, see Table 1). These values lie below values reported for other regions (22–27%, Ramo and Busto, 1984; Strahl and Schmitz, 1990; Domínguez-Bello et al., 1994). However, these authors calculated only simple percentages of successful nests instead of the more appropriate Mayfield estimates, which are known to be lower and avoid overestimating success due to nests found late in the season (Mayfield, 1961). In addition, previous studies were conducted in Gallery forests of the Llanos of Venezuela, while the Cuyabeno Lakes are embedded within a huge area of relatively pristine rainforest, where environmental factors including higher predation may contribute to a relatively low overall success.

### ***Hormonal response of chicks to experimental stress***

In response to a standardised stressor (capture) both hoatzin nestlings and juveniles showed the expected increase of the plasma corticosterone concentration (Wingfield et al., 1998), with initial and maximal values falling well within the range reported for other bird species (Romero et al., 2000). However, non-fledged juveniles from tourist-exposed nests responded much more strongly, showing higher maximum corticosterone values and a longer maintenance of the increased values. Thus, tourist-exposed juveniles demonstrated a higher sensitivity of their HPA axis to stressful events, which in vertebrates is known to be modulated based upon previous exposure to stressful stimuli like environmental stress or food conditions (Smith et al., 1994, Romero and Wikelski, 2001; Wikelski et al., 2001). Our results suggest that juvenile hoatzins experience tourist approaches as stressful and in consequence are sensitised by frequent visits. The hormonal response of nestlings, in contrast, was similar in individuals from undisturbed and from tourist-exposed nests indicating that tourism did not affect them measurably. These findings are highly consistent with the survival functions of the chicks, indicating that juveniles were in a particularly vulnerable life stage.

Fowler (1999) demonstrated that simple human presence at nest sites increased corticosterone concentrations in breeding Magellanic penguins (*Spheniscus* *magellanicus*) that are not accustomed to seeing humans, while birds exposed to high levels of human visitation did not respond to human presence as a stressor. Romero and Wikelski (2002) recently found similar results for Galapagos marine iguanas (*Amblyrhynchus cristatus*). However, penguins exposed to moderate levels of human disturbance did not show evidence of habituation over a period of several years and reacted with the same strong hormonal responses as entirely undisturbed birds (Fowler, 1999). In our study, tourist-exposed juveniles did not just react as strongly as undisturbed birds, but instead showed a much stronger corticosterone response. Such a sensitisation in the birds' response suggests that they had perceived the previous exposure to tourists as a stressful stimulus. Because the initial concentrations of plasma corticosterone were similar in juvenile hoatzins between undisturbed and tourist-exposed nest sites, tourism apparently did not result in chronically increased levels of corticosterone. However, it has to be considered that our juvenile sample is naturally biased towards surviving individuals who were able to cope with stress.

We could not detect a relationship between body weight and the hormonal stress response, either in nestlings or in juvenile hoatzins, which is consistent with data from other birds prior to fledging (Schwabl, 1999; Sims and Holberton, 2000). Thus, the strength of the hormonal reaction of the tourist-exposed juveniles cannot be explained by their lower body weights. On the contrary, we hypothesise that the lower body weights of tourist-exposed juveniles could most likely be a result of repeatedly elevated levels of corticosterone, which in turn are known to metabolise fat and protein reserves (Remage-Healey and Romero, 2001). Even if only one tourist boat per day elicits hormonal stress reactions, juveniles would accumulate a considerable number of such disturbance events in the weeks after they had left the nest. A reduced chick growth as a consequence of human presence could also be caused by reduced feeding rates by disturbed adults as documented for marsh harriers (*Circus aeruginosus*)(Fernández, 1993). This might apply to the hoatzins too, but the foraging bouts of the adults take place mostly in the morning and evening, avoiding the main visiting hours.

### *Habituation versus the maintenance of wariness*

Adult hoatzins incubating their clutches tolerated human presence at tourist-exposed nests to a much higher degree than their conspecifics in undisturbed territories. In contrast to their offspring, they apparently habituated towards human observers. This allowed them to continue incubation while at the same time preventing the exposure of nests to predators and explains why hatching success is indistinguishable between undisturbed and tourist-exposed nest sites. The suppression of flight reactions as a result of habituation to recreational activities is known from other bird species too (Keller, 1989; van Heezik and Seddon, 1990; Yorio and Boersma, 1992). However, for the hoatzins in the Cuyabeno Lakes it remains unclear whether the observed individuals had truly habituated to tourism activities, or whether tourism had instead driven away particularly sensitive individuals with intrinsically high flight distances (Gutzwiller et al., 1998; Fernandéz-Juricic, 2000; Rodgers and Schwikert, 2002).

The important question remains: Why do hoatzin chicks not adapt to “harmless” tourists? Regular handling, for example, habituated nestlings of night herons (*Nycticorax nycticorax*) (Parsons and Burger, 1982) and mallards (*Anas platyrhynchos*) (Heise, 1989). On the other hand, human activities near nest sites of gulls resulted in a stronger wariness of chicks (Burger, 1981). We expect the threshold for habituation to be much higher in non-flying hoatzin chicks than in adults because their predation risk is presumably much higher. A suppression of escape behaviour or a reduced wariness could have severe consequences when faced with natural predators, and may therefore be counter-selected. Because the primary rainforest at our study site possesses a full range of predators we doubt that this adaptation will take place. Recent studies clearly show that several constraints prevent some species in adapting to disturbances even if a plasticity in flight behaviour exists (Griffin et al., 2000; Blumstein et al., 2003).

Summarising, our data demonstrate that wildlife observation in the rainforest is not as harmless as often promulgated. Even a low number of ecotourists may have considerable negative effects on animals. Thus even ‘non-consumptive’ tourist activities should be carefully monitored and managed. Off-limit zones free of human disturbance must be an integral part for visitor management and represent an indispensable reference for how undisturbed conditions are. Birds in different life stages may be sensitive in different ways and conservation practices should consider the most vulnerable period. Area-specific guidelines for wildlife observation should be developed and promoted. Measuring hormonal responses to experimental stress has proved to be a rapid and cost effective correlate of survival probabilities. We suggest it as a useful tool to assess the stress-tolerance of birds under different human disturbances.

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# 3.3 Does ecotourism contribute to the protection of tropical biodiversity?

Antje Müllner

## Introduction

The 1992 UNCED conference in Rio de Janeiro was a turning point in international conservation strategy. The Convention of Biological Diversity signed in Rio declares sustainable use of biological diversity as one of its main objectives. This meant that the protection and use of biological diversity were no longer regarded as mutually exclusive.

Ecotourism is often called a paradigm case for a sustainable use of biological diversity because it can generate local income and replace more harmful uses of the environment. Large unutilised areas, which otherwise possess no lobby, become most valued by this tourism and can be effectively protected. Further, entry fees to the parks can be used to protect native species and to help finance the management of habitats.

In tropical countries in particular we still find large areas of pristine land with high biological diversity. Based on the potentially positive contributions of ecotourism to nature conservation, an often uncritical euphoria has occurred in these countries, which try to engage in any kind of ecotourism. Good intentions however, do not guarantee that tourist activities are environmentally benign. Experience in North America and Europe has taught us that uncontrolled ecotourism can damage fauna and flora. Sustainable tourism is only possible if it keeps its own attractions intact and is not harmful from a socio-economic and cultural point of view. Sustainable use of biological diversity is an important goal, but there is no consensus yet about how it can be achieved.

The aim of a multi-year study that I and several German and Ecuadorian colleagues conducted was to come closer to finding out how to achieve sustainability. The study took place in the Reserva Cuyabeno situated in the Amazon region in Ecuador and was sponsored by the Programa Forestal Sucumbíos (PROFORS) through the German Development Agency (GTZ) and by the Ecuadorian Department of Conservation.

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We investigated how tourism in the rainforest affects the fauna and which management measures must take place in order for a traditional form of ecotourism to be sustainable. The aims of our study were first to develop methods to detect the impact of tourism, and second to establish a concept for long term monitoring.

## Nature tourism in the Reserva Cuyabeno - risks and opportunities

The Cuyabeno Wildlife Reserve ("Reserva de Producción Faunística Cuyabeno") is situated in the north of the Amazonian lowlands of Ecuador. It contains an ecologically intact rainforest area with an impressive array of white and black water creeks and lakes. The 6000 $km^2$ conservation area, belonging to one of 10 centres of biodiversity world wide (Myers, 1988) is inhabited by extraordinarily diverse animal and plant communities. Well known for its animal diversity, the Reserva Cuyabeno is one of the best places in Ecuador to experience the rainforest and to observe monkeys, freshwater dolphins, anacondas and alligators or some of the over 500 bird species. After a boom in the 1990s, Cuyabeno attracts by now approximately 10,000 tourists per year. Excursions, which are only possible in guided groups, last three to five days, an important part of which are canoe trips and excursions through the rainforest.

Just as "ecotourists" in tropical countries usually travel a great distance to reach their destinations, over 90% of the visitors of Cuyabeno come from Europe or North America. On a global scale ecotourism can hardly be called ecological, as ecotourists come largely from overseas by plane. This damages the atmosphere directly and also contributes to the destruction of the rainforest where oil is produced. In a global 'eco-budget' ecotourism fares poorly for the rainforest. Strictly speaking, ecotourism ends at the airport of the tourist home country. However, the method by which tourism takes place on arrival in the Cuyabeno reserve can have a big local impact, and on a local and regional scale the "eco-budget" can look favourable.

Like many other conservation areas in Latin America the Reserva Cuyabeno is inhabited by indigenous people in co-operation with whom the park administration developed a management plan that designated areas for agricultural and traditional use, tourism, and strict conservation. Traditionally subsistence farmers, the people of Cuyabeno are now increasingly working in tourism related professions such as guides or cooks for travel organisations. Others rent out their boats or offer accommodation. For many inhabitants of Cuyabeno, tourism has become their main source of income, enabling them to refrain from other destructive uses of their environment such as hunting, or trapping and selling of parakeets. Many of the formerly hunted animals are also now presented as tourist attractions, the sighting of which can carry much weight in the success of a tourist's visit. The hunting pressure on large monkeys in the Cuyabeno Lakes ("Lagunas de Cuyabeno"), the most important tourism area in the reserve, has thus noticeably decreased. Whereas the price for the first outboard motor in the village of Puerto Bolívar at Rio Cuyabeno was once five ocelot furs, ecotourism now offers easier and more lucrative ways of earning money. Tourism can in this way contribute directly to the conservation of some species.

Ecotourism can also function to protect habitats as pristine natural areas, not regarded as particularly valuable within tropical countries, become appreciated for tourism. Conservationists and park administrators then have economical justifications to prevent destruction of these areas. The exploration activities of the oil industry, for example, are a permanent threat to the Reserva Cuyabeno, as they do not always respect the conservation area's borders. In 1993, however, conservationists and the tourism industry united to prevent oil drilling in the eastern part of the reserve. Tourism can help in the conservation of biological diversity and in process protection of ecosystems by making claims for large connected pristine areas.

Nature tourism also helps support park administrations which chronically suffer a lack of funding. In Ecuador the entry fees for the national parks represent an important source of income for the Department of Conservation and the Reserva Cuyabeno contributes, with a fee of $ 20 US per foreign tourist, a significant portion of the budget.

Even "eco"-tourism can, however, have a negative impact on the ecosystem and on particular species. Ecotourism could be the tip of a mass tourism iceberg, because the term ecotourism, admittedly vague, is often misused. Small numbers of tourists can do great damage to an ecosystem through the construction of roads and shelter as well as through the production of waste and sewage. Unsustainable use of the leaves of a palm tree (*Scheelia* spec.) as 'authentic' roofs for tourist accommodations, for example, has caused a dramatic decline of that species in certain areas of Cuyabeno. The increasing demand for canoes for the transport of tourists has led to the logging of the few remaining hardwood trees (mostly *Cedrelinga cateniformes*). Outboard motors have injured many dolphins and sea cows and led to a substantial increase in noise pollution. Despite these negative impacts, damages related to infrastructure can be detected relatively early and alternatives often found - at least theoretically –as long as regulating authorities exist. In Cuyabeno, for example, the EU-supported project PETRAMAZ investigates the construction of fibre glass boats for transport through the waters of the rainforest. For my own research I successfully used a solar powered motorboat.

More difficult to detect than these more obvious results of tourism, and in nature conservation almost always given minor relevance, are those direct impacts that tourist activities can have on wildlife. The maxim has been widely accepted that, as long as travellers collect and take out their own garbage, nothing is left behind except footprints. The tourist industry in Cuyabeno often emphasises that observation and photography of wildlife will not cause any problems. With arguments like these, the areas that are under complete protection are often undermined. There are, however, indications from areas in temperate regions that tourist activities like the ones described can indeed lead to changes in the behaviour of animals and have a negative impact on their feeding and reproductive success. During my investigation I found that disturbances by tourists led to a decline in breeding success in birds such as the Hoatzin.

## Case study Hoatzin

The bird Hoatzin (*Opisthocomus hoazin*), conspicuous for its size and colour, provides a good example for the study of tourist-animal interactions. Its breeding season lasts from May to August and its nesting habitats are areas along riverbanks and lakeshores. Canoe trips along the shores of the many rivers and lakes, the main activity to observe animals in Cuyabeno, can affect the behaviour and breeding success of the hoatzin. Reports from tour guides of a hoatzin population decrease led to a survey of nests and breeding success in 1996 and 1997. Tourist lakes were compared with sites closed to tourism and it was discovered that the flight distance of breeding hoatzins was lower in tourist areas than in undisturbed areas, indicating a habituation of adult birds to humans. It remains unclear whether the individuals became accustomed to tourists or whether after 10 years of tourism a selection process has taken place leaving the less sensitive animals in the area.

During the incubation period predation of eggs was 60% overall, with no difference detected between tourist lakes and undisturbed sites. The survival rate of chicks in the time between hatching and fledging at seven weeks of age, however, was lower in tourist areas than in undisturbed sites. The difference in survival rate was significant in 1996 and a trend could be confirmed for 1997. From their third day of age the young escape predators by jumping out of the nest into the water. They then climb up a tree within safe distance. Greater separation of three to six week old chicks from their nests was recorded in tourist lakes than in off-limit lakes, indicating that the animals are prompted to leave their nests more often when disturbed by boat traffic from tourism. Although the parents usually retrieve the young after their flight, the survival probability might be limited in the disturbed areas. In 1998 blood samples were taken from chicks in several lakes to measure the concentration of the stress hormone corticosterone, using the capture stress protocol (from Wingfield, 1995). It could be shown that chicks from tourist lakes responded to stress with a stronger increase and a higher maximum concentration of the hormone than chicks from prohibited areas. This permits the conclusion that birds in tourist areas suffer from stress inducing disturbances. A publication of the data on reproductive success and stress load is in press (Müllner et al., 2004, see chapter 3.2).

The results show that even non destructive tourist activities that are marketed with the label ecotourism can have a negative impact on wildlife. Tourism activities, particularly where demands of animals and tourism overlap, must be critically examined. Ecotourism should not be equated automatically with being beneficial for animals.

## Methods of channelling tourism

When the impact of tourism threatens the idea of conservation, park administrators and others who play a role in tourism must intervene. As tourism in the Cuyabeno Reserve plays a major role in generating revenue for the locals and in contributing to conservation, a reduction of visitors to lessen their impact is neither practical nor necessarily desirable. Nor is a “quality over quantity” - strategy feasible for Cuyabeno which focuses on fewer visitors while increasing fees and the quality of the tours. Too wide a spectrum of tourism activities with differing interests would make agreement on a changing distribution of revenue impossible. Options that influence the activities of tourists rather than their numbers must therefore be found. In the following I suggest measures that aim to reduce tourism related impacts on wildlife.

- Barring access to certain habitats is an important tool to ensure that plant and animals have freedom from human influence. Our results show that human presence alone can have consequences for the animals. Only strictly prohibited zones can create the refuges necessary here.

- By improving the infrastructure in tourist areas, i.e. building of hidden observation towers, the stress for animals can be reduced. A change in tourist behaviour can also provide relief. Interviews with visitors show that tourists conditioned in their homelands, especially those from Europe and North America, are inclined to and very prepared to behave considerately. Often, it is simply lack of knowledge that leads to behavior that can have dramatic negative effects for some animals, for example, the Hoatzin. To take advantage of these inclinations and provide information and tips for a sustainable visit, we developed a brochure entitled “A visit to the wildlife in the Cuyabeno Reserve” (Pfrommer et al., 2000) which each visitor, when admitted to the park, receives with their entrance ticket.

- Appropriate communication means and participation are necessary in order to integrate all tourism stakeholders into the visitor management. This is the only way to achieve acceptance and to implement proposed guidelines.

- The tourist guide plays a crucial role for the careful and ecological performance of a tour. His regular training is therefore a must. In the Cuyabeno Reserve tourist guides have to apply for a licence from the park administration. However, there is no need to regularly attend refresher courses in order to keep the licence  and in particular there is a lack of guidance regarding tourist – wildlife interactions and a lack of knowledge about the current status of animal populations. A long-term objective should be the establishment of a behavioural codex among the tourist guides.

- In the Cuyabeno Reserve, similar to many other protected areas in the tropics, capacities for law enforcement are not developed sufficiently and their strengthening is often not wished on a political level. Therefore it is important to seek consensus among the tourism stakeholders. Round-table meetings are an effective way of enhancing the communication between the stakeholders. In the Cuyabeno Reserve several workshops were held that were aimed at improving tourism management. All participants highly appreciated these meetings which revealed a definite lack of information flow between the stakeholders. Only small improvements have been achieved towards a prudent tour programme. However, even small changes will have a positive impact in the long run and may be the first steps towards a general change in attitudes.

- Proper management is only possible when the impacts of human disturbances on fauna and flora are scientifically investigated and clarified. This is particularly true for tropical rainforests where the knowledge gap is huge and where at the same time the ecological relationships are known to be very complex. Only the principle of precaution is acceptable in such cases.

- The ecological and economical sustainability of nature tourism can only be secured through monitoring animal and plant populations as well as tourism activities. Therefore, the development and implementation of a monitoring programme should be of high priority. The major aim of our study was to test and modify existing methods in order to make them suitable for use in a future monitoring programme and we have successfully elaborated a monitoring concept.

## Conclusions

“Eco”-tourism can also have negative impacts on rainforest wildlife and therefore may destroy its fundamentals. These impacts have to be investigated carefully. Even for a small number of visitors professional planning and implementation of tourism as well as public communication is necessary if this form of tourism is to be ecologically sustainable. Management measures have to be adapted in order to meet local needs and opportunities. In contrast to the widely made presumption, “eco-tourism” is not just simple tourism into nature which does not need to be regulated. In fact, intensive guidance and control is absolutely necessary because wildlife is the target of ecotourism activities. An indispensable management instrument is the regular and long-term monitoring of both the status of wildlife populations and the visitor activities. Only such monitoring together with the commitment to change present practice if necessary guarantees the sustainable use of biological diversity through ecotourism.

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# 4. Summary

The hoatzin (*Opisthocomus hoazin*) is an enigmatic bird that lives in the riparian lowlands of northern South America. Among its peculiar attributes are 1) microbial foregut fermentation, unique in birds, to convert plant cellulose in the foliage which it consumes into simple sugars, 2) an ongoing debate about the puzzling taxonomic position, although a relationship to the Cuculiformes appears likely, 3) adaptive wing claws in the young which are used for climbing, and 4) co-operative breeding behaviour. Despite the information available on digestive mode and taxonomy little has been published on its breeding biology and behaviour and until now almost all knowledge was based on a study in the savannah of Venezuela.

This is the first detailed study of the hoatzin’s nesting ecology in a rainforest habitat. From 1995-1998 and in 2000 I monitored a hoatzin population which consisted of approximately 700 individuals in an Amazonian rainforest in Ecuador situated in the Cuyabeno Wildlife Reserve (between 0°02’ N, 76°0’ W, 0°03’ S, and 76°14’ W). The area is composed of various black water lagoons and small rivers, flooded forests and *terra firme* forest. Primarily, I examined group composition and breeding pattern and success related to traits such as clutch and egg size, offspring sex ratio and the number of parents involved in a common breeding attempt. Apart from standardised observations and monitoring I took blood samples from chicks, which were later used for molecular sexing and for DNA fingerprints. Food plants were collected and determined and a rough habitat mapping was conducted. Since the impacts of boat tourism in the area became apparent I investigated the interactions of adult and young hoatzins with tourists and measured the plasma concentration of the hormone corticosterone in chicks as an indicator of stress.

The main findings of this study are:

- Despite living directly at the equator in a tropical environment the reproduction of the hoatzin was timed strictly following the bimodal rainy pattern in the area. The mean clutch size was two eggs with a range of one to seven eggs. The laying interval was one to two days and incubation started with the first egg laid; mean

incubation time was 32 days. Hatching and fledging success averaged 35% and 50%, respectively, on a per-nest basis at nest sites undisturbed by tourism, leading to an overall success of 18%. The major cause of nesting failure was predation, with birds and snakes being the most conspicuous predators. Although 86% of the clutches contained more than a single egg, one fledged young was the most common result of a successful hoatzin breeding attempt (75%). Two fledglings were only found in 24% of successful broods and in all of the study years we only once observed a breeding unit with three fledglings. Normally one breeding attempt per year was performed; renesting was rare (6-10% of all attempts in different years). Although it is very likely that the pheasant-sized hoatzin represents an isolated taxonomic lineage it exhibits a typical life-history pattern that is known from tropical passerines (Passeriformes). This indicates the role of effective and general environmental selection pressures in shaping these traits.

- Eggs hatched within an interval of 1-2 days and in the order in which they had been laid. This hatching asynchrony produced size hierarchies between the nestlings which sometimes remained until fledging. In broods with one fledgling it was almost always the A-chick (first-laid) that had survived; whereas in broods with two fledglings A- and B-chicks (first- and second-laid) were present. Consequently, fledged broods were composed of 75% A-chicks and only 25% B-chicks. The high loss of chicks according to their hatching rank suggests a brood reduction strategy of the female via hatching asynchrony. This conclusion is further supported by the observed *intra*-clutch egg-size variation which revealed a decrease in egg size with laying order and thus accentuated nestling size hierarchies. However, growth rates of A- and B-chicks did not differ and therefore it is unlikely that starvation accounts for the differential mortality. The proximate causes of the higher mortality of B-chicks remain unclear, but physiological or resource constraints of the female might play an important role. The *inter*-clutch egg-size variation supports this possibility and provides evidence for a trade-off between clutch and egg size. Considering the high predation during incubation, hatching asynchrony may have to be viewed as a non-adaptive and costly consequence of an enemy-induced incubation pattern.

- Approximately 80% of breeding attempts were carried out with auxiliaries; the most common group size was a trio (40%). Units with alloparentals had a higher percentage of surviving broods during both the incubation and the fledging period, but they did not produce more fledglings per successful nest than unaided pairs. The effect of helping was likely to be due to an increased predator detection and deterrence rather than to better food provision. Breeding success did not increase further in groups that consisted of more than three individuals. This indicates a neutral or even negative effect of additional group members, probably induced by more frequent and intense social interactions in larger groups. We therefore suggest a trade-off between helping and group size. Our results differ from the findings in Venezuela and demonstrate a variation in the social organisation of the hoatzin with respect to habitat and latitude.

- The offspring sex ratio was female-biased (59%) and there was no differential mortality between the sexes until fledging. Despite this population-wide bias singly breeding pairs only produced sons (potential helpers) from first-laid eggs. In contrast, pairs with helpers produced more daughters (potential dispersers) than would be expected by chance, and this percentage increased with increasing family size. This suggests that females adaptively manipulate the sex of their offspring in order to maximise individual fitness according to their current family size.

- DNA band-sharing comparisons revealed that nestmates from two-egg clutches were all full siblings, as were most nestlings from three-egg clutches. In contrast, chicks from clutches with more than three eggs were never all full siblings, but a mix of full siblings and half siblings or aliens, indicating up to three different parents. Three eggs appear the maximum number laid by a single female, and therefore bigger clutches must have been the result of jointly-nesting females. This result is supported by the observation that clutches with 4-7 eggs always came from breeding units with at least four adults. At our study site joint-nesting occurred in 16% of breeding attempts. The clutches of these joint-nests suffered severe egg loss during all stages of incubation, which is most likely explained by egg-tossing by other group members. Breeding success on a per-nest basis did not differ between single- and joint-nests. However, the fledgling to egg ratio was much lower in joint-female nests than in single-female nests with 0.3 fledglings per egg and 0.6 fledglings per egg, respectively. Likewise, when success was calculated for the individual female it was lower in joint-nests than in single nests. This disadvantage of jointly-nesting females is expected to be compensated for by other life-time benefits.

- In some parts of the study area hoatzins were confronted with rainforest tourism. The boat excursion along the nesting sites caused different responses in adult and young hoatzins: Whereas adults appear to have habituated to tourist boats and exposure to observers (mean flight distances of ca. 50m at undisturbed sites *vs*. 25m at tourist sites), hoatzin chicks at tourist sites had lower body weights, much stronger hormonal stress responses, and a lower survival (20%) than chicks from nests at off-limit sites (50%). Although rainforest tourism may contribute to the maintenance of biodiversity if managed properly, I demonstrated that it may also have both direct and indirect negative impacts on wildlife. Since wildlife is the main target of eco-tourists, rainforest tourism may also jeopardise its principal attraction and hence its economic base. Tourism and its potential impacts have to be monitored and it is necessary to develop and implement appropriate methods with which this can be done.

My investigations provide urgently needed data on the hoatzin in a rainforest habitat and additionally contribute to the knowledge of non-passerine tropical life-histories. Furthermore, my work provides more specific details of co-operative breeding, of clutch- and egg size variation, of hatching asynchrony and brood reduction hypotheses, of adaptive manipulation of sex ratio, and of the interactions between tourists and wildlife. However, several aspects are still worth further investigation in this species: We need more quantitative data from other sites in order to allow deeper insights into the revealed geographical variation in breeding pattern and social organisation. Secondly, further investigations of offspring sex ratios both on the population level as well as on the individual level should examine the evidence for adaptive manipulations. Finally, the clarification of the differential mortality of A- and B-chicks on the proximate level will be an interesting topic in the context of adaptive brood size reduction.

# Zusammenfassung

Der Hoatzin (*Opisthocomus hoazin*), im Deutschen auch Zigeunerhuhn genannt, ist ein ungewöhnlicher Vogel, der an den Ufern von Flüssen und Seen im Tiefland von Südamerika lebt. Zu seinen besonderen Eigenschaften gehören 1) seine für Vögel einmalige Vormagen-Verdauung, die sonst nur bei Wiederkäuern vorkommt, und die mit Hilfe von Mikroben die Zellulose aus seiner Pflanzennahrung in einfache Zucker abbaut, 2) seine noch immer unklare, heftig diskutierte Stellung in der Vogelsystematik, obwohl eine Beziehung zu den Kuckucken wahrscheinlich ist, 3) Flügelkrallen zum Klettern bei den Jungtieren und 4) sein kooperatives Brutverhalten mit „Helfern-am-Nest". Während über sein spezielles Verdauungssystem und seine rätselhaften Verwandtschaftsbeziehungen mehrere und zum Teil ausführliche Arbeiten vorliegen, war bisher nur sehr wenig über seine Brutbiologie und sein soziales Verhalten bekannt. Das gesamte Wissen darüber beruhte fast ausschließlich auf einer einzigen Studie aus der Feuchtsavanne von Venezuela.

Diese Arbeit stellt die erste detaillierte Studie über die Brutökologie des Hoatzins in einem Regenwald-Habitat dar. Von 1995-1998 und im Jahr 2000 untersuchte ich eine Population mit ca. 700 Hoatzin-Individuen im Cuyabeno Reservat im amazonischen Regenwald Ecuadors (0°02' N, 76°0' W, 0°03' S, and 76°14' W). Das Gebiet besteht aus verschiedenen Schwarzwasserseen und kleinen Flüssen, großen Überschwemmungszonen und *terra firme*-Wald. Kern meiner Arbeiten war die Bestimmung von Gruppenzusammensetzung und Bruterfolg im Zusammenhang mit dem Brutaufwand. Besonders behandelt wurden dabei die Themen kooperatives Brüten, Variation von Gelege- und Eigrößen, Schlupfasynchronie und Brut-Reduktions-Hypothesen. Neben standardisierten Nestkontrollen und Beobachtungen nahm ich Blutproben von Küken, die später für eine molekulare Geschlechtsbestimmung und für DNS-Fingerabdrücke verwendet wurden. Ich sammelte und bestimmte Futterpflanzen der Hoatzins und kartierte das Habitat. Da in Teilen des Gebietes Bootstourismus stattfand und es Hinweise auf dessen negative Einflüsse gab, untersuchte ich zusätzlich die Interaktionen zwischen Touristen und Hoatzins. Hierzu beobachtete ich Fluchtreaktionen und führte an Jungtieren Messungen der Plasmakonzentration des Stresshormons Corticosteron durch.

Die wesentlichen Ergebnisse meiner Arbeit sind:

- Obwohl Hoatzins direkt am Äquator in einer tropischen Umwelt leben, war ihre Fortpflanzung zeitlich strikt an das bimodale Muster der jährlichen Regenfälle gebunden. Die mittlere Gelegegröße betrug 2 Eier, bei einer Spanne von 1-7 Eiern. Das Intervall zwischen den Eiablagen betrug 1-2 Tage, und die Bebrütung startete mit dem ersten Ei. Die Brutdauer betrug im Mittel 32 Tage. In ungestörten, nicht touristisch genutzten Nistgebieten schlüpften im Mittel in 35 % der Nester Küken, und in 50 % dieser Nester wurden Jungvögel flügge. Damit waren insgesamt 18 % der Nester bei der Brut erfolgreich. Der Hauptgrund für einen Brutverlust war Prädation, mit Vögeln und Schlangen als vorherrschende Nesträuber. Obwohl 86 % der Gelege mehr als ein Ei aufwiesen, war ein einziges flügges Jungtier das häufigste Ergebnis eines erfolgreichen Brutversuchs (75 %). Nur in 24 % der erfolgreichen Bruten wurden zwei Jungtiere flügge, und sogar nur einmal in allen Untersuchungsjahren wurden drei Jungtiere beobachtet. Ein Brutversuch pro Jahr war die Regel; Zweitbruten machten in den verschiedenen Jahren nur 6-10 % aller beobachteten Brutversuche aus. Obwohl der fasanengroße Hoatzin sehr wahrscheinlich eine isolierte taxonomische Linie darstellt, zeigt auch er das typische Muster der Lebensgeschichte eines tropischen Sperlingsvogels (Passeriformes). Dies deutet darauf hin, dass generelle und wirksame umweltbedingte Selektionsdrücke diese Merkmale formen.

- Die Küken schlüpften in einem Intervall von 1-2 Tagen in der Reihenfolge der Eiablage. Diese Schlupfasynchronie schuf eine Größenhierarchie unter den Nestlingen, die teilweise bis zum Flüggewerden aufrechterhalten blieb. In Bruten mit einem flüggen Jungvogel war es fast immer das A-Küken, das überlebt hatte, während in Bruten mit zwei Flüggen neben dem A-Küken immer das B-Küken durchkam. Alle flüggen Jungvögel zusammen betrachtet bestanden daher zu 75 % aus A-Küken und nur zu 25 % aus B-Küken. Der hohe Verlust an Nestlingen in Abhängigkeit vom Schlupfrang deutet darauf hin, dass die Weibchen eine Brutreduktionsstrategie verfolgen und sich dabei des asynchronen Schlupfmusters bedienen. Diese Interpretation wird dadurch unterstützt, dass *innerhalb* der Gelege die Eigröße mit der Legefolge abnahm und damit die Größenhierarchie der Nestlinge noch verstärkt wurde. Allerdings unterschieden sich die Wachstumsraten der A-

Küken nicht von denen der B-Küken, und daher scheint ihre unterschiedliche Mortalität nicht durch Nahrungsmangel verursacht worden zu sein. Die proximaten Gründe für die erhöhte Mortalität der B-Küken bleiben unklar; es könnten auch physiologische Begrenzungen oder Ressourcenengpässe bei den Weibchen dabei eine Rolle spielen. Unterstützt wird die letztere Möglichkeit durch die Variation der Eigrößen *zwischen* den Gelegen unterschiedlicher Größe, die auf ein Abwägen des Weibchens zwischen Gelege- und Eigröße hinweist. Da während der Inkubation ein starker Räuberdruck herrscht, dient der Brutbeginn mit dem ersten gelegten Ei auch dem Schutz des Geleges. Die Schlupfasynchronie kann daher auch als eine kostspielige und nicht-adaptive Konsequenz eines räuberinduzierten Brutmusters interpretiert werden, die sich aus der frühen Bebrütung ergibt.

- Ungefähr 80 % der Brutversuche wurden mit Helfern ausgeführt, wobei die häufigste Gruppengröße ein Trio war (40 %). Brutgruppen mit Helfern hatten einen höheren Prozentsatz an erfolgreichen Nestern, sowohl in der Inkubationsphase als auch in der Aufzuchtphase der Nestlinge. Sie brachten jedoch pro erfolgreiches Nest nicht mehr flügge Jungvögel hervor als Paare ohne Helfer. Der Effekt des Helfens lag wahrscheinlich eher im früheren und häufigeren Entdecken von Räubern als in einer besseren Versorgung der Küken mit Futter. Bei Brutgruppen mit mehr als drei Individuen stieg der Bruterfolg nicht weiter an. Dies weist auf einen neutralen oder auch negativen Einfluss von zusätzlichen Gruppenmitgliedern auf den Bruterfolg hin, sehr wahrscheinlich hervorgerufen durch die häufigeren und intensiveren sozialen Interaktionen bei größeren Gruppen. Dieses Ergebnis lässt vermuten, dass die Eltern zwischen *mehr Helfern* und *zuviel Gruppenmitgliedern* abwägen müssen. Unsere Ergebnisse unterscheiden sich von Beobachtungen aus Venezuela und belegen eine Variabilität in der sozialen Organisation der Hoatzins je nach Habitat und geografischer Breite.

- Das Geschlechterverhältnis der Nachkommen war zugunsten von weiblichen Tieren (59 %) verschoben. Es gab keine Unterschiede in der Sterblichkeit zwischen den Geschlechtern bis zum Flüggewerden. Trotz dieses populationsweiten Trends zu Töchtern produzierten Brutpaare ohne Helfer mit ihrem erstgelegten Ei ausschließlich Söhne (potentielle Helfer am Nest). Im Gegensatz dazu produzierten Paare mit Helfern mehr Töchter (potentielle Auswanderinnen) als erwartet. Dieses

Ergebnis weist darauf hin, dass weibliche Hoatzins adaptiv das Geschlecht ihrer Nachkommen manipulieren, um damit ihre individuelle Fitness entsprechend der Familiengröße zu maximieren.

- Vergleiche der Bandmuster von DNS-Fingerabdrücken zeigten, dass Nestgenossen aus 2-Ei-Gelegen immer Vollgeschwister waren. Das galt auch für die meisten Küken in 3-Ei-Gelegen. Dagegen waren Küken in Gelegen mit mehr als 3 Eiern nie alle Vollgeschwister, sondern eine Mischung aus Vollgeschwistern und Halbgeschwistern oder unverwandten Tieren, mit bis zu drei verschiedenen Müttern. Drei scheint die maximale Anzahl an Eiern zu sein, die ein einzelnes Weibchen legt. Größere Gelege müssen das Ergebnis von gemeinschaftlich nistenden Weibchen sein. Dies wird durch die Beobachtung bestätigt, dass Gelege mit 4-7 Eiern immer nur in Brutgruppen mit mindestens vier adulten Hoatzins vorkamen. Diese gemeinschaftlichen Nester machten in meinem Untersuchungsgebiet 16 % der Brutversuche aus. Ihre Gelege zeigten auffällige Verluste an Eiern in allen Stadien des Brütens, höchstwahrscheinlich entfernten andere Gruppenmitglieder die Eier. Der Prozentsatz erfolgreicher Nester unterschied sich nicht zwischen Einzel- und Gemeinschafts-Nestern. Allerdings war das Verhältnis von Flüggen zu Eiern bei gemeinschaftlichen Nestern deutlich geringer als bei Nestern von einzelnen Weibchen (0,3 gegenüber 0,6 Jungvögeln pro Ei). Wenn die Anzahl an Flüggen pro Weibchen berechnet wurde, war der Bruterfolg von gemeinschaftlichen Weibchen noch ungünstiger im Vergleich zu einzeln brütenden Individuen. Wir erwarten, dass dieser Nachteil für gemeinschaftlich brütende Weibchen durch andere längerfristige Vorteile in der Lebensgeschichte aufgewogen wird.

- Hoatzins sind in Teilen des Untersuchungsgebietes mit Regenwald-Tourismus konfrontiert. Die Bootsexkursionen entlang der Brutgebiete riefen bei adulten und jungen Hoatzins unterschiedliche Antworten hervor: Adulte Tiere schienen sich an Touristenboote und an die Anwesenheit von Beobachtern weitgehend gewöhnt zu haben; ihre Fluchtdistanz war ca. 50 m in ungestörten Gebieten und 25 m in Tourismusgebieten. Hoatzin-Küken dagegen hatten in Tourismusgebieten ein geringeres Körpergewicht und eine wesentlich stärkere hormonelle Stressantwort als Küken aus touristenfreien Zonen. Dies war wahrscheinlich auch der Grund für ihre geringere Überlebenswahrscheinlichkeit. Nur 20 % der Küken überlebten in

Tourismusgebieten im Vergleich zu 50 % der Küken aus Nestern in den für Tourismus gesperrten Gebieten.

Tourismus kann zum Naturschutz beitragen, wenn er angemessen organisiert und überwacht wird. Meine Studie zeigt jedoch, dass Tourismus auch direkte und indirekte negative Auswirkungen auf Tiere haben kann. Da Tierbeobachtungen ein Hauptziel von Ökotouristen in Regenwäldern sind, kann Tourismus diese wichtige Attraktion folglich auch gefährden. Er untergräbt sich damit seine eigene ökonomische Grundlage. Tourismus und seine potentiellen Auswirkungen müssen daher überwacht werden.

Meine Untersuchungen liefern seit langem fehlende Daten aus einem Regenwaldhabitat über den berühmten, aber weitgehend unerforschten Hoatzin und tragen außerdem zum Wissen über die Lebensgeschichten von tropischen Nicht-Sperlingsvögeln bei. Es gibt jedoch einige Aspekte zur Biologie des Hoatzins, die es wert wären, weiter untersucht zu werden: Erstens benötigen wir mehr quantitative Daten von anderen Orten, welche die hier aufgezeigte geografische Variation des Brutaufwandes und der sozialen Organisation ergänzen. Zweitens sollten weitere Untersuchungen über das Geschlechterverhältnis der Nachkommen sowohl auf der Populationsebene als auch auf der Ebene des Individuums vorgenommen werden, um die von mir entdeckten Anzeichen für eine adaptive Manipulation des Geschlechts zu überprüfen. Im Kontext der adaptiven Brutgrößenreduktion wäre es schließlich äußerst interessant, die proximaten Ursachen für die unterschiedliche Sterblichkeit von A- und B-Küken aufzuklären.

# Appendices

## Appendix 1: Hoatzins and their habitat in Cuyabeno

(all pictures by the author)

**Figure A:** The Laguna Grande is the largest of 13 lakes that form the Cuyabeno Lake System located in the Cuyabeno Wildlife Reserve. Several camp sites and lodges are situated around the lake.

**Figure B:** Hoatzins live in the flooded forest along the watercourses. These birds attract scientists and tourists due to their considerable size, appearance and peculiar life style.

**Figure C:** All observations and data collections were carried out with a dugout canoe that allowed movement through the flooded forest without disturbance. The flooded forest of the Cuyabeno Lakes is of low diversity of plant species and is dominated by the characteristic Macrolobium trees (*Macrolobium acaciifolium),* the leaves of which form part of the hoatzin's diet.

**Figure D:** Hoatzins feed on leaves, buds and flowers from some of the epiphytic orchids. Hoatzin food seems superabundant throughout the year. However, the feeding of the young in July and August coincides temporally with the growth of the preferred young leaves.

**Figure E**. Hoatzins possess a special callus on their sternum that helps to support the large crop on a branch while resting and digesting.

**Figure F**: Hoatzins breed in family groups and often roost together.

## Appendix 2: Breeding hoatzins and their chicks

(all pictures by the author)

**Figure G**: A hoatzin incubating in a Huito shrub (*Genipa americana*, Rubiaceae), one of the most common plants of the flooded area.

**Figure H:** The most common size of a hoatzin clutch is two eggs. Because incubation always starts with the first egg, chicks hatch asynchronously, one to two days apart.

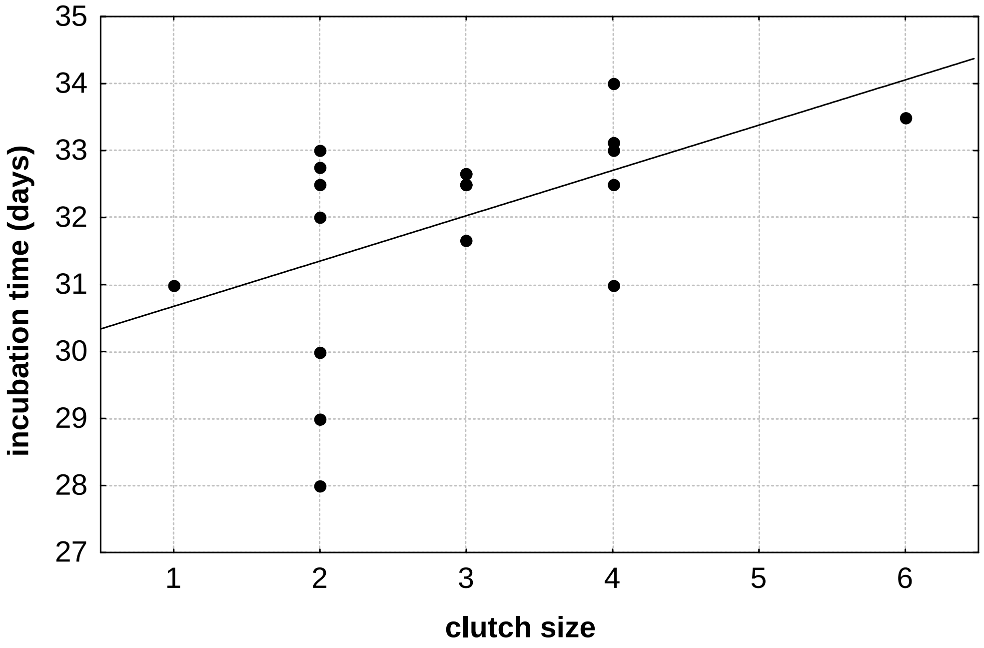
**Figure I:** Two hoatzin chicks resting on a branch of a Macrolobium tree (*Macrolobium acaciifolium*). When not disturbed the chicks leave the nest at the age of 14-20 days, climbing through the nest tree and picking for some leaves.

**Figure K:** When threatened, hoatzin chicks leave the nest and jump into the water – as early as an age of 4-6 days!

**Figure L:** They dive away and, if not eaten by a Piraña or another predator, they climb another tree in an inconspicuous manner. They do not come back to the nest. Normally the adults find and take care of them at the new site.

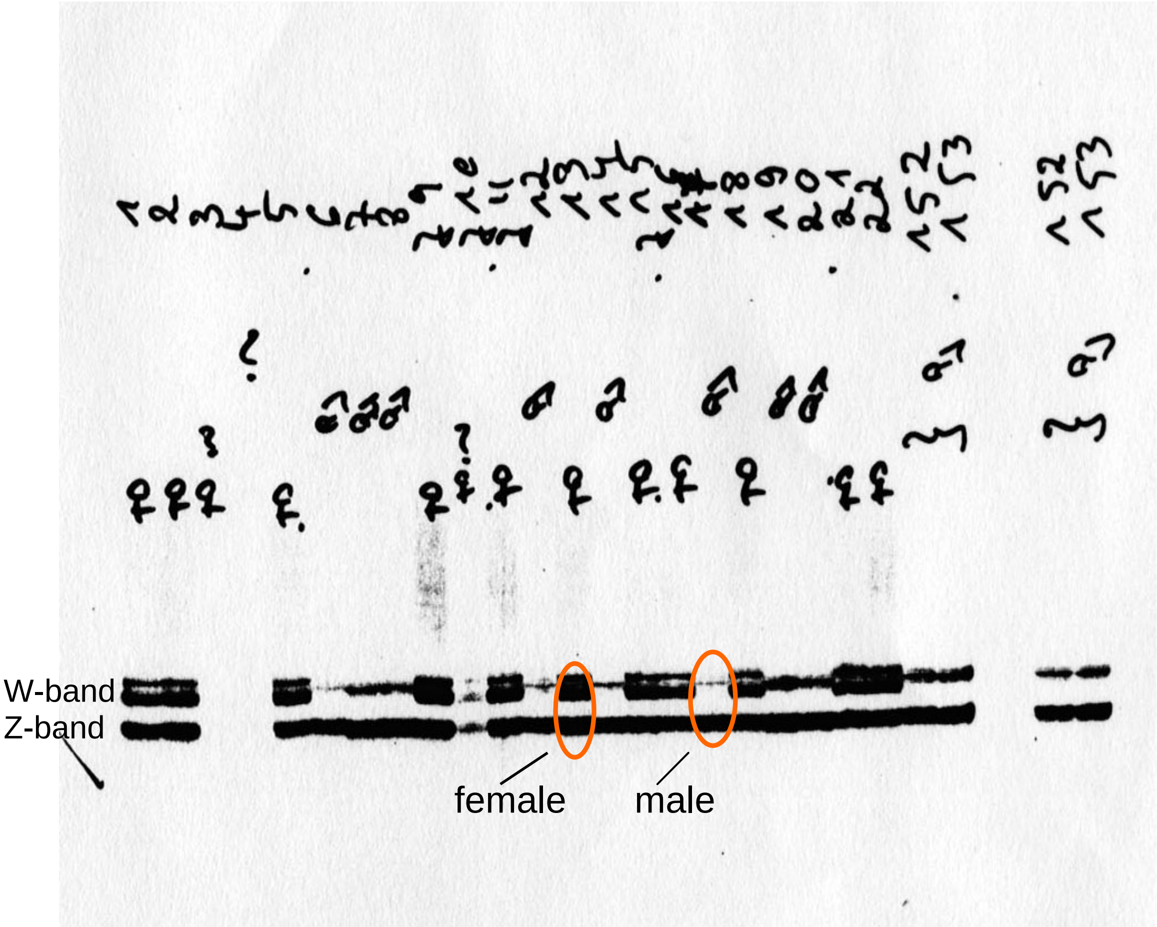
**Figure J:** Hoatzin chicks possess wing claws which help them to enter a tree after an escape into the water and to climb in the vegetation. Normally the claws are lost after fledging.

# Appendix 3



Mean clutch incubation time with respect to the number of eggs in the clutch.

# Appendix 4



PCR was performed with sex-specific primers and PCR products were separated on high resolution polyacrylamide gels and visualised autoradiographically.

**DNA sex identification in birds:** "The two PCR primers anneal to conserved exonic regions but then amplify across an intron in both CHD-W and CHD-Z. Because these introns are non-coding they are less conserved and their lengths usually differ between the genes. As a result, the PCR products vary in size. Therefore, gel electrophoresis immediately reveals a single CHD-Z band in males (ZZ), but a second, distinctive CHD-W band in females (WZ)", after Griffiths et al. (1998)*.

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* Griffiths, R., Double, M. C., Orr, K., Dawson, J. G. R., 1998. A DNA test to sex most birds. Molecular Ecology 7, 1071-1075.

## **Erklärungen**

Hiermit erkläre ich ehrenwörtlich, dass ich die vorliegende Arbeit selbständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Ich habe diese Dissertation weder in gleicher noch in ähnlicher Weise in einem anderen Prüfungsverfahren vorgelegt.

Ich erkläre ferner, dass ich bisher noch keinen weiteren akademischen Grad erworben oder zu erwerben versucht habe.

Würzburg, den 28. November 2004

Antje Müllner

# Lebenslauf

Antje Müllner
geboren am 13. April 1964 in Frankfurt/M
deutsche Staatsangehörigkeit
ledig

## Schule und Studium

| | |
|---|---|
| 1970 – 1983 | Grundschule und Gymnasium in Frankfurt/M., Abitur mit Note „1,5" |
| 1983 – 1987 | Universität Frankfurt/M, Grundstudium Diplom-Biologie, Vordiplom mit „sehr gut" (12/85), vertiefende Praktika in Zoologie und Biochemie |
| 1988 – 1991 | Universität Hamburg, Hauptstudium mit Spezialisierung in Verhaltensbiologie, Ökologie und Naturschutz, private Studienreise an die Ulu Gombak Field Station, University of Malaya, Malaysia (4-5/89), Tutorin für Kurse aus Grund- und Hauptstudium<br>Diplom mit „sehr gut" (3/91), Hauptfach Zoologie, Nebenfächer Botanik und Hydrobiologie/Fischereiwissenschaften, Freilandökologische Diplomarbeit „Zur Biologie von *Triturus cristatus* und *Triturus vulgaris* unter besonderer Berücksichtigung des Wanderverhaltens" |

## Studienbegleitende berufliche Tätigkeiten

| | |
|---|---|
| 1983 – 1988 | Pflanzenschutzforschung, Tierpathologie und Patentrechtsabteilung der Hoechst AG in Frankfurt/M., Studentische Assistentin (83, 86, 87, 88; insgesamt 10 Monate) |
| 1986 – 1988 | Naturmuseum Senckenberg in Frankfurt/M, Freie Mitarbeiterin im Museumspädagogischen Dienst, Führungen und umweltbildende Sonderveranstaltungen für alle Alters- und Ausbildungsstufen |
| 1990 | Naturschutzamt Hannover, Gutachten zur Entwicklung von Bewertungskriterien für Amphibienpopulationen |

## Weiterbildung und beruflicher Werdegang

| | |
|---|---|
| 1991 | Herpetologische Forschungsreise nach Malaysia, Aufenthalte an den Stationen „Integrated Conservation Research", Sabah Parks, Borneo und Ulu Gombak, University of Malaya, Westmalaysia (5-7/91)<br>Vorträge und Publikation über Amphibienschutz, Fernkolleg Humanökologie, Intensivkurse Französisch (Fremdspracheninstitut Hamburg) und Englisch (London) |
| 1992 – 1994 | Comoé-Nationalpark, Elfenbeinküste, Forschungsarbeiten im „projet biodiversité" der Universität Würzburg über Reproduktionsstrategien von Fröschen der tropischen Savanne, Gewässeruntersuchungen und Einsatz von Radiotelemetrie |

| | |
|---|---|
| 1994 – 1998 | Forst- und Naturschutzprojekt PROFORS (INEFAN/GTZ) in Ecuador, lokale Fachkraft zur Planung und Durchführung des Projektes „Entwicklung eines Monitorings des Ökotourismus zur nachhaltigen Sicherung der Reserva Cuyabeno” |
| | Forschungsarbeiten an Wirbeltieren unter Einsatz vielfältiger ökologischer Arbeitsmethoden und Einspeisung der Daten in GIS, Zusammenarbeit mit der Pontificia Universidad Católica del Ecuador und Betreuung nationaler Studenten; Beratung der Parkverwaltung im Tourismusmanagement und Durchführung von Workshops zur Tourismuslenkung; Präsentation der Ergebnisse auf internationalen Tagungen in Südamerika |
| 1999 - 2002 | Universität Würzburg, Dissertation über Brutaufwand und Sozialsystem des Vogels Hoatzin am Lehrstuhl für Tierökologie und Tropenbiologie, Publikationstätigkeit und Betreuung von tierökologischen Kursen |
| | Naturmuseum Senckenberg in Frankfurt/M, Planung und Durchführung umweltbildender Veranstaltungen |
| | Ressourcenschutzprojekt Gran Sumaco (INEFAN/GTZ) (4-9/2000) in Ecuador, Gutachtertätigkeit für die Entwicklung eines ökologischen Lernpfades und Förderung der Umweltbildung in der Pufferzone des Sumaco Parks; Konzept, Erstellung und Drucklegung einer Broschüre zur Tourismuslenkung in der Reserva Cuyabeno |
| seit 2002 | Zoologische Gesellschaft Frankfurt, Leiterin des Referats Lateinamerika & Südostasien, Initiierung, Betreuung und Begutachtung von Naturschutzvorhaben |
| **Sprachen:** | Spanisch und Englisch fließend in Wort und Schrift, Französisch gut |
| **Stipendien:** | Deutscher Akademischer Austauschdienst (1992-1994), Stifterverband der Deutschen Wissenschaften (1996), A.W. Schimper Stiftung (1998), Promotionsstipendium der Universität Würzburg (1999), Elfriede-Steinbacher-Stiftung (2000) |

Mitglied in der Senckenbergischen Naturforschenden Gesellschaft (seit 1985), im Bund Umwelt und Naturschutz Deutschland BUND (seit 1989), in der Deutschen Gesellschaft für Tropenökologie (seit 1992) und in der Deutschen Gesellschaft für Ornithologie (seit 1999).

Frankfurt, den 28. November 2004