SUITES OF BEHAVIORAL AND PHYSIOLOGICAL CHARACTERISTICS OF

JAPANESE QUAIL (*COTURNIX JAPONICA*)

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The concept of personalities is used as a way to understand suites of behaviors associated with how animals cope with change. Animal personalities can be described along a continuum from bold to shy and describe suites of behaviors unique to the type of personality. The relationship between a specific behavior and its underlying physiological state has been well studied, however, we do not have an understanding of the potential continuum of physiological traits associated with animal personalities. In this study, I evaluated the relationships between the behaviors and physiological traits associated with bold and shy personalities in Japanese quail (*Coturnix japonica*). I focused on metabolic and endocrine traits, which provide an integrated measure of energetic demand and use, and response to stress. My data show a continuum of bold to shy personalities, but do not correlate with the physiological traits.

ACKNOWLEDGEMENTS

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LITERATURE REVIEW

Personality Background

A personality is defined as a set of individual differences in behavior and physiology, which are consistent and stable over time (Cavigelli 2005, Groothius and Carere 2005), correlated (David et al. 2011), and repeatable (Wilson and Godin 2009). The study of human personalities has a long history, but the study of non-human personalities is relatively recent. To date, personalities have been identified and characterized in over 60 vertebrate species including great tits (*Parus major*; Groothius and Carere 2005), mice (*Mus musculus*; Gosling 2001, Koolhaas et al. 1999), bighorn sheep (*Ovis Canadensis*; Reale et al. 2000), hyenas (*Crocuta crocuta*; Gosling 1998), and pumpkinseed sunfish (*Lepomis gibbosus*; Coleman and Wilson 1997). The idea of individuals having a personality type that yields a suite of consistent behaviors is important, because, in the past, behaviors have been studied as a single act rather than an integrated package of behaviors that affect fitness and are subject to evolution by natural selection.

The relatively recent study of suites of animal behaviors has resulted in a number of terms all used in the literature to describe personalities. For animals, these suites of behaviors have been referred to as personality, coping styles, coping strategies, behavioral profiles, or behavioral syndromes. A coping style refers to the physiological response of an animal to stressful events, such as environmental fluctuations (Koolhaas et al. 1999). In contrast, a behavioral syndrome is a set of correlated behaviors consistently displayed in a number of different situations (Sih et al. 2004). A behavioral profile might include both the behavioral and the physiological response of an animal to novel situations (Groothuis and Carere 2005). Perhaps some of the confusion results from the established field of human personality and the care with

which researchers avoid anthropomorphic descriptors of their study animals. To simplify the language, I chose the term personality because as previously defined as it encompasses both behaviors and physiological traits.

Current theory predicts that personality types have evolved as a life history strategy to cope with changes in the environment (Cockrem 2007). Individuals within a population differing in personality types will react to the same environmental stimuli with different behaviors which may result in fitness consequences (Both et al. 2005, Dingemanse et al. 2003). Avian species display a continuum of personality types ranging from shy to bold. The terms shy and bold refer to the propensity of an individual to take a risk, especially in a challenging or novel situation (Dingemanse et al. 2003, Gosling 2001, Coleman and Wilson 1997). Similar to the number of terms used to describe personality, a number of terms have emerged to describe the two extremes of the personality spectrum: shy and bold (Carere and van Oers 2004), passive and active (Drent et al. 2003), slow and fast (Dingemanse et al. 2004), and reactive and proactive (Koolhaas et al. 1999). Again to simplify the language, I chose to use the terms shy and bold to describe the extremes of the personality spectrum because as described previously the terms refer to the individual's risk taking behavior.

Birds are important to the study of animal personality and personalities have been well documented and described in the avian literature compared to fish and mammals. Much is known about the ecology, life history, behavior and the mechanisms driving behavior of birds. Furthermore, individual variations in personalities have been studied and characterized in multiple species (Groothuis and Carere 2004). My literature review and research are focused on avian species unless otherwise noted.

Our current understanding suggests that shy birds readily recognize and respond to a change in the environment (Carere et al. 2001), and are usually non-aggressive, and are better able to adapt to social stress (Dall 2004, Sih et al. 2004a). Shy birds usually take longer to return to a food source after a mild startle (van Oers et al. 2004a) and are typically more cautious, tending to explore a novel situation more thoroughly (Groothuis and Carere 2005). Shy birds are occasionally classified as having an exploratory personality because of their thorough exploration behavior (Fucikova et al. 2009).

In contrast, bold birds tend to form routines and are slow to adjust to a change in the environment (Dingemanse et al. 2004). Bold birds tend to explore a new situation quickly with little or no hesitation (Groothuis and Carere 2005) and less thoroughly compared to shy birds (Verbeek et al. 1994). Bold birds are typically more active, more aggressive towards predators and conspecifics, and more competitive in a variety of situations (Dall 2004, Sih et al. 2004, Koolhass et. al. 1999), including nest defense (Hollander et al. 2008). Bold individuals are more likely to gain social dominance compared to shy individuals (Dingemanse et al. 2004). The degree of behavioral flexibility in response to an environmental change may explain why a shy individual is more likely to succeed in an unpredictable or variable environment compared to a bold bird (Cockrem 2005, Koolhaas et al. 1999).

The personality type of an individual will remain consistent across contexts and situations with slight variation at different life stages (Kralj-Fiser et al. 2006). A context is defined as a specific type of action such as foraging, mating or parental care while a situation is a set of conditions at a given point in time (Sih et al. 2004). For example, a bold bird is expected to show bold behaviors during dispersal, mating, feeding, and caring for offspring and will exhibit these bold behaviors throughout its lifetime.

Individual differences in personality may have fitness consequences, such as by influencing dispersal rates and social status in a flock. Studies of wild populations of great tits and European white storks (*Ciconia ciconia*) found bold individuals have higher dispersal rates and increased social status in the breeding population as compared to shy individuals (Dingemanse et al. 2003, Blas et al. 2007). Similar studies in other species found bold killifish (*Rivulus hartii*) dispersed further and grew faster (Fraser et al. 2001) and bold bighorn sheep have higher field survival rates as compared to shy individuals (Reale et al. 2000). Contrary to previous thinking, however, an individual may not display an optimal behavior (optimal indicated by maximizing fitness) in a given situation (Sih et al. 2004) and instead, will display the behavior predicted by its personality type. For example, if a predator comes across a bold bird, the bird may show aggression towards the predator rather than seek cover or freeze like a shy bird will do. A study in pumpkinseed sunfish found in some situations (foraging) it was adaptive for a bold individual to take risks, while being bold in other situations (predator present) can be maladaptive (Coleman and Wilson 1997).

Personality Testing

Methods of personality testing and its description varies in the literature. Reale and colleagues (2007) suggested animal personality can be divided into five broad categories: activity, risk taking, exploration, aggressiveness and sociability (dominance). While the study of animal personality is not as advanced as the study of human personalities, and lacks a consistent framework, the current research, including this study, takes at least one and usually more than one of these general categories into account when describing or categorizing individuals into a personality type. The most common tests found in the literature are exploration (novel

environment), neophobia (novel object), obstinacy (escape), activity level, and aggressiveness or dominance.

The exploration or novel environment test (as described in Verbeek et al. 1994, and Dingemanse et al. 2002) seems to be the standard personality test in passerine birds. The test involves releasing fasted birds individually into a large unfamiliar observation arena with five artificial trees, each with four horizontal branches. The movements of the bird, including flight and hops between the trees, branches and ground, are tallied and used as an index of exploratory behavior. The higher the score, the more bold the personality of the individual. Exploratory behavior testing has been shown to be repeatable and is unrelated to age, sex or body condition at measurement (Dingemanse et al. 2002, Hollander et al. 2008).

The startle test measures the latency of a bird to resume foraging after being startled by a loud noise or vibration while approaching food sources (van Oers et al. 2004, David et al. 2011). A neophobia or novel object test typically involves introducing a novel object such as a penlight, battery, or small toy in the home cage of a bird near the food source and quantifying the reaction of the bird towards the object on a scale of 0 to 5, with 0 being object avoidance and 5 being object manipulation (Dingemanse et al. 2003). The obstinacy or struggle rate test is more popular in mammal studies but has been used in a couple of avian studies. The birds are placed on a flat surface under a catch net and the number of escape attempts and movements in 30 seconds are recorded (Réale et al. 2000, David et al. 2010). The activity level test is the number of times a bird moves between perches in its home cage in a ten minute period after being fasted for an hour (David et al. 2010). The aggressiveness or social dominance test involves placing several birds in a large familiar testing arena and measuring the time it takes for birds to approach a single feeder after being fasted for an hour. The birds receive a score consistent with the order in

which they accessed the feeder and the level of aggressiveness shown towards other birds in the arena (Boogert et al. 2006, David et al. 2011). Using a combination of personality tests allows a researcher to better define the behavioral suite of a bird and place it on a bold to shy axis, however, most of the current literature relies on the use of one or two tests to define an individual.

Physiology Testing

Variation of physiological traits has been documented in a variety of species. Like personality traits, the physiology of individuals varies among and within species. Physiological traits which have been described and documented in the literature in a personality context include breathing rates, body temperature (Carere et al. 2004), endocrine profiles, immune response (van Oers et al. 2011), metabolic rates (Ronning et al. 2005), and stress response (Careau et al. 2008).

Shy and bold birds vary in a number of physiological factors. Shy birds tend to have higher breathing rates and higher body temperatures in response to handling tests and shy individuals take longer to return to a normal body temperature after the handling test (Carere et al. 2004). Shy birds also tend to have a higher level of baseline testosterone and a more active immune response as compared to bold birds (van Oers et al. 2011).

Metabolic Rates

Basal metabolic rates of individuals vary both within and between species and have been linked to personalities in recent literature (Biro and Stamps, 2010). Metabolic rates have been recorded in over 600 mammal and 300 bird species (Careau et al. 2008). The basal metabolic rate of an individual is the minimum amount of energy required for sustaining basic physiological processes at rest in a thermo-neutral environment, and in a post absorptive and non-reproductive

state (Speakman et al. 2004). The goal of metabolic rate research is to obtain a repeatable measure of the minimal metabolic level to maintain homeostasis (Hulbert and Else 2004).

Variation in basal metabolic rates can be explained by body mass, activity level, ambient temperature, climate, and diet (Careau et al. 2008, Lovegrove 2003, McNab 2002) with body mass being the main attributing factor (Careau et al. 2007). Variation in metabolic rate is repeatable across life stages in avian (Ronning et al. 2005) and fish species (Cutts et al. 2001) and can vary up to two-fold among individuals within the same species (Speakman et al. 2006). A large scale inter-species comparison of 530 plus species found 95% of the variation in metabolic rates was attributed to body mass (Lovegrove 2000), however, as body size decreases the variation in metabolic rates attributed to body mass decreases (Careau et al. 2008). Organ size may attribute to some of the variation. Daan at al. (1990) found heart and kidney lean mass could explain about 50% of variation of metabolic rates in 22 bird species. However at an intra-species level, the percent of variation decreases (Careau et al. 2008).

Because bold and shy personality types show varying degrees of activity level, personality may influence daily energy expense and metabolic rates. Because high activity levels are typically associated with bold individuals, the inference of bold individuals having a higher metabolic rate can be drawn. Individuals which are more active tend to be more bold and may require a higher metabolic rate to maintain homeostasis both during rest and activity. Daan et al. (1990) found avian species with a high daily energy expenditure for their relative size will have a higher metabolic rate. Multiple metabolic rate studies have shown higher metabolic rates are consistent with individuals with a higher daily energy expenditure (Biro and Stamps 2010). Increased basal metabolic rates have also been correlated with dominance, a trait consistent with

a bold personality type in dippers (*Cinclus cinclus*), willow tits (*Parus montanus*) and great tits (Bryant and Newton 1994, Vézina and Thomas 2000).

Stress Response

The ability of an individual to acclimate to a stressful situation is thought to be contingent on its personality type (Cockrem 2007) and individuals will show different responses when faced with the same stressful situation (Carere et al. 2010). Research has shown hormones from the glucocorticoid family mediate many of the effects of stress in avian species. Glucocorticoids are produced and released from the hypothalamic-pituitary-adrenal (HPA) axis as a consequence of a perceived stress (Rubolini et al. 2005, Kitaysky et al. 1999), and they elicit both behavioral and physiological changes (Washburn et al. 2002). The principal glucocorticoid found in birds is corticosterone, a twenty-one carbon steroid hormone released from the adrenal gland (Love et al. 2003). Corticosterone primarily serves a metabolic function at baseline levels but becomes elevated in response to a perceived stressor. The release of corticosterone as result of a perceived stress can affect many different homeostatic processes but the main function is to return the animal to its basal levels of circulating hormones (Baugh et al. 2012).

The behavioral response of a bold bird to a stressor usually involves avoidance and/or aggressive behaviors resulting in an increased heart rate, metabolic rate, glucose consumption and muscle activity (Careau et al. 2008, Koolhaas et al. 1999). A shy bird, however, primarily responds using the release of glucocorticoids resulting in immobility and the passive behavior of freezing (Careau et al. 2008). A study on great tits selected for different personalities found shy individuals have higher HPA sensitivity compared to bold individuals (Groothuis and Carere 2005) meaning shy individuals have a greater concentration of corticosterone released in response to a perceived stressor (Korte et al. 2005, Carere et al. 2003). A similar study on great

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tits selected for different personalities found the baseline levels of corticosterone were not significantly different between selection lines but the stress induced levels differed significantly between bold and shy individuals (Baugh et al. 2012) further suggesting a higher HPA reactivity in shy individuals.

Correlations between Physiology and Personality

A number of recent studies have looked for a potential link between personality and physiology in avian species. Some have found correlations between personality and physiology, some have found trends, and some have found personality and physiology vary independently. Artificially selected line populations are useful in correlation studies as the captive populations should reflect the extreme ends of the personality spectrum and may help clarify the physiological mechanisms behind the personality differences (Schjolden et al. 2005).

A Japanese quail selection line study found birds selected for low corticosterone showed less freezing behavior and started exploring more quickly after a startle test compared to quail selected for high corticosterone (Cockrem 2007). A similar study which selected for low, control and high levels of baseline corticosterone found that - as corticosterone concentration increased, risk taking and exploratory behaviors in zebra finches increased, suggesting a more bold personality (Martins et al. 2007) which conflicts with previous hypotheses, which suggest shy individuals have higher corticosterone levels.

A recent study on house sparrows found a correlation between the ability to solve a problem and the concentration of feather corticosterone. Bokoney et al. 2014 found the sparrows with the lower deposited corticosterone levels solved the problems quicker and seemed to learn faster as compared to sparrows with higher deposited concentration. A study on male house sparrows found levels of plasma testosterone were not correlated with exploration behavior

(Mutzel et al. 2011) which supports previous findings of shy individuals having higher circulating testosterone.

The link between personality and physiology may change in different environments as a study on dark-eyed junco (*Junco hyemalis*) populations suggested. The researchers found environmental stressors such as food availability or temperature changes can affect the relationship between personality and physiology. The research found a positive correlation between metabolic rates and dominance behaviors during the summer season but the relationship disappeared when the air temperature grew colder during the winter season (Vézina and Thomas 2000). A study on rufous-collared sparrows (*Zonotrichia capensis*) explored the correlation between exploratory behavior and two physiological factors: total evaporative water loss, which is considered an important physiological trait in passerine species; and basal metabolic rates in three different environments (desert, cold temperate and Mediterranean). The researchers found no association between exploratory behavior and total evaporative water loss for the birds from either of the sites. They found birds from the cold temperate site were more bold compared to the other two sites. They also found a positive correlation between exploratory behavior (bold individuals) and basal metabolic rate at the Mediterranean site. The study suggested perhaps ecological differences and environmental stressors have a role in the correlation between personality and metabolic physiology (Maldonado et al. 2012).

Exploring some of the changes between physiology and personality under an environmental stressor might help explain some of the correlation (or lack of) between personality and physiology. When faced with survival in an extreme environment an animal may alter its behavior or physiology to survive. For example, when food is scarce birds with higher basal metabolic rates will lose mass faster compared to animals with lower basal metabolic rates.

In response, the bird will increase its propensity to take a risk to find food, increasing the chance of predation as a result of the physiological need (Careau and Garland 2012).

A study on female zebra finches found breathing rates in response to handling stress were repeatable, however, the rates were not correlated with personality traits (David et al. 2011). Contrary to a study on great tits, which found breathing rates serve as a repeatable indicator or proxy of personality type, birds with lower breathing rates in a stress handling test showed less fear towards a novel object compared to those with higher breathing rates (Carere and van Oers 2004).

A field study on male pied flycatchers found individuals with lower breathing rates mobbed predators more frequently and approached the nest more readily after a perceived threat, suggesting more bold personalities compared to individuals with higher breathing rates which showed greater avoidance and longer latency to return to the nest. The researchers found a strong correlation between the nest defense bold behavior and breathing rates in males but did not see a correlation in females (Krams et al. 2014).

Similar work in fish species, like the Arctic charr, *Salvelinus alpinus*, have shown metabolic rates are repeatable, consistent over time and across multiple environments and correlated with aggression and dominance levels, both attributes of a bold personality (Cutts et al. 2001). A similar study, however, using European sea bass, *Dicentrarchus labrax*, found no correlation between boldness and routine metabolic rates except when food was scarce (Killen et al. 2011). As described in the literature, physiological traits, including metabolic rates and corticosterone responses vary within populations and may offer insight into the personality continuum.

Statement of Objectives

In this study, I investigated the relationship between metabolic and endocrine physiology of Japanese quail (*Coturnix japonica*) and their characterization along a behavioral continuum of bold to shy. My study had two main objectives. The first objective was to measure variation in behavior along a continuum from bold to shy personality types using six behavior trials. The trials were designed to place the personality of individual quail on a bold to shy continuum using observations and time measurements similar to that used on great tits (Groothuis and Carere 2005). The second objective was to measure both the metabolic physiology and stress response of the quail. The metabolic physiology of the individual quail was measured using a flow through respirometry system similar to that used on house sparrows (Martin II et al. 2002). The stress response of individual quail was measured by activating the HPA axis and measuring the amount of corticosterone produced similar to a study on common terns (Heidinger et al. 2008).

MATERIALS AND METHODS

Study System

Japanese quail (*Coturnix japonica*) are a classic model system for behavioral studies and much is known about their life history, behavior, physiology and development (Mills et al. 1997). The species, *Coturnix japonica*, is native to Japan, China, and Korea, and was introduced into Hawaii in 1921 (Mills et al. 1997). Japanese quail are terrestrial birds, generally inhabiting grassy areas. A captive-bred wild Japanese quail population was used for this study. These birds are descendants of the Hawaiian population, have been randomly bred in captivity, and the genetic stock was obtained from the Avian Genetic Research Laboratory, maintained by Agriculture and Agri-Food Canada.

Japanese quail mature in five to six weeks after hatching with mating attempts beginning as early as 35 days. These birds have a relatively short lifespan (2.5 years). Currently, the native mating system of Japanese quail is unclear, although research suggests they can either be polygamous or monogamous depending on their native region (Mills et al. 1997). Males and females have a similar fear response, consisting of avoidance behaviors such as crouching, freezing and withdrawing their head. These avoidance behaviors are innate and can be observed within hours of hatching.

I incubated 204 quail eggs between 24 October 2007 and 26 October 2007. The incubator was set to 37.5º Celsius and approximately 85-% relative humidity. Total incubation time for the eggs was between 17 and 19 days. The eggs were incubated for 15 days and moved to a hatcher for the last two. The hatcher was set at 37.2º Celsius with approximately 90% humidity.

After hatching, I weighed the chicks and placed a small color band on each leg. Each chick was banded with a unique color combination. They were housed together in a brood cage

(plastic baby pool) until they reach sexual maturity at approximately five weeks. The birds used in this study were housed in a common garden environment. After reaching maturity, the birds were moved to a large room and housed as a free-flight colony, maintained under short days (8 L: 16 D) to minimize aggressive interactions associated with reproduction. A commercial diet of gamebird crumble and water was provided to the quail *ad libitum*. The quail had access to shelter, plastic plants, shredded paper and a dustbath as a way of enriching their environment to avoid boredom behaviors like feather picking and fighting. Human contact with the quail was minimized to reduce stress.

Metabolic Measurements

I measured resting metabolic rates at three time points during my study. The first measurement was completed after the birds reached sexual maturity and prior to the first behavioral assessment. The second was completed when half of the behavioral trials were finished and the final trial was completed at the end of the study. Metabolic measurements were taken using an open respirometry system (see below) through the night (approximately 12 h period). The birds were removed from their holding room at approximately 8:00 PM and transported to the respiratory room in a crate. The birds were placed in a darkened respiration chamber and weighed to the nearest 0.1 g before being placed in the incubator. The respirometry chamber was made out of a quart-sized paint can with two barbed hose fittings in the lid, sealed with a silicone sealant. The chambers were maintained at a constant temperature in an incubator within the thermo-neutral zone of the quail, approximately 24° C. Birds were not fasted prior to being placed in the chambers and weighed, however the birds were collected from their holding three hours after their lights shut off and were usually asleep. I approximated post-absoptive metabolic readings by starting data collection approximately three hours into the night. Birds

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were returned to their holding room at approximately 8:00 AM the following morning and monitored for signs of distress.

Metabolic rates were quantified by measuring oxygen consumption per individual using a positive pressure flow-through respirometry system similar to that used on house sparrows (Martin II et al. 2002). Fresh air (20.95% O_2, dried and carbon dioxide free) was pumped into a temperature-controlled chamber at a constant flow rate high enough to maintain gas flow through eight metabolic chambers at a minimum rate of 515 ml/min. The air-flow was calibrated to within 10 ml/min before each trial.

Upon entering the chamber, the air line was split with the first tube leading to a mass flow meter (Model 840; Sierra Instruments, Amsterdam, Netherlands), controlled by a mass flow controller (TR-FC1; Sable Systems, Las Vegas, NV, USA), into an eight-channel multiplexer (TR-RM8 V2.0; Sable Systems, Las Vegas, NV, USA) and the second line entered the flush line of the multiplexer. The multiplexer controlled eight respiration chambers, with one being an empty baseline chamber. A single chamber was measured at a time with air from the first line flowing at a rate of at least 515 ml/min while air from the second line flushed through the other seven chambers at approximately the same rate. This flow rate ensured the oxygen levels in the chambers never reached a level to induce a hypoxic response from the birds in the chambers. After exposure to the birds in the chambers, the air from each chamber passed through a 30 ml syringe filled with drierite and a filter to remove water vapor and debris from the line. The air leaving the single measured chamber was plumbed directly into the oxygen analyzer (FC-1B; Sable Systems, Las Vegas, NV, USA) after being pushed through a column of gas scrubbers to remove water vapor and carbon dioxide. The gas scrubbers in the column and syringes were changed before each run.
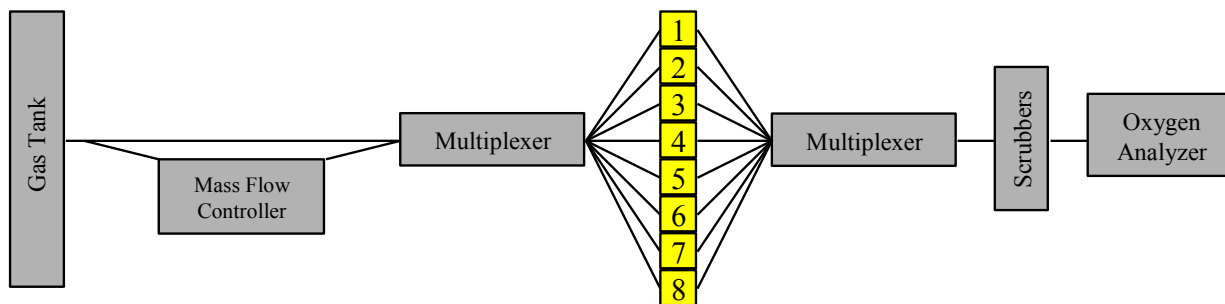
Figure 1. Respirometry system for measurements of metabolic rates. The boxes labeled 1-8 and in yellow represent the metabolic chambers with the first one being a baseline chamber. Only a single multiplexer was used, but each chamber was plumbed to an inlet and outlet line in the multiplexer (i.e. air flowed from the multiplexer to each chamber and then returned to the multiplexer before the oxygen analyzer).

Metabolic rates were recorded overnight to ensure that the baseline metabolic rates reflect basal metabolic rates. Using Expedata, I programmed the system to measure oxygen consumption for each individual at one-second intervals for 10 minutes per chamber including the baseline chamber before switching to the next chamber, with a four-minute flush between chamber measurements to remove latent gases. This process was repeated approximately seven times throughout the trial for a total of 4200 samples per individual. The first measurement per quail for the night was thrown out. These measurements were usually varied and unstable as the quail were settling in for the night. The equation used to calculate metabolic rates was $VO_2 = FR(FO_2{}^{IN} - F\,O_2{}^{OUT})/(1 - F\,O_2{}^{OUT})$. Where VO_2 was the rate of O_2 consumption, measured in milliliters per minute, FR was the flow rate of the air pushed through the respirometry system, FO_2 was the fractional concentration of oxygen where $FO_2{}^{IN}$ is the amount flowing into the chambers and the $F\,O_2{}^{OUT}$ was the amount flowing out of the chambers. Metabolic rates were corrected for standard temperature and pressure conditions (0°C, 101.3 kPa) and for individual body mass.

Behavior

Behavioral assessments were conducted in a room visually and acoustically separated from the quail colony. I conducted the behavior testing in an indoor arena approximately sixteen square feet with a screened top. The floor of the arena was a piece of laminate flooring with a square pattern of 64 total squares, each 6 inches by 6 inches. I tested each bird independently and transported the birds from their holding room to the testing room in a darkened crate. One wall of the testing arena had a door cut to fit the opening of the transport crate to reduce human contact. The quail were allowed access to the crate for shelter during the behavior trials. All trials were videotaped and reviewed upon completion. Before the behavior trials began, the quail were allowed to explore the testing arena in groups of five or six birds.

The birds underwent six behavioral trials of fifteen minutes each. The trials concentrated on three groups of behaviors: exploration, foraging, and anti-predator response. These behavior groups were chosen for their life history importance. For each trial, a number of measurements were recorded per individual: the time (in seconds) the bird spent in the shelter box, the time the bird spent in the testing arena, the total area of the testing arena the bird covered, the number of times the bird defecated, the number of times the bird interacted with the object (novelty, food or predator), the amount of time the bird spent interacting with the object, and the number of times the bird vocalized during the trial. How the bird interacted with the object (ingesting, manipulating, etc.) was also noted.

The first set of trials aimed to measure the exploration behavior of the individual. The trials consisted of presenting a novel environment and a set of novel objects to the quail. Prior to these trials, the quail were only exposed to wood shavings, plastic plants, shredded paper and a

dustbath (sand) in their home environment. The quail were presented with six pinecones in the first trial and a piece of sod in the second.

The second set of trials aimed to measure the foraging behavior of the individual. The quail were presented with two novel foods items (mealworms, crickets), which they had not seen prior to the trial. Insects would be a normal part of their diet in the wild. The trials took place during the morning and late afternoon hours as the frequency of feeding behaviors usually coincides with the start and end of the photoperiod.

The third set of trials aimed to measure the anti-predator response of the individual. The quail were presented with two live predators – a corn snake and a kestrel. The snake was contained in a glass aquarium and placed in the center of the arena. The arena was modified for the last trial with the kestrel. The kestrel was tethered and allowed to sit on the edge of the arena or on a perch. The quail were separated from the kestrel by a wire barrier. These trials were completed last to reduce any fear effects the predators may have had on the birds.

Stress Response

To measure the capability of an individual to handle stress, I activated the HPA axis to secrete corticosterone through an intramuscular (IM; breast muscle) injection of porcine adrenocorticotropin hormone (ACTH) (Heidinger et al. 2008, Wilson and Holberton 2001). To measure baseline corticosterone concentrations, a small blood sample (50-100 µl) was taken prior to injection using veinipuncture of the brachial vein and collecting the blood that welled from the wound in a 300 µl heparinized microvette blood collection tube. Next the birds received an IM injection of porcine ACTH (100 IU/kg, approximately 40ul). The birds were held in an individual dark chamber and held for approximately thirty minutes from the initial capture time. Thirty minutes is the approximate time of maximum corticosterone secretion in response to

handling and restraint stress (Heidinger et al. 2008). After the wait period, a second blood sample was collected as before to evaluate the maximal corticosterone secretion. The birds were returned to their holding room and monitored for signs of distress. Blood samples were centrifuged at 2000 g for 10 minutes to separate plasma from the red blood cells. Plasma samples were frozen at 4º C until analysis for corticosterone levels was completed. The stress response test was only done once because previous research done by Cockrem (2007) found the corticosterone response decreases when tested multiple times.

Corticosterone levels in plasma were quantified by radioimmunoassay (RIA). In brief, plasma samples were first spiked with a small amount of radio-labeled corticosterone (H^3) to estimate extraction efficiencies. Total corticosterone was extracted from plasma samples using dichloromethane as the solvent and then a competitive binding radioimmunoassay was used to quantify the corticosterone in the sample. A standard curve and two standard samples (i.e., known amounts of corticosterone) were run in a single assay.

ANALYSES

To analyze the data, I ran principal component analysis (PCA) using JMP software to quantify the relationships among the personality and physiology variables. To create the axes, I used the metabolic rates from the second trial, one or two measurements from each of the six behavior trials and the difference between the pre- and post-corticosterone levels from the stress response test (corticosterone response to ACTH challenge). For each behavior trial, I chose the area of the testing arena used during the trial as a measure of activity and for all trials, except the predator trials, I used the amount of time the quail interacted with either the food item or the novel item. All quail interacted with the predators the same, with no activity or variation among individuals in their interaction with the predator. For the feeding trials, I used the area of the testing arena the quail covered and the number of interactions the quail had with the food item. For the novel trials, I again used the area of the testing arena the quail covered and the amount of time the quail interacted with the novel item or novel environment. For the predator trials, I only used the area of the testing arena the quail was limited to during the trial because we did not allow for interactions with the predators for obvious reasons. I then used General Linear Models (Two-way ANOVA) to test the relationships among sex, body mass, metabolism, corticosterone levels, and behavioral suites as measured by the PCA scores.

RESULTS

Seventy-six chicks hatched between 10 November 2007 and 13 November 2007. Their weights varied from 4.08 grams to 6.48 grams at hatching. A total of 44 chicks reached adulthood and were used in the study. One quail passed away during the study and her numbers were excluded from the final analysis. Average weight of the adult quail ranged from 80.25 grams to 121.48 grams. Females were significantly heavier than males ($F_{1, 41} = 7.5$, $p = 0.0091$).

Table 1. Mean body mass sampled on three occasions during study.

Mass		n	Mean ± S.E. (g)
Pre Behavior Mass	All	43	99.5 ± 9.1
	Males	23	96.0 ± 1.8
	Females	20	103.5 ± 1.9
Mid Behavior Mass	All	43	97.5 ± 9.9
	Males	23	93.9 ± 1.9
	Females	20	101.6 ± 2.1
Post Behavior Mass	All	43	101.8 ± 11.2
	Males	23	99.5 ± 2.3
	Females	20	104.5 ± 2.5

Metabolic Rates

The mean metabolic rate increased from trial to trial with the metabolic rates from the third trial being the highest. I used the second metabolic rate trial for analysis in the PCA because the rates from the trials ran on the first day of testing were significantly lower compared to the other measurements and the third trial had the highest rates. The calculated repeatability of the metabolic rates ranged from 27.4% to 40.3% (the latter was calculated by excluding the first

day trials due to low readings). The equation used to calculate repeatability was $r = \frac{S^2A}{S^2+S^2A}$.

Where S^2A was the between group variance and S^2 is the within group variance. The variation in

metabolic rates among individuals was not explained by sex, body mass or an interaction

between sex and body mass (Pre Behavior Trails Metabolic Rate: $F_{3,39} = 1.73$, p = 0.18, Mid

Behavior Trials Metabolic Rate: $F_{3,39} = 1.14$, p = 0.34, Post Behavior Trails Metabolic Rate: $F_{3,39}$

= 0.62, p = 0.60).

Table 2. Mean metabolic rates sampled on three occasions during study.

Metabolic Trials		n	Mean ± S.E. (mL O₂/min/g)
Pre Behavior Metabolic Rates	All	43	2.92 ± 0.60
	Males	23	2.90 ± 0.13
	Females	20	2.93 ± 0.14
Mid Behavior Metabolic Rates	All	43	3.11 ± 0.44
	Males	23	3.15 ± 0.09
	Females	20	3.07 ± 0.10
Post Behavior Metabolic Rates	All	43	3.31 ± 0.60
	Males	23	3.31 ± 0.02
	Females	20	3.30 ± 0.13

a.
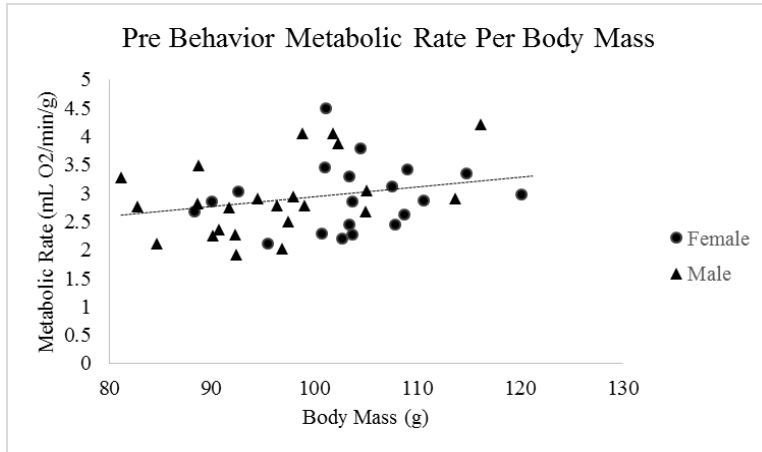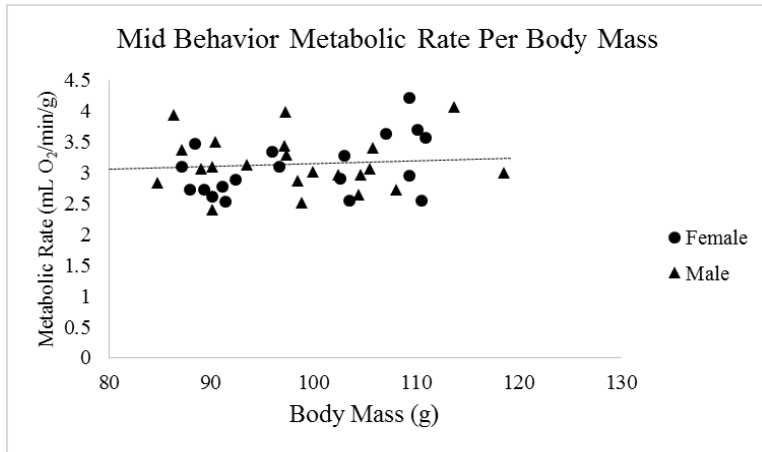
Figure 2. Metabolic rates plotted against body mass sampled on three occasions: a. Pre Behavior Test, b. Mid Behavior Test, c. Post Behavior Test. Males and females distinguished with different data points (females = circles, males = triangles). N = 43.

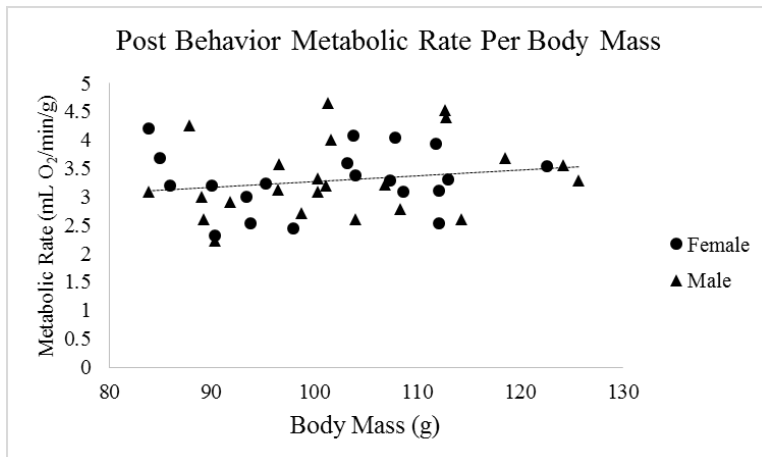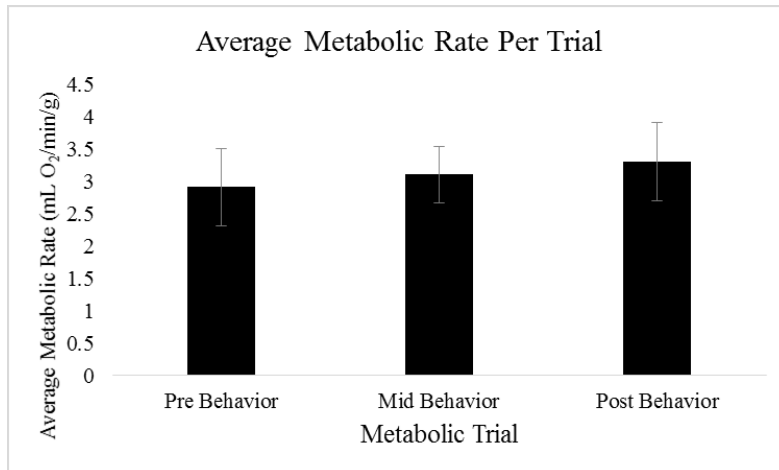Figure 3. Mean metabolic rates per trial. Data represented as mean ± S.E. (mL O_2/min/g), N = 43.

Stress Response

The difference between the pre- and post-corticosterone levels of the quail was used to analyze the stress response. The variation in the corticosterone baseline, post and response was not explained by sex, body mass or an interaction between sex and body mass, however, females have a trend of a higher corticosterone response (Baseline: $F_{3, 39}$ = 0.756, p = 0.526, Post: $F_{3, 39}$ = 0.745, p = 0.531, Response: $F_{3, 39}$ = 0.504, p = 0.682).

Stress Measure: ACTH Challenge

Table 3. Mean plasma corticosterone: baseline, post and the response.

	n	Baseline Mean ± S.D. (pg/µl)	Post Mean ± S.E. (pg/µL)	Response Mean ± S.E. (pg/µL)
All	43	10.24 ± 8.02	87.26 ± 28.65	77.02 ± 25.35
Males	23	8.81 ± 6.20	81.47 ± 30.20	72.66 ± 27.04
Females	20	11.88 ± 9.60	93.91 ± 25.91	82.03 ± 22.92

a.

Baseline v. Response Corticosterone

b.

Baseline Corticosterone v. Sex

c.

Post Corticosterone v. Sex
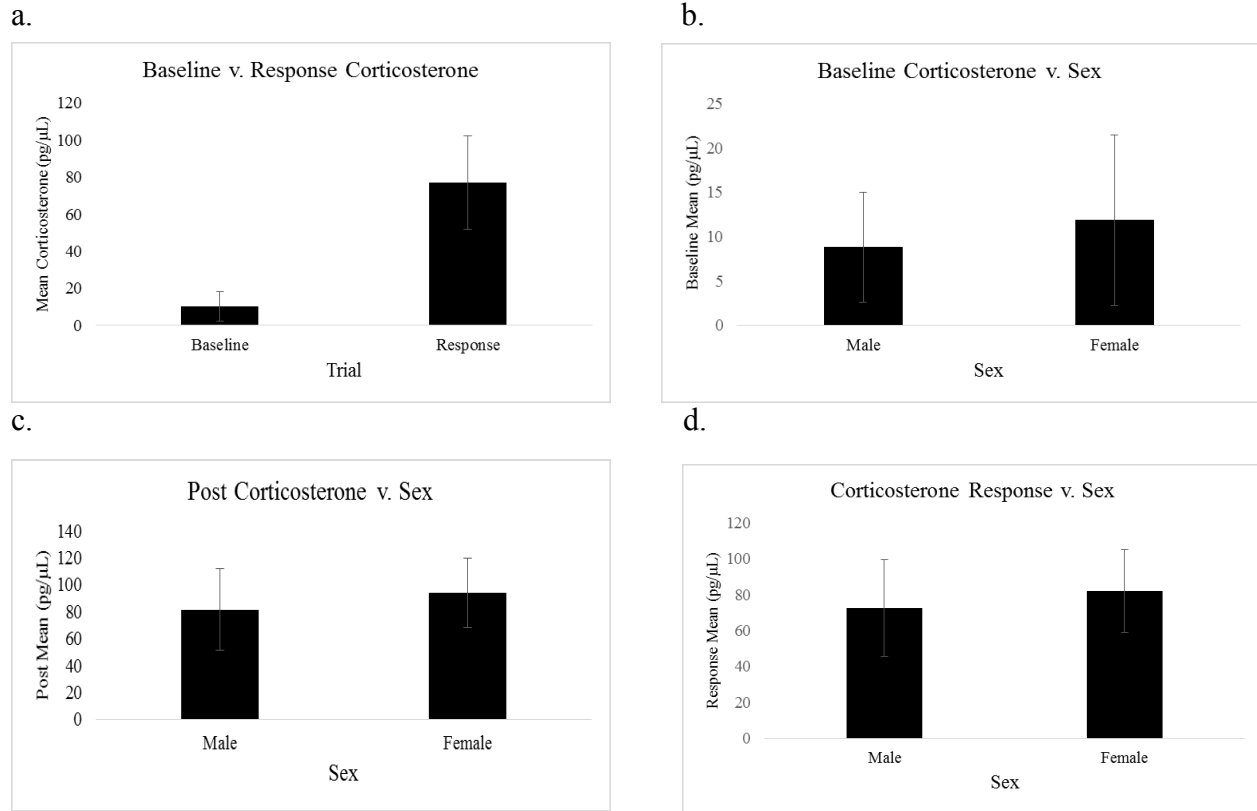
d.

Corticosterone Response v. Sex

Figure 4. Mean corticosterone level comparisons. a. Mean baseline corticosterone and response corticosterone of study population. b. Mean baseline corticosterone concentration per sex. c. Mean post corticosterone concentration per sex. d. Mean response (post minus baseline) corticosterone concentration per sex. Data represented as mean ± S.E., N = 43.

Behavior Trials

The mealworm trial was held in a different location (quail population was moved during the 2009 Fargo flood). A number of trials went missing from the trial due to equipment malfunction and the birds may have been affected by the move.

The number of squares explored in the kestrel trial was very low compared to other trials and 24 of the birds never left their shelter box. As an example, individual birds, Green/Green and Green/Magenta only left the shelter box for one trial and Pink/-- and Yellow/White only left the shelter box for two trials. The rest of the birds left the shelter for three or more trials. Pink/Green left the shelter for four of the total trials but she only explored one to two squares for those trials.

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The quail spent the most time interacting with the sod in the exploration trial. We saw zero interactions with the birds through the aquarium glass in the snake trial. We did not permit interactions during the kestrel trial, the birds were separated from the kestrel with a piece of mesh.

Behavior Measures: Area

Table 4. Comparison data for each behavior trial. The total number of squares changed depending on the set up of the particular trial.

Trial	N	Total Squares*	Mean Area ± S.E (Squares)	Median Area (Squares)	# of Birds who never left shelter
Exploration: Pinecones	42	64	22 ± 2	24	8
Exploration: Sod	42	64	34 ± 3	37	4
Foraging: Mealworms	39	64	20 ± 3	21	12
Foraging: Crickets	41	64	31 ± 3	36	6
Predator: Snake	43	56	28 ± 3	33	9
Predator: Kestrel	43	40	5 ± 1	0	24

a.

Average Area Explored Per Trial

b.

Number of Birds Who Hid Per Trial
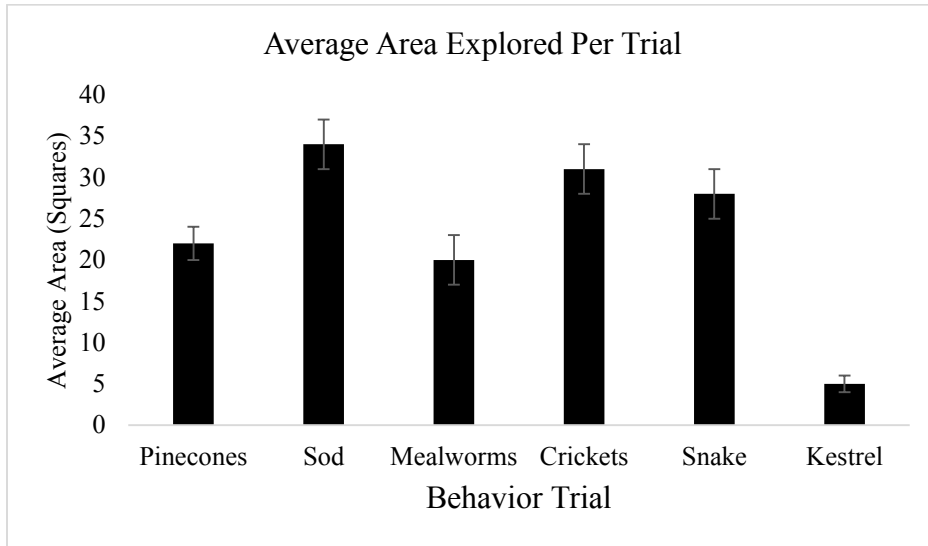
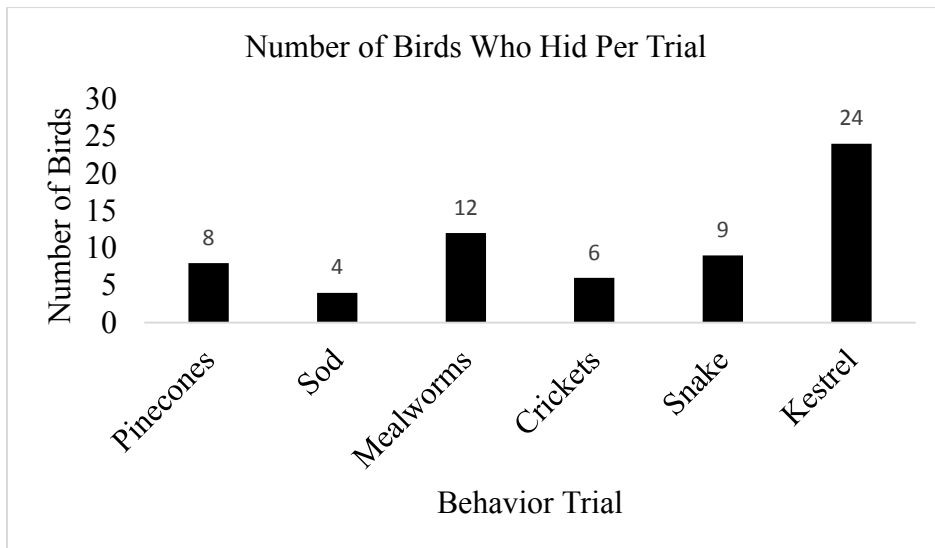Figure 5. Behavior Trial Comparisons. a. Mean area explored in behavior arena for study population per behavior trial. Data represented as mean ± S.E., N = 43. b. Number of birds who hid in the shelter box per behavior trial. N = 43.

Behavior Measures: Interaction Time

Table 5. Mean and median interaction time of study population for each behavior trial.

Trial	N	Mean Interaction Time ± S.E. (Seconds)	Median Interaction Time (Seconds)
Exploration: Pinecones	42	2 ± 1	0
Exploration: Sod	42	268 ± 40	237
Foraging: Mealworms	39	1 ± 0	0
Foraging: Crickets	41	1 ± 0	0
Predator: Snake	43	0	0
Predator: Kestrel	43	N/A	N/A

Principal Components

I used components with eigenvalues greater than one for the PCA. The first four principal component scores for each individual act as a summary for the original ten scores in the analysis. These four components account for about 71% of the variation in the behavior.

Table 6. First four principal components with eigenvalue and percent variation explained.

Principal Component	Eigenvalue	% Explained	% Cumulative
1	2.88	28.76	28.76
2	1.81	18.08	46.83
3	1.34	13.36	60.20
4	1.09	10.94	71.14

The loadings for the first principal component analysis are all positive and correlated to each other (see Table 7: Loading Matrix). From a biological standpoint, the first principal component represents the bold to shy continuum of personalities in the quail and was used further in the hypothesis testing.

Principal Component Loading Matrix

Table 7. Principal component loading matrix for all ten factors.

| Trial	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Pine Area	0.85	0.03	-0.21	-0.06	-0.09	-0.16	-0.07	0.19	-0.39	0.06
Pine Time	0.51	-0.55	-0.15	-0.05	-0.10	0.63	0.10	-0.02	-0.03	-0.05
Sod Area	0.47	0.46	-0.43	-0.13	-0.47	0.03	-0.16	0.18	0.28	0.04
Sod Time	0.40	0.29	-0.12	-0.16	0.80	0.09	-0.04	0.21	0.10	-0.05
Mealworm Area	0.50	-0.15	0.48	0.57	-0.05	-0.03	-0.36	0.06	0.05	-0.15
Mealworm Time	0.41	-0.82	-0.03	0.09	0.16	-0.19	-0.03	-0.00	0.17	0.25
Cricket Area	0.68	0.48	0.15	-0.13	0.10	0.07	-0.14	-0.48	-0.01	0.09
Cricket Time	0.60	-0.31	0.24	-0.54	-0.12	-0.28	0.21	-0.05	0.09	-0.20
Snake Area	0.24	0.36	0.80	-0.01	-0.11	0.15	0.27	0.21	0.02	0.15
Kestrel Area	0.47	0.23	-0.33	0.63	0.03	-0.11	0.44	-0.08	0.05	-0.05
EV	2.88	1.81	1.34	1.09	.95	0.58	0.50	0.40	0.29	0.17

Principal Components
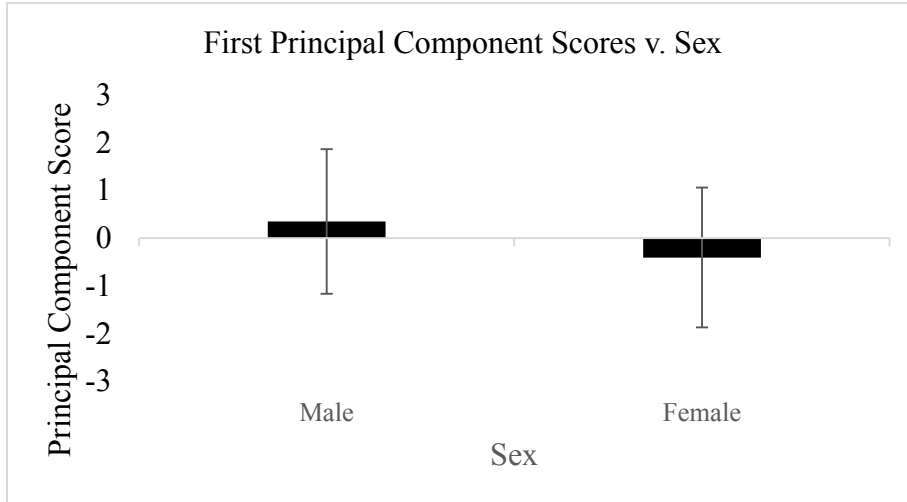
Figure 6. Plot of the first individual PCA scores against sex. Data represented as mean ± S.E., N = 43.

Interactions between Behavior and Physiology

Using the PCA scores from the behaviors and an ANOVA to evaluate the axes, I found no significant relationship between the axes of personality and physiology. There was, however, a trend of males personality being driven by their physiology. The personality variation was not explained by the sex of the quail ($F_{1, 35}$ = 2.796, p = 0.103), the mass of the quail ($F_{1, 35}$ = 0.102, p = 0.752) or the interaction of the sex and mass ($F_{3, 33}$ = 1.164, p = 0.338) The personality variation was not explained by the metabolic rates of the quail ($F_{1, 35}$ = 0.03, p = 0.86) or the stress challenge (Baseline: $F_{1, 35}$ = 0.67, p = 0.42, Post: $F_{1, 35}$ = 2.27, p = 0.14, Response: $F_{1, 35}$ = 2.20, p = 0.147). To analyze the full model, I used a log transformation of the metabolic rate and CORT data to normalize it. The personality variation was not explained by the sex, mass, or the interaction between the sex of the quail or the physiological measures but the males had a trend to be more bold (Metabolic Rate: $F_{4, 32}$ = 1.306, p = 0.289, CORT: Post: $F_{4,32}$ = 1.097, p = 0.375, Response: $F_{4,32}$ = 0.976, p = 0.434).

DISCUSSION

Variation in physiological traits and personalities exists within avian populations, but the mechanisms driving the variation are not well understood. My data are consistent with other studies, which show a continuum of personalities, and individual variation and repeatability among metabolic rates within avian populations, however my data do not indicate a correlation between personality and physiological traits.

My data show a continuum in personality along a bold shy axis. I used six behavior tests focused on three traits chosen for their life history and fitness importance to characterize personality. Most of the current research on animal personality uses one test to categorize an individual as bold or shy. Personality by definition is a suite of behavior traits but a number of studies fail to use multiple behavior tests when categorizing individual personalities, instead relying on the novel environment test or the novel object test as a single test or proxy for personality.

My metabolic rate data support previous research suggesting metabolic rates within a population vary and are repeatable over time. I did not, however, see a correlation between the metabolic rates and personality within the study population. A number of publications suggest an individual with a higher daily energy expenditure and/or a higher metabolic rate should have a more bold personality. However, very few empirical studies have examined the relationship between the two (Careau et al. 2008). Biro and Stamps (2010) reviewed a number of studies focused on avian, fish and mammal species examining personality and metabolic rates and found 20 out of 27 studies resulted in a positive correlation. Upon closer examination, however, only 9 of the studies used behavioral tests measuring personality traits as previously defined (Reale et al. 2007).

My stress response data showed variation in the quail population but we did not see a correlation between the corticosterone response and the personality of the quail. I did, however, see a trend for females to have higher levels of plasma corticosterone. Plasma corticosterone increases as a response to the stress of being captured and human handling. Studies have shown glucocorticoids rise within three minutes of a perceived stressor (Silverin 1998) and our initial blood draws occurred between 6:01 and 8:24 minutes after the capture time. While I took measures to decrease the amount of time it took to catch the quail and the amount of time before drawing blood, we probably missed the true baseline reading. A similar study on behavior and fear response in Japanese quail chose to decapitate the quail immediately after presenting a stressor to reduce the added increase in corticosterone human interaction may cause (Richard et al. 2008).

Fecal and feather corticosterone testing is relatively new to the field but could be a less stressful option for future studies, especially field studies. Using fecal samples as a measure of corticosterone could be limiting because of the short time frame the corticosterone represents and individual identification of the animal in question might be difficult (Cabezas et al. 2007). Feather corticosterone on the other hand can provide a reliable measure of baseline corticosterone and has been consistent with plasma corticosterone levels taken during the same time frame even during stressful life events (Bortolotti et al. 2008).

Because of its importance to an individual's life history, multiple studies have looked at the correlation between the corticosterone response and personality traits. Cockrem (2007) and Korte et al. (2005) suggested circulating corticosterone is one of the mechanisms behind personality variation in populations, however, the current research has not substantiated that relationship. Results within a study have shown correlations between lines selected for shy and

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bold personalities but failed to show correlations in birds from a non-selected line using the same research methods. A study on baseline corticosterone concentration in great tits selected for shy to bold personalities found the shy line had higher plasma corticosterone concentration (higher stress response) and slower response times in exploratory and behavioral tests during personality testing. The same study, however, did not find a correlation between plasma corticosterone concentration and personality traits in birds tested from a non-selected line (Baugh et al. 2012).

Current literature focusing on the interaction between physiology and personality is contradictory. Table 8 is a comparison of my research and published research concerning the association between physiological traits and personality in birds including my study. Of the fifteen previous studies presented, six studies observed a significant correlation, six studies observed a lack of or insignificant correlation and three studies observed correlation in one sex or one environment but not the entire study population.

Table 8. Comparison of research studies concerning physiology and personality.

Species	Study Type	Physiology Test(s)	Personality Test(s)	Correlation (Y/N)	Author/Date
Blue Tits	Field	Breathing Rate, Immune Response	Obstinacy	N	Kluen et al. 2013
Dark Eyed Junco	Wild Caught	Plasma Corticosterone	Exploration	Y	Atwell et al. 2012
Dark Eyed Junco	Wild Caught	Metabolic Rate	Dominance	N	Vezina and Thomas 1999
Dippers	Wild Caught	Metabolic Rate	Dominance	Y	Bryant and Newton 1993
Great Tits	Wild Caught	Breathing Rate	Exploration	Y	Carere and van Oers 2004
Great Tits	Wild Caught	Breathing Rate	Exploration	Y	Fucikova et al. 2009
Great Tits	Captive: Selected and Non	Plasma Corticosterone	Exploration	Y (Selected) and N (Non)	Baugh et al. 2011
Great Tits	Captive: Selected	Plasma Testosterone, Immune Response	Exploration	Y	van Oers et al. 2011
Greenfinches	Wild Caught	Deposited Corticosterone	Fear	N	Sepp et al. 2014
House Sparrows	Wild Caught	Plasma Testosterone	Exploration	N	Mutzel et al. 2011
Japanese Quail	Captive: Selected	Plasma Corticosterone	Fear	N	Richard et al. 2007
Japanese Quail	Captive	Plasma Corticosterone, Metabolic Rates	Exploration, Neophobia, Fear	N	Current Study
Pied Flycatcher	Field	Breathing Rate	Exploration, Fear	Y (Males – Fear Only), N (Females)	Krams et al. 2014
Rufous-collared Sparrows	Wild Caught	Metabolic Rate, TEWL	Exploration	Y (1 Site), N (2 Sites)	Maldonado et al. 2012
Zebra Finches	Wild Caught	Breathing Rate	Neophobia, Exploration	N	David et al. 2011
Zebra Finches	Captive: Selected	Plasma Corticosterone	Neophobia, Exploration	Y	Martins et al. 2007

My study shows a similar trend for male personality and physiology to be correlated although the correlation was not statistically significant. Upon reviewing literature, very few studies have been conducted on the differences between males and females. The personality and physiology of males show a tendency to be more correlated as compared to females, Schuett et al. (2010) suggested perhaps consistency in male behavior is a sexually selected trait.

CONCLUSION

My data show a continuum of bold to shy personalities within the quail population but did not show a significant correlation between the personality and physiology of the quail. My results show metabolic rates are variable within a population and repeatable, and my results suggest personality of the males had a trend to be driven by physiology.

Variation in individual personality and physiology within a population exists and has been shown in multiple studies across species. Current literature suggests perhaps a correlation between personality and physiology exists, however, the mechanisms behind the relationship are poorly understood. My study examined multiple behavior traits and two physiological traits but our data failed to find a significant correlation between the personality and physiology. As presented above, the literature is fairly split on whether or not personality and physiology are correlated. The idea is well researched and presented, however, the empirical data are inconsistent and my research is no different.

My data add to the understanding of metabolic rate variation and stress response in a quail population. I did see trends for male personality to be driven by physiology and for females to have higher levels of plasma corticosterone. More studies are required to flush out the rest of the personality versus physiology relationship, including field studies to determine if perhaps captive populations limit the relationship observed. Future research should be focused on like species to compile a more comprehensive understanding of the relationship between physiology and personality. The current literature is very passerine species focused and lacks study populations with similar life history traits of quail.

Moving forward, animal personality research requires a more structured framework similar to the study of human personality. The personality traits need to be classified and more

thoroughly defined. I suggest using multiple behavior tests to place individuals on a continuum. Physiological testing is popular but, like personality testing, varies in methods. Less invasive methods and field methods should be explored for future research.

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