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Embryonic heart rate predicts pre-natal development rate, but is not related to post-natal growth rate or activity level in the zebra finch (*Taeniopygia guttata*)

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Abstract

Inter-individual variation in behaviour has been the focus of much recent work, yet the underlying mechanisms that cause and maintain this variation are unclear. It has been proposed that consistent individual differences in metabolism could be related to inter-individual variation in behaviour and development throughout life. Here, we tested this idea in the zebra finch (*Taeniopygia guttata*), by investigating whether embryonic heart rate (a proxy for metabolic rate), is associated with pre-natal developmental rates, post-natal activity levels, and post-natal growth rates. Embryonic heart rate and post-natal activity level were significantly repeatable throughout an individual’s development, such that consistent individual differences in these traits could be distinguished. We detected a significant, negative relationship between embryonic heart rate and incubation duration. However, we did not detect any relationship between embryonic heart rate, post-natal activity levels or post-natal growth rates. Our findings are significant because they identify consistent individual differences in embryonic metabolic phenotype, and post-natal traits. However, we were unable to identify any correlation between the pre-, and post-natal phenotypes suggesting that either intrinsic metabolic differences do not persist across the developmental boundary of hatching, or that such differences become obscured by parental or environmental effects after hatching. Our findings raise a number of questions about possible selection on metabolic phenotypes, both before and after hatching, and why developmental trajectories before and after hatching are apparently unlinked.

Key words: Metabolic rate, consistent individual differences, behavior, incubation duration, ontogenic boundaries, inter-individual variation.

Introduction

Traits that differ across individuals but are temporally and contextually consistent within individuals are known as 'consistent individual differences' (Biro and Stamps, 2010). The existence of consistent individual differences in behaviour, (commonly referred to as personality) and developmental rates are well established across a wide range of species (Ronning, et al, 2005; McCowan et al, 2015; White et al, 2016). Relatively little is known about how/when these consistent differences emerge during development, or how they change across different life stages (Wuerz and Kruger, 2015; Trillmich et al, 2015). However, in a number of taxa, it has been demonstrated that consistent individual differences can persist across different ontogenic boundaries (Niemela et al, 2012; Sprenger et al, 2012; Wilson and Krause, 2012b; David et al, 2012).

It has recently been proposed that consistent individual differences in energy metabolism could promote and maintain consistent individual differences in behaviour and developmental rate through life (Biro and Stamps, 2010; Bouwhuis et al, 2013). Metabolic rate has the potential to influence a suite of developmental and behavioural traits due to the fact that energy, and the processing of energy, is fundamental in fueling the physiological processes that generate activity and growth (Schmidt-Nielson, 1991). Consistent individual differences in metabolic rate have been identified in a diverse range of animal taxa (Nespolo and Franco, 2007; Moe et al, 2009; Broggi et al, 2009), yet, a coherent framework linking behaviour, developmental rate, and energy metabolism remains unclear (Biro and Stamps, 2010; Careau et al, 2008).

The limited research available that has linked metabolism with behavioural and developmental variation has largely focused on associations within the same life stage (e.g.

whether variation in *adult* metabolism co-varies with variation in *adult* activity level) (Vezina et al, 2006; Mathot et al, 2009; White et al, 2016). However, many taxa have ontogenic boundaries separating life-stages that differ dramatically. Despite this, the extent to which metabolic rate from a previous life stage can influence the phenotype of a later life stage (e.g. whether variation in *embryonic* metabolism co-varies with variation in *adult* activity level) has received relatively little attention (Hall et al, 2016). This idea is worthy of consideration because individual variation in behaviour and developmental rates might be expected to shift between different life stages in association with the often profound changes in environments, physical constraints, and life history priorities across ontogenic boundaries.

Here, we explore the potential for consistent individual differences in embryonic metabolism to affect consistent individual differences in behaviour and developmental rates in the nestling stage in wild zebra finches (*Taeniopygia guttata*). This work extends an earlier study that demonstrated the correlation between individual differences in activity levels across two life stages (nestling and adult) in a captive population of this species (McCowan and Griffith, 2014). We use embryonic heart rate as a proxy for embryonic metabolism due to its strong, positive correlation with oxygen consumption (metabolic expenditure) in other oviparous species (Du et al. 2010b, Piercy et al, 2015; Ward et al, 2002; Owen, 1969; Butler et al., 2004; Dechmann et al., 2011). We use activity level as a proxy for nestling behaviour, as in the earlier study (McCowan and Griffith, 2014).

Embryonic heart rate can be measured non-invasively using a relatively new device - the Buddy digital egg monitor (Vetronic Services, Devon, UK) (Rivera et al, 2018, Sheldon et al. 2017a). The acquisition of short-term heart rate measurements using the digital egg monitor has been used to demonstrate plasticity in embryonic heart rate in response to a range of environmental and social cues (Du et al. 2010a; Du and Shine, 2010; Colombelli-Négrel et al. 2014). However, use of the egg monitor for characterizing consistent individual

differences in heart rate (i.e. the extent to which some individuals have consistently, relatively low heart rates and others comparatively high heart rates throughout development) is limited to date. Consequently, before we explore the association between embryonic heart rate and activity/development, we first aim to assess whether heart rate measures from the digital egg monitor are repeatable over early and late stages of pre-natal development, such that they can be used to provide a proxy for consistent individual differences in embryonic metabolic rates (embryonic heart rate has previously been used as a proxy for embryonic metabolic rate due to the its relationship with embryonic development time in both reptiles (Du et al. 2009), and birds (Vedder et al. 2017)). The specific goals of our study were (i) to characterize the extent to which both embryonic heart rate and nestling activity level measures are repeatable throughout development and (ii) investigate the extent to which variation in embryonic heart rate is associated with embryonic development rate, nestling growth rate and nestling activity levels.

Methods

Data collection

Fieldwork was conducted at Fowlers Gap Arid Zone Research Station during the main part of the Austral breeding season: September-November 2016. We quantified heart rate variation of zebra finch embryos from eggs that were laid in nest boxes (details on nest boxes and study site given in Griffith et al. 2008), and active nests were monitored daily to ascertain the laying date, clutch size and the date on which incubation is likely to have started (further details below). Embryonic heart rates were taken in a shaded position close to the natal nest box using a digital egg monitor (Buddy, Vetronic Services, UK). This device generates heart rate data by tracking infra-red light absorption changes owing to embryonic blood flow. To follow an embryo through development, we labelled each egg with a unique

ID (with a fine soft-tipped permanent marker pen). On the day of measure, the entire clutch was removed from the nest and placed in a standardised container; a 'soft-box' filled with cotton wool that shaded the eggs from the sun and wind and reduced the risk of eggs cracking. On each day of sampling, we took three repeated measures of each embryo's heart rate (where possible) over a ~ 2 minute period and took the average of these measures to characterize heart rate for that day of development. As the time out of the nest has been shown to exert significant effects on the heart rate of zebra finch embryos (Sheldon et al, 2017a), we excluded singular heart rate measures that did not allow us to control for effects of time out of the nest (Table 1).

We took heart rate measures at 114 zebra finch nests (for information on sample sizes, see Table 1), and aimed to take heart rate measures at six time points across pre-natal development (from day ~ 5 to ~ day 12 of incubation) (zebra finch eggs are expected to hatch at day ~12 of incubation, and the heart rate monitor cannot detect heart rate in zebra finch embryos before ~ day 5 of embryonic development (Sheldon et al, 2017a). We attempted to take three heart rate measures for both 'early' (before and including day 8 of incubation), and 'late' (after and including day 9 of incubation) pre-natal development. However, in some cases the monitor was unable to detect a reliable heart rate when the embryo was moving (see Sheldon et al 2017a and Table 1), and the actual number of heart rate measures ranged from 2 to 4 at each stage (early and late) of development. We excluded embryos with less than two heart rates at each (early and late) stage of development, so that we could attain an average heart rate value for each embryo for each half of development. We calculated the average heart rate during the early and late part of development so that we could clearly explore the correlation between the two in a simple parametric analysis that permitted the visualisation of the raw data (shown in Figure 1). This approach was specified in our pre-registered analysis plan (Sheldon and Griffith 2017b).

Short term (<30 seconds) heart rate measures collected with the Buddy heart rate monitor are extremely sensitive to environmental fluctuations, and capture a significant degree of plasticity in response to biotic and abiotic conditions prior to, and during sampling (Sheldon et al, 2017a). 'One-off', short-term heart rate measures in wild birds thus have limited value for predicting consistent individual differences in the metabolic rates of embryos measured under different sampling conditions. Use of the egg monitor for long-term heart rate monitoring (10's of minutes) is also problematic, because the infrared energy that the instrument emits heats the egg, and potentially interfere with embryonic development (Sartori et al. 2015). As we used wild birds in their natural environment in our study, we were unable to standardise the pre-natal sampling conditions (i.e. put all eggs in an incubator of the same temperature). However, here we tested a potentially overlooked solution to this problem; we acquired multiple, short-term heart rate measures across an individual's development to test whether these measures were significantly repeatable. If so, we assumed these short-term measures would represent a useful estimate of an individual's 'metabolic rate' (Du et al. 2010b, Piercy et al, 2015).

In the wild, we have previously shown that parent zebra finches only initiate incubation on the day that the last egg was laid (Gilby, et al 2013). Consequently, we used the day of the last laid egg to represent the first day of embryonic development (although the earliest laid eggs may start to develop before this, given the high ambient temperatures (Griffith, et al. 2016)). It has previously been estimated that zebra finch embryos take ~12 days to hatch after the onset of incubation, and we used this estimation to predict clutch hatch date. Two days prior to a clutch's estimated hatch date, we monitored the nest three times per day between 06.00-17.00hrs; our first nest check was at ~06.00hrs, our second nest check was at ~11.30hrs and our third nest check was at ~17.00hrs. We used these nest check time points to estimate the number of hours the egg had been incubated before it hatched. We did

not include in our sample eggs that hatched between 17.00 and 06.00hrs (unless the chicks were still wet at 06.00 which indicated a very recent hatch at the time of first check in the morning).

After hatching, each chick had a small, unique patch of down feathers trimmed for post-natal identification within the nest. In cases where multiple eggs hatched synchronously we were unable to identify which chick hatched from which egg (see Table 1). These individuals were not used to investigate the relationship between embryonic heart rate and post-natal growth rates and activity levels, however they were used to investigate the relationship between embryonic heart rate and developmental rate (Table 1). After hatching, we visited the nest at day 3 and day 11 of post-natal development and collected measures of nestling mass and tarsus length using a Pesola spring balance, and digital callipers, respectively. Mass was unable to be accurately measured for a portion of day 3 chicks, as the balances were unstable due to the windy conditions and the low mass of young nestlings (see Table 1).

On days 5 and 7 (after hatching), we visited the nest to conduct our assay of nestling activity. This activity assay involved removing the hatchling from the nest, and placing it in a shaded open-box 'arena' (approximately 15cm x 15cm) that was divided equally into 9 marked squares. A stopwatch was started after the chick had settled in the center square of the arena for 20 seconds. The behaviour of the chick was then monitored every 5 seconds for 60 seconds. We recorded two behaviours - movement and begging (these were binary observations; yes or no at each 5 second time point), and the number of times the whole head of the chick entered a different square in the box. Three proxies for individual activity level were thus attained, referred to hereafter as; 'time spent moving', 'time spent begging' and 'number of squares reached'.

Daily atmospheric temperature data were obtained from the Australian Bureau of Meteorology's Automated Weather Station at Fowlers Gap, located within 20 km of the study sites. Temperature was averaged over embryonic development (from the first day of incubation to the day of hatch, for each nest in the sample).

Statistical analysis

Consistent individual differences in embryonic heart rate and nestling activity levels

To detect the existence of consistent individual differences in embryonic heart rate, we evaluated whether multiple, short-term, heart rate measures were repeatable over early and late pre-natal development. We averaged the embryonic heart rate measures attained from early (day 5-8 of incubation), and late (day 9 of incubation – hatching day) pre-natal development. In our analyses we only included individuals that had more than one heart rate measure during each period of development to attain an average measure for each period (see Table 1). For embryos that had an average heart rate for both the first and second half of development (total number of individuals for which there was data in the 1st and 2nd half of development, N=98), we assessed whether these averages were repeatable using a linear mixed model (LMM), with Nest ID as a random factor to account for between clutch variation in developmental conditions (that may influence heart rate across development). LMMs were run using the package lmer in R (Bates et al. 2015) with the package lmerTest (Kuznetosova et al. 2016) to calculate degrees of freedom and *p*-values. Statistics were run in R version 3.3.1 (Core Development Team, 2015).

For each measure of activity: 'time spent moving', 'time spent begging' and 'number of squares reached', we tested whether the data were normally distributed using a Shapiro-Wilk Test. A separate LMM was then used to test for repeatability between the activity measures at day 5 and day 7, again Nest ID was used as a random factor. The measure of

activity found to be the most repeatable across development was used as our only representation of activity for the analysis relating activity to metabolism. This was to reduce the number of statistical tests conducted and the likelihood of a type one error.

Post-natal growth rate

A body condition index was calculated as the residuals from a least-squares linear regression analysis between body mass (dependent variable) and tarsus length. Growth rate was considered as the increase in residual mass per day (i.e. the difference between day 3 and day 11 measures, divided by 8). The change in body condition per day will be referred to as 'growth rate' hereafter.

Relationship between embryonic heart rate, incubation duration, activity and growth rate

To model the effects of the fixed factor 'embryonic heart rate' on the response variables 'activity level', 'incubation length' (number of hours from first day of incubation to the estimated hour of hatching (see 'data collection' for estimations)), and 'growth rate', we ran three separate LMMs. In each model, we accounted for 'ambient incubation' by featuring average maximum ambient temperature during an individual's development as a fixed effect. We also included brood size as an additional fixed effect in the two linear models that could be affected by the conditions of the post-natal environment (response variables: growth rate and activity level (brood size was not considered to affect pre-natal response variables, as brood and clutch size often differed due pre-natal mortalities)). We also accounted for familial effects by including the identity of the chick's natal nest as a random factor in all models. We calculated marginal R^2 and ICC values for all LMM using the method described in Nakagawa & Schielzeth (2013). We also used a Pearson's correlation analysis to test for a

relationship between incubation length (i.e. pre-natal developmental rate) and post-natal growth rate.

Pre-registration with the Open Science Framework

We pre-registered our analyses with the Open Science Framework which facilitates reproducibility and open collaboration in science research (Foster and Deardorff, 2017). Our pre-registration: Sheldon and Griffith (2017b), was carried out to limit the number of analyses conducted and to validate our commitment to testing a priori hypotheses. Our methods are consistent with this pre-registration (Sheldon and Griffith 2017b).

Results

Embryonic heart rate measures were normally distributed: (Shapiro-Wilk=0.955, p=0.071). We found a significant positive relationship between heart rate averages from the first and second half of embryonic development, after controlling for variation arising from clutch identity (estimate = 0.99, t_{97} = 6.58, SE=0.25, p<0.001, R^2=0.316, ICC=0.34). The model explained 55% of the variance in heart rate in late development; heart rate early in development explained 32% of this variance and nest ID explained 23% of the variance explained by the model. Given that heart rates are significantly repeatable across development, we used the heart rate from the second half of detectable development for the final estimation of individual embryonic heart rate (we chose data from the second half of development because we had a larger sample size for this measure (Table 1)).

Data for the time spent moving was the only measure of activity that was normally distributed (Shapiro-Wilk=0.974, p=0.437) (Table 2), consequently we used the individual average measure of this behavioural parameter to quantify individual activity. We found a significant positive relationship between activity level at day 5 and day 7 of post-natal

development, after controlling for the variation arising from clutch identity (i.e. between nest differences) (estimate = 0.63, t_{147} = 8.96, SE=0.07, p<0.001, R^2= 0.35, ICC=0.17). The model explained 46% of variance in activity later in development; activity level at day 5 of development explained 35% of this variance and nest ID explained 11% of the variance.

We found a significant negative relationship between embryonic heart rate and incubation duration (Table 3c, Figure 3), such that embryos with a consistently lower heart rate took a longer time to develop (before they hatched). The LMM model explained 62% of the variance in the length of incubation before hatching; heart rate and temperature explained 17% of this variance, however natal nest ID explained more (45%) of the variance in development time (Table 3c).

Using a linear mixed models we found no effect of embryonic heart rate, ambient temperature, or brood size on nestling growth rates (see Table 3a), although developmental nest ID explained a significant proportion (60%) of variance in nestling growth rates (Table 3a). Using another LMM we found no significant effect of embryonic heart rate, temperature, or brood size on the time spent moving by a nestling during the trial (Table 3b). Again, developmental nest ID explained a significant proportion (27%) of variance in the data from the nestling movement assay (see Table 3b). Finally, there was no correlation between the duration of development in the egg and nestling growth rate (Pearson's correlation, r=0.049, p=0.676, n=81).

Discussion

Behaviour and development are particularly responsive to changes in social and environmental cues throughout life, and indeed in our study we found significant influences of development nest identity on growth rates and nestling activity levels. Whilst we found clear evidence for consistent individual differences in our proxy for embryonic metabolic rate

(embryonic heart rate), we found no evidence that this individual characteristic influenced the behavior or growth of nestlings in their next phase of development (as a nestling after hatching) (a result that differed from our a priori hypotheses (Sheldon and Griffith 2017b)). Interestingly, we also failed to detect any relationship between pre-natal developmental rate and post-natal growth rate. We are reasonably confident that embryonic heart rate is a good proxy for metabolic rate, because those individuals with a high heart rate developed at a faster rate during pre-natal development than those with relatively low heart rates (an organism's metabolic rate limits the rate at which it processes energy, which in turn determines its development rate (Rosenfeld, 2014; Ton and Martin, 2016; McCarthy, 2000)).

Incubation temperature has a strong positive effect on embryonic heart rate and a strong negative effect on embryonic development time (Du et al. 2009 Vedder et al. 2017). Consequently, the relationship between embryonic heart rate and the duration of development in the egg (incubation duration before hatching), might be confounded by ambient incubation (i.e. ambient conditions might be hot enough to trigger development of some embryos before the clutch is complete and parental incubation begins (i.e. Griffith et al 2016)). Whilst this is certainly a possibility that we are unable to completely exclude with the present data, in our previous work, conducted during a hotter period of the year, we found that ambient incubation accelerated the growth of the first laid eggs by only 0.9 days (Griffith et al 2016). As a result, we are confident that the relationship between embryonic heart rate and the duration of incubation is largely unconfounded by this. Differences in the temperature of the pre-natal environment can also be influenced by differences in parental incubation behaviours. Future work should account for differences in nest incubation temperatures (as well as ambient incubation temperatures). This will allow us to decipher the extent to which the negative correlation between heart rate and incubation duration, and the repeatability of

embryonic heart rate found in this study are explained by intrinsic variation or temperature induced variation.

Together, our results suggest that consistent individual differences in metabolic rate do not persist across ontogenic boundaries, or if they do, their relationship with behaviour and developmental rate varies between pre- and post-natal life stages. This suggestion is consistent with the hypothesis that different selection pressures and priorities at different life stages promote shifts in the regulation of behaviour and development (White et al, 2016). However, we acknowledge that in our study of wild, 'desert dwelling' zebra finches, we were unable to control for the effects of ambient incubation (Griffith et al, 2016), and differential provisioning within and between nests that likely influenced our estimations of 'intrinsic' pre-natal and post-natal developmental rate. Indeed, embryonic metabolism and growth rates are known to be correlated in commercial poultry (where food intake is more controlled) (Tona et al, 2004). Future work on birds developing under controlled conditions are thus necessary to confirm whether the effects of embryonic metabolic rate on growth persist across ontogenic boundaries.

A clear framework linking metabolism, life history and behaviour remains to be fully established (Biro and Stamps, 2010), and this is reflected by the inconsistent findings of studies that support (Hall et al, 2015; McCowan and Griffith, 2014) and do not support (Petelle et al, 2013; Bell et al, 2004) the maintenance of consistent individual differences across developmental boundaries. A limitation of our study was our failure to measure levels of pre-natal activity levels, and post-natal metabolic rates. Thus the degree to which metabolism affects variation in behaviour in the same ontogenic stage, and the degree to which metabolic variation persists across ontogenic stages, remains to be thoroughly examined in the zebra finch. Although more conventional methods for gathering data on metabolic rates could be used for both embryos and nestlings (e.g. respirometric measures

(Lierz er al, 2006)), such methods are rather intensive and difficult to achieve with large sample sizes. Assessing whether metabolic rates influence activity level within the same ontogenic stage, and whether metabolic rates persist across hatching, will allow us to clarify whether our results indicate that (i) metabolism is not related to activity levels in the zebra finch, or (ii) metabolism is related to activity levels within the same ontogenetic stage (as in other avian species, Ton and Martin (2016)). Addressing these limitations will not only enable us to better interpret the results of our study, but also help to more generally clarify the role of metabolic rate in maintaining consistent individual differences across an individual's life.

Although the link between inter-individual variation and metabolic rate requires further investigation, our study has demonstrated the ability of the Buddy heart rate monitor to detect inter-individual differences in heart rate, and confirmed a degree of individual consistency in embryonic heart rate over time. These findings suggest that multiple, short-term heart rate measures are able to capture inter-individual variation in embryonic metabolism, despite the sensitivity of heart rates to a wide range of environmental and sampling variables (Sheldon et al. 2017a). This finding opens up new opportunities for this device to explore the effects of metabolic variation on animal personality and the pace of life-history traits.

In conclusion, our results suggest that inter-individual variation in metabolism is related to development within the same life stage, but further work is necessary to clarify whether this relationship persists across the important development milestone of hatching in a developing bird. This finding contrasts with earlier work on the same species across the next ontogenetic boundary (nestling to adult) in which it was shown that variation in activity levels in the nestling stage persisted through to adulthood (McCowan & Griffith 2014). The contrasting results of this current study and the previous one may reflect differences in resource acquisition between different life stages: development and metabolism in an egg is

largely determined by incubation temperature and the fixed resources and hormones allocated to the egg by the mother when it is created, but upon hatching, a nestlings' development can dramatically change trajectory dependent on its ability to acquire resources. We did find effects of the family ID on pre- and post-natal developmental rate, and activity levels. Parental influences such as egg resource allocation (Buchanan et al, 2001), nest environment (Sheldon et al, 2017a), and genetic make-up (Sadowska et al, 2009) have previously been shown to effect metabolic rate, which may underlie the familial effects detected in our study. Further work to clarify the causes of metabolic variation would thus be useful in understanding the proximate role of metabolic rate in maintaining consistent individual differences across different stages in an individual's life.

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Author contributions: E.L.S. and S.C.G. formulated the research, designed the methodology, and wrote the paper. E.L.S. conducted the research in the field, and analyzed the data.

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Tables

Table 1. An overview of sample sizes in our study. Heart rate (HR) measures with <2 repeats per sampling session were excluded from the study to mitigate the effects of time out of the nest on HR.

	Eggs/chicks	**Families**
Initial sample size (viable clutches, not abandoned)	518	114
≥2 HR measures at 1st ½ detectable development	202	58
≥2 HR measures at 2nd ½ detectable development	288	74
≥2 HR measures at 1st & 2nd ½ detectable development	98	37
Activity assay at day5 & day7	148	51
Chick & egg ID	62	26
Heart Rate & Chick ID & Body condition (day 3 & 11)	57	23
Heart Rate & Chick ID & Activity Level	61	26

Table 2. Normal distribution values for the three activity measures, (normally distributed measures are indicated with an (*)).

Activity Measure	**Shapiro-Wilk**	***p***
(%) time spent moving	0.974	0.437*
(%) time spent begging	0.870	<0.001
Number of squares reached	0.531	<0.001

Table 3: The effect of heart rate, brood size and temperature on a) growth rate, b) percentage of time spent moving, and c) incubation duration. The conditional R^2 represents the variance explained for the total model, and the marginal R^2 represents the variance explained by the fixed effects. The ICC value represents the degree of similarity between body condition values from the same family (an ICC close to 1 indicates high similarity).

***a)* Growth rate**	**Estimate**	**SE**	**d.f.**	**t-value**	**p-value**
Intercept	0.38	0.05	35.21	7.38	<0.001
Heart rate	-0.01	<0.01	32.07	-0.56	0.578
Temperature	<0.01	<0.01	27.75	1.26	0.217
Brood size	-0.01	<0.01	21.42	-0.98	0.338
	Variance	**SD**	***n***		**ICC**
Marginal R^2	0.07				
Developmental nest	<0.01	0.03	23		0.63
Residual	<0.01	0.03	57	Conditional R^2	0.67

***b)* Activity Level**	**Estimate**	**SE**	**d.f**	**t-value**	**p-value**
Intercept	17.46	27.91	48.52	0.63	0.534
Heart rate	-0.11	0.11	42.17	-1.04	0.306
Temperature	0.99	0.65	51.98	1.53	0.133
Brood size	1.40	3.46	32.80	0.41	0.688
	Variance	**SD**	***n***		**ICC**
Marginal R^2	0.06				
Developmental nest	146.20	12.09	26		0.28
Residual	364.20	19.08	61	Conditional R^2	0.33

c) **Incubation duration**	**Estimate**	**SE**	**d.f**	**t-value**	**p-value**
Intercept	357.90	16.49	57.26	21.71	<0.001*
Heart rate	-0.12	0.04	92.73	-2.75	0.007*
Temperature	-1.02	0.70	62.13	-1.45	0.153
	Variance	**SD**	***n***		**ICC**
Marginal R^2	0.17				
Natal nest	256.90	16.03	74		0.56
Residual	201.10	14.18	288	Conditional R^2	0.62

Figures

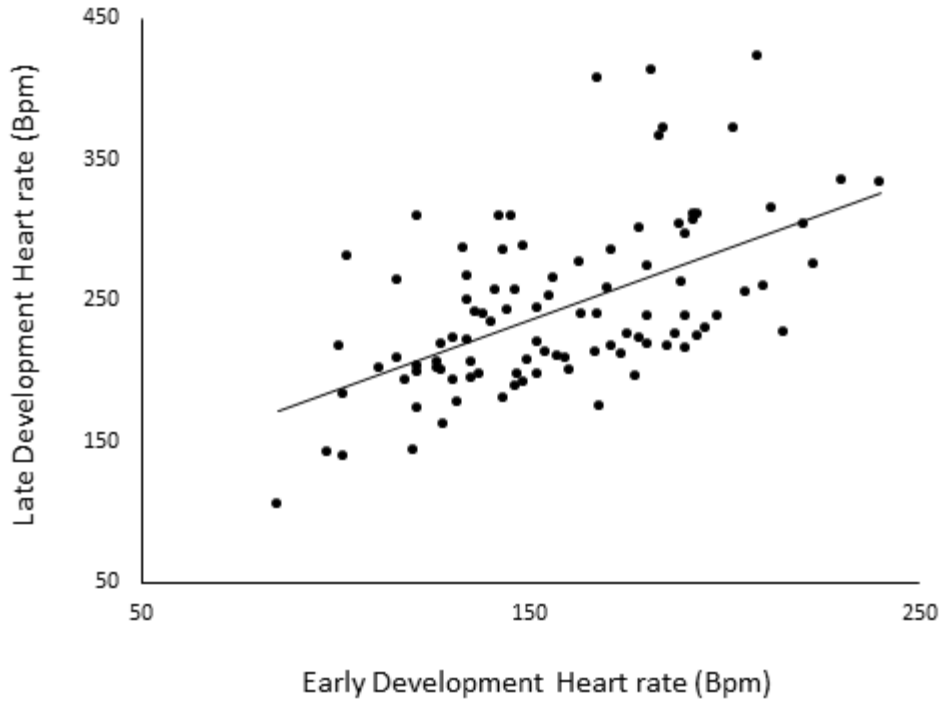

Figure 1. Significant, positive correlation between embryonic heart rate ranks for the early and late stages of pre-natal development (r=0.548, p<0.001, n=98, r^2=0.3105).

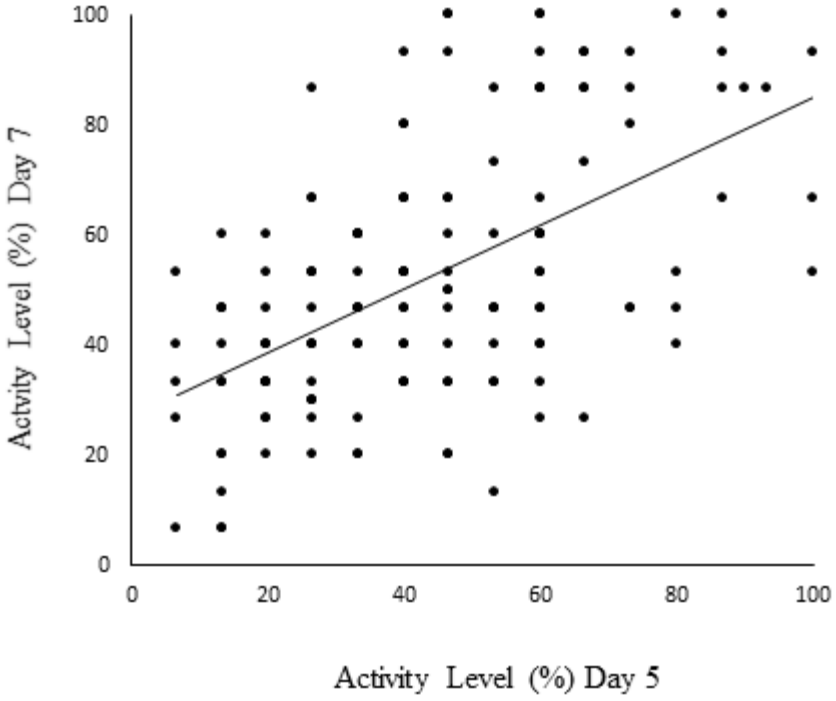

Figure 2. Significant, positive correlation between Activity levels (the percentage of time moving) at day 5 and day 7 of development, (r=0.425, p=0.005, n =148, r^2=0.3072)

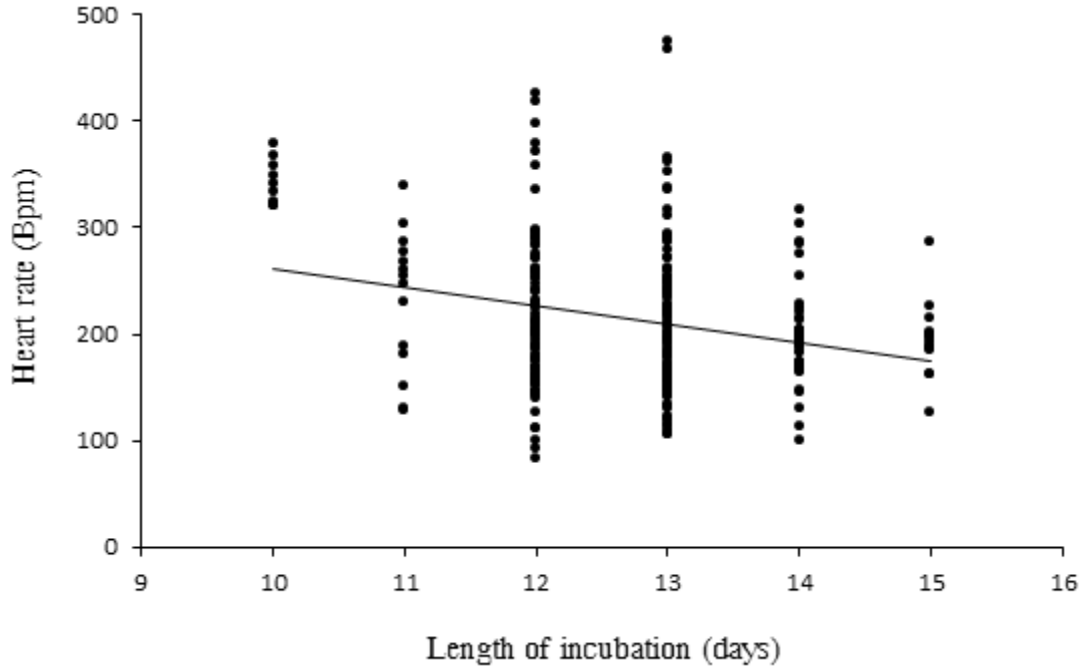

Figure 3. A significant, negative relationship between late-stage embryonic heart rate and length of incubation (days) (r^2=0.08, *n*=288).