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„Disentangling the effects of metabolism on activity levels and boldness: a study of between-individual differences in heart rate and behaviour from embryo to nestling in Northern Bald Ibis (*Geronticus eremita*)“

verfasst von / submitted by

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Kathrin Arbeithuber

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Zusammenfassung (Abstract in German)

Stoffwechsel und Verhaltensreaktionen im Bezug auf äußere Reize können stark verbunden sein, jedoch gibt es große Lücken im Wissensstand ob und wie diese verknüpft werden können. Das Pace-of-life Syndrom sagt, dass Individuen innerhalb einer Population konstant zwischen Unterschieden in Stoffwechsel, Aktivität und Verhalten schwanken können. Das Ziel dieser Arbeit ist, den Zusammenhang zwischen Metabolismus, Aktivitätsniveau (Anzahl an Schritten) und Reiz-induziertem Verhalten (Loslassen von Angst, Exploration) in Waldrappen (*Geronticus eremita*) zu finden. (1) Es wurden Herzfrequenzen in Embryos und Nestlingen gemessen, um zu testen, ob der Stoffwechsel bei einem Individuum während verschiedener Lebensphasen gleichbleibt. (2) Es wurden Aktivitätsniveau (Anzahl an Schritten) sowie Verhaltensreaktionen (Zeit bis zum Umdrehen, Anzahl entdeckter Feldern) während eines standardisierten Tonic-Immobility-Tests und Novel-Arena-Tests gemessen. Die Ergebnisse zeigen zwei Erkenntnisse. Zum einen, dass Individuen während der unterschiedlichen Lebensphasen nicht denselben Stoffwechsel aufrechterhalten, und zum anderen, dass es eine Verbindung zwischen Metabolismus und Aktivität, jedoch nicht zwischen Metabolismus und anderer Verhaltensweisen gibt. Wie erwartet zeigte sich bei konstanten Verhaltensreaktionen eine Verbindung zwischen Loslassen von Angst und Exploration, welche von der Herzfrequenz unabhängig war. Diese Studie ist eine von wenigen, welche testet, ob das Niveau an Metabolismus über Lebensphasen aufrecht bleibt, diese Behauptung wurde jedoch nicht bestätigt. Diese Studie ist auch eine der Ersten, welche gesondert den Effekt von Metabolismus auf das Aktivitätsniveau im Vergleich zu Verhaltensreaktionen auf Umwelteinflüsse misst. Dadurch wurde eine teilweise Befürwortung für das Pace-of-Life Syndrom gefunden, weil man eine Verbindung zwischen Metabolismus und Aktivitätslevel belegt, jedoch andere Verhaltensweisen wie Angst und Exploration unabhängig von Metabolismus auftreten.

Abstract

Metabolism and behavioural differences to external stimuli may be very interconnected, but there are large gaps in knowledge to understand if and how they could be associated. The pace-of-life syndrome predicts that individuals within a population may vary consistently in having slower or faster metabolism, less or more activity, and weaker or stronger behavioural response intensity, but has not been studied much. The aim of this thesis is to measure the association between metabolism, activity level (number of steps) and stimulus-induced behaviour (fear release, exploration) in Northern Bald Ibis (*Geronticus eremita*). (1) We measured heartrate in embryos and nestlings to test if metabolism per individual is consistent across life stages. (2) We measured activity level (number of steps) and behavioural response (time to turn over, number of fields explored) during a standardized tonic immobility test and a novel arena test. The results show two main findings, first, individuals do not maintain the same metabolism across life stages. And second, there was an association between metabolism and activity, but not between metabolism and other behaviour. As expected, if behavioural response profiles are consistent, there was an association between fear release and exploration that was independent of heartrate. In conclusion, this study is one of few studies to test if metabolic rate is maintained across life stages and was not supported. Also, this is one of the first studies to separately measure effects of metabolism on activity level versus behavioural response to external stimuli and found partial support for the pace-of-life syndrome because there was an association between metabolism and number of steps taken, but other behaviour such as fear release and exploration may occur independently of metabolism.

1. Introduction

Individuals within a group can differ greatly in traits, and characteristics like boldness, shyness, activity or exploration can be very different between individuals but very stable within one individual (Stamps 2007). This individual variation can be the target of sexual or natural selection, because differences in environment and development can lead to a range of individual differences in behaviour (Dall, Houston et al. 2004). Such variation is of great interest to evolutionary biologists not least because one can test effects of individual differences on various measures of fitness. At the individual level, individuals may have high consistency in the expression of a particular trait in different contexts (metabolism, personality) (Sih, Bell et al. 2004). There are gaps in knowledge about whether there is consistency between physiological and morphological traits, e.g., slow metabolism and passive personality versus fast metabolism and active personality (Biro and Stamps 2008) and also across life stages, e.g. prenatal metabolism versus post hatch metabolism (Herde and Eccard 2013). Understanding such patterns of association is also useful when managing threatened species. For example, one can make predictions about how the behaviour of a threatened species will respond to management intervention. Some researchers have suggested that 'personality' (a short-hand term for consistent individual differences in behavioural response to external stimuli) is a 'pace of life syndrome' that spans passive to active, e.g., in different traits like individual behaviour, hormonal, metabolic or immunological (Reale, Garant et al. 2010). No study has disentangled the effects of metabolism on activity level versus the boldness and exploration components, which this study aims to do.

Heart rate is considered a proxy for baseline metabolic rate (Butler, Green et al. 2004) and can also be measured in embryos (Colombelli-Negrel, Hauber et al. 2014). The consistency of heart rate as a proxy for metabolism has been tested in studies with zebra finches (*Taeniopygia guttata*) (Sheldon and Griffith 2018) and great tits (*Parus major*) (Dechmann, Ehret et al. 2011). Metabolism could influence a range of attributes including development, activity or growth, and may be the result of impacts on behaviour (Schmidt-Nielsen 1991). In some cases, metabolism and behaviour were found to be correlated but showed different patterns in males and females (Biro and Stamps 2010, Bouwhuis, Quinn et al. 2014). But on the whole, while theoretical frameworks have been proposed, few studies have empirically tested for effects of metabolism on behaviour (Careau, Thomas et al. 2008, Biro and Stamps 2010). One challenge is that metabolism may change across life stages, such as when an individual changes from embryo inside an eggshell to hatchling or adult. Sheldon and Griffith (2018) tested if embryonic heart rate was associated with prenatal developmental rates, post-natal activity levels and post-natal growth rates. They found a significant, negative relationship between prenatal heart rate and incubation duration but no significant relationship between embryonic heart rate, post-natal activity levels or growth rates in the zebra finch (Sheldon and Griffith 2018).

A correlated suite of individual behavioural differences has sometimes been referred to as ‘pace of life syndrome’, with some individuals having a consistently faster or slower behavioural response across different activities (Careau, Thomas et al. 2008). According to this syndrome, there are consistent individual-level associations between activity and exploration with aging and onset of reproduction (Reale, Garant et al. 2010). Biro and Stamps (2008) suggested a key role of metabolism in this pace of life syndrome, proposing that metabolism could be the causative factor for activity level that then affects growth rate and food intake (Biro and Stamps 2008). The studies to date that have examined correlations between the physiology and development of individuals have found associations between hormone concentration, personality, metabolism and life-history (Reale, Garant et al. 2010, Dammhahn, Dingemanse et al. 2018). However, metabolism, physiology and behaviour have different patterns of association across species. For example, body-armoured mammals (a study into 70 different terrestrial, non-flying species) had lower basal metabolic rate and lower activity than individuals without a body-amour (Lovegrove 2001). Furthermore, there was a positive correlation between investigative behaviour and the onset of reproduction but a negative correlation between these both aspects and basal metabolic rate (Lovegrove 2000, White and Seymour 2003). Thus, these previous findings highlighting that the pace of life syndrome brings together physiology, behaviour and life-history in a conceptual framework (Reale, Garant et al. 2010) but that experimental approaches are needed, where possible, to better understand causal explanations for the different patterns.

The Northern Bald Ibis is suited to an examination of the association between prenatal and post-hatch metabolism and hatchling to fledgling individual behavioural differences because this species has accessible nests and hatchlings for measurement at the Konrad Lorenz Research Center for Behaviour and Cognition (KLF) (Puehringer-Sturmayr, Wascher et al. 2018). A population of Northern Bald Ibis have been studied at the KLF since 1993. The entire adult population is individually colour-banded (Puehringer-Sturmayr, Wascher et al. 2018, Frigerio, Puehringer-Sturmayr et al. 2019). Furthermore, the birds nest in an open access free-flight aviary connected to the outdoor, allowing adult birds to forage in the wild and researchers to measure eggs, hatchlings and juveniles (Puehringer-Sturmayr, Stiefel et al. 2020). The prenatal measurement of heart rate has been standardized in other systems (Colombelli-Negrel, Hauber et al. 2014), and a range of standard measures of individual behavioural differences including tonic immobility (Jones 1986, Jones 1992) and novel arena test (Sih, Bell et al. 2004) have been developed.

The aim of this study is to measure physiological and behavioural consistency within and across life stages in Northern Bald Ibis. We use heart rate (HR) as a proxy for metabolic rate (Butler, Green et al. 2004) and two personality tests (tonic immobility, novel arena) (Jones 1986, Sih, Bell et al. 2004) to measure behavioural response profiles to experimental conditions. For the personality tests, we separate our analysis into a measure of activity level (number of steps during test) and 'personality' level (fear recovery score, exploration score). We test five hypotheses: (1) Individuals vary in their prenatal metabolism, measured as prenatal HR, which increases with age during incubation, (2) HR changes with age during the nestling phase, (3) There is an association between embryo and nestling HR, (4) if personality is a pace of life syndrome, then individuals with faster metabolism and higher HR will have stronger activity profiles, and (5) if personality is not related to metabolism, but still shows individual differences, then different personality tests should be correlated with each other but not with metabolism. We test the following predictions: (i) a positive increase in HR with egg age, (ii) variation in prenatal HR across individuals, (iii) a positive increase in HR with nestlings age, (iv) controlling for age, embryo HR predicts nestling HR, (v) a positive correlation between HR and number of steps during a tonic immobility test and number of steps during a novel arena test, and (vi) a positive correlation between time to turn over in a tonic immobility test and number of lines crossed in a novel arena test, and no effect of HR.

2. Methods

2.1. Study site and study species

Data were collected during the nesting season of the Northern bald Ibis (Geronticus eremita), during 2019 (April) and 2020 (March to June). The colony of the Northern Bald Ibises from this study nests in free-flight and open-access aviaries managed by the Konrad Lorenz Research Center for Behaviour and Cognition in Grünau im Almtal, Upper Austria (47°48'50.2"N 13°56'50.8"E). This colony was founded in 1997 and was the first free flying settlement in the north of the alps, after they were extinct in Central Europe. The animals are flying free the whole year, they are returning to the aviary just for sleeping and breeding. The aviary is located in the Cumberland Wildpark Grünau and the dimensions are 20x15x7 meter. The nests for breeding are created like they are in the natural habitat of the sandstone cliffs. Each individual is marked with an individual-colored ring. The species is breeding in colonies but is socially monogamous. Both parents take part of building the nest, incubating the eggs and feeding the young. Clutch size varies from 1-4 eggs and incubation lasts about 28 days (Figure 1). The breeding starts with the first laid egg, so the offspring hatch one after the other with up to 3 days difference. The feeding phase of the hatchings lasts about 40-50 days until fledgling.

Figure 1: Adult Northern Bald Ibis with two eggs

2.2. Heart rate

In this study, we measured heart rate using different approaches during the prenatal and post-hatch period. The dates for heart rate data collection were April, 3rd 2019 until April, 24th 2019 and March, 11th 2020 until June, 25th 2020. In 2019 we tested heart rate in 34 offspring and in 2020 in 38 offspring.

To measure heartrate in embryos, we used the digital egg monitor system “Buddy” (Avitronics, Cornwall, UK, https://www.avitronics.co.uk/; Figure 2). This device generates heart rate data by tracking infrared light absorption changes owing to embryonic blood flow. Each egg was marked and numbered with pen immediately on the day of egg laying, which was possible because of daily nest monitoring. During 2019, and once eggs were five days old, we measured heart rate every two days. During 2020, we measured heart rate every five days.

Figure 2: Heart rate measurement of an embryo with the digital egg monitor system “Buddy”

To measure heart rate in hatchlings, we used a microphone placed externally on the hatchlings' chest to record the sound of the beating heart. The heartrate recording was analysed with the software program "Audacity" (iWeb Media Ltd., Marco Mercieca, Birkirka, Malta, www.audacity.de). During 2019, we measured post-hatch heartrate on one day between d 1 and d 6 after hatching. During 2020, we measured heart rate three times, with the first recording between d 1 and d 3 after hatching, the second recording on d 15, and the third recording on d 30. At the time of making the heart rate recoding, we noted the egg ID, nest ID, date, time, and ambient temperature from a portable thermometer placed next to the chick at the time of measurement.

2.3. Personality

We used two approaches to measure individual differences in behavioural response to external stimuli: tonic immobility test and novel arena test.

Tonic Immobility

We did the tonic immobility test when chicks were d 15. The tonic immobility test is a standard test to measure fear in birds (Jones 1992). Tonic immobility considered an unlearnt catatonic state and there is evidence that it is the final stage in a chain of anti-predator behaviour patterns (Jones 1986, Mills and Faure 1991). The shorter an individual remains in the catatonic state, the less fearful it is. First, a chick was removed from the nest, placed in a bag (to reduce additional external stressors), and carried to the empty experimental room (301cm x 404cm) located within 10-20 m form the nest. The handling was done by VPS or KA to minimize differences in handling. The chick was left to acclimatize for 5 minutes in the bag; the video camera was started, and the chick was removed from the bag and placed on its back on a cardboard surface (measurements: 79.5cm x 58.5 cm). The handler (VPS or KA) applied their hand to the breast of the chick for 10 sec and used their other hand to cover the eyes of the chick. After 10 seconds the chick was released, and the experimenter stepped away 2 m to oversee the filming for 5 min (Figure 3). There was always only one person in the room with the chick. Any human and chick eye contact was avoided. In case the chick moved immediately after removal of the human hand, the procedure was repeated another four times and still filmed by the camera. The videos were coded with the software Solomon Coder (Copyright © 2017 by András Péter https://solomoncoder.com/; Version: beta 17.03.22) and the following behavioural responses were analysed: latency to roll over (release from tonic immobility) and number of steps taken during 5 min (activity level). The sample size was 36 (21 in 2019 and 15 in 2020).

Figure 3: Time laps of the tonic immobility test

Novel Arena

We did a novel arena test when chicks were d 30. This test measures exploration behaviour whereby exploration score is considered to be consistent with a strong boldness response (Verbeek, Drent et al. 1994, Sih, Bell et al. 2004). As above, chicks were handled in the same way: removed from the nest, placed in a bag to reduce external stressors, and carried to the novel arena chamber located within 20 m of the nest. The novel arena was a rectangular patch on the floor (measurements: 204cm x 205cm x 39.5cm) demarcated by four panels to form the external walls of the enclosure; the floor was subdivided into nine equal-sized squares with tape markings to denote ‘lines’’ (Figure 4). The experimenter placed the chick in the right lower corner of the arena; the chick was left alone in the experimental room. The video camera was placed in the left lower corner of the room and had a full field of view of the arena. The behaviour of the chick was recorded for ten minutes. The videos were coded with Solomon Coder and the following behaviors were scored: the number of crossed lines (exploration behaviour) and the number of steps (activity level). We tested 33 individuals (19 in 2019 and 14 in 2020). Exploration of a novel environment is well-established as a consistent behavioural trait with implications for behaviour in the wild (Verbeek, Drent et al. 1994, Herborn, Macleod et al. 2010) and has been used in birds as a proxy for personality in general (Krause and Naguib 2011, Titulaer, van Oers et al. 2012).


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Figure 4: Experimental set-up of the novel arena test

3. Statistical methods

The statistical analysis was carried out with two programs, R Studio Version 3.5.1 (R Studio, Boston, MA, USA) and SPSS (SPSS Inc., Chicago, IL, USA). For the possibility to compare the heartrates of the eggs, the data was connected in average heartrate and maximum heartrate for two different days. Day 1 was around day 20 and day 2 was about day 25 of incubation. For post-hatched analysis the unedited data was used.

4. Results

4.1. Individual variance and a positive increase in HR with egg age

Heart rate did not increase in a linear manner with embryo age (r= -0.088, P = 0.18) (Figure 5).

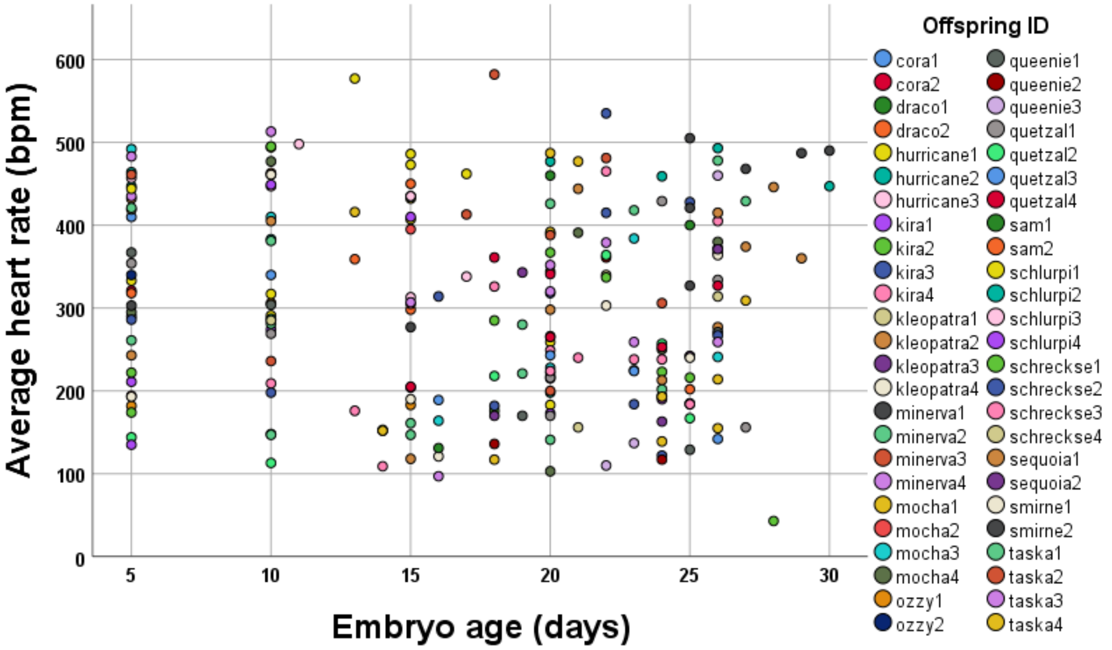

Figure 5: The average heart rate (beats per minute) in embryos measured on different days (d 5 to d 30) across the 30-day incubation phase in Northern Bald Ibis. Data are shown for 71 embryos from 30 nests. HR was measured for 60 sec; the average HR was calculated for HR measured every 10 sec for 1 min in each embryo.

Using GLMM with average HR as the dependent variable and the fixed factor age and the random factor embryo ID, only embryo ID was significantly associated with prenatal HR (GLMM: age t= -1.225, P = 0.222; offspring ID: Z = 2.194, P = 0.028). There was a significant positive correlation between HR in the same embryo measured on d 20 and on d 25 (r = 0.511, P = 0.004, N = 29) (Figure 6). An overview about the specific HR values in trial 1 and trial 2 is given in Table 1.

Table 1. Summary data for heart rate measurements in the sample population during trial age 1 (19.4 ± .5 days) and trial age 2 (24.4 ± .6 days). Inspection of the values shows that the overall HR patterns were comparable across the two sampling periods, which we also tested in the same embryo with correlation test (Figure 6).

HR values	Trial 1	Trial 2
Minimum	117	103
Percentile 25	198	194
Median	434.0	373.5
Mean ± SE	283 ± 16	279 ± 14
Percentile 75	361	371
Maximum	582	505

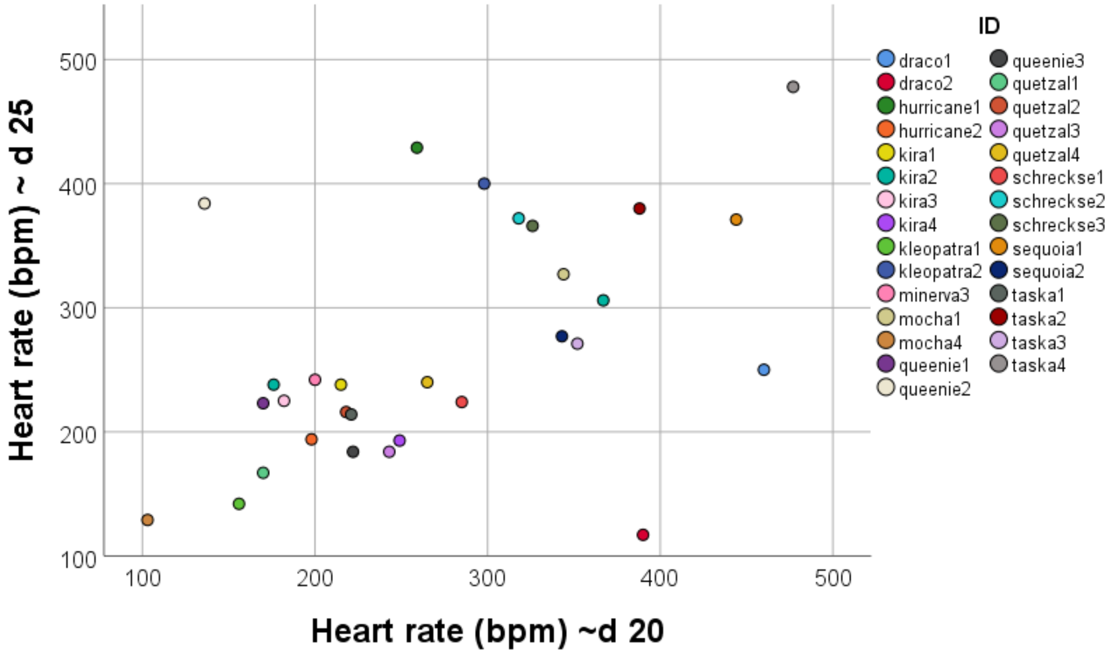

Figure 6: The association between heart rate on d 20 (range d 19 to d 22) and heart rate on d 25 (range d 23 to 26) per embryo. An embryo with lower heart rate on d 20 tended to have lower heart rate on d 25.

We explored individual variation in more detail and found a significant correlation between average HR and maximum HR per individual (r = 0.946, P = 0.001) (Figure 7, Table 2).

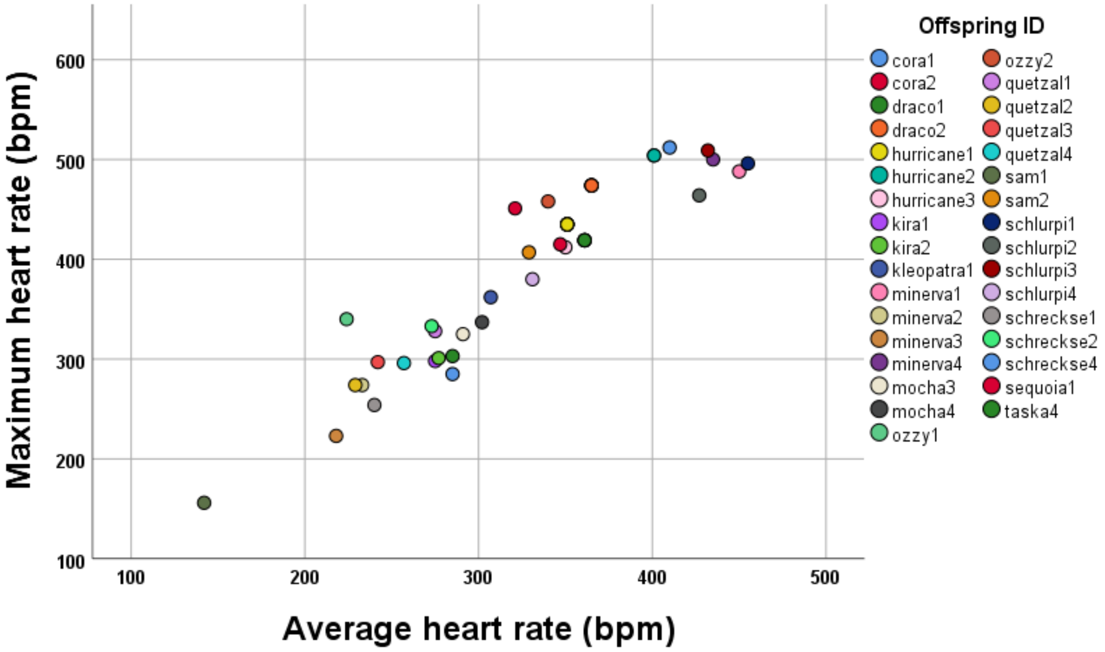

Figure 7: The positive association per embryo (N = 33) between an embryos average and maximum heart rate measured as beats per minute. HR was measured with a digital recorder and the average was calculated form measurements every 10 sec for 1 min per embryo.

Table 2. GLMM results for effects of ‘Egg ID’, ‘embryo age’, ‘trial age’ (ca d 19 versus ca d 24) during incubation, and the random factor ‘maximum heart rate’. The test statistic was t for fixed factors and Z for random factors. All convergence criteria were satisfied. Only Egg ID was a significant predictor of average heart rate during the final trimester of incubation in Northern Bald Ibis.

Effects on average heart rate (beats per minute)

Variables	Final Model	Coefficients	Estimate	Test statistic	P-value
Egg ID **Embryo age** **Trial age** **Maximum HR**	Intercept	108.791	119.4033	.911	.369
	Egg ID	2.407	33	32	**.007**
	Embryo age	-.085	7.0124	-.012	.990
	Trial age	11.399	37.7555	.302	.765
	Maximum HR	.557	.801	.694	.487

4.2. A positive increase in HR with nestling age

HR was lower and more variable during d 1 post-hatch but increased and remained stable between d 15 and d 30 (Figure 8).

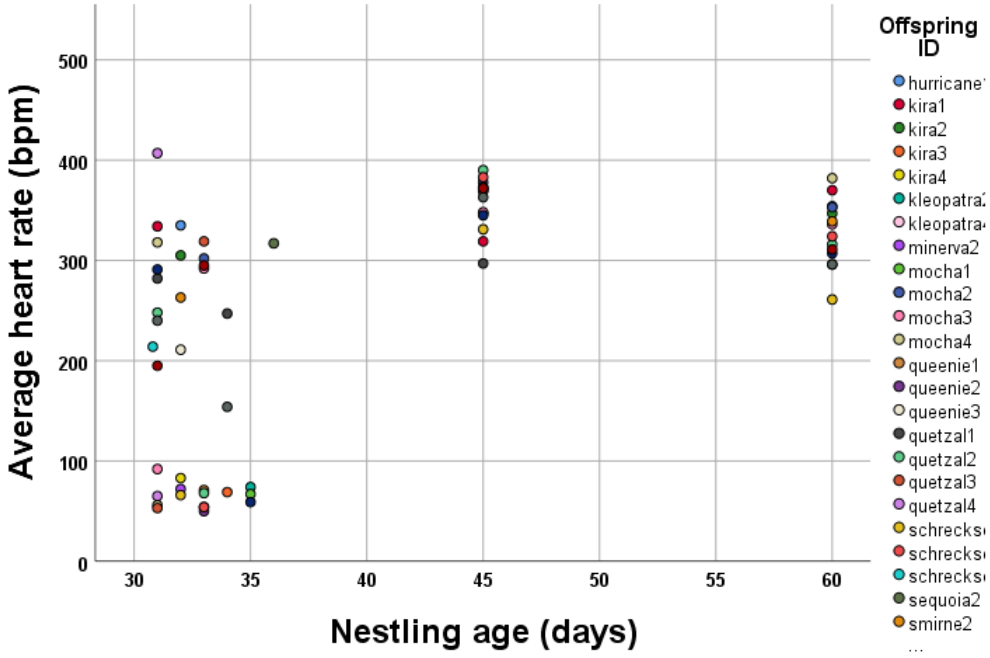

Figure 8: Post-hatch heart rate in 36 Northern Bald Ibis.

Using GLMM with average HR as the dependent variable and the fixed factor nestling age and the random factor nest ID, only <u>nestling age was significantly associated with post-hatch HR</u> (GLMM: age t = 4.380, P < 0.001; nest ID: Z = 1.388, P = 0.165) (Figure 9).

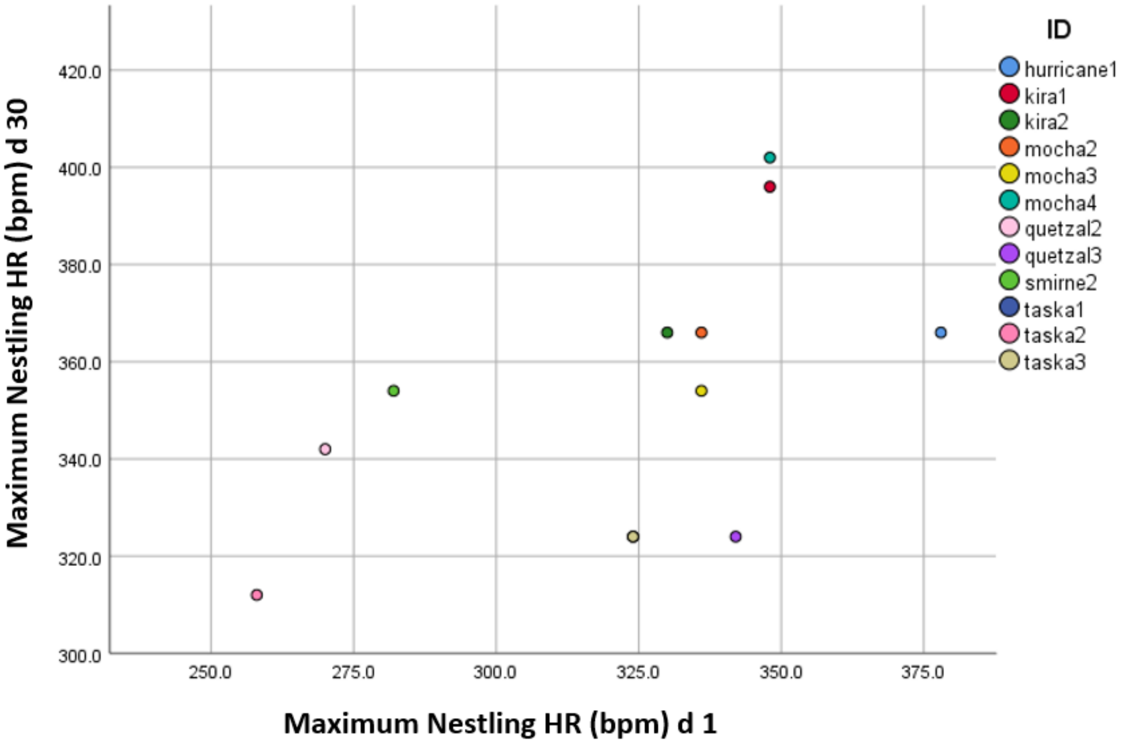

Figure 9: A positive correlation between heart rate in chicks in d 1 and d 30 post-hatch.

4.3. Embryo HR and nestling HR

<u>Prenatal HR predicted nestling HR at d 1 of hatch, but not d 15 or d 30 post-hatch.</u> Using average HR per embryo, there was a significant correlation between prenatal HR and d 1 nestling HR (r = 0.389, N = 26, P = 0.045) but not between prenatal HR and d 15 nestling HR (r = 0.119, N = 11, P = 0.713) or d 30 nestling HR (r = 0.501, N = 10, P = 0.116). Across the nestling phase, the correlation between d 1 nestling HR and d 30 nestling HR was not significant (r = 0.527, N = 11, P = 0.078).

4.4. Nestling HR and nestling activity

There was a significant negative effect of HR on number of steps during TI (defined as number of steps using available time calculated as 300 – ‘time to turn’; #steps/available time x 100) taken during the tonic immobility (TI) test (Figure 10). Nestlings with higher HR had fewer steps during TI. We used GLMM with number of steps during Tonic Immobility as the dependent variable, d 1 nestling HR and d 30 nestling HR as fixed factors and nestling ID as a random factor (d 1 nestling HR t = 1.065, P = 0.315; d 30 nestling HR t = -2.920, P = 0.017; nestling ID: Z = 1.061, P = 0.289; ID of mother Z = 1.211, P = 0.226).

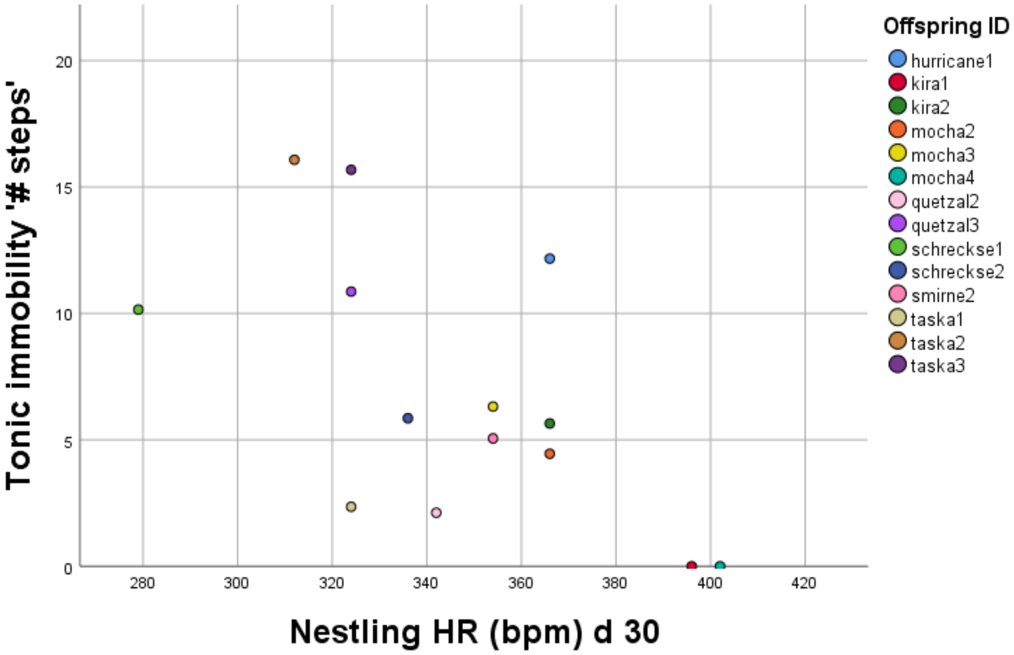

Figure 10: The significant negative effect of HR on number of steps during TI.

There was a significant positive effect of HR on number of steps during NA. Nestlings with higher HR had more steps during NA (Figure 11). We used GLMM with number of steps during Novel Arena test as the dependent variable, d 1 nestling HR and d 30 nestling HR as fixed factors, the number of fields explored in the novel arena test, and nestling ID and mother ID as random factors (d 1 nestling HR t = 0.232, P = 0.822; d 30 nestling HR t = 2.350, P = 0.047; nestling ID Z = 0.647, P = 0.517; ID of mother Z = 1.068, P = 0.286).

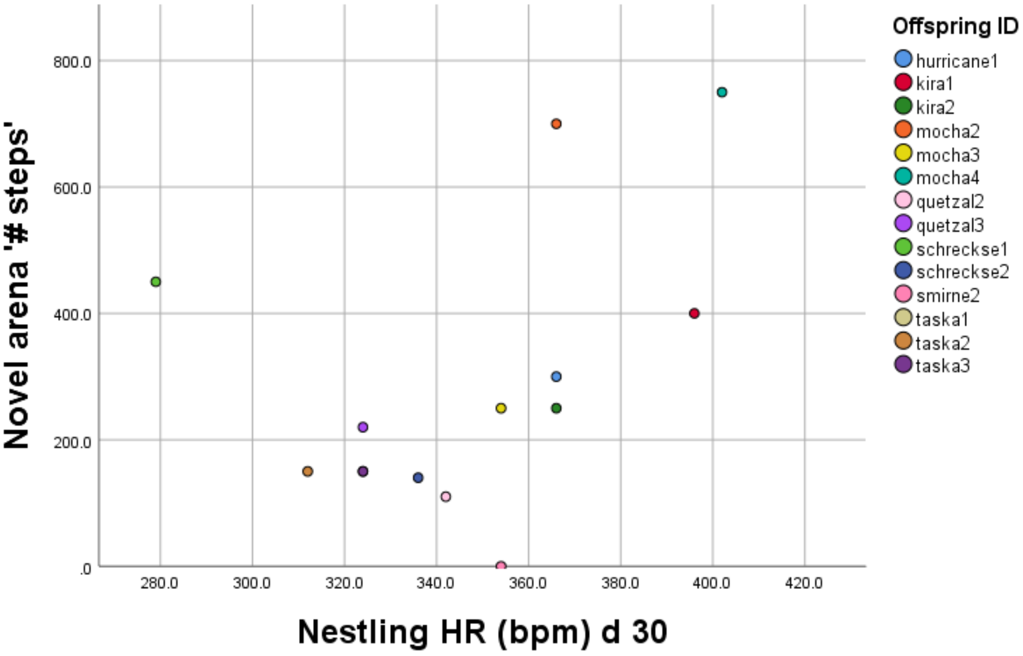

Figure 11: The significant positive effect of HR on number of steps during NA.

4.5. Nestling HR and nestling ‘personality’

There was no significant effect of HR on ‘time to turn’ during a TI test. We used GLMM with time to turn (sec) during Tonic Immobility as the dependent variable, d 1 nestling HR and d 30 nestling HR as fixed factors, and nestling RD as a random factor (d 1 nestling HR t = -0.624, P = 0.548; d 30 nestling HR t = 2.009, P = 0.075; nestling ID: Z = 1.061, P = 0.289; ID of mother Z = 0.319, P = 0.750).

There was no significant effect of HR on 'number of lines crossed' during a novel arena experiment. We used GLMM with number of lines crossed during Novel Arena test as the dependent variable, d 1 nestling HR and d 30 nestling HR as fixed factors and nestling RD as a random factor (d 1 nestling HR t = 0.651, P = 0.532; d 30 nestling HR t = 22.123, P = 0.063; nestling ID: Z = 1.061, P = 0.289; ID of mother Z = 0.740, P = 0.460).

4.6. Consistency in nestling personality across tests

There was a positive correlation between the 'time to turn' during TI and 'number of lines crossed' during NA (multiple regression: r = 0.521, N = 32, P = 0.004) (Figure 12). Nestlings that took longer to roll over during the TI test were more exploratory and crossed more lines during the novel arena test.

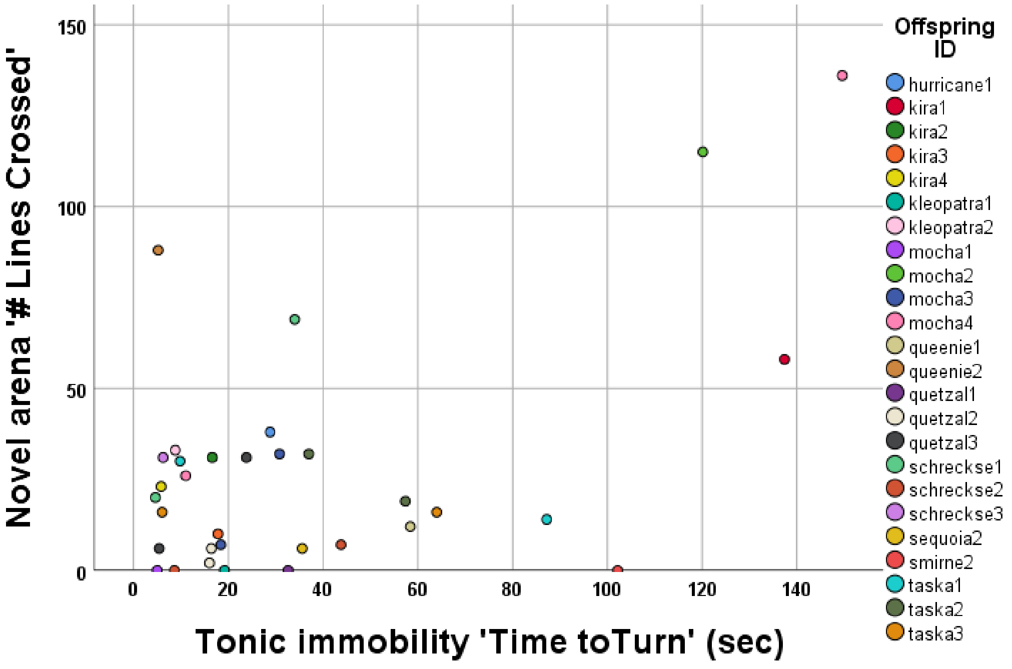

Figure 12: The positive correlation between the 'time to turn' during TI and 'number of lines crossed' during NA. Both tests were done with 33 individuals.

There was no significant correlation between number of steps during TI and NA (r = -0.200, N = 32, P = 0.264) (Figure 13).

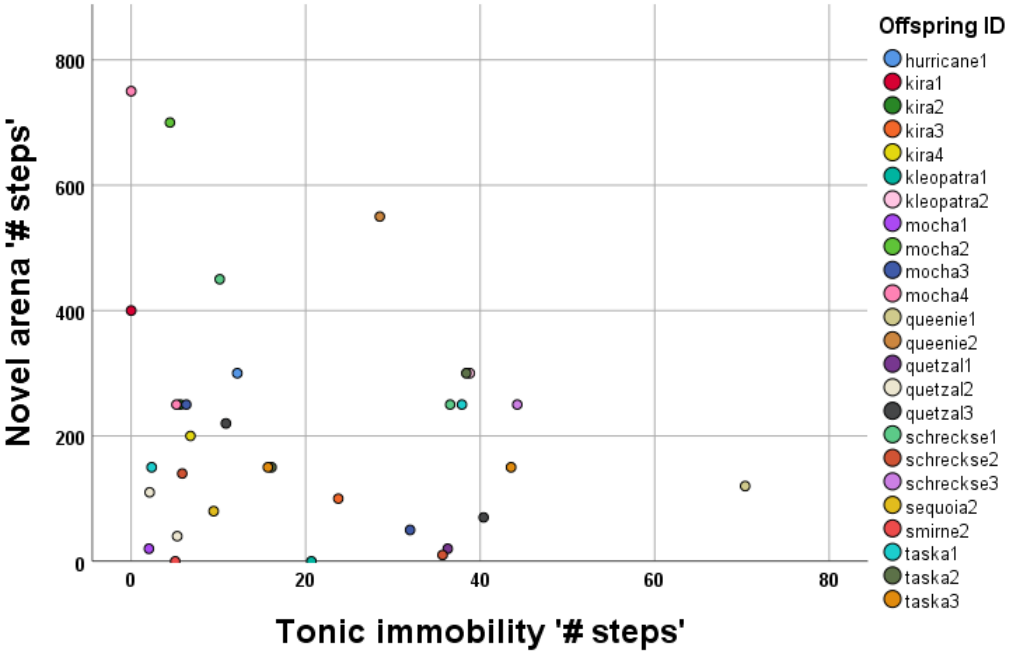

Figure 13: No significant correlation between the number of steps during tonic immobility and novel arena.

5. Discussion

This study found individual differences in prenatal metabolism and individual differences in post-hatch metabolism. There was an effect of post-hatch (but not prenatal) metabolism on movement activity during experimental trials, but no effect of metabolism on measures of fear release or exploration score per se. These findings underscore the fact that metabolism in birds may change across life stages (embryos, nestling) and that nestling metabolism predicts activity profiles but not fear release or exploration behaviour in standardised tests of individual behavioural differences. Specifically, we found that (i) embryo ID was significantly associated with prenatal HR, (ii) nestling age was significantly associated with post-hatch HR, (iii) prenatal HR predicted nestling HR at d 1 of hatch, but not d 15 or d 30 post-hatch, (iv) there was a significant negative effect of HR on number of steps during the tonic immobility test, (v) there was a significant positive effect of HR on number of steps during the novel arena test, (vi) there was no significant effect of HR on 'time to turn' during a tonic immobility test, (vii) there was no significant effect of HR on 'number of lines crossed' during a novel arena experiment, and, as expected if there are individually consistent differences in behavioural response, (viii) there was a positive correlation between the 'time to turn' during the tonic immobility test and 'number of lines crossed' during the novel arena test.

In this study, there was a change in baseline metabolism across life stages from egg to hatchling. The heartrate did not increase with age during the embryo stage and did not correlate at an individual level with baseline metabolism after d 15 post-hatch. Interestingly, the prenatal heartrate predicted the nestling heartrate at day 1 of hatch at an individual level. One possible way to explain this is, that day 1 hatchlings were still changing to their new metabolic phenotype. The environment around the individuals and the individuals themselves are in transformation. In the moment between pre- and post-hatching, the embryo has to change among living inside an egg to a way of life outside the eggshell as a nestling. This suggests that prenatal heartrate may be more similar to the nestling heartrate at day 1, because there the transition from one stage of life to the next just begins. This situation is not only occurring in birds and other oviparous individuals but may also occur in individuals generally that go through different stages during life. For example, in mammals, the first phases of development in the uterus may require different metabolic phenotypes than after birth. In such cases, there could be selection for different metabolic phenotypes for different life stages even within the same individual. There has been limited research into possible variation in metabolism within individuals across different life stages. All studies to date that we are aware of investigated the impact of metabolism on other variables like behaviour or development or the progression of metabolism within one life stage, but disregarding the fact that metabolism could change across life stages (Tazawa, Watanabe et al. 1994, McCowan and Griffith 2014, Sheldon, Cronin et al. 2018). In this regard, Sheldon et al. (2018) specifically mention in their study that the missing measurement of post-natal metabolic rate is a limitation of their work (Sheldon and Griffith 2018). In contrast in our study, we used the chance to measure both pre-hatch and post-hatch basal metabolic rate and so gained interesting information about changes in metabolism across life-stages that raises questions about the importance and biological significance of such changes within an individual.

In this study, we found positive and negative patterns of association between nestling heartrate and the activity level of the nestlings. There was a negative correlation between nestling heartrate and the number of steps during the tonic immobility test. And there was a positive correlation between nestling heart rate and number of steps during the novel arena. This opposite finding for heart rate and activity level between activity during tonic immobility and novel arena tests is especially surprising because we found a positive correlation between time to fear release response during the tonic immobility test and the number of fields explored in the novel arena tests. According to the pace of life syndrome, metabolism could be the causative factor for activity level that affects growth rate and food intake (Biro and Stamps 2008, Reale, Garant et al. 2010), but the opposite patterns found here across tests does not provide consistent support for this view. Not many other tests of individual behavioural difference have separately tested the role of activity from other behavioural response measures. In North American red squirrels (*Tamiasciurus hudsonicus*), activity levels of the mothers were shown to influence offspring growth in the nest. The survival of the juvenile squirrels did not depend on the activity of the mother, but on her aggressiveness (Boon, Réale et al. 2008). Koolhaas et al. (1999) showed a correlation between activity level and boldness in a range of species (e.g., Great tit *Parus major,* Mouse *Mus musculusdomesticus*), which is consistent with the pattern we found if one accepts that boldness and exploration occur on the same axis (Koolhaas, Korte et al. 1999). Perhaps fear release occurs on a different behavioural axis and is therefore differently affected by metabolism. Overall, connections between metabolism and pace-of-life and their impacts on activity level and individual behavioural differences reveal the importance of particular pathways that may be specific to particular behavioural axes.

In the Northern Bald Ibis we studied, there was a positive correlation between the ‘time to turn’ during the tonic immobility test and the ‘number of lines crossed’ while participating in the novel arena. Thus, we found consistency in behavioural response profiles across different behavioural response to external stimuli. However, it is possible that the two tests were measuring different behavioural axes (aggression and aggression avoidance versus boldness/exploration). On the one hand, in the tonic immobility test, we measured fear release (and the activity level of the individuals), which could be a measure of aggression avoidance. On the other hand, in the novel arena, we measured exploration behaviour (and the activity level). In summary, nestlings that took longer to roll over during the tonic immobility test (a longer time before ‘fear release’) were more exploratory and crossed more lines during the novel arena test, which suggests that there is a positive correlation between fear release (possible proxy for low aggression) and exploratory behaviour. Previous studies had mixed results about the association between fear release and exploration behaviour. For example, Budaev (1997) showed in a study with European wrasses (*Symphodus ocellatus*) that exploration of individuals was not associated with fear or escape behaviour. But in Trinidadian guppies (*Poecilia reticulata*) (Budaev 1997). Budaev and Zhuikov (1998) found that less exploratory guppies had higher fearfulness (longer time to emerge) (Budaev and Zhuikov 1998). This is opposite to the pattern we found. Other studies have also found that more explorative (and active) animals had less fear response (Smith and Blumstein 2010). Interestingly, these behavioural axes tend to correlate in predictable ways. Bell (2005) suggested that despite correlations within individuals in many studies, the correlation between individual behavioural response often differs between populations of the same species, which they reviewed between boldness and aggression. It is suggested that behavioural syndromes (suites of correlated behaviours) may be free to evolve independently and don’t always act as evolutionary constraints (Bell 2005).

To my knowledge this is the first study to measure prenatal heartrate in relation to post-hatch activity and personality, which is very exciting and motivating. From the results, Northern Bald Ibis have a wide range of prenatal physiological phenotypes (large variance in individual prenatal HR) that did correlate with individual HR on d1 of hatch but did not correlate with post-hatch HR after d15. For future research, one could measure fitness benefits that may arise from having a particular prenatal HR (more rapid consumption of limiting resources in the egg? First to hatch?) or a particular post-hatch HR (faster movement to new food sources?). One could use experimental approaches such as experimentally changing the laying date within clutches to test if later laid eggs are more penalised in hatching success if they have a slower HR for example. Therefore, later laid eggs may have a fitness benefit if they have faster HR and earlier laid eggs may benefit if they have lower HR if hatching synchrony is beneficial. Or perhaps selection occurs on d1 post-hatch if begging intensity correlates with prenatal HR to give hatchlings a head start over their nest mates.

With regard to the pace-of-life syndrome, because post-hatch HR predicted activity level but not personality response scores, the pace-of-life syndrome is only partially supported by my research. This study shows some links between physiology and movement activity level, and also showed that behaviour, in our case most notably fear release and exploration, may occur independently of metabolism. For further research, it would be exciting, to amplify the knowledge about behaviour, metabolism and physiology with tests in other systems to examine the generality of this pattern.

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