Cardiorespiratory responses upon increased metabolism in the Ornate Tinamou, *Nothoprocta ornata*

Isabella Gasparini
LiTH-IFM-A-Ex--12/2647--SE

Supervisor: Jordi Altimiras, Linköping University
Examiner: Mattias Laska, Linköping University
Cardiorespiratory responses upon increased metabolism in the Ornate Tinamou, *Nothoprocta ornata*

**Författare**
Author: Isabella Gasparini

**Sammanfattning**
Abstract

The Bolivian Ornate Tinamou, *Nothoprocta ornata*, lives higher than 3300 m above sea level and must constantly deal with a restricted availability of atmospheric oxygen, i.e., chronic hypoxia. Interestingly enough, the Ornate Tinamou has a small heart to body ratio, which implies a reduced ability in transporting oxygenated blood to the tissues. In order to increase knowledge about the cardiorespiratory response of the Ornate Tinamou, heart rate (HR) and ventilation frequency (VR) were monitored during resting at 25 °C. The values were compared with those obtained in conditions known to elevate metabolism, i.e., lowered temperature and graded exercise. This was later compared with domestic chickens, *Gallus gallus*. Results showed a significant increase in HR at 4 °C, 305±42 bpm in the Ornate Tinamou when compared with HR at 25°C, 241±48 bpm (330±42bpm and 239±32bpm in chicken). A significant increase in VR was only observed in chicken. As expected, with a progressive increase in running speed, a significant increase in HR in both species was observed. At 1.5 km h⁻¹, HR in the Ornate Tinamou was 327±5.6 bpm and 342±8.5 in chicken. At 3.0 km h⁻¹ HR was 383±15 bpm and 404±7.9, respectively. However, HR was not significantly higher in the Ornate Tinamou than in chicken, indicating that there must be other physiological adaptations involved in the sufficient oxygen delivery to tissues, e.g. a high blood oxygen affinity or a preference for anaerobic metabolism due to living in a chronic hypoxic environment.

**Nyckelord**
Keyword: Chronic hypoxia, Ornate Tinamou, small heart, HR, VR, lowered temperature, exercise
1. Abstract

The Bolivian Ornate Tinamou, *Nothoprocta ornata*, lives higher than 3300 m above sea level and must constantly deal with a restricted availability of atmospheric oxygen, i.e., chronic hypoxia. Interestingly enough, the Ornate Tinamou has a small heart to body ratio, which implies a reduced ability in transporting oxygenated blood to the tissues. In order to increase knowledge
about the cardiorespiratory response of the Ornate Tinamou, heart rate (HR) and ventilation frequency (VR) were monitored during resting at 25 °C. The values were compared with those obtained in conditions known to elevate metabolism, i.e., lowered temperature and graded exercise. This was later compared with domestic chickens, Gallus gallus. Results showed a significant increase in HR at 4 °C, 305 ±42 bpm in the Ornate Tinamou when compared with HR at 25°C, 241± 48 bpm (330 ±42bpm and 239 ±32bpm in chicken). A significant increase in VR was only observed in chicken. As expected, with a progressive increase in running speed, a significant increase in HR in both species was observed. At 1,5 km h^{-1}, HR in the Ornate Tinamou was 327 ±5,6 bpm and 342 ±8,5 in chicken. At 3,0 km h^{-1} HR was 383 ±15 bpm and 404 ±7,9, respectively. However, HR was not significantly higher in the Ornate Tinamou than in chicken, indicating that there must be other physiological adaptations involved in the sufficient oxygen delivery to tissues, e.g. a high blood oxygen affinity or a preference for anaerobic metabolism due to living in a chronic hypoxic environment.

Keywords:
Chronic hypoxia, Ornate Tinamou, small heart, HR, VR, lowered temperature, exercise

2. List of abbreviations

HR – Heart rate  VR – Ventilation frequency

3. Introduction

Animals living at high altitude have to constantly deal with a restricted availability of atmospheric oxygen, i.e., hypoxia (Ellerby et al., 2003; Bertelli et al.,2002; Sheafor, 2003). In general birds at high altitude have a large heart relative to their body size in order to deal with a chronic hypoxic environment. By increasing cardiac output it is possible to ensure a sufficient delivery of oxygen to tissues and organs. This is crucial for producing enough energy that is required in order to be able to fly long distances or escape from potential predators (Ellerby, 2003; Sheafor, 2003).

At the same time, studies in the highland Ornate Tinamou (Nothoprocta ornata) a ground dwelling bird belonging to the Tinamidae family and closely related to the flightless ratites (e.g., ostriches and emus), has revealed that it has a small heart in comparison to its body size but also in comparison to chicken (G. gallus) (Altimiras personal communication). The Ornate Tinamou is a primitive species as the ratites and therefore share similar traits such as a paleognathous palate and a reduced keel, which explains their reduced flight ability (Harshman et al., 2008).
However, why it has maintained a small heart relative to its body size while living in a chronic hypoxic environment is not fully known. But it is most likely that it has been maintained because it is a primitive trait and hence, does not necessarily mean that it is more beneficial to have a small heart than to have a large heart when living in a chronic hypoxic environment (Altimiras personal communication; Harshman et al., 2008). A small heart implies a reduced ability in transporting oxygenated blood to the tissues, in comparison to the ability of a larger heart, not only because of the small heart size, resulting in a decreased cardiac output but also, due to the fact that the Ornate Tinamou is living in a chronic hypoxic environment (Altimiras personal communication; Magnan, 1922; Bertelli et al., 2002; Garitano-Zavala, 2010). Therefore, having a small heart suggests that the Ornate Tinamou had to develop physiological adaptive mechanisms involved in the cardiorespiratory system, to ensure a sufficient distribution of oxygen to the tissues (Sheafor, 2003). This is crucial during strenuous locomotion, e.g., when escaping a predator (Sheafor, 2003). Furthermore, this finding suggests a lower capability of performing strenuous daily activities, e.g., flying or running, as oppose to an animal with a larger heart.

Nevertheless, there have not been many physiological studies on highland animals but many studies on lowland animals (Brackenbury, 1982; Sheafor, 2003). These studies have mainly investigated the involvement of ventilation, heart rate (HR), oxygen consumption, metabolites such as glucose and lactate, the aerobic citrate synthase and the anaerobic lactate dehydrogenase, respectively, which also are indicators for the potential effects that exercise training in a hypoxic environment will have on the physiology of the animal under study (Brackenbury, 1982; Butler, 1970; Ellerby et al., 2003; Sheador, 2003). Studies in lowland animals have also shown that during exercise in a hypoxic environment, ventilation frequency (VR) and HR increases significantly, since an increased demand of oxygen for distribution to the tissues will be needed (Brackenbury et al., 1982; Butler, 1978; Nassar et al., 2001). Also, a significant increase in HR and VR has been observed during treadmill running or flying in wind tunnels, during induced hypoxia to simulate the effects occurring in highland birds (Butler et al., 1978). Research on highland animals has mainly focused on the effects on oxygen consumption and oxidative enzymes, while the effects on the cardiovascular system in highland animals during exercise, have yet not been studied to the same extent (Brackenbury et al., 1987; Sheafor, 2003).

Based on previous studies on cardiorespiratory responses in birds, I hypothesize that the Ornate Tinamou will have a significantly higher HR than the HR of chicken, both at a lowered ambient temperature and during graded exercise. This is because its small heart will result in a decreased cardiac output, due to a
decrease in stroke volume, which should instead be compensated by an increased HR which in turn increases cardiac output and hence, an efficient distribution of oxygen to the tissues can still be done even in the presence of chronic hypoxia.

Therefore, the aim of this study was to investigate the physiological effects on the cardiorespiratory system by monitoring HR and VR in the Bolivian, highland Ornate Tinamou, *Nothoprocta ornata*. Firstly, by measuring HR and VR during resting at 25°C. The values were compared with those obtained in conditions known to elevate metabolism, i.e., lowered temperature, graded exercise and after exercise. These results would later be compared with observed effects in chicken and hence, increase understanding of the ability of the Ornate Tinamou, to deal with a chronic hypoxic environment even though it has a small heart relative to its body size.

4. Materials and Methods

4.1 Animal holding facilities
The study was conducted at the Cota-cota university campus, Universidad Mayor de San Andrés, in La Paz, Bolivia, which is located 3300 m above sea level. The studied Ornate Tinamous’ were held in 6 aviaries (4 m x 2 m x 1.80 m). The floor was made of soil and in every cage, hay was provided as nesting material for the animals. Furthermore, food and water was provided on a daily basis. Chicken were separated from the Ornate Tinamou and placed individually in 4 cages (1.20 x 0.50 x 0.60 m). They were also provided with nesting material, food and water on a daily basis.

4.2 Study subjects
Four Ornate Tinamou and 4 adult female chickens were used in this study. Ornate Tinamou was lent from Professor Alvaro Garitano at the Universidad Mayor de San Andrés while chickens were bought from a local market in La Paz, Bolivia. All subjects were, throughout the study, kept in cages where they were provided daily, with food and water. Ornate Tinamou had a body weight of 470 ± 8g (mean ± stdev) and the chickens had a mean body weight of 450 ± 18g.

4.3 Training protocol
Two months before measurements of HR and VR took place, the subjects were trained to run on a motorized treadmill (YK-ET0902, YeeKang, China). They were individually placed inside a plastic box (46 cm x 28 cm x 30cm; 38.6 Liters) that was hanging from a table placed over the treadmill in order for it to be 1 cm of distance from the treadmill. The purpose of the plastic box and its placement was to maintain the subject on the treadmill while running. The
exercise regime consisted of the subject running for 3 min at 1.5 km/h, 2.0 km/h, 2.5 km/h and 3.0 km/h, with 30 s resting between the run velocities (fig.1). The animals were trained until all could complete the complete the entire running protocol (Ellerby et al., 2003). The animals that were not able to do that were excluded from the study.

4.4 Surgery and transthoracic implantation of ECG electrodes
Before starting the surgery, the anesthetics, xylazine (Alfasan, Woerden-Holland; 2 %, 10 mg/kg for G. gallus, 15 mg/kg for N. ornata) and ketamine (Cristália, Sao Paulo, Brazil; 40 mg/kg for G.gallus and 25 mg/kg for N. ornata, were injected intramuscularly with a needle syringe (U-100 Insulin 1ml, Terumo, Tokyo, Japan). Surgical equipment (Aesculap, Melsungen, Germany), including, scissors, tweezers, scalpels and suture threads (ETHILON II, Polyamid 6, EH7144, Johnson & Johnson, New Jersey, USA), were used throughout the surgery. Iodine (Alcohol Yodado 1 %, industrias torrico antelo, Cochabamba, Bolivia) and ethanol (70 % alcohol, La Paz, Bolivia) were used to clean the cuts of the animals to prevent infections. The surgery was initiated when a surgical plane of anesthesia was achieved after as attested by the lack of corneal reflexes. A double thread electrode, consisting of two insulated stainless steel wires soldered to a small connector were implanted subcutaneously on the sides of the thoracic cavity of the animal (Bopelet, 1974). After the surgery was finished, the subject was monitored until being awakening and walking normally.

No postoperative use of antibiotics was required and animals recovered readily without signs of infection. All procedures were approved by an ethical permit from the Ethical Committee of Universidad Mayor de San Andrés granted to Dr.Álvaro Garitano and Dr.Jordi Altimiras.

4.5 Measurement of heart rate and ventilation frequency during resting and after flying and running
The measurements of HR and VR began 24 hours after surgery. The animal was placed for 2 hours into an airtight plastic container (20 cm height and 10 cm radius; 6 Liters), in order to measure HR and VR during resting conditions. A cable went into the chamber, which was connected to the female connector, placed on the subject. At the other end, the cable was connected to an impedance meter (model 2991, impedance converter, Morro Bay, California) in series to a connection through the bioelectric amplifier of a Powerlab 4ST unit (ADInstruments Ltd.). The output of the impedance converter was also connected to the Powerlab amplifier so that both signals (impedance ventilation and electrocardiogram) were acquired using LabChart v 7.0.3 (ADInstruments, 2012) in a Latitude D610 laptop computer.
Resting HR and VR were obtained during 2 hours while the animal was placed inside the chamber in an incubator (YONAR Incubadoras, Buenos Aires, Argentina) in which the temperature was regulated to 25 °C. After two hours of resting measurements the animal was subjected to a flying protocol which consisted of the subject being chased to fly and run inside the cage, until being tired enough and started to open its beak. The beak opening was an indication of fatigue since afterwards the animal did not put as much effort in flying or running or basically refused to fly. This took between 1-2 min, including approximately 12-25 flights, depending on the subject. Immediately after that the subject was placed back in the chamber for measuring HR and VR after flight for 2 hours inside the incubator at 25 °C. The resting protocol was both utilized for chicken and Ornate Tinamou at 25 °C and at 4 °C inside a refrigerator. However, the postflying protocol was excluded for the Ornate Tinamou, due to lack of time, whereas the postflying part at 4 °C, was measured in chicken. Directly after the measurements took place, the subject was removed from the chamber, and moved to its cage to rest for the coming running protocol the following day.

4.6 Measurement of HR and VR during graded exercise
The following day, the subject was placed inside the plastic box on the treadmill, where it had to run according to the fixed running protocol, i.e., 3 min for each of the following velocities, 1.5, 2.0, 2.5, 3.0 km h⁻¹. A 30 s resting period at the end of each 3 min running period was implanted to give the animal a short break. This was done after it was shown that short breaks allowed the animal to perform better at increasing speeds. The electrodes of the animal were coupled to a cable, directly connected to the impedance meter so HR and VR could be measured. After the running session, the cable was removed and the cable that was inside the chamber was instead coupled to the electrode. The subject was placed inside the chamber and measurement of VR and HR during postrunning took place. This protocol was repeated twice for every studied chicken, once at 25 °C and at 4 °C but only measured at 25°C for Ornate Tinamou. Thermochrons (Dallas Semiconductors Inc.), devices that measures temperature, were placed inside the incubator and refrigerator in order to monitor the temperature and ensure that it was stable during the runs of measurements. Finally, the subject was removed from the chamber and returned to its cage.

4.7 Statistical analysis
All statistical analysis were performed with Excel 2010 and SPSS v.20. A student’s t-test was used to compare HR and VR during ambient temperature changes between the species. A repeated measures ANOVA was used in order to compare HR between species during graded exercise.
5. Results

5.1 Heart rate during resting at 25 °C and 4°C
During the 2 hour resting period, the animals recovered from the handling procedures and reached stable conditions in the last 30 min of measurement, as shown in fig.1-2 and more in detail as shown in fig. 3. Chicken tended to have a slightly higher mean HR during resting compared to the Ornate Tinamou at both temperatures. The mean HR value during the last 30 min of resting at 25 °C and 4°C in chicken was 239 bpm ± 32 bpm and 331 bpm ± 29 bpm, respectively (Fig.3). The Ornate Tinamou had, on the other hand a similar mean HR value during the last 30 min of resting at 25 °C, i.e., 241 bpm ± 48 bpm but not at 4 °C, i.e., 305 bpm ± 42 bpm, which was lower than for chicken (Fig.3). The mean HR values were higher at 4 °C for both species (Fig.2-3).

![Graph A](A) Heart rate (HR) during resting at 25 °C in chicken and in the Ornate Tinamou, respectively. A mean value have been chosen for every 10 minutes and standard deviation error bars have been included in all graphs. Graph A shows the mean HR values at 10 minute intervals in chicken at 25 °C during a 2 hour resting period.
Graph B shows the mean HR values for every 10 minutes was obtained in Ornate Tinamou at 25 °C during a 2 hour resting period.

Fig. 2. Heart rate (HR) during resting at 4 °C in chicken and in the Ornate Tinamou, respectively. A mean value have been chosen for every 10 minutes and standard deviation error bars have been included in all graphs. Graph C shows the mean HR values for every 10 minutes in chicken at 4 °C during a 2 hour resting period. Graph D shows the mean HR values for every 10 minutes in Ornate Tinamou at 4 °C during a 2 hour resting period.
Fig. 3. Mean HR during the last 30 min of resting in chicken and in the Ornate Tinamou obtained from fig. 1 and fig.2. The left bars show the mean HR during the last 30 min of a 2 hour resting period in chicken at 4 °C and 25°C with standard deviation error bars, i.e., 330 ±29 and 239 ±32, respectively. The two asterisks indicate a significant difference between the temperatures, p=0.007<0.05. The right bars show the mean HR during the last 30 min of a 2 hour resting period in the Ornate Tinamou at 4 °C and 25°C with standard deviation error bars, i.e., 305 ±42 and 241 ±48, respectively. The two asterisks indicate a significant difference between the temperatures, p=0.004<0.05.

5.2 Ventilation frequency during resting at 25 °C and 4°C
During the 2 hour resting period, all graphs shown in fig. 4-5, showed in general a similar pattern, with a successive decrease during the first 40 min, followed by a rather stable pattern for the last 30 min of the resting period (Fig.4-5). In chicken the mean VR values were higher at 4°C between 30-40 bpm than at 25 °C, which was also the case for Ornate Tinamou but with a much smaller difference (Fig.4-5). In the Ornate Tinamou, VR at 25°C was overall higher but not significantly different than in chicken (Fig.4). However, the last 30 min of resting in both species, the VR stabilised but still a higher VR at 4 °C then at 25 °C was observed (Fig.4-6).
Fig. 4. Ventilation frequency (VR) during resting at 25 °C and 4 °C in chicken and Ornate Tinamou, respectively. A mean value has been chosen for every 10 minutes and standard deviation error bars have been included in all graphs. Graph A shows the mean VR values for every 10 minute intervals in chicken at 25 °C during a 2 hour resting period. Graph B shows the mean VR values for every 10 minutes in Ornate Tinamou at 25 °C during a 2 hour resting period.
Fig. 5. Ventilation frequency (VR) during resting at 25 °C and 4 °C in chicken and Ornate Tinamou, respectively. A mean value has been chosen for every 10 minutes and standard deviation error bars have been included in all graphs. Graph C shows the mean VR values for every 10 minutes in chicken at 4 °C during a 2 hour resting period. Graph D shows the mean VR values for every 10 minutes in Ornate Tinamou at 4 °C during a 2 hour resting period.
5.3 Postrunning HR and postflying HR at 25 °C

Postrunning HR at 25°C in chicken showed a rather stable HR the first 11 min, varying between 336-367, followed by a clear decrease during the 11-12 min, until reaching a even more stable HR resting value the remaining 18 min, around 315 bpm (Fig.7A). Postflying HR at 25°C in chicken showed a similar pattern as with the postrunning measurements with a rather stable but much lower HR, around 300 bpm, the last 14 min (Fig.7-8). Postrunning HR at 25°C in the Ornate Tinamou was a bit higher in the beginning compared with chicken i.e., around 400 bpm the first 15 min and, decreasing successively until stabilizing around 320 bpm, the last 15 min of postrunning (Fig.7).
Fig. 7. Postrunning heart rate in chicken and in the Ornate Tinamou at 25 °C. A mean value has been chosen for every minute and standard deviation error bars have been included in the graphs. Graph A shows the mean HR values for every min in chicken at 25 °C during 30 min of postrunning. Graph B shows the mean HR values for every min in Ornate Tinamou at 25 °C during 30 min of postrunning. In fig.7 and fig.8 a significant difference in HR was observed, $p=0.01$, between postrunning and postflying in chicken.
Fig. 8 Postflying heart rate in chicken at 25 °C. A mean value has been chosen for every minute and standard deviation error bars have been included in the graphs. Graph C shows the mean HR values for every min in chicken at 25 °C during 30 min of postflying. In fig.7 and fig.8 a significant difference in HR was observed, p=0.01, between postrunning and postflying in chicken.

5.4 Graded exercise
The animals ran for a total of 12 min in which they had to run for 3 min at each given velocity (fig.9A & 9B). Throughout the running, a progressive increase in HR was observed in chicken and Ornate Tinamou, which was significantly increased between 1.5 km/h and 3.0 km/h, p= 0.007 and p=0.009, respectively (fig.9A & 9B). Also, a significantly lower HR in the Ornate Tinamou was observed when compared with chicken, p= 0.006.
Fig. 9. Heart rate during running at ambient temperature in chicken and in the Ornate Tinamou. The subjects ran for 3 min at each given speed, 1.5 km h\(^{-1}\), 2.0 km h\(^{-1}\), 2.5 km h\(^{-1}\) and 3.0 km h\(^{-1}\). A mean HR value had been chosen for every run minute at every speed and standard deviation error bars have been included in both graphs. Graph A shows HR values during running in chicken and \(p=0.007\). Graph B shows HR values during running in Ornate Tinamou and \(p=0.009\). The asterisks indicates a significant difference between velocity 1.5 km/h and 3.0 km/h. \(p=0.006\) was observed between the species.

6. Discussion

6.1 The implications of having a small heart in a chronic hypoxic environment

The purpose of this study was to increase understanding on the cardiorespiratory response upon increased metabolism in the Tinamou. This is the first time to my knowledge that HR and VR have been documented for this species. There is a better understanding of the Tinamou metabolism based on two studies, the Whiters el al. paper from 1987 on the Chilean Tinamou and the recent companion study on the Ornate Tinamou, by Ekström, 2012. During chronic hypoxia, the cardiovascular system works harder than in a normoxic environment, i.e., HR and VR are elevated, which will in turn result in an increased cardiac output and hence ensure a sufficient amount of oxygen reaching the tissues and organs (Bishop, 1997; Kiley, et al., 1979; Sheafor, 2003). It has also been observed that high altitude birds, such as Pine siskins (Spinus spinus) have an 11% higher heart weight than its lowland relatives (Carey and Morton, 1970). A larger heart in highland birds indicates an increased ability in pumping oxygenated blood to the tissues than the pumping ability of a smaller heart due to a higher cardiac output. All Tinamou have a small heart relative to their body size, since they occasionally only fly short distances and therefore do not require the high energy demand of long-distance flying birds (Hartman, 1955). This applies both to lowland and highland Tinamou. Except for the highland Tinamou species, most animals
living at high altitude have a larger heart than their lowland relatives in order to increase their cardiac output and hence ensure an efficient oxygen delivery to tissues and organs (Hartman, 1955). It is most likely that the Ornate Tinamou has maintained a small heart at a high altitude because it is a primitive trait and perhaps have not been able to develop a larger heart. It is also clear that due to the heart size, several adaptive physiological mechanisms had to be developed in order to allow a sufficient distribution of oxygen in the cardiovascular system, have had to be developed in response to the implications of having a small heart while living in a chronic hypoxic environment (Bertelli et al., 2002; Garitano-Zavala, 2010; Hartman 1955; Magnan, 1922; unpublished data by Altimiras & Garitano-Zavala, 2010).

6.2 Heart rate increases when ambient temperature decreases in both species

Studies have revealed that at resting conditions during normoxia, ventilation frequency (VR) in *G. gallus* ranges between 13-25 bpm (breaths/min) and HR ranges between 210-350 bpm (beats/min) (Butler et al., 1977; Calder, 1968; Gleeson, 1985; Kharin et al., 2006). The high variation between studies must be due to the effect of size, age and experimental conditions. During hypoxia, HR and VR in resting conditions increase significantly as oppose to a normoxic environment. During graded exercise, HR and VR will continue to increase significantly, both during running and flying due to an increased oxygen demand (Brackenbury et al., 1982; Butler, 1978; Nassar et al., 2001).

Even if it was predicted in my hypothesis that the Ornate Tinamou would significantly increase HR and VR more than the chicken, due to its smaller heart and hence lowered cardiac output, the Ornate Tinamou was unable to significantly increase HR more than the chicken (Fig.1-3). However, as expected, the Ornate Tinamou was able to significantly increase HR at 4 °C and in exercise conditions (Fig.1-3 & 9).

Previous studies on cardiorespiratory responses have shown that when *A. platyrhynchos* is exposed to an ambiental temperature of 20 °C and later to an ambiental temperature of -20 °C, its cardiac output increases from 173, 7 ml x kg⁻¹ min⁻¹ to 431,4 ml x kg⁻¹ min⁻¹ which is caused by an increase in HR (Bech et al., 1984). In this study, both the Ornate Tinamou and the chicken maintained HR at a significantly higher level at 4 °C, than at 25 °C (Fig.1-3). This occurs as expected in endotherms because a lower ambiental temperature forces the animal not only to increase its HR which in turn increases the metabolism, in order to maintain the body temperature but it also activates the thermoregulatory mechanism which initiates shivering of the skeletal muscles, mainly the flight muscles (Chaffee & Roberts, 1971).
Ventilation rate showed a similar trend in both species during resting as what was observed with HR (Fig.1-5). With a high VR during the beginning of the resting period, which might have been caused by the animals being stressed, but that progressively decreased until reaching a stable level approximately the last 30 min (fig.3-4). The last 30 min of resting did not show any significant difference in VR between the species only a significant increase in VR at 4 °C in the chicken was observed. This indicates that the Ornate Tinamou is not able to increase the VR further than the chicken at 4 °C, but in chicknens VR increased at 4 °C in comparison to 25°C, in order to increase the oxygen delivery to tissues and organs.

6.3 Graded exercise increases HR significantly but the Ornate Tinamou has a lower HR than chicken
Studies have shown the effects on the cardiorespiratory system in different avian species, during treadmill running and flying in wind tunnels, in comparison with resting (Brackenbury et al., 1987; Peters et al., 2005). Studies in Branta leucopsis (geese) have been done with implanted radio transmitters, in order to monitor HR and VR during flight, not only before and after, something that was not possible before using radio transmitters (Butler and Woakes, 1980). Published studies on fixed flying protocols are rather scarce due to the difficulty in studying the animal during flight (Butler et al., 1977). Because of this reason, only postrunning data has been documented in my study for chicken and hence showing how fast the animal recovers from the exercise (Fig.5). When progressive hypoxia is induced in order to simulate what could occur physiologically in birds at high altitude during exercise, the HR and VR in e.g. A. platyrhynchos and Columbia livia (pigeon) increases (Butler, 1970; Kiley et al., 1979).

As expected, HR increases progressively both in chicken and in Ornate Tinamou, while running in a chronic hypoxic environment (Fig.6; Butler, 1970; Ellerby et al., 2003). Also, a significant difference in HR between the species was observed since HR in the Ornate Tinamou was slightly lower throughout the running (Fig.6). This indicates that the Ornate Tinamou is unable to increase HR further than the chicken which also suggests that there are other adaptive, physiological mechanisms that are fundamental in regards to allowing that Ornate Tinamou survives at high altitude while having a small heart.

6.4 Other physiological adaptations than increasing HR in the Ornate Tinamou in order to deal with a chronic hypoxic environment
According to my study, the Ornate Tinamou is able to significantly increase HR in order to ensure a sufficient oxygen delivery to tissues and organs, while still having a small heart (Fig.1-9). As expected, there was a significant increase in HR and also an increase in metabolism when ambient temperature decreased
(Fig.2-3; the resent companion study by Ekström, 2012). Also, a progressive increase in HR occurred in both species due to graded exercise (Fig.7). These findings clearly show that the Ornate Tinamou is able to increase HR upon increased metabolism, both during ambient temperature changes and during graded exercise but the increase in HR is not larger than in chickens (Butler, 1967; Butler, 1970; Fig.2 & 4). Based on this there must be other physiological, adaptive mechanisms involved in the cardiorespiratory system that are fundamental when having a small heart in a chronic hypoxic environment (Ellerby et al., 2003; Bertelli et al., 2002; Sheafor, 2003). It would also be relevant to investigate the relationship between heart rate, stroke volume (SV), arteriovenous difference and oxygen consumption (VO₂) since all these factors are involved in the cardiorespiratory system (Fick principle, VO₂ = SV x HR x arteriovenous difference) (Barnas et al., 1984). This would increase knowledge of the physiology of the Ornate Tinamou and the implications of having a small heart in a chronic hypoxic environment. Since, this study revealed that the Ornate Tinamou is unable to increase HR significantly when compared to chickens meanwhile it also has been shown, in the recent companion study by Ekström, 2012, that VO₂ is lower in the Ornate Tinamou than in chicken. It would be of great interest to study the arteriovenous oxygen difference of the Ornate Tinamou and how it responds to chronic hypoxia while the animal under study has a small heart (Black & Tenney, 1980; Storz, 2007). These mechanisms need further study. Due to the small heart size of the Ornate Tinamou a lower stroke volume should be expected as oppose to approximately 0.7-1.2 ml x kg⁻¹ in the chicken (Barnas et al., 1984). If this is the case, together with a low VO₂, as observed in the recent companion study by Ekström, 2012 and a heart rate that is not higher than in chicken, the arteriovenous difference should be much lower than what is observed in chickens. This is not only because of the chronic hypoxic environment which results in a lowered arterial blood oxygenation but also due to the small heart size (Altimiras personal communication; Ellerby et al., 2003; Bertelli et al., 2002).

Because of this, it would be highly relevant to increase understanding of the physiology of the Ornate Tinamou by investigating other involved factors such as blood oxygen affinity in the hemoglobin. Previous studies have revealed that high altitude birds, e.g. the bar heeded goose (Anser indicus) that are living in a chronic hypoxic environment will have a high blood oxygen affinity in as oppose to a similar goose species living at lowland, the Pekin duck (Anas platyrhynchos) (Black & Tenney, 1980). This means that their oxygen dissociation curve will be shifted more to the left, since they will reach oxygen saturation at a lower oxygen partial pressure because their hemoglobin have a higher binding affinity to oxygen (Storz, 2007). This is fundamental when...
living in an environment with chronic hypoxia and a quick and sufficient oxygen delivery to tissues and organs is crucial (Sheafor, 2003). It is therefore possible, that an increased blood oxygen affinity might be one of the fundamental physiological adaptations present in Ornate Tinamou, which might compensate not only for a low cardiac output but also for a decrease in the arteriovenous difference due to living in a chronic hypoxic environment while having a small heart (Storz, 2007).

However, if it is determined in future studies that neither blood oxygen affinity nor arteriovenous difference are high in the Ornate Tinamou in comparison to other hypoxic birds with larger hearts (Black & Tenney, 1980) it is possible that instead there is a preference for anaerobic metabolism, because it might be more beneficial for the Ornate Tinamou to rely on the anaerobic system when living in a chronic hypoxic environment while having a small heart, e.g., when having to escape a predator quickly. This could also explain the fact that VO₂ is significantly lower in the Ornate Tinamou than in the chicken according, but this needs to be further studied in order to be verified.

7. Conclusion
According to my studies, the Ornate Tinamou was able to increase HR significantly upon increased metabolism during ambient temperature changes but also during graded exercise. But not increase it significantly higher than HR in chicken (Fig.3 & Fig.9). This indicates that increasing HR is not a physiological adaptation in the Ornate Tinamou in response to an increased metabolism when living at chronic hypoxia while having a small heart. This indicates instead that to increase understanding on the implications of having a smaller heart while living in a chronic hypoxic environment other physiological adaptive mechanisms, i.e., a high blood oxygen affinity or a preference for anaerobic metabolism, would increase understanding of the physiology of the Ornate Tinamou and how it deals with having a small heart while living in a chronic hypoxic environment.

8. Acknowledgements
I would like to show my immense gratitude to my supervisor, Dr. Jordi Altimiras, for supporting and helping me throughout my thesis but also for allowing me to do the practical work in La Paz, Bolivia. I would also like to thank Dr. Alvaro Garitano for lending me Ornate Tinamou and for allowing me to work in his laboratory. Finally, I would like to thank any of my fellow students at Universidad Mayor de San Andrés that helped me throughout my study.

9. References


