Title

Started From the Egg, Now We’re Here: Examining the Development of Consistent Individual Variation in Growth and Behavior of Captive-Reared, Precocial Wood Ducks (Aix sponsa)

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Started From the Egg, Now We’re Here: Examining the Development of Consistent Individual Variation in Growth and Behavior of Captive-Reared, Precocial Wood Ducks (*Aix sponsa*)

By

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DISSERTATION

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Abstract

Individuals within a population often vary in the range and kind of behaviors that they exhibit. This variation, when consistent across time and/or context, has been termed animal personality. How and why consistent individual differences are maintained within populations when they have far-reaching fitness consequences remains a fundamental inquiry. Links between individual state and behavior have been hypothesized as an important component in the maintenance of this variation. Furthermore, understanding the development of consistent differences in state, behavior, and their relationship is of pivotal importance in addressing this question. With this dissertation we aimed to longitudinally examine the relationship between growth and personality across ontogeny in captive-reared Wood Ducks (*Aix sponsa*). In 2016 and 2017, we collected Wood Duck eggs from wild populations in the Central Valley of California and artificially incubated them at the University of California, Davis. We then reared hatched individuals in a controlled environment, collecting morphometric and behavioral data on a weekly basis using standardized assays. We found that individual differences in behaviors associated with sensitivity to predation risk (tonic immobility as well as activity, space-use, and response to a startling stimulus in a modified Open Field Test) remained relatively consistent across development. Repeatability of these behaviors qualitatively increased as ducklings aged while within-individual variance in behavioral expression decreased significantly, suggesting canalization. Duckling size also consistently varied and was significantly related to behavioral expression among-individuals, though the relationship was complex and age dependent. In a common garden setting, we found no evidence of feedback between size and behavior within-individuals as a driver of behavioral differentiation, suggesting that the observed variation in behaviors is not proximately driven by covariance with size during development. Experimentally increasing the perception of predation risk within the developmental environment significantly decreased size and growth rates as ducklings aged but had a limited effect on expression of select behaviors and their relationship with duckling size throughout ontogeny. Experimental treatments also appeared to generate negative feedback between a metric for Activity & Space-use and body mass within-individuals, potentially leading to lowered repeatability and convergence of this behavioral type. Herein we discuss the methodology of measuring consistent individual differences in behavior, the possible causes of the relationships we discovered, the implications of our findings for Wood Ducks (a precocial waterfowl species), and the broader implications of our study for the understanding of personality, its development, and its ecological impacts.

Dissertation Overview and Contents

Broad Question: Are behavioral differences in juvenile Wood Ducks consistent across ontogeny and how do we measure these differences?

Broad Question: Does behavior change as ducklings develop?

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Introduction to the study of Animal Personality

Despite a consensus that variability drives evolution by natural and social selection, many investigators in the past have treated apparent variation as noise around a statistical mean (Williams 2008; Réale et al. 2010a; Stamps and Groothuis 2010a&b). Within the last 15 years, however—due, in part, to recent strides in computational, statistical, and methodological approaches—many are starting to focus on the far reaching ecological and evolutionary implications of consistent behavioral variation (Bolnick et al. 2003, Sih et al. 2004a&b; Réale et al. 2007; Sih et al. 2010). Evidence in a variety of taxa suggests that individual variation in behavioral characteristics (e.g., sociability or exploratory tendency) can be consistent across time and/or context (i.e., animal personality) and that these behavioral characteristics can be correlated (Bell and Sih 2007). Traits that are typically of focus include boldness (risk sensitivity), exploration (speed or proclivity), sociability (tendency to be social), activity, and aggression (Réale et al. 2007). Behavioral syndromes occur when there is covariation among various behavioral traits (Sih et al. 2004a&b) and may also include correlations among morphological (Luttbeg and Sih 2010) and/or physiological (Careau et al. 2009) traits.

Currently, there is evidence for the importance of personality in social interactions (Bergmüller and Taborsky 2010; Kozlovsky et al. 2014), reproduction and life history productivity (Biro and Stamps 2008; Réale et al. 2010b; Dingemanse and Wolf 2010; Mutzel et al. 2013; Nicolaus et al. 2015), predator-prey and disease dynamics (DiRienzo et al. 2013; Ezenwa et al. 2016), foraging optimality (Biro and Stamps 2008; Bergvall et al. 2010), dispersal and invasion (Cote et al. 2010; Chapple et al. 2012), and response to human induced rapid environmental change (HIREC; Sih et al. 2012a). A number of literature reviews (Careau et al. 2008; Sih et al. 2010; Réale et al. 2010a; Dingemanse and Wolf 2010; Dochtermann et al. 2014;

Cabrera et al. 2021; along with many others) cover an extensive collection of compelling theoretical and empirical studies that exemplify the role of personality in ecology and evolution.

A major question emerges when considering animal personality and its potentially far reaching ecological and evolutionary implications: *how and why is this variation maintained within populations*? Although there may be some plasticity in behavioral responses, individuals generally appear to be limited in the range of behavioral responses they can effectively exhibit. Behavioral carryover effects (i.e., correlations among behavioral traits that carry across context and/or time) due to these limitations on behavioral plasticity or flexibility have been invoked as a possible explanation for the observation of seemingly suboptimal behavior in a given context (Sih et al. 2004a&b; Sih et al. 2010). It is unlikely that suboptimal traits can remain within a population over evolutionary time, but when considering carry over effects, individual differences in behavioral type or correlations in behavioral traits might represent alternate strategies for dealing with ever-changing environmental stimuli when faced with a variety of constraints at multiple levels.

Elucidating the proximate mechanisms that might drive the emergence of personality within populations is pivotal in understanding how personality is maintained as well as its fitness consequences (Tinbergen 1963; Stamps and Groothuis 2010a&b; Trillmich and Hudson 2011). Though the importance of ontogeny is widely understood and has been acknowledged in the literature, gaps in our knowledge of personality development remain (Cabrera et al. 2021). With this dissertation we examine the development of consistent individual differences in behavior among two cohorts of captive-reared Wood Ducks (*Aix sponsa*). We do so by longitudinally assessing consistency in behaviors associated with sensitivity to predation risk and their relationship to duckling growth in a common garden environment (Chapters 1-3) and under experimental manipulation of the developmental environment (exposure to simulated predation and increased required foraging effort; Chapter 4).

General Methods

In this section we describe in detail the general methods applied throughout the dissertation. This includes a brief background overview of our study system, justification for the use of this system in addressing our overarching questions, specimen collection, rearing protocol, and measurement protocols (both morphological and behavioral). The first three chapters of this dissertation involve data collected during the 2016 field season, while the fourth chapter revolves around data collected in 2017 and includes an experimental treatment protocol. Specimen collection, rearing, and measurement protocols differed slightly between seasons; we include thorough description of these adjusted methods in Chapter 4.

Study System

Wood Ducks (WODU; *Aix sponsa*) are a small-medium sized species of dabbling duck with precocial offspring. Though mature plumage is broadly characterized by a crest of feathers on the posterior of the head, adults are sexually dimorphic with males exhibiting a vibrant and colorful breeding plumage and females exhibiting a generally drab coloration with a starkly contrasting white eye patch. Their range is broad, spanning most of the continental United States, southern Canada, and northern Mexico (Hepp and Bellrose 2013). Overall habitat preferences typically consist of a range of freshwater habitat including riparian (rivers, creeks, and streams) and wetland (bottomlands, swamps, marshes, and in some cases ponds) habitat (Hepp and Bellrose 2013; Bellrose and Holm 1994). They are often referred to as "tree ducks" due to their high flight maneuverability (relative to other waterfowl) in forested areas and because they are secondary-cavity nesters. Hens provide the majority of reproductive effort, exclusively acquiring nest sites, incubating eggs, and rearing broods. Historically, female WODU establish nests in found tree cavities, but more recently they have become reliant on broadscale, artificial nest-box programs (Semel and Sherman 1995), particularly in California where >90% of suitable wetland habitat and old-growth trees have been removed or fragmented in the past century (Ferren et al. 1996; Dahl and Allord 1997). Wood Ducks are dietary generalists and have been documented consuming a large variety of plant-based foods and invertebrate species; dietary preferences often varying ontogenetically and seasonally (Bellrose and Holm 1994, Hepp and Bellrose 2013). Wood Ducks are well known for their 'skittish' and 'secretive' behavior, displaying large Flight Initiation Distances in most scenarios (*personal observation*). Due to their general risk aversion, they tend to prefer dense emergent vegetation, likely as a means of refuge from predators but also potentially as a source of food-rich foraging opportunity (Bellrose and Holm 1994).

In the central valley of California (within our field sites; see below), hens begin prospecting for nest sites/boxes in January and February. Egg deposition within California typically begins around February or March and incubation begins in late March or early April (with considerable variation among hens as well as across populations and seasons; *unpublished data*). Clutch sizes range from 4 to 50 eggs due to variable Conspecific Brood Parasitism (a behavior in which some hens lay their eggs in the nests of other WODU hens; Lyon and Eadie 2008). Incubation durations are typically around 28-30 days in length, although there is potential for intrinsic and/or incubation temperature mediated developmental variability (DuRant et al. 2012). The highly precocial ducklings hatch relatively synchronously and exit the nest together within approximately 24 hours of hatch (often referred to as 'nest exodus'; Bellrose and Holm 1994) in response to a specific vocalization from their brood mother. Subsequently, ducklings brood with the nesting hen for a period of roughly 4-8 weeks; ducklings begin molting in their first set of primary and secondary remiges (flight feathers of the wing) around week 5 of age and gradually become fully flight-capable between weeks 5-8 (*personal observations*; Bellrose and Holm 1994). Though brooding hens provide a physical buffer from temperature fluctuations and guide ducklings to foraging patches, the young feed themselves and can thermoregulate; WODU hens do not appear to provide active defense from predators, typically flushing before guarding their broods (*personal observation*). It is broadly unknown whether juveniles remain in close social contact with brood members, mothers, or other relatives post-fledging, but adult WODU can often be found in social groups (Bellrose and Holm 1994). The average number of breeding years for adult WODU is 2-3 years, although some hens breed for up to 6–8 years (*unpublished data*).

Justification for Use of This System

The Wood Duck provides an ideal study system for addressing questions of ontogenetic growth and early life behavioral development for two important reasons. The first is that WODU have a precocial mode of development. Much of the empirical work that has examined development of personality in birds has revolved around a select few model study systems (i.e., Great Tits [*Parus major*], Eurasian Blue Tits [*Cyanistes caeruleus*], and Zebra Finches [*Taeniopygia castanotis*]; Cabrera et al. 2021). While these studies provide significant insight into how personality might change or become established throughout ontogeny, we contend that examining development in a precocial system can provide a unique and important perspective.

Altricial birds hatch from their eggs helpless, blind, and wholly dependent on parental provisioning. Though timing of fledging can vary from species to species, altricial birds typically don't leave the nest for up to 2-4 weeks after hatching (Starck and Ricklefs 1998). Wood Duck hatchlings, by comparison, emerge from the egg fully capable of sight, with a downy plumage, developed motor skills and leave their nest within 24 hours post-hatch. Although ducklings brood with the mother for roughly 4-8 weeks before fledging, she typically only provides a buffer for extreme weather conditions (i.e., aiding in duckling thermoregulation by directly providing body warmth and refuge from the elements) and leads her brood to potential foraging patches. As such, WODU ducklings are much more directly responsible for their own fitness outcomes, actively feeding themselves and investing in predator avoidance and escape behaviors that the mother does not provide. Within a day of hatching, WODU duckling behavioral expression mediates their growth and survival.

This brings us to the second reason that we consider Wood Ducks to be a model system for growth and behavioral development research; young WODU face a daunting early-life gauntlet. Duckling survival tends to be low in the wild, ranging from 15-50% depending on the environment (Davis et al. 2007; Davis et al. 2009). A majority of the mortalities of young Wood Ducks occurs within the first two weeks of life, and most mortality events are driven by an extensive array of predators. In the Central Valley of California, these predators include (but are not limited to) Great Blue Herons (*Ardea herodias*), Black-crowned Night-Herons (*Nycticorax nycticorax*), other Herons/Egrets, American Crows (*Corvus brachyrhynchos*), California Scrub-Jays (*Aphelocoma californica*), various Owls and Raptors, River Otters (*Lontra canadensis*), American Mink (*Neovison vison*), Raccoons (*Procyon lotor*), house cats (*Felis catus*), Snapping Turtles (*Chelydra serpentina*), Bullfrogs (*Lithobates catesbeianus*), and Large-mouth Bass

(*Micropterus salmoides*) (Bellrose and Holm 1994). This intense selective pressure is likely to have far reaching implications for the evolution of life history traits and early-life behavioral development in WODU and, accordingly, makes this system an ideal candidate for examining potential relationships between growth and behavior across ontogeny (see Chapter 3 for a discussion of the growth-mortality trade-off; Stamps 2007).

Specimen Collection, Rearing, and Housing

In 2016 we collected 397 eggs (244 of which were viable) from four long-term field sites in the Central Valley of California, successfully hatching and rearing 125 ducklings. In 2017, we collected 469 eggs (265 of which were viable) and successfully hatched and reared 150 ducklings (we present data collected from this cohort in Chapter 4). The breeding season of Wood Ducks in this region is generally between February and July (hens begin prospecting potential nesting sites in February if not earlier, first eggs are deposited around March, and the last clutches hatch around mid-July). We collected eggs from a variety of nesting situations; a majority of the eggs were from abandoned nests (a frequent occurrence in our system as many hens respond to sufficient perturbations near the nest by ceasing incubation) or when unhatched eggs that did not hatch synchronously with the rest of the brood were left behind. We collected a subset of eggs prior to incubation. It should be noted that small fluctuations in incubation temperature have been shown to produce physiological and behavioral consequences for hatchlings (DuRant et al. 2010; DuRant et al. 2012; DuRant et al. 2013; Hope et al. 2018); we recorded stages at which eggs were collected but did not consider this to be particularly detrimental for our purposes as we aimed to assess post-hatch behavioral development regardless of the initial post-hatch behavioral baseline.

As quickly as possible after collection, we recorded morphometrics (length/width, weight, and a rough estimate of embryonic stage) and relocated eggs to our artificial incubator (Rcom MARU Digital Incubator and Hatcher) on campus at the University of California, Davis. We incubated eggs at a consistent temperature of 35.9 degrees Celsius and humidity of 60%, within the acceptable range for Wood Ducks (DuRant et al. 2010), with the incubator set to automatically rotate eggs at regular 60-minute intervals to simulate egg turning by females in the wild. We also provided eggs with two 30-minute cool down periods per day by turning off incubator heating elements and allowing eggs to cool to room temperature, roughly simulating incubation breaks taken by wild hens in our population. When individual eggs internally pipped (a process by which the pre-hatchling breaks its internal membrane to access a pocket of air within the egg, usually occurring a few days prior to full hatch), we individually isolated eggs (to match egg to hatchling post-hatch) and moved them to a hatcher unit ceasing regular rotation and changing temperature to 36.2 degrees Celsius and humidity to 70-80% to allow greater ease of hatching.

When hatchlings emerged from the egg, we conducted initial in-hand processing (see-below) and implanted PIT tags (Passive Integrative Transponders) subcutaneously in the scapular region (following protocols for separate studies conducted by our lab in the field; Stair et al. *In prep.*) to reliably identify individuals across the study, which is essential for collecting repeated measurements. Retention of PIT tags was high (10 of 125 rejected in 2016 and 3 of 150 rejected in 2017), but to ensure we did not lose track of individuals in the event that a PIT tag was rejected, we fastened temporary plastic identifying tags to hatchlings' backs (which usually fell off within 10 days post-hatch) and began applying leg bands with color codes after the first week post-hatch. In 2017 we also applied color coded leg bands at hatch (appropriately re-sizing bands as ducklings grew).

We assigned birds to brood groups based on hatch timing. In the wild, WODU hatch synchronously, but due to the nature of our study (eggs continuously collected during the reproductive season from a variety of nests across multiple field sites at various stages of incubation) hatch timing was very asynchronous. Even for eggs that we collected from the same nest at the same time, hatches did not necessarily occur on the same day. Furthermore, we we established broods of ducklings that were no more than 4 days apart in age to ensure that brood sizes were within the natural range for WODU (approximately 3-25) and that older ducklings did not attack and even kill younger brood-mates (a behavior that can occur if there are significant age differences among ducklings; *personal observation*). In 2016, we successfully reared 17 broods in captive brood sizes ranging from 3 to 11 ducklings. In 2017 (again, data used in Chapter 4), we successfully reared 18 broods (one was not included in our experiment) and captive brood sizes ranged from 5 to 11 ducklings.

For the first week post-hatch, we kept hatchlings in small, indoor brooding bins with a ceramic over-head heat element providing heat (one of the essential responsibilities of brooding hens in the wild). We altered first week brooding protocol in 2017 (see Chapter 4 methods). At this stage, we also supplemented ducklings with live mealworms to promote feeding behavior. At all stages, Mazuri waterfowl starter (Formula #5641) was provided *ad libitum* in a centralized location (distribution of food was experimentally manipulated in Chapter 4). At one week of age, we moved broods into outdoor housing pens (3x6 meters) with soil substrate where we provided them hides for refuge and pools. We collected behavioral data until week 5, and we released a majority of our birds back into their site of origin when flight capable for monitoring in future studies (between the ages of 8 and 14 weeks). We kept a subset of birds in captivity for further research outside the scope of this study. In total, we reared 275 individuals across two years; 23 of which we kept for long term studies.

Behavioral Assays

In-hand Processing

At hatch and approximately every week thereafter for the first 7 weeks of life, we collected individuals and processed them in-hand (Figure 1.1). In 2016, we processed eight of our broods at hatch and weeks 1, 3, 5, and 7 due to time constraints. In 2017, we processed every individual every week from hatch through week five. When processing, we collected individuals of the same brood from their home enclosure simultaneously and isolated them in small opaque bins. We determined processing sequence randomly to account for the potential for sequence effects in our behavioral assays. After providing the birds a short period of time (~10-30 minutes at minimum) to recover from capture, we began processing them one at a time. We collected traditional morphometrics (Dzubin and Cooch 1992) including body mass in grams, tarsus length (i.e., tarsometatarsus; the fused bone between the toes and the ankle joint), culmen (upper mandible) length, and culmen width in millimeters. Using a digital scale, we recorded body mass by placing individuals upside down in an opaque tube with a tight enough diameter to prevent them from repositioning (a standard approach to weighing birds with a digital scale).

While birds were in-hand, we conducted two behavioral assays; a Struggle Test and Tonic Immobility assay, both designed to assess the birds' response to being handled. We conducted the Struggle Test when the ducklings were set within their weighing tube; we started a timer for 30 seconds and we recorded the number of kicks that the bird produced during that time

(Tube Struggle). After taking morphometric measurements we then turned birds onto their back on a standardized surface and gently pressed the bill into their breast while covering their eyes. For a majority of ducklings (and adult WODU) this will induce a state of Tonic Immobility, a state in which the individual is relatively unresponsive to external stimuli and remains motionless. Tonic Immobility has been documented in a variety of species as a response to a specific stimulus, usually in situations that might mimic capture/handling by a predator (Gallup 1977; Jones 1986; Campbell et al. 2019).

We designed our assay with four metrics. First, we recorded the number of attempts—up to a maximum of five—required to get the bird into a state of Tonic Immobility (herein Tonic Attempts). Second, we assigned a score (from 0-4) for the degree to which the bird was alert (focusing on expressions of attentiveness and body position) while in Tonic Immobility. A score of 0 indicates that both of the bird's eyes were closed and it was in a relaxed position. A score of 3 indicates that the bird's eyes were open and its neck was erect; often this was paired with some movement of the legs. If the bird never entered Tonic Immobility, we gave it a score of 4 (in 2017 we capped the maximum score at 3 rather than 4; see Chapter 4). Third, we recorded the latency for the bird to break out of its state of Tonic Immobility to a maximum of 60 seconds (Tonic Immobility). We considered breaking Tonic Immobility as any attempt from the bird to right itself.

Open Field Tests

At weeks 1, 3, and 5 we conducted modified Open Field Tests (OFT) in an arena comprised of a wooden box ~1.8x1.8x0.9 meters, painted white with a visible black grid on its floor (Figure 1.2). In 2016, we were unable to obtain data for Open Field Tests for three of our broods (21 birds) but do include them in the in-hand and morphological analyses in Chapters 1-3. We recorded Open Field assays with an overhead security camera storing the video files with a Digital Video Recording device (LaView HD Security DVR System), later attaining behavioral metrics via manual video transcription. We filled the arena with approximately 5-12 centimeters of water such that ducklings were swimming throughout the test. In an attempt to keep the arena relatively novel for each repeated assay, we used an array of plastic plants placed in a specific pre-determined pattern which we rearranged every week, keeping arrangements for each week standardized across broods.

After processing birds in hand, we placed them back in their isolation bins and conducted OFTs in the same sequence that we processed the birds just prior. While conducting the assay, we first placed the individual within an opaque acclimation chamber inside of the arena. Birds were then given approximately 2-5 minutes to acclimate, after which an observer opened a door on the acclimation chamber remotely with a pulley system. Birds were then allowed up to 7 minutes to exit the chamber of their own volition (reduced to 2 minutes in 2017; see Chapter 4 Methods). We recorded latency to emerge from the chamber in seconds. As this metric was highly skewed (with most individuals remaining in the chamber for the entire duration), we did not include it in our analyses. After 7 minutes, whether or not the birds had exited the acclimation chamber, we remotely lifted the entire chamber out of the arena using a remote pulley system. For most birds this was a startling event, and we recorded the intensity of the birds' response to lifting the acclimation chamber on a subjective scale of 0-5 (Response to Startle Stimulus). A score of 0 indicates that the bird responded in no noticeable way while a score of 5 indicates that the bird fled the stimulus with extreme vigor.

We then observed the birds' free moving behavior for the next 5 minutes before terminating the assay. We quantified activity as the number of grid line crosses within this period. In 2016, we recorded a subjective score for the birds' 'jitteryness'—instantaneous sampling of the number of 30 second intervals in which erect posture and rapid head movements were observed; this behavior might be interpreted as a measure of ‘neuroticism’—herein referred to as Rapid Head Movement. We also quantified space-use via instantaneous sampling of recording location (the grid cell in which the bird was located) in 5 second intervals for the 5-minute duration. A majority of the time, ducklings tended to remain close to the walls of the arena which we interpreted as a display of thigmotaxis. Thigmotaxis, specifically within the context of an Open Field Test, has been well defined in rats and mice as relating to anxiety and anxiety associated behaviors (Simon et al. 1994; Treit and Fundytus 1988; Seibenhener and Wooten 2015). It has also been observed in a variety of other systems including zebrafish (*Danio rerio*, Shams et al. 2015), cockroaches (Blattodea: Blattidae, Salazar et al. 2018), amphipods (*Echinogammarus marinus* and *Gammarus pulex*, Kohler et al. 2018), and cuttlefish (*Sepia officinalis*, Tonkins et al. 2015). Here, we quantified the degree to which individuals appeared willing to stray from these locations of relative perceived safety (which we designate anti-Thigmotaxis) as a proportion of the 5-second intervals within the 5-minute period spent in grid boxes away from the arena walls. We also measured the proportion of 5-second intervals that ducklings spent in grids near the original location of the acclimation chamber which we refer to as Startle Stimulus Revisitation. Finally, at the conclusion of the assay, an observer approached the arena to retrieve the duckling with a net; we recorded the intensity of the individual's response to capture on a scale of 0-5 similar to our Response to Startle Stimulus scale described above. We refer to this score as Response to Observer. We include in this manuscript tables of all behavioral metrics with brief descriptions (Tables 1.1 and 4.1).

Chapter 1

Validation and Reliability: A Holistic Approach to Quantifying Behavioral Differences in Captive-reared and Developing Wood Ducks (*Aix sponsa*)

Introduction

Despite an elevated interest in understanding animal personality and its fitness consequences and advancement in both computational capabilities and statistical approaches, the field continues to grapple with conceptual, logistical, and methodological challenges associated with quantifying personality and assessing its biological importance. Carter et al. (2013) lay out a guideline for behavioral ecologists interested in questions of personality. They emphasize biological relevance of behavioral measures and discuss two of the core complications of measuring personality (both in humans and non-human animals): (1) there are 'many tests for one trait' and (2) there is often 'one test for many traits' (i.e., certain assays might have a lack of specificity). In the past empiricists have often studied animal personality by measuring one or two behaviors using one or two assays and then interpret these measures to be direct representations of core personality traits such as 'boldness' or 'exploratory tendency' without explicitly addressing the process by which those particular behaviors/assays were chosen and why they represent said traits.

In practice, however, personality traits (boldness, activity, aggression, sociability, and exploratory tendency) are nebulous and difficult to define, making it extraordinarily difficult to encapsulate them with one or even a few specific behaviors or behavioral assays. “Personality” is an emergent, umbrella concept that likely incorporates a multitude of specific behaviors. Moreover, in many cases, there is correlation among different personality traits and there is often overlap in which personality traits specific behaviors represent. Compounding these issues is that terms and concepts are applied generally across species, yet this is often inappropriate; - for example, behaviors measured in one species might be excellent representatives of 'boldness' but could have a completely different biological meaning in another species (or might not be present at all). Indeed, it is impractical and likely incorrect to assume that behavioral tests can be standardized across species due to a plethora of logistical and biological factors.

So, what is to be done? Clearly, context is important. However, a foundational aspect of behavioral ecology involves explaining the fitness consequences of behavioral patterns broadly observed in nature. Moreover, personality has been clearly documented (to some degree) in almost every species observed. Many of the aforementioned problems are not new and behavioral ecologists are not the first to encounter them. Psychologists and psychometric analysts studying human personality have been struggling with these same issues for decades (Hogan 2005; Carter et al. 2013). Although there are clearly challenges with cross-communication between our fields, we can learn much from human-centric research. With this in mind, Carter et al. (2013) suggest some useful steps for behavioral ecologists studying animal personality. First, test design must be carefully considered, including *a priori* assessment of relevance. In other words, does the test actually measure the trait(s) of interest in the species being studied? Second, multiple tests should be used for each trait being studied. This is essential for the third step, validation (validity being the 'degree to which a test measures the targeted trait') of tests being used. Validation requires assessment of repeatability (reliability of tests), ecological validity (tests measure behaviors that are meaningful for the questions of interest), convergent validity (assessment of correlations between two tests that should be measures of a single trait), and discriminant validity (no correlation among multiple tests that are assumed to measure different personality traits) (Carter et al. 2013).

In this study, we begin to assess personality in precocial, developing captive-reared Wood Duck ducklings (WODU; *Aix sponsa*). Our overall goal lies in studying the development of behavior and its potential fitness consequences in the early life of Wood Ducks (see Chapters 2-4) but to do so, we first need to focus on the methodological process of quantifying behavior and personality to provide a foundational springboard for further research with this system. We argue that in-depth validation of measured behaviors is essential before making any inferences regarding biological impacts or fitness consequences of personality. Our goal in this chapter is not to criticize prior research efforts in this field or dismiss their findings; previous empirical studies of animal personality are indeed relevant and iteratively contribute to a broader understanding of this phenomenon. Rather, our goal lies in holistically approaching our own research questions and providing transparent validation for our methods, inferences, and assertions.

To develop a robust assessment of traits associated with personality, we recorded 10 behavioral variables intended, *a priori*, to quantify aspects of reactivity, activity, and sensitivity to predation risk (both in terms of avoidance and capture prevention) in two standardized contexts. We aimed to validate our behavioral measures by meeting two goals. Our first was to **elucidate and describe relationships among these behavioral variables** to better understand and interpret their biological meaning and relevance. Dealing with a large number of behavioral variables can be difficult, and each variable only represents a portion of the underlying traits that could be of interest. Our second goal was to determine whether **behavioral differences among individuals remained consistent across ontogeny**. While we might expect to find some degree of plasticity in these young precocial birds as the grow and develop, there is a reasonable expectation that the behaviors we examined differ consistently among individuals from hatch through to early maturity (Cabrera et al. 2021), particularly in precocial species (see Chapter 2).

Developing a Study About Personality Development

Recently, Dingemanse and Wright (2020) produced a comprehensive set of "criteria for acceptable studies of animal personality and behavioural syndromes"; they discuss a number of terminological, study design, analytical, and dissemination issues that continue to challenge behavioral ecologists researching animal personality. Here we explicitly address and incorporate their suggestions, as well as those of Carter et al. (2013), in our own empirical research. We had four major considerations while determining which behaviors to measure and how to measure them.

First, we focused our efforts on a captive-rearing study because it both allowed us a degree of control over the developmental environment and it allowed us to conduct standardized behavioral testing (which is important when examining how behavior changes across ontogeny). Additionally, it is exceptionally difficult to track wild ducklings, let alone acquire multiple measures of a specific behavior across time (mostly due to low duckling survival rates; see below); thus, sample sizes in such a scenario would be exceptionally low.

Second, we had to determine which personality traits to assess. A majority of animal personality studies examine aspects of boldness, exploratory behavior, sociability/sociality, aggressiveness, and/or activity. It is our view that these 'axes' of personality are system specific (i.e., 'boldness' for Wood Ducks is not necessarily the same as 'boldness' for American Crows or three-spine stickleback). Again, WODU experience extremely low survival rates during the first few weeks of life post-hatch. Not only do young ducklings have to deal with environmental pressures such as temperature fluctuations and potentially severe weather conditions, but they also have to contend with a number of potential predators. In the wild, ducklings may face depredation from a variety of species including, but not limited to, Herons (Black-crowned Night Herons, Great Blue Herons, etc.), Corvids (California Scrub-Jays and American Crows), Large-mouth Bass, Bullfrogs, River Otters, American Mink, Raccoons, Snapping Turtles, and more. In one study, WODU duckling survival rates ranged from 15-58% (differences likely driven by habitat type) and avian and aquatic predators alone accounted for ~70% of all recorded duckling mortalities (Davis et al. 2007; Davis et al. 2009). With this in mind, we focused on aspects of sensitivity to predation risk, activity (due to a possible link between activity, growth, and depredation), and exploratory behavior.

Third was the question of how to quantify the traits we were interested in measuring. Boldness, exploration, and even activity are difficult to measure directly. Though it is appealing to assess traits like 'boldness', it would be insufficient to simply select one or two specific variables and consider them representatives of 'boldness' (Carter et al. 2013). Therefore, we aimed to measure a number of behaviors in two contexts and assess their relationships to one another in an exploratory analysis, followed by statistical variable reduction that incorporates information from each variable (see methods) The assays and behavioral variables we chose were informed by prior studies in other systems, Wood Duck biology, and logistical limitations (see methods for further details).

Last, we needed to determine how frequently to measure the behaviors of interest. This decision was a balance of logistic considerations and statistical/analytical requirements. Assessing personality (i.e., calculating repeatability, see Statistical Methods below) requires two or more measurements per individual. Because we were interested in assessing change across development as well as comparing early and late developmental stages, it was important to record each behavior (and morphological metrics) a minimum of three times during what would be considered the 'brooding' or 'pre-fledging' stage of life. Logistically it was not possible for us to conduct each assay twice within a week, however we ensured that we met this minimum three-measure requirement for each individual and each variable. Wood Ducks are on the cusp of molting their first set of remiges (flight feathers on the wing) at week 5, and tend to fledge (leave the hen) between weeks 4 and 8 (Bellrose and Holm 1994). Structurally, juveniles reach adult size at this time, although body mass continues to increase for the next year or so. As such, we ensured that our minimum of three measurements per individual all occurred within the first 5 weeks of life. We provide further detail on specific methodology in the General Methods section above.

Statistical Methods

Multi-response Correlation Analysis and Eigenvalue Decomposition

As Dingemanse and Wright (2020) discuss in detail, many researchers have approached animal personality with variable reduction methods, particularly through Principal Component Analysis. PCA can provide a convenient way to reduce the number of variables to a small subset of orthogonal variables while maintaining variance structures. However, this analysis does not incorporate an assumption of repeated measures (i.e., repeated observations on the same individuals and variables) and thus is subject to concerns of pseudoreplication. Accordingly, we conducted multi-response Generalized Linear Mixed Models (GLMM) using a Bayesian framework with the *brms* package (Bayesian Regression Models using Stan ver 2.13.0 in R;

Bürkner 2017) to assess relationships among 10 behavioral variables recorded in both the contexts described above. The *brms* package uses a Markov Chain Monte Carlo (MCMC) algorithm to draw samples randomly from the posterior distribution as the model runs (Bürkner 2017).

We ran all multi-response models with Gaussian distributions and flat, uninformative priors. We built models with 1000 warmup runs and 8000 overall iterations with four total chains. We assessed health of our models by checking Rhat values (not accepting any output with Rhats > 1) and visually examining posterior distributions of each parameter and posterior chains. In our first model, we included only individual identity as a 'random' effect and response variables included all 10 of our behavioral metrics as measured at weeks 1, 3, and 5; we only recorded OFT metrics on those weeks, therefore we removed in-hand variable observations accordingly for exploratory analysis. We then conducted the same analysis separating our data into two sets: in-hand metrics and OFT metrics. These models provide both within-individual intercept correlation estimates as well as residual correlation coefficient estimates (which more closely resemble traditional correlation analysis) between every dyadic pair of behavioral variables.

We then constructed residual correlation matrices and conducted eigen decomposition using the eigen function in R (we do not explicitly incorporate the credibility intervals of residual correlation estimates in our eigen decomposition analysis, instead only using the average estimate). This approach is similar to a PCA, but it allows us to explicitly account for repeated measures therefore addressing the pseudoreplication issue. We calculated eigenvalues for two components (i.e., two new, representative variables analogous to components in PCA) accounting for a majority of the variance in our data. For each observation, we manually calculated our two component scores. First, we centered each raw behavioral variable (using the ‘scale’ function in base R) and then multiplied each variable by its respective eigenvalue. Lastly, we summed across adjusted variables for each observation within each component to generate a new score.

For comparative validation, we supplemented our multi-response correlation and eigen decomposition analyses with the traditional Principal Component Analysis (using a frequentist correlation matrix with the *FactoMineR* ver 2.3 package in R ver 4.0.2). As for the multi-response GLMMs above, we also conducted this analysis including all 10 variables as well as splitting the variables into two groups (In-hand and OFT).

Repeatability Across Ontogeny

To calculate repeatability we constructed a series of varying intercept Generalized Linear Mixed Models in *brms* (Bayesian Regression Models using Stan ver 2.13.0; Bürkner 2017) for each individual behavioral variable and eigen component (Table 1.2). We included individual identity and brood as ‘random’ effects. We more thoroughly consider the influence of age, sex, and body mass in a separate chapter, but we wanted to account for them in our models and so we included them as ‘fixed’ effects along with an interaction term between age and body mass (but see Chapters 2-4 for more in-depth analyses).

For the OFT (which was recorded in an outdoor pen) models, we also included wind intensity, testing sequence, and arena temperature as fixed effects to control for their potential influence. We then ran posterior distributions through the *sjstats* package (ver 0.17.4) in R to produce a Bayesian equivalent of the adjusted Intraclass Correlation Coefficient (ICC) as a measure of repeatability with a 95% Highest Density Interval (HDI) (Nakagawa and Schielzeth

2010). For all our Bayesian models we interpreted significance of estimates based on HDI and Credibility Interval overlap with zero, a standard for Bayesian inference; terms that overlap zero are not considered to be significant.

Results

Assessing Relationships Among Behavioral Variables

Multi-response Correlation Analysis

To better understand how our measured variables related to each other and to provide context (and the first step) for variable reduction analysis, we examined dyadic correlations among all of our behavioral variables across and within two measurement contexts. In both contexts (in-hand and OFT), we observed correlational structure among our behavioral variables. For in-hand variables, all of the residuals were significantly correlated with one another (Figure 1.3). Similarly, nearly all OFT variables were significantly correlated with one another; exceptions were the dyads Activity- Startle Stimulus Revisitation, and Anti-Thigmotaxis - Startle Stimulus Revisitation (Figure 1.3). The latter pair (space-use metrics that measure different aspects of space-use) trended towards a positive correlation. Rapid Head Movement, though trending towards a relationship, was not significantly correlated with either space-use metrics. When all variables, across context, are considered together in a single multi-response model, there were several trends and significant correlations among in-hand and OFT variables (Figure 1.3). Interestingly, response in-hand was negatively correlated (as a whole) with reactivity in the Open Field Test (Figure 1.3).

Eigen Decomposition

For in-hand measures, eigen decomposition using residual correlation coefficients from the multi-response correlation analysis revealed two components with eigenvalues of 2.378 (eigen component 1) and 0.946 (eigen component 2). Additional components were far below eigenvalues of 1 (accounting for much less of the variation in our data), and we do not include them here. Tonic attempts, tonic alertness, and tonic immobility all loaded heavily on the first component, while only the Struggle Test metric loaded heavily on the second component (Table 1.3). We interpret the first in-hand component as an index of ‘Tonic Intensity’ (higher scores indicating that individuals were less likely to enter tonic immobility, showed indications of high alertness, and did not remain tonically immobile for long) and the second component as an index of ‘Struggle Intensity’ (higher scores indicating birds kicked more frequently during the Struggle Test); we refer to these names in all further analyses. For OFT measures, eigen decomposition revealed two components with eigenvalues of 2.391 (eigen component 1) and 1.133 (eigen component 2); remaining components had eigenvalues well below 1 and we do not include them here. Rapid Head Movement, Activity, Startle Response, and Response to Observer loaded heavily on the first component, while both space-use metrics loaded heavily on the second component (Table 1.3). We interpret the first component as a measure of ‘Reactivity’ and the second component as an index of ‘Space-use’ and, again, we refer to these names in further analyses.

Principal Component Analysis

Results from Principal Component Analysis (PCA) qualitatively matched the results from the multi-response GLMM correlation analysis and eigenvalue decomposition (Table 1.3). For in-hand measures, the first principal component (PC1) had an eigenvalue of 2.32 and accounted for 58.1% of the variation while the second PC had an eigenvalue of 0.96 and accounted for 24% of the variation. Tonic Attempts, Tonic Immobility, and Tonic Alert all loaded heavily on the first PC. Struggle Test, alone, loaded more heavily on the second PC. This was the same loading structure as in the eigen decomposition analysis. For OFT, the first PC accounted for 39% (with an eigenvalue of 2.34) of the variation and the second PC accounted for 22.2% of the variation (with an eigenvalue of 1.33). Startle Response, Response to Observer, Activity, and Rapid Head Movement all loaded heavily on the first principal component as was also found in the eigen decomposition analysis. Anti-thigmotaxis and Startle Stimulus Revisitation (both space-use metrics), as well as Activity, loaded heavily on the second principal component. Thus, both analyses—one accounting for repeated measures and one not—produced very similar loading structures and suggest that the variable reductions represented by the first two components in each analysis are comparable and robust.

Individual Differences in Behavior are Consistent Across Ontogeny

To validate repeatability measurements for our eigen components, we calculated Intraclass Correlation Coefficients for each raw behavioral variable separately. All but two of our behavioral variables were repeatable (Figure 1.4) The two variables that were not repeatable were Startle Stimulus Revisitation and Response to Observer; both of which had 95% Highest Density Intervals that reached zero.

Overall, in-hand measures appear to be less repeatable than Open Field Test measures, with mean estimates between 0.10 and 0.17 (Figure 1.4). Activity in OFT was highly repeatable with an ICC of 0.39; Anti-Thigmotaxis and Response to a Startle Stimulus were also fairly highly repeatable with 0.27 and 0.26 respectively.

We also calculated repeatability for each of the eigen decomposition components described above. Tonic Intensity (our first in-hand eigen component) had a significant repeatability of 0.15 (95% HDI: 0.07, 0.22), while Struggle Intensity (our second in-hand eigen component) had a significant repeatability of 0.16 (95% HDI: 0.09, 0.23) (Figure 1.4). For our OFT eigen decomposition scores, Reactivity had a repeatability of 0.34 (95% HDI: 0.21, 0.47) and Space-use had a repeatability of 0.28 (95% HDI: 0.15, 0.41) (Figure 1.4).

Discussion

Validity of the approach to quantifying consistent individual differences in behavior remains an important consideration for studies of animal personality, although this step has often been lacking or is minimal in existing literature. We were primarily interested in how consistent individual differences in behavior develop in captive young Wood Ducks with the ultimate intention of drawing inferences regarding the possible fitness consequences of said variation in their wild counterparts; however, to do so, we first had to assess the biological relevance of the behaviors we chose to measure as well as their robustness as indicators of personality. Following recommendations by Carter et al. (2013), we selected behavioral metrics that were intended to represent sensitivity to predation risk given the large selective role that predators likely play during duckling development in this species. Wood Ducks in the wild are extremely skittish; adults often flush in response to approaching observers from up to 50 or more meters (*personal observation*). We also observed that, in captivity, individual and group responses to observer presence or sudden noise and movement, were often intense and characterized by active escape behavior (i.e., attempts to get away from the current disturbance as quickly as possible). Knowing this, we developed two separate standardized tests—an in-hand assay and a modified

Open Field Test—and recorded 10 separate behavioral metrics to capture these behavioral responses more fully.

A core requirement for our study, then, was to validate that these tests and behavioral metrics do represent some aspect of sensitivity to predation risk (predation events often being characterized by sudden bursts of sound and movement produced by a larger animal). Moreover, we sought to determine whether these behavioral assays were repeatable (an obvious, but important indicator of reliability as indices of animal personality; Carter et al. 2013). Our results show that individual variation in behavioral expression of young, captive WODU ducklings remains relatively consistent across development for most of the variables we measured (i.e., most behaviors measured were significantly repeatable; Figure 1.4). In other words, individual differences in behavioral expression at hatch are largely maintained through fledging age, suggesting that there may be underlying constraints or limits to the degree of individual flexibility in behavioral expression. Though not surprising (it has been found in multiple other systems; see review by Cabrera et al. 2021), this result is important for developing the groundwork for future studies in this species and when making inferences regarding the fitness, ecological, and social consequences of these behaviors.

In addition to demonstrating repeatability of behavioral metrics, we also wanted to better understand how each variable was related to the others, thus verifying what Carter et al. (2013) termed convergent validity. To more holistically assess relationships among our measured behaviors, we conducted multi-response GLMMs with an eigen decomposition analysis as well as a traditional, frequentist Principal Component Analysis (PCA) for comparison. Though Dingemanse and Wright (2020) suggest that conducting PCA with repeated measures to assess and analyze 'personality' is problematic (potentially representing pseudoreplication due to violation of model assumptions), we found that results from PCA qualitatively matched results from a multi-response GLMM residual correlation and eigen decomposition approach. As such, accounting for individual differences (i.e., including a random effect in the multi-response model as opposed to assuming no random effects in the PCA) did not alter the pattern of relationships among our variables. Additionally, there was significant repeatability of eigen decomposition scores matching those of the raw behavioral metrics (Figure 1.4). In our study, at least, variable reduction methods did not substantially impact our ability to assess repeatability in these behavioral variables and the relative differences among individuals remained consistent for at least the first 5-7 weeks of development (the critical post-hatch developmental period for these birds).

Overall, we found evidence of correlational patterns among our measured behaviors across two standardized testing contexts. Our multi-response correlation analysis revealed residual (accounting for individual differences) correlations among nearly all of our in-hand measures such that birds that took longer to induce a state of tonic immobility, also broke out of tonic immobility more quickly, were more 'alert' while in tonic immobility, and struggled more intensively during the weight-tube struggle test (Figure 1.3). In both our eigen decomposition and PCA, the effort required to induce tonic immobility, latency to break out of tonic immobility, and ‘alertness’ while in tonic immobility all loaded heavily on the first component, reinforcing these significant correlations. The degree to which the bird struggled during the struggle test loaded heavily on the second component possibly suggesting a more complex relationship among in-hand variables.

Predation risk appears to be a major selective pressure in the wild for Wood Ducks and ducklings are frequently captured by a variety of predators; in these instances, the ducklings' response to capture might influence predator success (depending on the size/age of the duckling and the type of predator). Variation along the Tonic Intensity spectrum might represent different tactics that could be successful depending on the predator and/or the general context of capture. Individuals that easily enter and/or maintain Tonic Immobility might be capitalizing on advantages conferred by ‘lulling’ a captor into investing less attention towards their prey. Behaviors that take advantage of this are often referred to as 'Playing dead', thanatosis, catalepsy, or death-feigning (dos Santos et al. 2010; Greene 1988; Sargeant and Eberhardt 1975; Miyatake et al. 2004). Our own personal experiences handling ducklings during this study reinforce the potential utility of such an escape response–ducklings that “played” dead while in the hand were often able to escape observers by suddenly lunging away when the handlers grew slightly more complacent with a calm or tonically immobile duckling. On the other end of the Tonic Intensity spectrum, ducklings that struggle relentlessly while being held might be using a more direct approach to evading a predator’s grasp post-capture.

Further analysis of physical characteristics (such as structural size and body mass) that might be associated with these behaviors could provide a better understanding of Tonic Immobility in this species (see Chapter 3). For instance, we predict that larger individuals might take the more direct escape approach, and therefore be less likely to enter and/or maintain tonic immobility because they have greater potential capability to physically break away from captors. Nonetheless, we recognize that these behaviors might not directly match any set of responses that these birds might exhibit in a wild/natural context. Rather, repeatable in-hand behaviors might simply be indicative of a separate underlying behavioral axis; a series of behavioral correlations that constrain the birds to behave in a consistent fashion even within this non-natural context. These correlations could be connected via a number of genetic, neurological, or physiological pathways as has been suggested by other authors (Sih et al. 2004a&b; Réale et al. 2010a&b; Stamps and Groothuis 2010a&b).

The modified Open Field Test is a slightly less artificial context, but it is still quite different from what birds would experience in the wild. Multi-response results indicated strong correlation among activity, response to a startle stimulus, response to an approaching observer, and a subjective score for jittery behavior (rapid head movement). Although the two space-use metrics were not strongly correlated with each other, a duckling's willingness to revisit the origin of a startling stimulus was negatively correlated with both response scores while anti-thigmotaxis (willingness to explore the open area of the arena) was positively correlated with both response scores. Together, the dyadic relationships among all of these variables, and the eigen decomposition, indicate a general 'reactivity' characteristic in captive WODU ducklings. Highly responsive individuals tend to be more active, display greater intensity of rapid head movement, respond more intensely to the startle stimulus and observer presence, are less likely to explore spaces close to the area in which the startle stimulus originated, but possibly display less thigmotaxis (spend less time along the walls of the arena).

Eigen decomposition and PCA corroborate these dyadic relationships with all but the space-use metrics loading heavily on the first component (Table 1.3). Both Space-use scores loaded heavily on the second component for the eigen decomposition and PCA revealing a positive correlation between the two once variance structures for the other variables were accounted for (Table 1.3). Activity loaded heavily on *both* the first and second eigen components (albeit not as heavily on the second), perhaps suggesting that there is more than one way for a duckling to be active. Anecdotal observations support this interpretation; some individuals appeared to calmly but quickly explore their environment and respond to potentially stressful situations more calmly, while others seemed to move erratically around the arena while avoiding open spaces and responding intensely to potentially stressful situations.

Biologically, these behaviors might indicate how WODU ducklings in the wild navigate a complex and dangerous environment with a plethora of potential predators that can approach from water, land, or air (see General Methods above). Wood Ducks show a general preference for locations that have dense emergent vegetation and are often jittery and responsive to noise and movement (Bellrose and Holm 1994; *personal observations*). We often observe wild WODU hens exhibiting large flight-initiation distances when approached by observers, flushing from nests, and even deserting their broods when startled.

This chapter represents the first step in a series of studies examining personality development in a precocial bird. Our goal here was to provide a thorough evaluation and validation of both the individual behavioral measures we selected, as well as to evaluate the variable reduction approach we employed following recent guidelines and critiques of animal personality studies (Carter et al. 2013 and Dingemanse and Wright 2020). In the next two chapters of this dissertation, we use the four variables produced by this eigen decomposition analysis—Tonic Intensity, Struggle Intensity, Reactivity, and Space-use—to address questions regarding their longitudinal development throughout ontogeny and their relationship to growth. This approach allows for some simplification and greater ease of inference and interpretation. We also note that, while there was some relationship between behaviors across the two assay contexts, the relationship was mostly negative. Birds that displayed greater intensity in-hand, generally displayed lower activity, rapid head movement, anti-thigmotaxis, and response intensity in the Open Field Test. This finding is somewhat counterintuitive and difficult to interpret; we explore this relationship as it relates to changes in duckling behavior across ontogeny and the dynamic relationship between duckling growth and these behaviors in the following chapters of this dissertation.

In conclusion, our results are consistent with an interpretation that, together, our behavioral metrics quantify aspects of responsiveness to potentially dangerous situations (i.e., situations in which predation risk is perceived to be present or capture has already occurred). Most importantly, we provide evidence that individuals differ in how they respond to these potentially dangerous situations and that these differences are maintained throughout post-hatch ontogeny, further supporting several empirical studies reviewed by Cabrera et al. (2021). In a precocial system—where hatchlings leave the nest within 24 hours—these significant differences could have immediate fitness implications within a short time-frame post hatch, especially considering the high rates of depredation young Wood Ducks generally face (Davis et al. 2007; Davis et al. 2009).

Table 1.1: Overview of behavioral variables measured as well as how they are associated with eigen and principal component variables (see Statistical Methods) and which behavioral assay they are associated with.

Assay Context	**Component Variable**	**Behavioral Variable**	**Description**
In-hand	**Tonic Intensity**	Tonic Attempts	Number of attempts to induce Tonic Immobility
		Tonic Alert	Score for the degree that the bird is alert while in Tonic Immobility
		Tonic Immobility	Latency to break out of Tonic Immobility; maximum 60 seconds
	Struggle Intensity	Tube Struggle	Number of kicks produced while constrained in an opaque weighing tube
Open Field Test	**Reactivity**	Rapid Head Movement	Subjective score for jittery behavior; erect posture and 'nervous' repetitive head movement paired with vocalization
		Startle Response	Subjective score for intensity of response to lifting of the acclimation chamber
		Response to Observer	Subjective score for intensity of response to approach of observer at the conclusion of the assay
	Space-use	Activity	Number of gridlines crossed during the 5-minute testing period
		Anti-Thigmotaxis	Proportion of observation intervals in which the focal duckling ventured away from the walls of the arena
		Startle Stimulus Revisitation	Proportion of observation intervals in which the focal duckling was near the location of the startle stimulus (lifting Acclimation chamber)

Figure 1.1: Photos depicting in-hand processing of ducklings conducted throughout the study. Photo (A) depicts a duckling in a state of tonic immobility, photo (B) depicts both the struggle test and weighing protocol, and photo (C) depicts measurement of the tarsus using digital calipers.

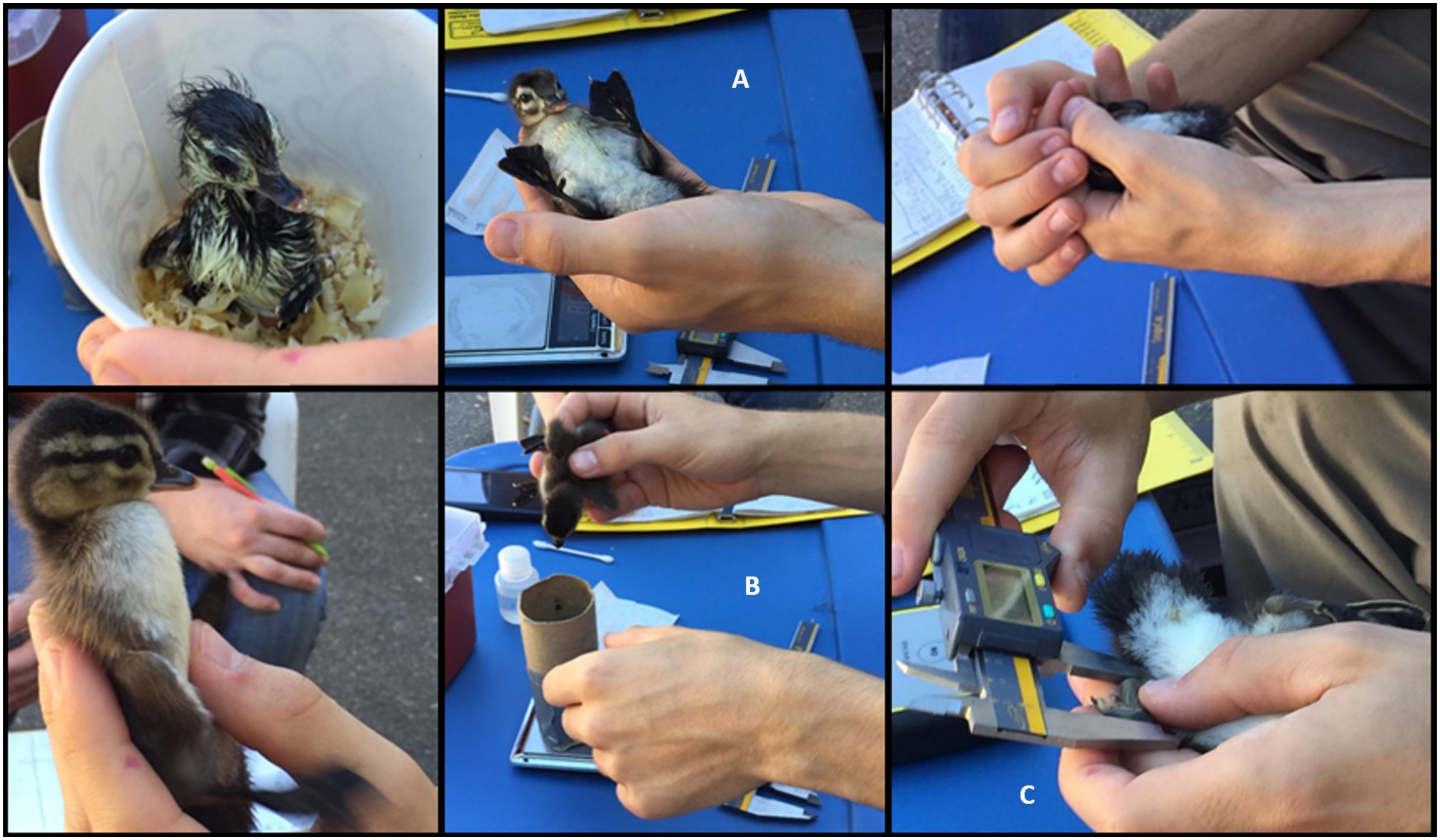


Figure 1.2: An overhead still-frame from one of our Open Field Test video recordings. This duckling chose to emerge from the acclimation chamber (blue bin in the center bottom of the frame) before it was remotely lifted out of the arena using a pulley system. Food was provided during the OFT in 2017 (but not in 2016), however foraging behavior was exceptionally rare.

Table 1.2: Model specifications (simplified version of code used in the *brms* package for R) and brief description of the models' intended purpose.

Model specification	Purpose
Behavior ~ (1|Individual) + (1|Brood) + Age + Sex + Confounds [for OFT]	Random intercepts model to calculate Intraclass Correlation Coefficient (Repeatability)

Figure 1.3: Correlogram showing the correlational structure among measured behavioral variables. Results were produced by three multi-response models: one with only in-hand behaviors as response variables (lower left), one with only Open Field Test behaviors (upper right), and one with all behaviors across both contexts (lower right). Values are residual correlation estimates after accounting for repeated measures by individual. Blue squares denote a positive correlation while red squares denote a negative correlation. Boldness of color indicates the strength of the correlation (higher correlations showing bolder color). Significance is denoted with the duckling symbol as shown in the figure key. In our Bayesian approach, significance is not determined with a p-test, but by determining how much of the posterior distribution of effect estimates overlap zero; we considered an effect estimate (here a correlation estimate) to be significant if 95% of the posterior distribution did not overlap zero.

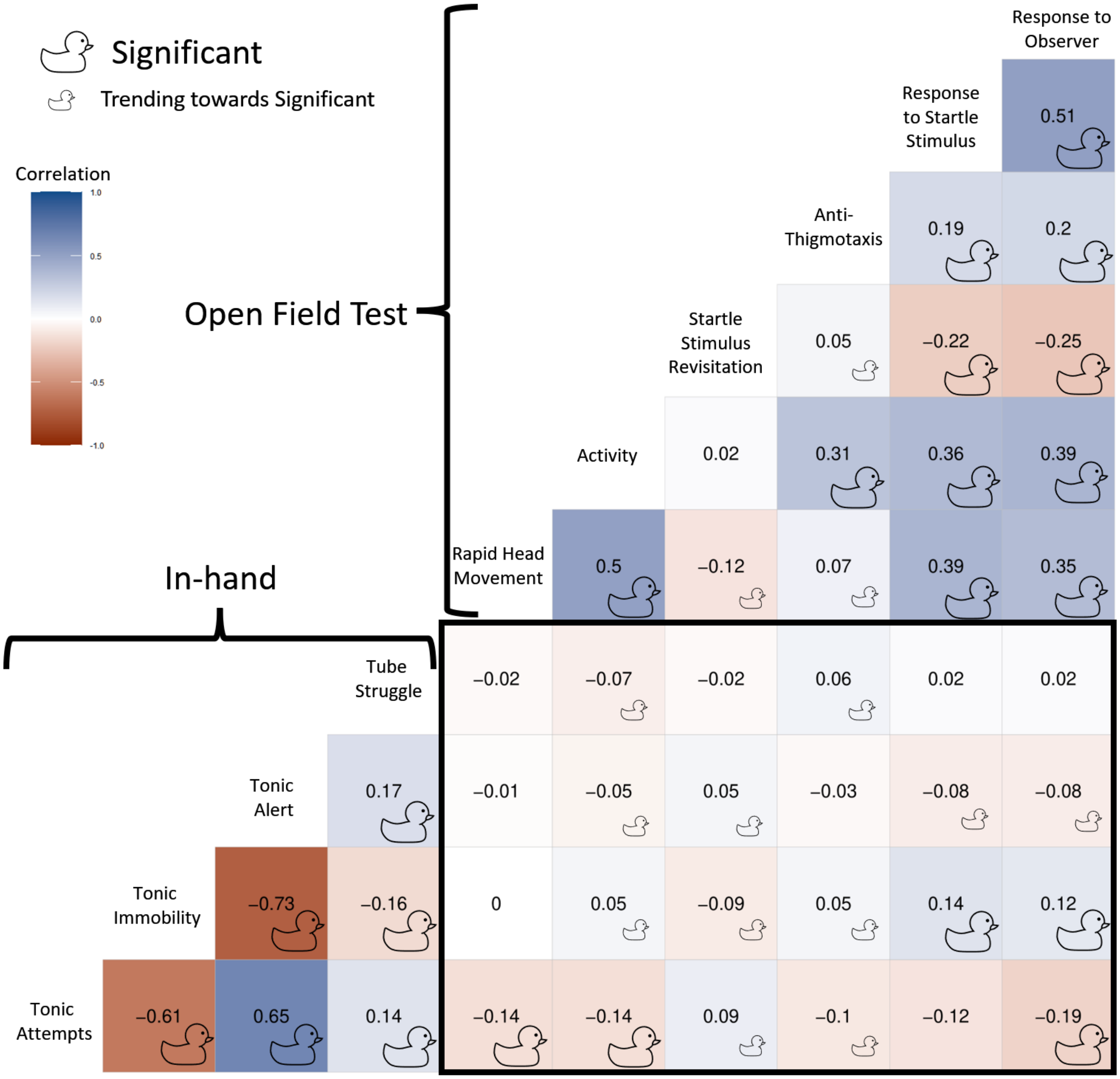


Table 1.3: Eigenvector loadings for each eigen decomposition (top) and PCA loadings (bottom). In-hand and Open Field Test analyses conducted separately. Eigenvector loadings (the values depicted under each component row) show how much each variable influenced our eigen components; we considered loadings to be important (i.e., heavily influence the eigen component) if their absolute value exceeded 0.3.

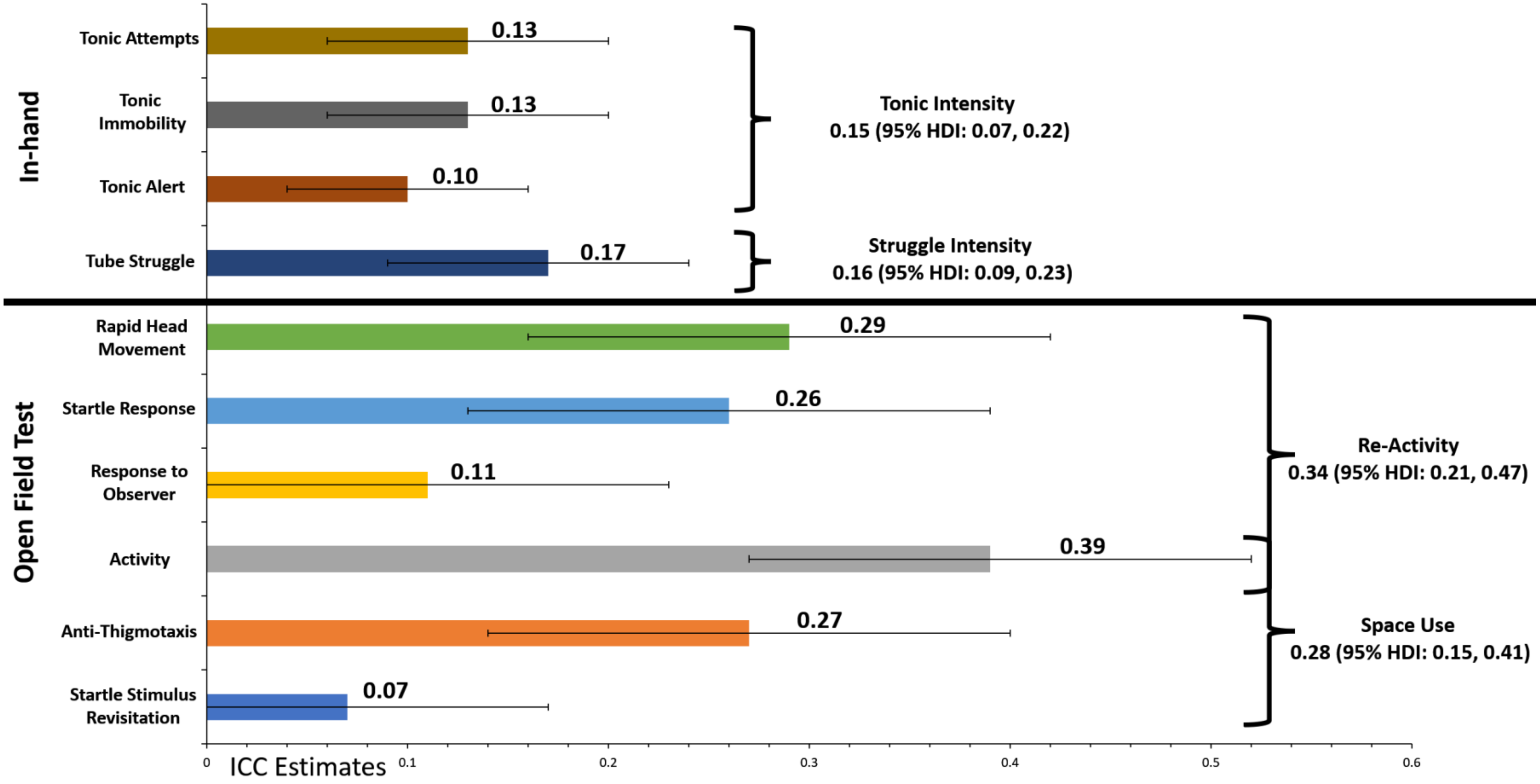
In-Hand Eigen Decomposition: Behaviors ~ (1|ID)		
	Component 1	**Component 2**
Tonic Alert	***0.581***	0.1
Tonic Attempts	***0.546***	-0.133
Tonic Immobility	***-0.571***	0.105
Struggle Test	0.195	***0.98***

OFT Eigen Decomposition: Behaviors ~ (1|ID)		
	Component 1	**Component 2**
Rapid Head Movement	***0.453***	0.002
Startle Stimulus Revisitation	-0.186	***0.735***
Anti-Thigmotaxis	0.251	***0.529***
Activity	***0.473***	***0.351***
Startle Response	***0.486***	-0.165
Response to Observer	***0.487***	-0.171

In-Hand PCA		
	Component 1	**Component 2**
Tonic Alert	***0.589***	-0.101
Tonic Attempts	***0.546***	-0.119
Tonic Immobility	***-0.569***	0.087
Struggle Test	0.176	***0.984***

OFT PCA		
	Component 1	**Component 2**
Rapid Head Movement	***0.503***	0.0547
Startle Stimulus Revisitation	-0.219	***0.61***
Anti-Thigmotaxis	0.141	***0.621***
Activity	***0.46***	***0.417***
Startle Response	***0.502***	-0.204
Response to Observer	***0.465***	-0.154

Figure 1.4: Bar chart illustrating the Intraclass Correlation Coefficient estimates for each behavioral metric we recorded across both assay contexts in relation to each other. Error Bars show the 95% Highest Density Interval (HDI) for ICC estimate posterior distributions calculated using the *sjstats* package in R. We also show here the ICC estimates for each Eigen Component variable for both assay contexts as well as their associated variables.



Chapter 2

Exploring Developmental Trajectories: Evidence for the Canalization of Sensitivity to Risk

Introduction

Animal personality is a phenomenon in which broad behavioral traits vary consistently among individuals, across time and/or context (Sih et al. 2004a&b; Réale et al. 2010a). These consistent behavioral differences can have far reaching ecological and evolutionary implications. For example, animal personality has been linked to early life dispersal tendencies which facilitate habitat selection that can then influence survivorship (Hall et al. 2015; Haage et al. 2017), access to food and other essential resources (Biro and Stamps 2008), and reproductive success (Both et al. 2005; Mutzel et al. 2013; Vetter et al. 2016). Notably, individuals can vary in risk sensitivity which can have significant implications for life-history and demography by influencing foraging and predation rates within a population (Mangel and Stamps 2001; Stamps 2007; Biro and Stamps 2008). Yet, despite almost two decades of elevated interest in animal personality and behavioral syndromes (correlations among consistent behavioral characteristics within populations), the field continues to develop, and we still have much to learn about how and why individual behavioral variation occurs and persists within populations.

Until recently, many studies have examined only a ‘snapshot’ of personality using a minimal number of repeated measurements of adult animals. Although such studies are valuable, they do not provide deep insight into the mechanisms underlying the maintenance of the variation (Stamps and Groothuis 2010a&b). Examining how behavioral differences develop during ontogeny is fundamental to understanding the proximate mechanisms that contribute to trait divergence and the maintenance of variation within populations (Tinbergen 1963; Stamps and Groothuis 2010a&b; Trillmich and Hudson 2011). Still, the ontogenetic development of behavioral variation and its carry-over into later life-stages remains poorly understood. (literature reviewed by Cabrera et al. 2021).

For most species, early life-stages tend to be demanding, and developing organisms are almost constantly in a state of flux. Developmental trajectories are subject to both endogenous and exogenous influences which interact to affect somatic growth, sexual maturation, physiology, and behavior both during ontogeny and potentially well into adulthood (Gilbert 2012; Stamps and Groothuis 2010a&b; Starck and Ricklefs 1998; Mangel and Stamps 2001). Further, the array of environmental and biological pressures often shift as individuals undergo developmental changes across time. Changes in cognition, neural circuitry, and musculoskeletal structure can influence the range of behaviors that can be expressed (Düttmann et al. 1998; Starck and Ricklefs 1998) while changes in environmental pressures like seasonal fluctuations in temperature or precipitation, food availability, and predator abundance/activity (e.g., shifts in predator activity with seasonal timing or alteration of the potential coterie of predators due to increasing sizes of individuals; Nowlin et al. 2006; Montaña et al. 2011) might alter optimal behavioral patterns (Charnov 1976; Brown et al. 1999; Hamilton 2010 & Nonacs and Blumstein 2010). Within a cohort, average behavioral trait expression might shift across ontogeny to accommodate these fluctuations, particularly if they are consistent experiences across cohorts over evolutionary time and these trait shifts are adaptive.

Growing animals often exhibit some degree of adaptive developmental plasticity (i.e., the ability to adjust developmental trajectories in response to environmental stimuli as an evolutionary response to historic environmental oscillation/change/etc.; Nettle and Bateson 2015) which can function at the individual level allowing some flexibility in responding to a landscape of varying pressures. Often, canalization of expressed traits is observed in species that display plasticity; cognitive, neural, and musculoskeletal development, along with experiential factors, can constrict the possible range of potential behaviors as predictable, behavioral phenotypes emerge that better match the environment individuals were exposed to as juveniles (Gottlieb 1991a&b; Stamps and Krishnan 2017). Canalization has been studied with regard to development of song in passerine birds and other aspects of vertebrate cognition (Waddington 1942; Gottlieb 1991a&b; Beecher and Brenowitz 2005; Flatt 2005) but is only recently appearing in discussions of consistent individual differences in behavior (e.g., Careau et al. 2014 and Kok et al. 2019).

Despite the complexity of factors influencing its development, behavioral expression remains detectably repeatable from hatch/birth through ontogeny in many young animals (Cabrera et al. 2021), suggesting some degree of constraint on individual behavioral expression and its adjustment during the early life developmental stage. However, even if behavior is repeatable across the entire juvenile stage (and potentially into the adult stage), the degree to which behavioral traits are repeatable can change throughout ontogeny (Careau et al. 2014; Kok et al. 2019). Surprisingly, few studies have closely examined consistency and changes in repeatability of behavioral expression within the early-life developmental stage. In fast growing invertebrates (e.g., arthropods like field crickets) acquiring the necessary number of observations within a tight timeframe can be difficult while vertebrates tend to require more logistical effort to follow across development both in captivity and the wild. Of those studies that have conducted longitudinal analyses, most do not explicitly address whether changes in repeatability are due to changes in among-individual variation and/or within-individual variation (briefly reviewed by Kok et al. 2019).

Another consideration that has important implications for understanding developmental changes in personality, but that appears to be rarely addressed, is the natal mode of development. Where an organism lies on the Altricial-Precocial spectrum can have major ramifications for developmental rates, parental care patterns, and early life selective pressures both pre- and postnatally (Gill 2006). The spectrum of developmental mode is particularly well studied in birds. In avian species, precocial hatchlings are more or less fully capable of self-sufficient movement and recent hatchlings forage with relatively little direct parental provisioning (in some extreme cases hatchlings are completely autonomous; Starck and Ricklefs 1998). A majority of the neural development and brain growth that occurs in precocial species occurs prior to hatch, likely to provide the cognitive functions necessary for independence once out of the egg (Starck and Ricklefs 1998). On the opposite end of the spectrum, altricial young hatch out relatively helpless, blind, and even lack the capacity for muscle thermogenesis (shivering; Starck and Ricklefs 1998); most neural development for these species occurs post-hatch.

Although the young of altricial species often display a variety of behavioral and morphological strategies to manipulate parental care and/or deal with nestling competition, altricial hatchlings are entirely dependent on direct parental provisioning for growth, survival and anti-predator defense (the loud and persistent calls for food may even attract predators in the nest; *personal observation* and Dearborn 1999). Consequently, researchers working with altricial species have needed to consider the influence of parental phenotype on post-natal growth and development of their young (e.g., van Oers et al. 2015). Indeed, much of our understanding of personality development in terrestrial vertebrates stems from studies of altricial species (Cabrera et al. 2021). We posit that, to a much greater extent than in altricial systems, early life phenotypic differences in precocial animals may have particularly pronounced 'downstream' effects on phenotypic development, survival, and reproduction during early life history. This isn't to say that parental effects are not important in these systems; parents may play a role in providing temperature regulation (i.e., brooding) and leading young to foraging sites in addition to influence through incubation behavior (Hepp et al. 2015) and deposition of yolk hormones (Groothuis et al. 2005; Daisley et al. 2005; this also seems to occur in altricial species, Ruuskanen and Laaksonen 2010). However, a more focused examination of personality development in precocial systems may serve to provide a broader base of understanding for the development and maintenance of behavioral variation within populations.

In this study, we empirically examine the developmental trajectory of behaviors associated with sensitivity to predation risk in captive-reared, wild Wood Ducks (WODU; *Aix sponsa*) within a common garden environment. Wood Ducks, like all waterfowl, are a precocial species; hatchlings leave the nest within 24 hours of hatching, fully capable of sight, motor function, and foraging (albeit under loose supervision of their brooding mother; but see Methods). With our prior work in this system, we established moderate to high repeatability in standardized behavioral traits associated with predation risk sensitivity (Chapter 1). The current study aims to build on this foundation by more closely examining potential changes in average (within-cohort) behavioral expression, repeatability, and within-individual variance (i.e., predictability) as hatchlings develop.

Specifically, **we were interested in assessing whether this set of behaviors associated with sensitivity to predation risk become canalized as ducklings develop**. If so, we expected to find both lower relative repeatability and greater within-individual variance at younger ages and a reduction in within-individual variance as ducklings grow older and solidify their consistent behavioral expression. The underlying premise is that plasticity in behavioral expression is much higher during more sensitive developmental periods, while the brain and body are still growing and maturing. For instance, Delval et al. (2020) found evidence that personality traits do not seem to establish for multiple years after birth in wild yellow-breasted capuchin monkeys (*Sapajus xanthosternos*). Interestingly, for precocial birds, a majority of early life neural development occurs prenatally (as discussed in greater detail above); for example, in the Muscovy Duck (*Cairina moschota*), the optic lobe volume in hatchlings is approximately 82% that of adults (Starck and Ricklefs 1998). It might follow, then, that precocial chicks are less capable of adjusting behavioral expression in early life than their altricial counterparts. It is important to note, however, that a considerable amount of musculoskeletal growth occurs post-hatch, and in the wild there appears to be greater predation pressure on younger ducklings (the majority of mortality, mostly due to depredation, occurs within the first two weeks of life). Accordingly, changing selective pressures across ontogeny within this system might interact with aspects of somatic growth and maturation.

Statistical Methods

Assessing Behavior Across Development

We ran Bayesian Mixed Models using the *brms* package in R (Bayesian Regression Models using Stan ver 2.13.0 in R ver 4.0.2; Bürkner 2017) with weakly informative priors. In this chapter we build on initial analysis from Chapter 1 where we conducted Eigenvalue Decomposition of residual correlation coefficients as a means of variable reduction. We did this using multi-response Bayesian Generalized Linear Mixed Models for in-hand behaviors and OFT behaviors separately, while including a term for individual identity to account for repeated measures. We then generated new weighted scores for each observation in a fashion similar to

Principal Component Analysis. This method of variable reduction accounts for repeated measures in the correlation coefficients among variables (which is not the case with traditional Principal Component Analysis) while allowing us to reduce the number of behavioral variables to four interpretable and logical components (eigen components 1 and 2 for both in-hand assays and OFT assays; Table 1.3). In Chapter 1 we demonstrated strong qualitative similarities between this approach and the output of a traditional PCA and we discussed interpretation of these weighted variables. For all further analyses, we refer to in-hand component 1 as Tonic Intensity, in-hand component 2 as Struggle Intensity, OFT component 1 as Reactivity, and OFT component 2 as Space-use. Each of these variables were significantly repeatable (Figure 1.4).

To assess changes in average behavior across ontogeny, we conducted a series of single-response Bayesian Generalized Linear Mixed Models with both varying slopes and varying intercepts, including a 'random' effect term for individual identity. This allowed us to account for potential <u>within-individual</u> correlations among slopes (behavior over time) and intercepts (initial behavioral expression). To account for potential group effects, we included Brood as a 'random' effect. We included age and sex as 'fixed' effects and we examined variation among individual slopes for behavior by age (i.e., we explicitly modeled each individual's behavioral trajectory across ontogeny). Because our Open Field Tests were recorded in outdoor pens, we also included a score for wind intensity and air temperature as those were determined to be potential confounding influences in an exploratory step-wise analysis conducted for each raw behavioral variable. We ran models with Gaussian distributions, weakly informative priors, four parallel chains, and 1000 warmup and 6000-8000 total chain iterations. We visually checked model chains and posterior distributions to ensure smooth run. No models we include here exceeded Rhat scores of 1.

Age-based Differences in Repeatability and Within-Individual Variation of Behaviors

To assess the within-individual variance and relative changes in variance of behavioral responses as ducklings aged, we divided our data set into two age categories representing the 'Early' developmental stage (Hatch-Week 3) and the 'Late' developmental stage (Week 3-Week 7). We then conducted varying intercept models as above (we did not do so for slopes as this can be problematic for Intraclass Correlation Coefficient estimates; Nakagawa and Schielzeth 2010). We used the *sjstats* package (ver 0.17.4) to calculate a Bayesian equivalent of the adjusted Intraclass Correlation Coefficients (ICC), but for each new age category. Though comparison of these paired sets of ICC estimates is qualitative, it is useful for description and exploration, and the *sjstats* package provides a confidence distribution (Highest Density Interval which we set to 95%) around the ICC estimate.

To assess average changes in within-individual variation of behaviors over time, we calculated the Coefficient of Relative Plasticity (CRP; see Dingemanse et al. 2010) for each variable at both Early and Late stages. The Coefficient of Relative Plasticity is calculated as [within-individual variance]/[total variance] allowing us to adjust for potential changes in cohort behavioral variation as ducklings aged. We then conducted simple Bayesian Generalized Linear Mixed Models with CRP scores as the response variable and stage (early/late) as a fixed effect. We ran models with Gaussian distributions, weakly informative priors, four parallel chains, and 1000 warmup and 6000-8000 total chain iterations, visually checking model chains and posterior distributions to ensure smooth run. Again, none of the models included here exceeded Rhat scores of 1.

Results

Average Expression of Behavior Changes with Age

Generalized Linear Mixed Models revealed a negative effect of age on Tonic Intensity, with older birds showing generally lower Tonic Intensity (fewer attempts to induce tonic immobility, higher latency to break out of tonic immobility, and lower levels of alertness with more relaxed body positioning during tonic immobility; Figure 2.1). Struggle Intensity, however, did not vary significantly with age. We also found no evidence of a correlation between intercepts and slopes within-individuals for either of these metrics (i.e., initial behavior was not associated with a change in behavioral trajectory across ontogeny within-individuals, Table 2.2). Reactivity was also significantly associated with age, with older birds generally increasing in activity and intensity of response to both the startle stimulus and approach of an observer. Unsurprisingly, wind intensity was significantly associated with reduced Reactivity responses (Table 2.2). Space-use was not correlated with age on average. Somewhat unexpectedly, wind intensity was also significantly associated with an increase in Space-use (Table 2.2). Again, there was no evidence of a correlation between intercepts and slopes within-individuals (Table 2.2).

Repeatability and Within-individual Predictability Increase with Age

We next separated our dataset and conducted simple, varying intercepts models to qualitatively compare repeatability (ICC) between early and late stages of development. We found potential increases in repeatability for all of our behavioral variables from early to late developmental stage (Figure 2.2). We also calculated a proxy for within-individual variation, called the Coefficient of Relative Plasticity (CRP; Dingemanse et al. 2010), for both early and late stages of development. Generalized Linear Mixed Models revealed that the Coefficient of

Relative Plasticity, on average, decreased from early to late-stage development for Tonic Intensity, Reactivity, and Space-use (Figure 2.3). The Coefficient of Relative Plasticity for Struggle Intensity tended to decrease with age as well, but this relationship was not significant (Figure 2.3).

Discussion

While interest in the development of animal personality has increased within the past decade, there remain important gaps in our understanding of how consistent individual differences manifest, change (or remain consistent) over developmental time, and are maintained within a population (Cabrera et al. 2021). In this study, we sought to examine the stability of behavioral expression (specifically sensitivity to predation risk), both within- and among-individuals, across post-hatch ontogeny in captive-reared WODU.

Our findings provide compelling evidence of canalization of behavior of young Wood Ducks throughout post-hatch ontogeny (e.g., Kok et al. 2019).Repeatability scores increased for most behaviors between early (week 0 - week 3) and late (week 3 - week 5) stages of ontogenetic development. Furthermore, there were statistically significant decreases in the Coefficient of Relative Plasticity (a measure of within-individual variance) between early and late stages for three of four behavioral variables (with a negative but not significant trend for the fourth). Given that the early life of WODU in the wild is challenging and extremely dangerous, especially in the first two weeks post-hatch, a greater degree of flexibility may be required to enable newly hatched ducklings to respond effectively to environmental pressures during this critical life stage. Early-life flexibility might also provide devlopmental scope to adjust to environmental pressures that these birds or their progeny might then face in a later season or life-stage (Stamps and Krishnan 2014). Predation pressure might be sufficiently high and unpredictable for young ducklings that flexible behavioral expression in an early stage provides the greatest overall selective advantage in an uncertain landscape. Additionally, physiological, neurological, and cognitive constraints might limitr consistency in behavioral expression early on (Düttmann et al. 1998). Canalization of behavioral traits might also reflect learning and neural pathway formation necessary for developing the ability to consistently exhibit certain behaviors.

Counter to our predictions, **we also found average shifts in behavioral expression across time**, even though individuals remained relatively consistent in their differences. On average, Tonic Intensity in-hand decreased, and Reactivity within an Open Field arena increased as ducklings aged (Figure 2.1). The relative differences between these behaviors in how they change across time are difficult to interpret but are consistent with our robust correlation analysis from Chapter 1, which showed negative correlations (or tendencies towards negative correlations) in the residuals between various in-hand and OFT measures. The mirrored change across ontogeny suggests that we might be measuring different aspects of the same underlying behavioral traits (or personality axis if one chooses to be so bold). Possibly, high responsivity during free movement reduces the likelihood of capture by a predator and could be indicative of aversion to predation risk or a willingness to accept potential predation risk. If a duckling is captured, however, lack of intense response (in-hand) might confer an advantage, particularly when a large amount of energy has already been invested in predator avoidance. Playing dead (also known as thanatosis, catalepsy, or death-feigning) is a known response of prey that can be effective by lulling predators into a reduced level of control of a prey item, therefore providing an opening for escape (dos Santos et al. 2010; Greene 1988; Sargeant and Eberhardt 1975; Miyatake et al. 2004; this even worked on observers in this study!). Below we consider three major explanations for average shifts in expression of measured behaviors across ontogeny both within- and among-individuals; each might be a distinct factor, or they might all work in concert.

First, shifts in behavioral expression as ducklings age could be linked to physiological and/or neurological developmental processes. These underlying processes could be environmentally and/or genetically mediated; though, birds in our study were exposed to extremely similar developmental conditions (save for potential differences in temperature and light-cycles due to the asynchronous hatch times across broods; see General Methods). As they grow, birds experience changes in neuro-muscular systems, brain structure, neural pathways, cognition, and endocrine systems across ontogeny (Starck and Ricklefs 1998), each of which govern various aspects of behavioral expression. For instance, maturation of neuro-muscular systems could enhance locomotive capacity allowing for an increase in rapidity or intensity of responses to predation risk. This could provide an explanation for the increase in Reactivity with age, but it does not seem to fit a broad decrease in Tonic Intensity.

Organizational effects of developmental endocrine processes could also play a role in the trajectory of behavioral change with age, although most of the research in this area has focused on sex-based differences (Beatty 1984; Adkins-Regan 2012). Cognitive development related to information processing and experiential learning could lead to increases in Reactivity and decreases in Tonic Intensity as ducklings become more capable of processing and responding to the stressors associated with our Open Field Test and capture/handling by an observer (we discuss experiential learning in more detail below). The exact, pre-fledging, developmental processes that play a role across multiple weeks after hatching in precocial systems are unclear. Future longitudinal research investigating shifts in cognition, physiological shifts in stress response, development of the Central Nervous System, and changes in neurologically driven performance in this system would help to elucidate some of the potential drivers.

Second, changes in behavioral expression (that also increases in repeatability) across ontogeny might represent age-dependent, adaptive responses to a historically shifting selective environment experienced by ducklings. For example, the suite of potential predators might be very different for a 1-week old duckling than for a 5-week old duckling that is roughly 10-20 times the size it was four weeks prior. Likewise, food availability generally tends to decrease over the summer and temperatures increase along with reduced precipitation; lack of access to food can have ramifications for personality expression directly (e.g., Wauters et al. 2021). If such changes are consistent among years, the predictable shift in selection pressures could lead to an adaptive behavioral shift later in ontogeny if those behaviors are associated with increased foraging efficiency or success. As such, an increase in Reactivity, specifically activity, might be necessary when ducklings must expend greater foraging effort (Stamps 2007; Biro and Stamps 2008; DiNuzzo and Griffen 2020).

The social environment is another important factor that influences behavioral expression (Pearish et al. 2019; Jäger et al. 2019; Krause et al. 2010) and behavioral development (e.g., Taborsky et al. 2012) and, in the wild, social environment can also change over time. For Wood Ducks (and other waterfowl), there is a shift in the extent to which young ducklings rely on their brood mother as they fledge. Although fledging ages can be variable (Wood Ducks can fledge as early as 4 weeks while others might remain with the mother for as long as 8 weeks; Bellrose and Holm 1994), there is certainly a point at which the mother's presence no longer substantially influences access to foraging patches and predator avoidance. Additionally, wild WODU broods experience changes in brood social structure as broodmates die during the growing period.

Removal of even single individuals could have far-reaching impacts on personality and behavioral expression (i.e., "keystone" individuals; Modlmeier et al. 2014). Reduction in brood size (which is almost guaranteed in our system) might also confer a disadvantage with regard to effective predator avoidance as young individuals potentially rely on "safety-in-numbers" or a dilution effect to improve survivability (i.e., benefits of group formation; Lehtonen and Jaatinen 2016), necessitating behavioral adjustments for the surviving individuals. Endogenous changes (locomotor and neurological development) might interact with these exogenous factors to influence the relative 'success' of different behavioral expressions during different phases of ontogeny. All of these interactions are undoubtedly dynamic and may be specific to the location, time, population, and individuals in question.

Third, the changes in behavioral expression we observed in our study could be the result of experiential learning and/or practiced response. Extended exposure to captivity and repeated exposure to behavioral assay testing conditions could reasonably lead to habituation, or an attenuation of response over time (e.g., Houpt et al. 2010). Habituation would be a simple and plausible explanation for reduction in Tonic Intensity with age. However, an increase in Reactivity across the study is not consistent with an effect of habituation. Possibly, there might be different degrees or types of habituation (habituation in one context and lack of habituation in another). Still, older birds that displayed very low levels of Tonic Intensity often attempted to escape observers when provided the chance (indicating that they still perceived in-hand processing as dangerous or undesirable). Furthermore, these birds exhibited strongly negative responses to an observer’s presence regardless of frequent exposure to observers for extended periods of time. We have maintained a small number of adult captive-reared WODU in captivity for nearly 7 years. Yet, despite daily encounters with caretakers (providing food and completing daily maintenance protocols) and intermittent testing over the years, these birds continue to exhibit strong avoidance and escape responses to observer/caretaker presence and handling *(personal observation*). We have also noted near-immediate reduction in body mass in response to repeated capture and handling among captive-reared adults (*unpublished data*; Figure 4.2), possibly suggesting extended physiological and/or behavioral responses and therefore a lack of habituation.

Perhaps, then, these changes indicate a learned response over time. Flight Initiation Distance, a common metric for perceived predation risk, has been shown to generally increase in response to repeated experiences of predators or 'dangerous' situations (Meta-analysis conducted by Stankowich and Blumstein 2005). In other words, animals that experience a frightening situation often respond with an increased sensitivity to risk, which could explain both the increase in Reactivity and decrease in Tonic Intensity over time (assuming that low Tonic Intensity represents an escape tactic). Furthermore, learning is a driving factor in canalization (Gottlieb 1991a&b) which appears to be occurring within this system. The link between learning and animal personality is complex and highly variable across species (meta-analysis conducted by Dougherty and Guillette 2018), but we clearly need a greater understanding of the role of cognition in generating and/or maintaining consistent individual behavioral differences (Carere and Locurto 2011; Sih et al. 2012b).

Concluding Remarks

Overall, our findings suggest that the behavior of juvenile Wood Ducks, at least in our specific testing contexts, undergoes changes both within- and among-individuals during the pre-fledging period of early life (the first 5-8 weeks post-hatch). The changes we observed within-individuals indicated a reduction in variability of behavioral expression paired with an apparent increase in repeatability, strongly suggesting canalization (Gottlieb 1991a&b; Stamps and Krishnan 2017) of behaviors associated with mitigating the risk of predation. Studies of canalization within a personality context are surprisingly uncommon and we are aware of only two other empirical studies (Careau et al. 2014 and Kok et al. 2019) that have explicitly attempted to examine both changes in repeatability and changes in within-individual variance across developmental time; both studies provide evidence of canalization for specific consistent behavioral traits. Yet, determining the manner with which consistent differences arise during sensitive developmental periods is an important element in understanding how and why individual differences in behavior are maintained within populations (Stamps and Groothuis 2010a&b). Canalization of consistent individual differences in behavior is an intuitive explanatory framework for describing the trajectory of behavioral development in young animals as environmental information (e.g., activity of potential predators) informs endogenous developmental processes leading to stable phenotypic expression later in life (Stamps and Krishnan 2014).

Selection for stable behavioral phenotypes that differ among individuals within a population might arise from a number of factors. In conjunction with increasing repeatability and within-individual variance, we found that the entire study population shifted their responses to behavioral assays across the duration of our study. These shifts might indicate an evolutionary response to changes in selective pressures as ducklings age, and/or they may simply be a reflection of endogenous developmental processes. Furthermore, even though they are highly precocial, our findings suggest that young ducklings are still neurologically and/or cognitively developing throughout early life post-hatch.

We suggest that future studies designed to examine within-individual consistency across ontogeny focus efforts on acquiring multiple measures within time points where possible. This will be difficult for animals that have very short windows of development; such is the case with birds, which tend to have rapid/short developmental periods in relation to some other vertebrate systems. Additionally, future work should include analysis of repeatability across the transitional boundary between juvenile and adult stages (as suggested by Cabrera et al. 2021). Unpublished data with a subset of our captive-reared birds from this study suggest that both in-hand and OFT behaviors remain repeatable for several years into adulthood.

Another vitally important component of our study system is the social environment. In our research, we were unable to examine the dynamics of within-brood social interactions or explore how the brood-level social environment might influence individual behavioral development. From our observations and experience with WODU ducklings, it is clear that they respond to brood-mate social cues very strongly. Ducklings almost always prefer to follow or move towards brood-mates, suggesting strong selection for maintaining brood cohesion during the developmental period. There are a number of reasons for such a strong repsonse (e.g., anti-predator benefits of group formation; Lehtonen and Jaatinen 2016). We also completely eliminated the presence of the brood mother in our study, but we did so intentionally to better control and standardize the developmental environment for ducklings. The extent to which brood mother behavior might influence duckling development requires more attention, and maternal personality does seem to influence chick behavior in other species, such as Japanese Quail (*Coturnix japonica*) (Pittet et al. 2014).

One final consideration is the influence of size and somatic growth on behavioral developmental processes. A fundamental relationship between behavior and individual state is often invoked as a theoretical explanation for the maintenance of individual differences in behavior within populations (Dingemanse and Wolf 2010; Wolf and Weissing 2010; Sih et al. 2015). Lower Reactivity at younger ages could simply represent a sit-and-hide tactic of dealing with predation when conservation of energy is essential during a demanding period of somatic tissue development (i.e., following an asset-protection behavioral strategy; McElreath et al. 2007; Wolf et al. 2007; Wolf et al. 2008; Sih et al. 2015). By week 5, growth rates decline as ducklings begin to reach adult-size, indicating a reduction in energetic demand and possibly freeing up energy to be allocated towards increased Reactivity. This explanation, however, doesn't easily explain the change in Tonic Intensity and it should be noted that, around week 5, these birds are beginning to molt in their first primary and secondary flight feathers and are likely beginning to recruit and develop the pectoral musculature necessary for flight which can be energetically intensive (Hohtola and Visser 1998; Starck and Ricklefs 1998). In chapters 3 and 4 we explicitly examine the relationship between duckling size and behavior as a potential explanation for individual differentiation and the maintenance of the individual differences in behavior we established in this and the prior chapter.

Table 2.1: Model specification (simplified version of code used in the *brms* package for R) and brief description of the models' intended purpose.

Model specification	Purpose
Behavior ~ (1+Age|Individual) + (1|Brood) + Age + Sex + Confounds [for OFT]	Random intercepts and slopes model to assess change in average behavior across development
Coefficient of Relative Plasticity for Behavior ~ (1|Individual) + (1|Brood) + Stage	Model to estimate the binary effect of stage of development (early vs. late) on within-individual variability

Table 2.2: Summary of important model estimates and their 95% Credibility Intervals for our Behavior response Generalized Linear Mixed Models run using *brms* in R. We show results from individual models for Tonic Intensity, Struggle Intensity, Reactivity, and Space-Use. Estimates for the effect of Sex are males in relation to females (e.g., a significant positive effect estimate indicates that males showed a greater behavioral score than females). Likewise, the term for Wind was modeled as a categorical variable with moderate and high shown in relation to low. Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but the bulk of the posterior distribution lies on either side of zero).

In-hand				**Open Field Test**			
Response	**Effect**	**Effect Estimate**	**95% C.I.**	**Response**	**Effect**	**Effect Estimate**	**95% C.I.**
Tonic Intensity	Within-Individual: Intercept ~ Slope	-0.49	-0.98, 0.77	Reactivity	Within-Individual: Intercept ~ Slope	0.041	-0.82, 0.94
	Age**	**-0.04**	**-0.046, -0.035**		**Age****	**0.052**	**0.04, 0.064**
	Sex (Male)**	**0.28**	**0.031, 0.53**		Sex	-0.011	-0.5, 0.29
Struggle Intensity	Within-Individual: Intercept ~ Slope	0.13	-0.63, 0.95		**Wind (Moderate)****	**-0.86**	**-1.32, -0.4**
	Age	-0.0017	-0.0059, 0.0024		**Wind (High)****	**-1.02**	**-1.56, -0.47**
	Sex (Male)	0.008	-0.18, 0.2		Temperature	-0.017	-0.039, 0.0054
				Space-use	Within-Individual: Intercept ~ Slope	-0.24	-0.82, 0.88
					Age	-0.0011	-0.011, 0.0091
					Sex*	-0.32	-0.65, 0.011
					Wind (Moderate)	0.15	-0.23, 0.53
					Wind (High)**	**0.61**	**0.15, 1.07**
					Temperature	-0.011	-0.029, 0.0081

Figure 2.1: Spaghetti plots depicting change in behavioral expression across development (~0-49 days) for each eigen component with in-hand behaviors in the top two boxes and OFT behaviors in the bottom two. Each colored line represents a single individual. The bold central line represents the average while the light gray shaded region outlines the 95% Credibility Interval. Significance is denoted with the duckling symbol as shown in the figure key.

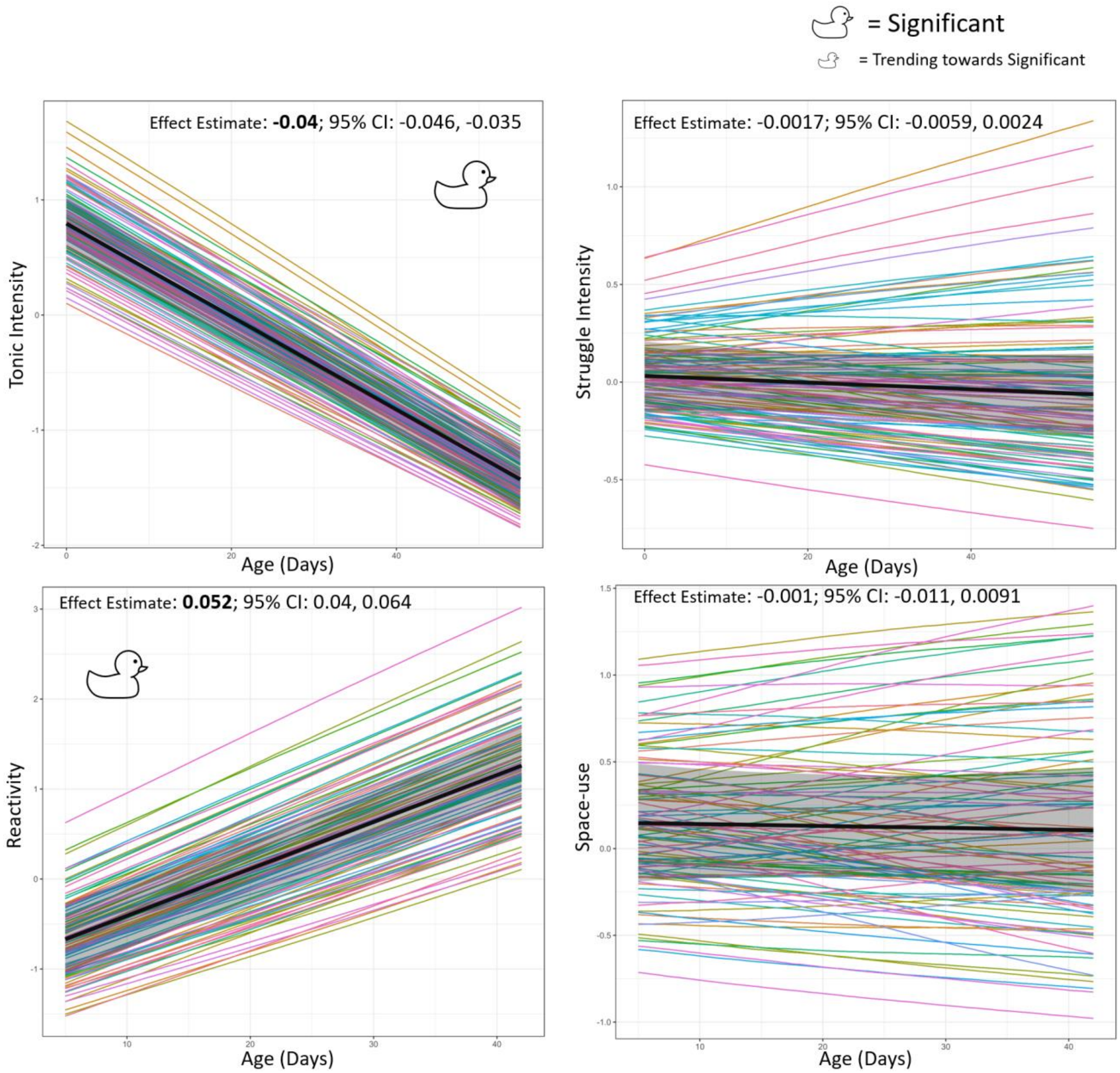


Figure 2.2: Bar graph showing the qualitative change in Intraclass Correlation Coefficient from Early (black stripes) to Late (solid gray) stages of duckling development. As indicated in the figure key, the Early stage accounts for weeks 0-3 while the Late stage accounts for weeks 3-7. Error Bars show the 95% Highest Density Interval (HDI) for ICC estimate posterior distributions calculated using the *sjstats* package in R.

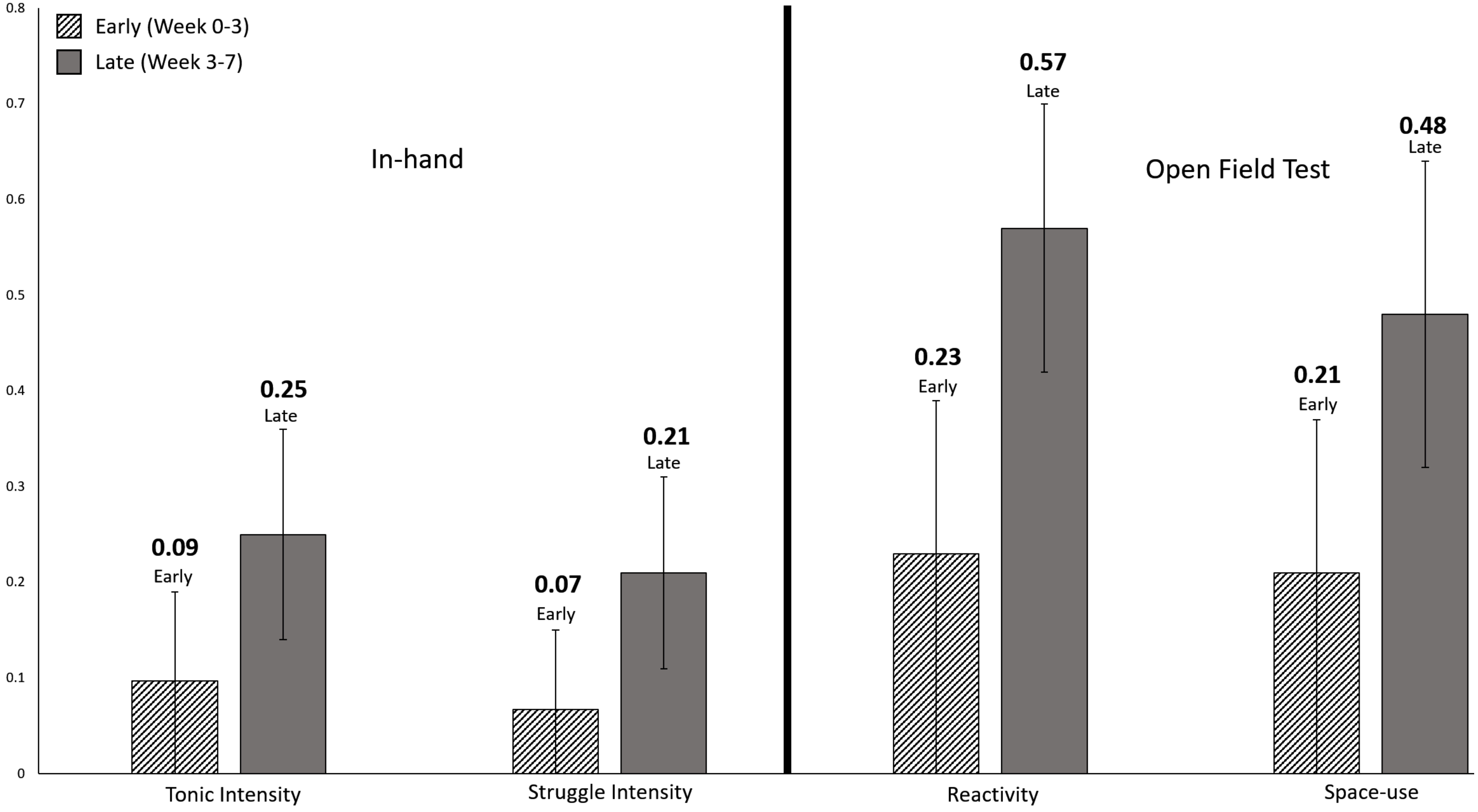
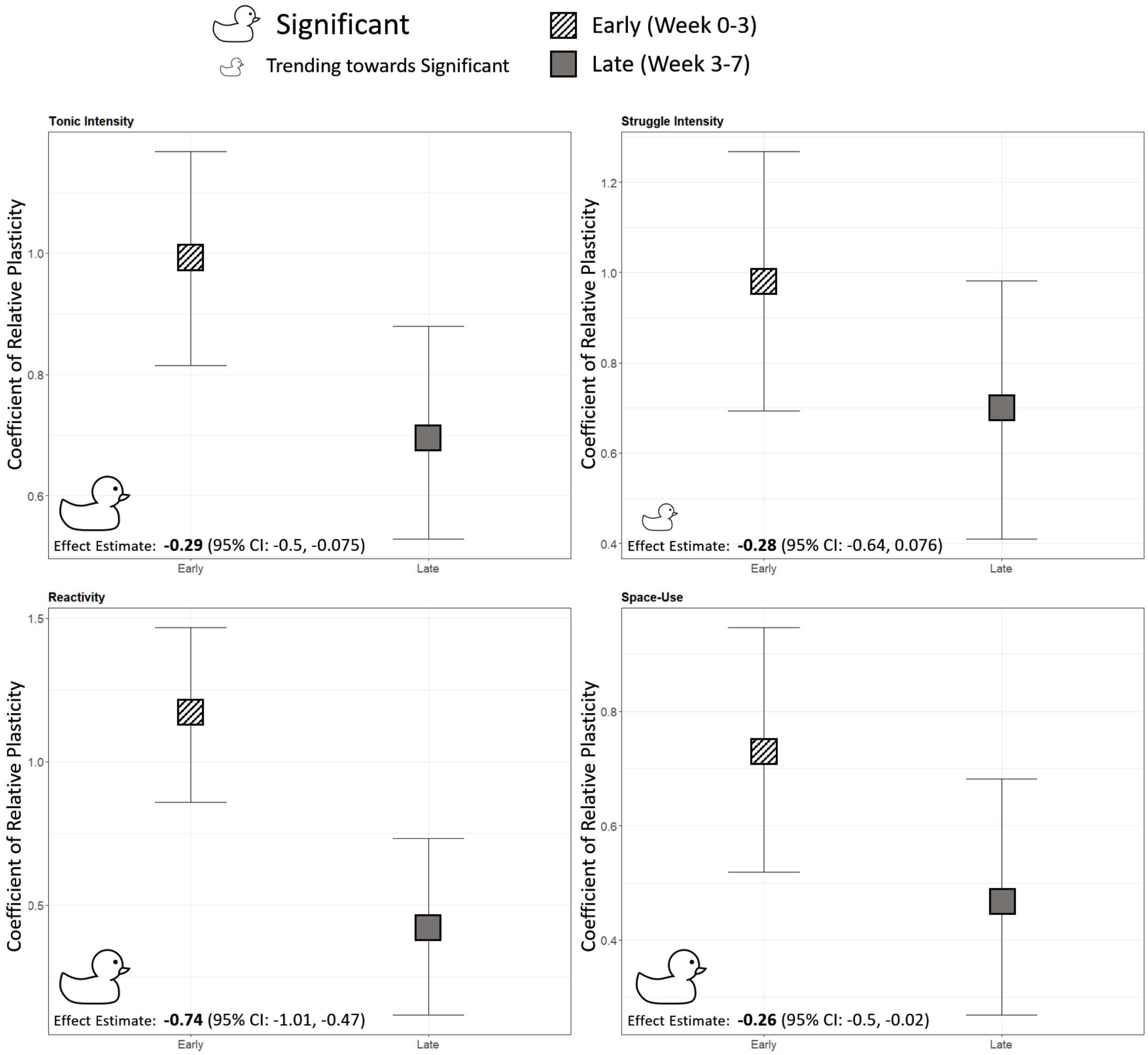


Figure 2.3: Graphs showing the change in the Coefficient of Relative Plasticity (a measure of within-individual variance) from Early to Late stage of development for each eigen component. In-hand behaviors are shown in the top two boxes and OFT behaviors in the bottom two. Significance is denoted with the duckling symbol as shown in the figure key. The Early stage comprises weeks 0-3 while the Late stage comprises weeks 3-7.



Chapter 3

Examining the Relationship Between Behavioral Type and Growth across Development in Young, Precocial Wood Ducks (*Aix sponsa*)

Introduction

Organismal life history is defined by patterns of ontogeny (growth), mortality, and reproduction (fecundity) across multiple life stages. Each of these major components of an individual's life—elements of fitness that are evolutionarily important—are driven by a plethora of factors both endogenous and exogenous. Life history theory attempts to address the large-scale patterns in these factors that dictate population and species level differences in the ways that organisms go about life (Stearns 1989). Growth is of particular interest to ecologists studying life-history due to its immediate and long-lasting fitness implications which have been demonstrated both theoretically (e.g., Rice et al. 1993; Mangel and Stamps 2001; reviewed by Dmitriew 2011) and empirically (Travis 1984; Bobyn and Brooks 1994; Nicieza and Metcalfe 1997; Gregory and Wood 1999; Johnsson et al. 1996; Dunham et al. 1999; Björkman and Larsson 1991).

Considerable variation in developmental growth rates is a widespread natural phenomenon. Mangel and Stamps (2001) review some of the factors that are influence whether an individual will (or should) grow rapidly or slowly. These include: predation risk, mature function (tissue that is actively growing cannot concurrently perform 'mature' function at maximum efficiency), somatic development (rapid development of somatic tissue can lead to increased probability of malformation, lesions, and general physical vulnerabilities), immune function, and ability to cope with physiological stressors (e.g., DuRant et al. 2013 show that

Wood Duck hatchlings that developed more rapidly as embryos were less capable of dealing with thermal stress in their first week of life post-hatch). Even in common-garden environments many researchers observe variation in the rate of somatic growth (Mangel and Stamps 2001). This variation implies the involvement of either intrinsic and/or social processes independent of actual food and nutrient availability.

What many life-history studies neglect is widespread, and in many cases adaptive, intraspecific and intrapopulation behavioral variation. Broad interest in consistent individual differences in behavior has led to the development of theory regarding the possible connections between consistent behavioral traits, physiological traits (such as hormonal responses and metabolism), morphological traits, and life history traits (Réale et al. 2010b). Multiple state-dependent models (see Dingemanse and Wolf 2010) have addressed the possibility that behavioral differences can arise from, and be maintained by, differences in energy reserves (Andersson and Höglund 2012), body size (McElreath and Strimling 2006), and physiology (Careau et al. 2008; Biro and Stamps 2008; Houston 2010).

For developing animals facing a multitude of physiological and time constraints, differences in the rate of growth might be a powerful explanation for the emergence and/or maintenance of behavioral differences via a trade-off between growth and mortality (Stamps 2007; Dingemanse and Wolf 2010; Wolf and Weissing 2010; Stamps and Groothuis 2010 a, b). Foraging behaviors necessary to maintain accelerated growth rate can increase the probability of depredation for fast-growing individuals (Mangel and Stamps 2001). Likewise, the physiological expense of maintaining rapid growth can have short and long-term effects on locomotor, cognitive, immune, and general somatic tissue function (Arendt 1997; Mangel and Stamps 2001; Stamps 2007; Dmitriew 2011). Despite these costs, rapid growth relative to other conspecifics might confer adaptive benefits (or impose further costs) depending on the context (e.g., benefits associated with aggressive dominance or temporal constraints imposed by cycles of food availability; Royle et al. 2005; Descamps et al. 2008).

Sih et al. (2015) suggest that one way a positive association between behavior and one or more state variable(s) results in divergence and maintenance of particular behavioral types is through positive feedback between behavior and those state variable(s) across ontogeny. Imagine a scenario in which size and behavior are linked and two individuals begin life with reasonably similar behavioral profiles and sizes, but one of those individuals is slightly more bold (insensitive to perceived/potential predation risk) than the other. If this behavioral profile leads to more efficient or successful acquisition of resources (because foraging is inherently risky business), and the individual does not succumb to predation or parasitism, then that bold individual could be set on a trajectory in which increased acquisition of higher quality resources reinforces the expression of bold behavior which in turn improves the animal's ability to acquire more and/or higher quality resources. Conversely, the less 'bold' individual would have relatively lower proficiency at resource acquisition, and either be incapable of maintaining or choose to avoid risky foraging behavior (e.g., forego foraging opportunity to spend greater time in refuge, invest in vigilance, and exhibit pre-emptive escape behavior); the trade-off here being increased potential for immediate survival. These alternate strategies may not confer equivalent fitness benefits (Luttbeg and Sih 2010), but their relative success is likely dependent on environmental context (e.g., actual prevalence and activity of predators as well as availability of food and location of foraging patches).

Negative feedback could also drive initially dissimilar phenotypes to converge along a similar behavioral type, in accordance with the ‘asset protection principle’ (Wolf et al. 2007;

McElreath et al. 2007; Wolf et al. 2008; Sih et al. 2015). Here, individuals in a lower state (in the above example, low energy reserves or nutrition) have nothing to lose and therefore should display risky behaviors in order to achieve any possible gain, while individuals in a high state should avoid risk since they are not in any immediate danger of starvation and have more to lose. Other factors, not associated with risk sensitivity or exploratory tendencies, could further influence variation in growth; for instance, if food acquisition rates do not co-vary with anti-predatory behaviors, but rather co-vary with the level of skill required to seek and consume food items.

In the current study we endeavor to empirically assess the relationship between growth and behavior throughout ontogeny in captive-reared Wood Ducks (WODU; *Aix sponsa*) within a common-garden. Due to the potential trade-off between mortality and growth (Stamps 2007), we focused on quantifying behaviors that relate to predator avoidance and post-capture escape (see Chapter 1 for details). Here, we assess the trajectory of growth and behavioral development as WODU ducklings develop, addressing three main goals:

First, we aimed to determine whether **individuals consistently differ in size across ontogeny** (in other words, whether individual differences in size and growth are maintained as ducklings develop). In many waterfowl systems, egg size tends to be correlated with hatchling size (Krist 2011), and hatchling size tends to be correlated with recruitment success (Wells et al. *In Prep.*; Dawson and Clark 1996; Pelayo and Clark 2003), suggesting lasting fitness consequences of these individual differences. As noted, there are many examples of organisms exhibiting considerable variation in growth rates across individuals, even when exposed to the same environmental pressures (i.e., common-garden and anecdotally, we have observed variation in individual size and apparent growth in our study system. We predict that initial differences in size at hatch remain consistent across ontogeny as individuals grow.

Second, we aimed to determine whether **developmental size affects behavioral expression and behavioral expression, in turn, affects future development and size among-individuals**. From previous research with this system, we know that behavioral differences appear to be maintained across ontogeny (Figure 1.4). Again, one potential explanation for the maintenance of behavioral variation within populations is that it is (at least partially) driven by a connection to some state variable (Dingemanse et al. 2010; Wolf and Weissing 2010; Moiron et al. 2019; Morandini and Ferrer 2019). For developing young organisms, growth is pivotal. Furthermore, there is a potential trade-off between growth and various aspects of behavior (Mangel and Stamps 2001; Stamps 2007). Behavioral expression necessitates expenditure of energy, particularly for behaviors that are physically demanding such as anti-predator behaviors (i.e., evasion and/or escape). Furthermore, anti-predator behaviors might entail an opportunity cost; foraging often requires exposure to higher predation risk due to allotment of attention away from vigilance as well as potential location of profitable foraging patches. Sensitivity to this risk may lead to reduced foraging rates and therefore smaller rates of growth and smaller sizes. As such, we predict that behaviors associated with avoiding or alleviating predation risk will be negatively correlated with size as ducklings develop. Alternatively, according to the asset protection principle (McElreath et al. 2007; Wolf et al. 2007; Wolf et al. 2008; Sih et al. 2015), associations between growth and behavior might be correlated such that small individuals are willing to take greater risks (they have little to lose) while large individuals are incentivized to reduce risk (they have little to gain and have sufficient energy reserves to fall back on).

Finally, we hypothesized that **positive feedback between behavior and size within-individuals leads to divergence in traits and maintenance of variation across ontogeny.** Here we take one further step to address the mechanism by which a [state]x[behavior] relationship might lead to and/or maintain divergence in behavioral traits. Specifically, tackling this hypothesis necessitates a close look at the relationship between initial (at hatch) behavior/state and the trajectory of behavior/state over time on an individual basis. For positive feedback to occur, there must first be a relationship between size/growth and behavior. Furthermore, we predict that: (i) larger hatchlings will display lower predation risk sensitivity than smaller hatchlings, (ii) larger hatchlings will show a greater rate of growth (slope of size across age), (iii) risk prone hatchlings will become more risk prone with age, and (iv) larger hatchlings will show an increase in risk prone behavior with age (following the theoretical framework established by Sih et al. 2015). In statistical terms, this hypothesis predicts positive correlations among intercepts and slopes of size/growth and behavior (see Statistical Methods below).

Statistical Methods

Consistent Individual Differences in Size

To determine whether individuals in our study consistently varied in their size or rate of growth throughout ontogeny, we conducted a series of Generalized Additive Mixed Models (GAMMs) using *brms* in R (Bayesian Regression Models using Stan ver 2.13.0; Bürkner 2017) for each size and growth variable (Table 3.1). We included individual identity and brood as, functionally, random effects. We also included sex and age as fixed effects. We fit the effect of age with a smoothing spline (akin to a polynomial function) to account for the curvilinear nature of WODU growth patterns. For each response variable we then calculated the Bayesian equivalent of the adjusted Intraclass Correlation Coefficient (ICC) for individuals using the *sjstats* package (ver 0.17.4) in R (ver 4.0.2), corroborating outputs with manual calculations of ICC (as the within-individual variance over the sum of within-individual variance and residual variance).

Trajectory of Behavior Across Development and its Relationship with Size and Growth

To assess the consistency of, and potential change in, behavior across time, as well as the potential influence of size and growth on behavioral expression, we constructed a series of single-response Generalized Linear Mixed Models using the *brms* package in R for each of our eigen component response variables calculated in Chapter 1 (Tonic Intensity, Struggle Intensity, Reactivity, and Space-Use, Table 1.3). We ran two separate models for each behavioral metric to reduce potential for multicollinearity between our two size metrics, one for body mass and one for tarsus length (Table 3.1). For each model, we included individual identity and brood as random effects, as well as sex and an interaction term for age and size (running separate models for both body mass and tarsus length). For Open Field Test (OFT) variables, which were recorded in an outdoor enclosure, we also included a score for wind intensity and air temperature. We ran models with Gaussian distribution, weakly informative priors, four parallel chains, and 1000 warmup and 6000-8000 total chain iterations.

The Effect of Current Behavior on Future State

To determine whether behavior had an influence on future state we conducted a series of single-response Generalized Linear Mixed Models as above, but with body mass and tarsus length as the response variables (measured at the subsequent observation period relative to age and behavior). To reduce potential for overfitting and multicollinearity, we constructed four models for each size metric: one with each behavioral metric as a fixed effect for a total of eight models. In these models we included individual identity and brood as random effects and sex as a fixed effect. We also included age and behavior as measured during the previous observation period and included a term for their interaction (i.e., response variables were measured at observation/time [t+1] while fixed effect variables were measured at observation/time [t]). As above, we ran models with Gaussian distribution, weakly informative priors, four parallel chains, and 1000 warmup and 6000-8000 total chain iterations.

Assessing Feedback Between Size and Behavior Across Ontogeny

To directly assess the potentially dynamic relationship between size and behavior and to study the potential for feedback between the two, we constructed a series of multi-response Generalized Linear Mixed Models including behavioral variables and size variables as responses. Due to model fitting issues, we elected to run a model for each dyadic pair of behavioral and size metrics. These models all included age and sex as fixed effects and brood and individual identity as random effects. They also included varying slopes (and intercepts) and terms allowing for calculation of correlations between intercepts and slopes across response variables. For example, we were interested in whether starting size was correlated with initial behavioral expression and we also were interested in whether starting size was correlated with the trajectory of behavioral development. These models also provide estimates for residual correlations among response variables (i.e., covariance among response variables after accounting for all parameters of the model) which we could use to corroborate findings from our above models with regard to among-individual relationships between behavior and size. For these models, we used flat, uninformative priors or weakly informative priors. As above, we ran all models as Gaussian with four parallel chains, as well as 1000 warmup and 6000-8000 total iterations.

Results

Consistent, Among-individual Variation in Size Across Ontogeny

Within-individual, growth curves (calculated via GAMM) qualitatively revealed consistent variation in size and growth across ontogeny, even when individuals were exposed to similar environmental conditions (Figure 3.1). Generalized Additive Models demonstrated repeatability (a Bayesian equivalent of adjusted Intraclass Correlation Coefficient) of both body mass (0.27, 95% HDI: 0.19, 0.37) and tarsus length (0.33; 95% HDI: 0.22, 0.44) suggesting individuals consistently varied in size across ontogeny. Moreover, the consistent differences in growth rates resulted in increasingly divergent body mass among individuals over time (Figure 3.1).

Behavior is Correlated with Size while Controlling for Age

We constructed a series of univariate GLMMs for each behavioral metric (eigen components) to assess the effect of age, size, and their interaction on behavior. In this analysis, age was a significant factor for Tonic Intensity, Struggle Intensity, Reactivity, and Space-use in all of our size models, corroborating our findings from models reported in Chapter 2 that did not include terms for size or growth (Table 3.2). While body mass alone was not a significant factor in most of our models (though for Tonic Intensity and Reactivity, it was close to significant), the effect of tarsus length was significant for Tonic Intensity and Reactivity such that birds with larger tarsi displayed lower Tonic Intensity and greater Reactivity (Table 3.2).

Notably, the interaction between age and size was a significant factor in all of our models (Table 3.2). Among older birds, Tonic Intensity was significantly positively correlated with body mass and tarsus length, even while Tonic Intensity scores decreased, on average, with age (Figures 3.2 and 3.3). Similarly, Struggle Intensity was significantly positively correlated with body mass and tarsus length among older birds. We did not find a significant relationship between Struggle Intensity and age with our prior work (Chapter 2), but when including the interaction term between age and size, a negative relationship between age and Struggle Intensity emerged; this relationship was significant in the model with body mass and close to significant in the model with tarsus length (Table 3.2).

Though Reactivity generally increased with age, it was significantly negatively correlated with body mass among older birds (Figure 3.2). Reactivity was positively correlated with tarsus length at younger ages but not at older ages (Figure 3.3). For Space-use, older birds also showed a significantly positive correlation with body mass and tarsus length while it decreased with age overall (Table 3.2; Figures 3.2 and 3.3). Also of note, for our Space-use models, the estimate for effect of sex was significant with males showing lower scores than females. Sex did not have a significant effect for any of our other behavioral variables (Table 3.2).

See Table 3.5 for a broad summary of significant effects.

Behavior is Correlated with Future State while Controlling for Age

We also wanted to test the hypothesis that current behavior would be related to future size (a component of significant behavior-state feedback). Our models revealed no significant relationship between in-hand behaviors (Tonic Intensity and Struggle Intensity) and body mass in a future observation period (Table 3.3). However, Tonic Intensity was a significant, negative predictor of tarsus length in a future observation period, the effect becoming increasingly positive as ducklings aged. This relationship appeared to be age dependent; younger birds showed a negative relationship between tonic intensity and future tarsus length while older birds showed a positive relationship between tonic intensity and future tarsus length (Table 3.3; Figure 3.5). Struggle Intensity was not a significant predictor of tarsus length in a future observation period but was close. The interaction between Struggle Intensity and age did not have a significant effect on tarsus length (Table 3.3).

Reactivity was significantly positively correlated with future body mass and future tarsus length (Table 3.3). However, this relationship was also significantly dependent on age for both size metrics. Younger birds showed a positive relationship between Reactivity and size while older birds showed a negative relationship between Reactivity and size (Table 3.3; Figures 3.4 and 3.5). Space-use in a modified open field test had a significant effect on future body mass, but its effect was only close to significant on future tarsus length (Table 3.3). The effect of an interaction between Space-use and age on future body mass (but not tarsus length) was significantly positive, with older birds showing a positive correlation between Space-use and future body mass (Table 3.3). Broadly, relationships shown with these models corroborated relationships in our behavioral response models above.

See Table 3.5 for a summary of significant effects.

No Evidence of Behavior-state Feedback Driving Differentiation Within-individuals

To determine whether feedback between size and behavior within-individuals was likely a proximate driver of behavioral differentiation, we constructed a series of multi-response GLMMs with behavioral and size metrics as response variables. If positive feedback between state and behavior was occurring, we expected to find positive correlations among individual intercepts and slopes across behavior and size metrics (a fanning out of individual behavior and size trajectories across time; Sih et al. 2015).

Across all of our behavior and size multi-response models we found no evidence of correlations among intercepts and slopes between behavior and state (Table 3.4). Dyadic residual correlations between body mass and Tonic Intensity, Struggle Intensity, and Reactivity corroborated relationships found at older ages in our single-response models above (Table 3.4). Interestingly, dyadic residual correlations between tarsus length and the same behaviors aligned with the relationships between these behaviors and tarsus length at earlier ages in our single-response models (Table 3.4).

Discussion

In this study we assessed growth and its relationship with behaviors that denote sensitivity to predation risk by addressing three goals: (1) examining whether differences in size are repeatable across ontogeny and individual growth varies in a common garden setting; (2) examining whether individual state (specifically size) affects behavior (specifically risk sensitivity) and vice versa; and (3) testing the hypothesis that feedback between behavior and state drives behavioral differentiation and the subsequent maintenance of behavioral differences later in life. Examination of individual growth curves revealed striking variation in growth among individuals and we found relatively high repeatability for both body mass and tarsus length across ontogeny despite *ad lib* access to high quality food for all individuals (Figure 3.1). Further analysis revealed that, on average (among individuals), size is dynamically associated with risk sensitivity in multiple contexts; current size was a significant predictor of behavior and behavior, in turn, was a significant predictor of size at a future measurement period (Tables 3.2 and 3.3). Interestingly, relationships among size and behavior were not straight-forward, showing a consistent and convincing age-dependence and potential differences between behaviors across contexts (Figures 3.2, 3.3, 3.4, and 3.5). While we found support for our first two hypotheses, we found no evidence of feedback between behavior and state occurring within-individuals throughout ontogeny. This suggests that the relationship between state and behavior is not proximately driving behavioral differentiation in this system under our controlled conditions (i.e., common garden and *ad lib* food access). More likely, size and behavior in Wood Ducks are linked by other, evolutionarily driven ecological or developmental processes.

Behavioral Expression Changes as Birds Age

Average changes in behavior across ontogeny corroborated our findings from Chapter 2. Including size in our models increased the significance of this relationship for Struggle Intensity and Space-use in an Open Field arena. While changes in Struggle Intensity matched those of Tonic Intensity, Space-use decreased significantly with age, coinciding with an increase in Reactivity, corroborating that birds less likely to display intense responses to startle stimuli were also more likely to venture away from the walls of the arena and revisit the origin of the startle stimulus. This average shift in behavioral expression is difficult to explain considering high predation risk for younger birds. If predation rates tend to be higher among younger ducklings, why would younger ducklings display lower reactivity and greater willingness to explore? One possibility is that the energetic and nutritional demands of early growth and development are an immense pressure for younger birds. A lack of energetic reserves would likely decrease the ability of an individual to invest in active predator avoidance or escape behavior; the need to rapidly acquire energy resources could, in turn, incentivize exploration that could be essential for resource acquisition. Although prioritizing foraging related behaviors might increase the risk of predation, predation pressure might be at such a high and unavoidable level within the first two weeks of life for Wood Ducks that investing too heavily in predator avoidance might be selected against at the cost of slow growth (i.e., growth/mortality trade-off; Moore 1999; Stamps 2007). In other words, it might not pay off to prioritize predator avoidance over food acquisition when predator avoidance behavior only marginally reduces the probability of predation, due to the extreme abundance of potential predators, and when food acquisition is extremely important and may enable ducklings to more rapidly escape the high-risk predation window. We discuss other explanations for these shifts in behavior across ontogeny with greater detail in Chapter 2 and in the context of growth below.

Size and Risk Sensitivity are Related, but Their Relationship Across Development is Complex

The potential importance of foraging requirements in mediating anti-predator behavior leads us to the central focus of this study. During ontogeny, young vertebrates must acquire a relatively large amount of nutrients and energy to invest in somatic tissue growth and neural organization (Starck and Ricklefs 1998). Wood Ducks, increase their body mass at hatch by roughly 2000% across ontogeny, and represent an excellent example of an organism that must balance these developmental needs with extreme predation risk and high rates of depredation (Davis et al. 2007; Davis et al. 2009). Thus, we expected to find evidence of a relationship between size and behaviors associated with sensitivity to predation risk (i.e., responsiveness to startling stimuli in two standardized contexts away from the safety of brood-mates) representing the trade-off between growth and mortality (Stamps 2007) and supporting either asset-protection (such that individuals with sufficient resources tend to exhibit risk aversion) or a state-dependent safety effect (such that individuals with excess resources can afford to be more risk prone) (McElreath et al. 2007; Wolf et al. 2007; Wolf et al. 2008; Luttbeg and Sih 2010; Sih et al. 2015).

Although we did find a relationship between behavior and size, it was much more complex than a simple positive or negative correlation, shifting as ducklings developed. The correlation between size and behavior either significantly increased or significantly decreased as birds got older (depending on the behavior), with correlations at older ages generally showing stronger negative or positive signals (Table 3.5). The correlations between size and in-hand behaviors (both Tonic Intensity and Struggle Intensity) became significantly more positive among older ducklings; larger ducklings exhibited more intense responses to being handled. This pattern coincided with a relationship between size and open field behaviors among older ducklings; larger ducklings exhibited less reactive behavior (lower activity rates, less frequent rapid head movement, and lower responses to startle stimuli) and a greater tendency to explore both the center of the arena as well as revisit the location of a prior startle stimulus. While these relationships were all significant for our behavioral response models (Table 3.2), they were not all significant for the future size response models (Table 3.3). Notably, neither in-hand component appeared to impact future body mass, and the effects of Struggle Intensity and Space-use on future tarsus length were not significant (though Space-use was close to significant). Still, the general tendency for increasing or decreasing relationship across age also appeared in our future size response model estimates, matching output from our behavior response models (but see below).

There are several possible explanations for the apparent shifts across development, most of which we discussed as possible drivers of average change in behavior across time in Chapter 2; mainly, physiological/neurological developmental processes and historical ontogenetic shifts in selective environment. Another important consideration here, however, is that the scale of differences in size among ducklings does increase across ontogeny even though size (and the behavior we measured) is repeatable. Hatchlings have a range of body masses from approximately 17g to 30g, but by the time ducklings are 5 weeks of age the range of size is dramatically larger with the lower limit around approximately 200g and upper limits well above 400g. We attempted to control for this in our behavioral response models by log transforming body mass and tarsus length, but the difference of scale remained (albeit much more-so for body mass than tarsus length). Furthermore, our prior analysis with the same data set (see Chapter 2) suggested an increase in repeatability and within-individual consistency of these behaviors across ontogeny, potentially further increasing our models' ability to detect a signal among older birds. What might be a fundamental underlying relationship between mass and behavior from hatch onward could simply be more detectable at older ages. Interestingly, the effect of Reactivity on future body mass was detectibly positive at early ages (becoming increasingly negative at older ages) while the effect of Space-use at early ages was somewhat negative. Possibly, increased Reactivity and reduced Space-use are related to increased foraging behavior and food intake in home enclosures at early ages, though this is unlikely. More likely, larger individuals can afford to divest from risky behaviors at younger ages (e.g., asset protection principle) and can afford to take greater risks at older ages (e.g., state-dependent safety).

While our models indicate a weak or no relationship between body mass and behavior at younger ages (with the exception of the influence of Reactivity and Space-use on future body mass at younger ages), the influence of tarsus length on both Tonic Intensity and Reactivity reversed in the direction of effect as birds aged. Younger birds showed either a negative or positive effect while older birds showed either a positive or negative effect respectively (Figures 3.3 and 3.5), the relationship at older ages matching that of body mass at older ages. Increasing scale across time is not sufficient to explain this pattern. Residual correlations from our multi-response models with tarsus length matched the relationships observed at younger ages in our single-response models, suggesting that the effect of tarsus length is more important for younger ducklings.

Selective pressures on tarsus length and growth might differ from those of body mass, particularly at younger ages in precocial birds when the structure of the leg can have greater implications for mobility; longer leg lengths and improved swimming ability could certainly enhance both foraging efficiency and predator avoidance (Prange and Schmidt-Nielsen 1970; Hohtola and Visser 1998; Anderson and Alisauskas 2001). As such, behavior is likely linked to physical capability and/or remaining energy reserves while investing in tarsus growth during the earliest phases of post-hatch development. Birds that have the energy to invest in tarsus growth early on might have enough energy in reserve to exhibit more energy intensive behaviors (i.e., higher rates of activity and greater intensity of responses to startling stimuli) or might be employing a behavioral strategy that takes advantage of their physical capability. The negative correlation between tarsus length and Tonic Intensity at young ages is more difficult to explain; the apparent inverse association between Tonic Intensity and Reactivity as they relate to changes over time and differences in size is discussed below (and in Chapter 2). Still, external factors have been shown to be more important in determining locomotor function for young WODU than structural size. Hopkins et al. (2011) found that incubation temperature was a significant predictor of locomotor performance but found no relationship with tarsus length in 2-3 week old WODU ducklings. Future research specifically testing the link between tarsus length, performance (both in foraging efficiency and predator evasion), and energy reserves within this system would help to elucidate these potential connections and provide greater context for the relationships we observed.

It is also possible that shifts in correlations between size and behavior across ontogeny either represent a biologically driven, temporal change in expression of a common developmental pathway or hint at the existence of different, temporally specific components of the developmental pathway(s) for these traits. If the correlation between behavior and size is adaptive, the high degree of age-dependence likely suggests a history of shifting exogenous and/or endogenous pressures as young ducklings grow. If so, then what do the actual relationships between size and behavior indicate when they appear at the latter stages of development? Our results here support a state-dependent safety effect (particularly in the Open Field Test) at later stages of duckling development. By the point ducklings are 3+ weeks of age, their size differences within their respective age class might matter more when it comes to reducing the pool of potential predators as well as mounting an effective anti-predator response. Among very young ducklings, size differences are small enough as to not impact the pool of potential predators. However, past a certain age threshold the increase in scale of differences could be impactful, with larger ducklings generally facing reduced risk of predation by limiting the pool of potential predators that can physically consume them (similar in concept to gape limitation which is well studied in fish; e.g., Ramamonjisoa et al. 2018). Bullfrogs, Herons, and Large-mouth Bass are examples of potential predators that might have greater difficulty capturing and consuming larger ducklings. If this is the case, larger birds (at older ages) could possibly afford to display lower Reactivity and increased Space-use (exploratory tendency). They might also have the energetic reserves and physical ability to exhibit higher Tonic Intensity during handling (a more direct approach to escape than feigning death; see discussion in Chapters 1 and 2).

Some form of 'gape limitation' in our system might exert strong selection on rapid growth, although such rapid growth would likely come at the expense of higher risk of predation at earlier ages when the pool of potential predators is larger (and could also have physical and physiological drawbacks; Mangel and Stamps 2001). Variation in how ducklings approach this trade-off might serve as a buffer when predation rates are either very high or very low (such that certain individuals are likely to be more successful when there are many predators and others when there are very few). Reduced expression of Reactivity among larger ducklings later in life supports the potential influence of 'gape limitation' (or at least size-dependent selective predation); smaller individuals experience higher predation pressure which might select for higher degrees of Reactivity. However, the potential for size-dependent safety in this system is a bit more complicated when we also consider that, overall, there is an average increase in Reactivity and decrease in Space-use with age (discussed in Chapter 2). Future experiments directly assessing predator capabilities with regard to duckling size, paired with a more detailed examination of specific duckling responses to actual predator presence, might be necessary to elucidate the selective influence of both predator and duckling capabilities on behavioral expression of ducklings across ontogeny.

In a previous study (Chapter 2) we found an inverse association between changes in Tonic Intensity and Reactivity across development. The same pattern was observed in this study with regard to age-dependent behavior-state correlations among individuals. Intensity of response to being handled and willingness to leave areas of perceived safety in an Open Field Test were positively associated with size among older birds, while intensity of response (Reactivity) in an Open Field Test was negatively associated with size among older birds. Assuming that our behavioral variables measured biologically relevant traits (see Chapter 1), these two assays could be measuring different aspects of the same underlying behavioral phenotype. High responsivity during free movement in a wild context, for instance, could be associated with a reduction in the likelihood of capture by a predator and would therefore be beneficial for highly reactive individuals in an environment with relatively high predation risk. In the event of capture, however, a muted response to being handled by a predator might provide an advantage (i.e., playing dead and waiting for a lapse in the captor's attention to escape). Therefore, these seemingly different responses in two contexts might be representative of tendency to accept potential predation risk and/or some underlying physiological or neural mechanism that results in seemingly different approaches. Yet, capture is capture, and intense struggle might also confer benefits for individuals that are large enough to physically break out of a predator's grasp, possibly explaining the positive association between in-hand struggle intensity and size.

Concluding Remarks

One major hypothesized explanation for the existence and maintenance of consistent individual differences in behavior within populations is state-dependence (theoretical models reviewed by Dingemanse and Wolf 2010; Wolf and Weissing 2010). Due to the enormous demands of growth during early life development, we chose to focus on size (both body mass and tarsus length as a measure of structural size) during ontogeny as the state variable of interest.

We provide evidence in support of a relationship between somatic growth and behaviors associated with aversion to predation risk, and we show, as well, that the relationship itself is dependent on duckling age (Table 3.5). However, we found no evidence of feedback between growth and behavior within-individuals, despite finding a relationship among individuals between size and behavior as well as a relationship between behavior and future size. This suggests that variation in the behaviors we measured is not proximately driven by differences in size and growth when ducklings are raised in a common garden. We provided a detailed discussion of some factors that might explain these relationships, but it is important to acknowledge the complexity inherent in developmental processes, even in a heavily controlled environment. This complexity reinforces the importance of more holistic, longitudinal approaches to questions surrounding ontogeny and behavioral development. Indeed, if we had relied exclusively on residual correlations from multi-response models, we would have missed the deeper age-dependance of these relationships.

Whether the controlled context of our captive study can provide insight into the wild Wood Duck system, or behavioral development in a wild context more broadly, remains a valid concern. While simplification of the system allowed us to isolate factors of interest, it does leave questions regarding extra-focal factors. In the current study, we explicitly reduced or eliminated variation in a number of environmental factors, most notably social environment, food availability, and risk of predation. In the wild, social factors like the behavior of the brooding mother (social transmission of behavioral types; Pittet et al. 2014), number of ducklings in the brood (influence on predator avoidance behavior due to the benefits of group formation; Lehtonen and Jaatinen 2016), brood dynamics (possible aggression or competitive exclusion from resources; Britton 1989), and depredation of particularly influential individuals as time progresses (i.e., “keystone” individuals; Modlmeier et al. 2014) can all impact focal individual behavior both in the short and long-term. Although we statistically controlled for the effect of brood (i.e., differences among broods that varied in number of ducklings from 3-11 individuals), we were unable to detect any differences among broods in duckling size, behavior, or their interaction. How social factors might interact with developmental growth to influence behavioral phenotype, while complex, is a fascinating question that requires detailed exploration.

Food availability is also of obvious importance for growing animals, possibly having lasting impact on behavioral developmental processes (as discussed above). In a wild setting, not only does access to food vary among ducklings or broods, but it can change across time for any given brood due to a variety of unpredictable factors. Our finding that size during ontogeny is correlated with behavior, even when birds are provided *ad lib* access to high quality food, further reinforces a greater need to understand the influence of varying access to essential nutrients and energy. Furthermore, predation risk and prey’s perception of it often varies across an ecological landscape. Understanding how differences in the perception of predation risk (the so-called "landscape of fear"; Laundré et al. 2010; Bleicher 2017; Gaynor et al. 2019) and how different ducklings respond to the possible growth-mortality trade-off in a variable landscape also remains pivotal in understanding behavioral differentiation and the maintenance of personality within a population. The lack of external pressures acting (perhaps differentially) on ducklings in this study could serve as an explanation concerning the lack of evidence for feedback between growth and behavior within-individuals (Sih et al. 2015). In the final chapter, we experimentally address the effect of differing developmental environments (exposure to simulated predation events and effort required to access food) on growth, risk-associated behaviors, and their interaction both among- and within-individuals.

Table 3.1: Model specifications (simplified version of code used in the *brms* package for R) and brief description of the models' intended purpose.

Model specification	**Purpose**
Size ~ (1|Individual) + (1|Brood) + s(Age) + Sex	Random intercepts Generalized Additive Mixed Model to estimate Intraclass Correlation Coefficient (Repeatability) - s(Age) indicates a curvilinear specification of the effect of Age (modeled spline)
Behavior ~ (1+Age|Individual) + (1|Brood) + Size*Age + Sex + Confounds [*for OFT*]	Random intercepts and slopes model with an interaction term for size across time to assess relationship between size and behavior across development
Behavior ~ (1+Age|Individual + (1|Brood) + Age + Sex + Confounds [*for OFT*]	Multi-response random slopes and intercepts model to estimate within-individual correlations among slopes and intercepts – examine evidence of feedback between size and behavior
Future Size ~ (1+Age|Individual) + (1|Brood) + Behavior*Age + Sex	Random intercepts and slopes model to estimate the effect of current behavior and age on size at the next observation period

Figure 3.1: Spaghetti plot of modeled individual growth curves within the first 50 days of life post-hatch showing variation in body mass (y-axis) that remains (and increases in scale) throughout development (age in days, x-axis). This figure depicts output from a varying intercepts and slopes General Additive Mixed Model with Body Mass as the response variable run using the *brms* package in R. The bold central line represents the average while the light gray shaded region outlines the 95% Credibility Interval. An estimate for Intraclass Correlation Coefficient from a version of the same model only specifying varying intercepts (and not slopes as this can complicate ICC interpretations) is also presented with a 95% Highest Density Interval calculated using the *sjstats* package in R.

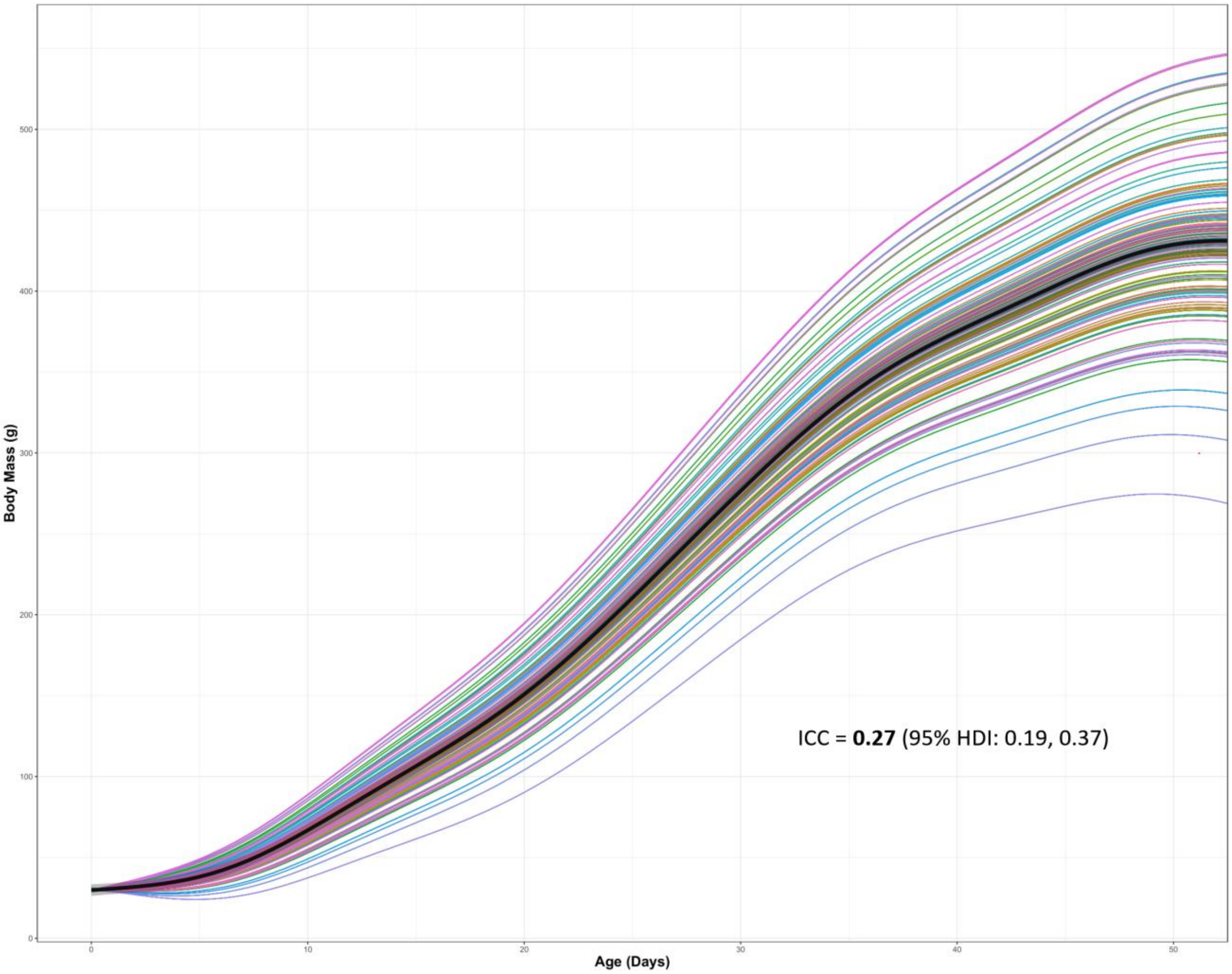


Table 3.2: Summary of important model estimates and their 95% Credibility Intervals for our Behavior response Generalized Linear Mixed Models using *brms* in R. We show results from individual models for Tonic Intensity, Struggle Intensity, Reactivity, and Space-use. Two separate models were run for each behavioral metric to reduce potential for multicollinearity between our two size metrics, one for body mass and one for tarsus length. Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero).

In-hand			
Response	**Effect**	**Effect Estimate**	**95% C.I.**
Tonic Intensity	Within-Individual: Intercept ~ Slope	-0.63	-0.98, 0.45
	Ln(Body Mass)*	-0.33	-0.69, 0.016
	Age**	**-0.34**	**-0.42, -0.25**
	Ln(Body Mass):Age**	**0.05**	**0.039, 0.063**
	Sex	0.22	-0.036, 0.48
Struggle Intensity	Within-Individual: Intercept ~ Slope	0.094	-0.62, 0.94
	Ln(Body Mass)*	0.17	-0.1, 0.44
	Age**	**-0.098**	**-0.16, -0.032**
	Ln(Body Mass):Age**	**0.014**	**0.005, 0.023**
	Sex	-0.0031	-0.19, 0.19
Tonic Intensity	Within-Individual: Intercept ~ Slope	-0.65	-0.98, 0.32
	Ln(Tarsus Length)**	**-2.48**	**-3.39, -1.56**
	Age**	**-0.92**	**-1.13, -0.71**
	Ln(Tarsus Length):Age**	**0.24**	**0.18, 0.29**
	Sex	0.18	-0.07, 0.44
Struggle Intensity	Within-Individual: Intercept ~ Slope	0.14	-0.61, 0.94
	Ln(Tarsus Length)	-0.36	-1.08, 0.35
	Age*	-0.16	-0.33, 0.0021
	Ln(Tarsus Length):Age**	**0.043**	**0.0013, 0.085**
	Sex	-0.0061	-0.2, 0.19

Open Field Test			
Response	**Effect**	**Effect Estimate**	**95% C.I.**
Reactivity	Within-Individual: Intercept ~ Slope	-0.51	-0.83, 0.58
	Ln(Body Mass)*	0.75	-0.033, 1.53
	Age**	**0.51**	**0.29, 0.73**
	Ln(Body Mass):Age**	**-0.079**	**-0.12, -0.049**
	Sex	-0.078	-0.48, 0.33
	Wind**	**-0.67**	**-1.18, -0.16**
	Temperature	-0.011	-0.031, 0.01
Space-use	Within-Individual: Intercept ~ Slope	-0.34	-0.83, 0.83
	Ln(Body Mass)	0.16	-0.51, 0.83
	Age**	**-0.28**	**-0.47, -0.085**
	Ln(Body Mass):Age**	**0.042**	**0.015, 0.068**
	Sex**	**-0.34**	**-0.66, -0.15**
	Wind*	0.46	-0.0089, 0.91
	Temperature	-0.013	-0.032, 0.0054
Reactivity	Within-Individual: Intercept ~ Slope	-0.54	-0.83, 0.45
	Ln(Tarsus Length)**	**5.73**	**3.84, 7.56**
	Age**	**0.73**	**0.15, 1.29**
	Ln(Tarsus Length):Age**	**-0.19**	**-0.34, -0.047**
	Sex	-0.092	-0.51, 0.32
	Wind**	**-0.68**	**-1.2, -0.17**
	Temperature	-0.012	-0.033, 0.0082
Space-use	Within-Individual: Intercept ~ Slope	-0.45	-0.84, 0.73
	Ln(Tarsus Length)*	-1.09	-2.68, 0.49
	Age**	**-0.77**	**-1.29, -0.24**
	Ln(Tarsus Length):Age**	**0.2**	**0.065, 0.33**
	Sex**	**-0.36**	**-0.69, -0.03**
	Wind*	0.42	-0.039, 0.89
	Temperature	-0.013	-0.031, 0.0051

Table 3.3: Summary of important model estimates and their 95% Credibility Intervals for our future size response Generalized Linear Mixed Models run using *brms* in R. We show results from individual models for body mass and tarsus length as measured at a future observation period. Four separate models were run for each size metric to reduce potential for overfitting; one model each for Tonic Intensity, Struggle Intensity, Reactivity, and Space-use. Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero).

Response	Effect	Effect Estimate	95% C.I.
Future Body Mass	Tonic Intensity	0.15	-2.65, 2.95
	Age**	**10.54**	**10.22, 10.86**
	Tonic Intensity:Age	-0.03	-0.2, 0.14
	Sex	2.55	-5.2, 10.18
Future Body Mass	Struggle Intensity	0.01	-4.44, 4.47
	Age**	**10.55**	**10.26, 10.84**
	Struggle Intensity:Age	-0.0091	-0.24, 0.22
	Sex	2.45	-5.26, 9.99
Future Body Mass	**Reactivity****	**14.33**	**8.45, 20.11**
	Age**	**9.67**	**9.25, 10.09**
	Reactivity:Age**	**-0.57**	**-0.85, -0.3**
	Sex	5.65	-4.26, 15.5
Future Body Mass	**Space-use****	**-7.84**	**-15.18, -0.47**
	Age**	**9.9**	**9.51, 10.29**
	Space-use:Age**	**0.44**	**0.11, 0.78**
	Sex	5.43	-4.75, 15.42
Future Tarsus Length	**Tonic Intensity****	**-1.41**	**-1.65, -1.16**
	Age**	**0.39**	**0.37, 0.42**
	Tonic Intensity:Age**	**0.063**	**0.051, 0.075**
	Sex	0.68	0.18, 1.19
Future Tarsus Length	Struggle Intensity*	-0.39	-0.79, 0.019
	Age**	**0.44**	**0.42, 0.46**
	Struggle Intensity:Age	0.0034	-0.015, 0.022
	Sex	0.63	0.1, 1.15
Future Tarsus Length	**Reactivity****	**1.71**	**1.29, 2.11**
	Age**	**0.22**	**0.19, 0.24**
	Reactivity:Age**	**-0.056**	**-0.071, -0.041**
	Sex**	**0.9**	**0.38, 1.44**
Future Tarsus Length	Space-use*	-0.43	-0.92, 0.07
	Age**	**0.26**	**0.24, 0.28**
	Space-use:Age*	0.015	-0.005, 0.035
	Sex	**0.74**	**0.17, 1.31**

Figure 3.2: Graphical representation of change in the relationship between behavior and body mass across time. We show linear estimates of the effect of body mass on behavior when age is held constant at 7 days (left column, blue shading), 21 days (center column, green shading), and 35 days (right column, pink shading) for Tonic Intensity (top-most row), Struggle Intensity (top-middle row), Reactivity (bottom-middle row), and Space-use (bottom-most row). Shaded regions denote the 95% Credibility Interval for each effect estimate when age is held constant at a given point. We also provide the effect estimate for the [body mass]x[age] interaction term which informs how much the effect of body mass on behavior changes and in which direction the relationship changes across time. Positive effect estimates indicate that the relationship becomes increasingly positive while negative effect estimates indicate that the relationship becomes increasingly negative as ducklings age. Significance of the interaction term is indicated by the duckling symbol as shown in the figure key. Note that the range of sizes changes at each time point as ducklings grow.

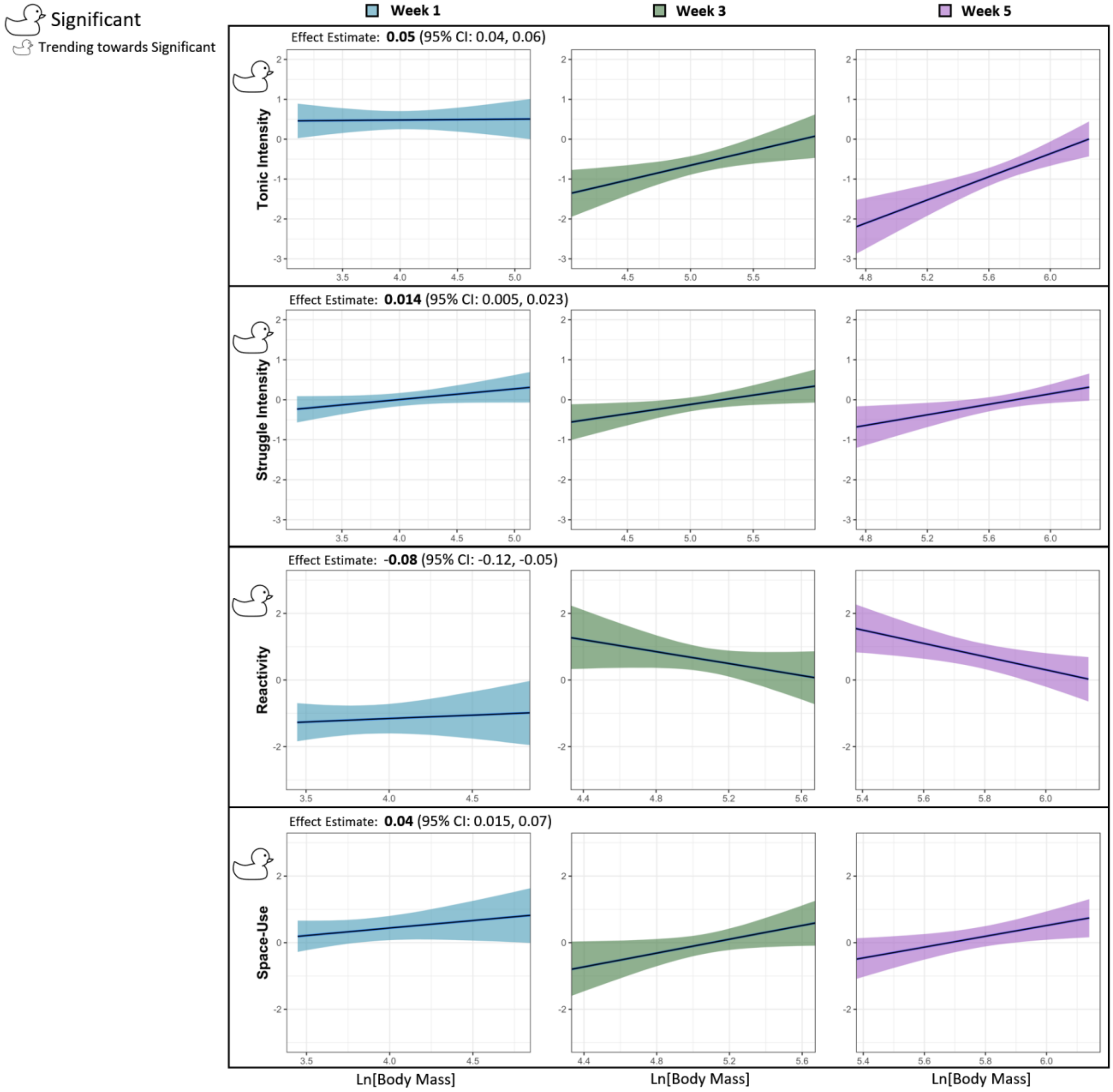


Figure 3.3: Graphical representation of change in the relationship between behavior and tarsus length across time. We show linear estimates of the effect of tarsus length on behavior when age is held constant at 7 days (left column, blue shading), 21 days (center column, green shading), and 35 days (right column, pink shading) for Tonic Intensity (top-most row), Struggle Intensity (top-middle row), Reactivity (bottom-middle row), and Space-use (bottom-most row). Shaded regions denote the 95% Credibility Interval for each effect estimate when age is held constant at a given point. We also provide the effect estimate for the [tarsus length]x[age] interaction term which informs how much the effect of tarsus length on behavior changes and in which direction the relationship changes across time. Positive effect estimates indicate that the relationship becomes increasingly positive while negative effect estimates indicate that the relationship becomes increasingly negative as ducklings age. Significance of the interaction term is indicated by the duckling symbol as shown in the figure key. Note that the range of sizes changes at each time point as ducklings grow.

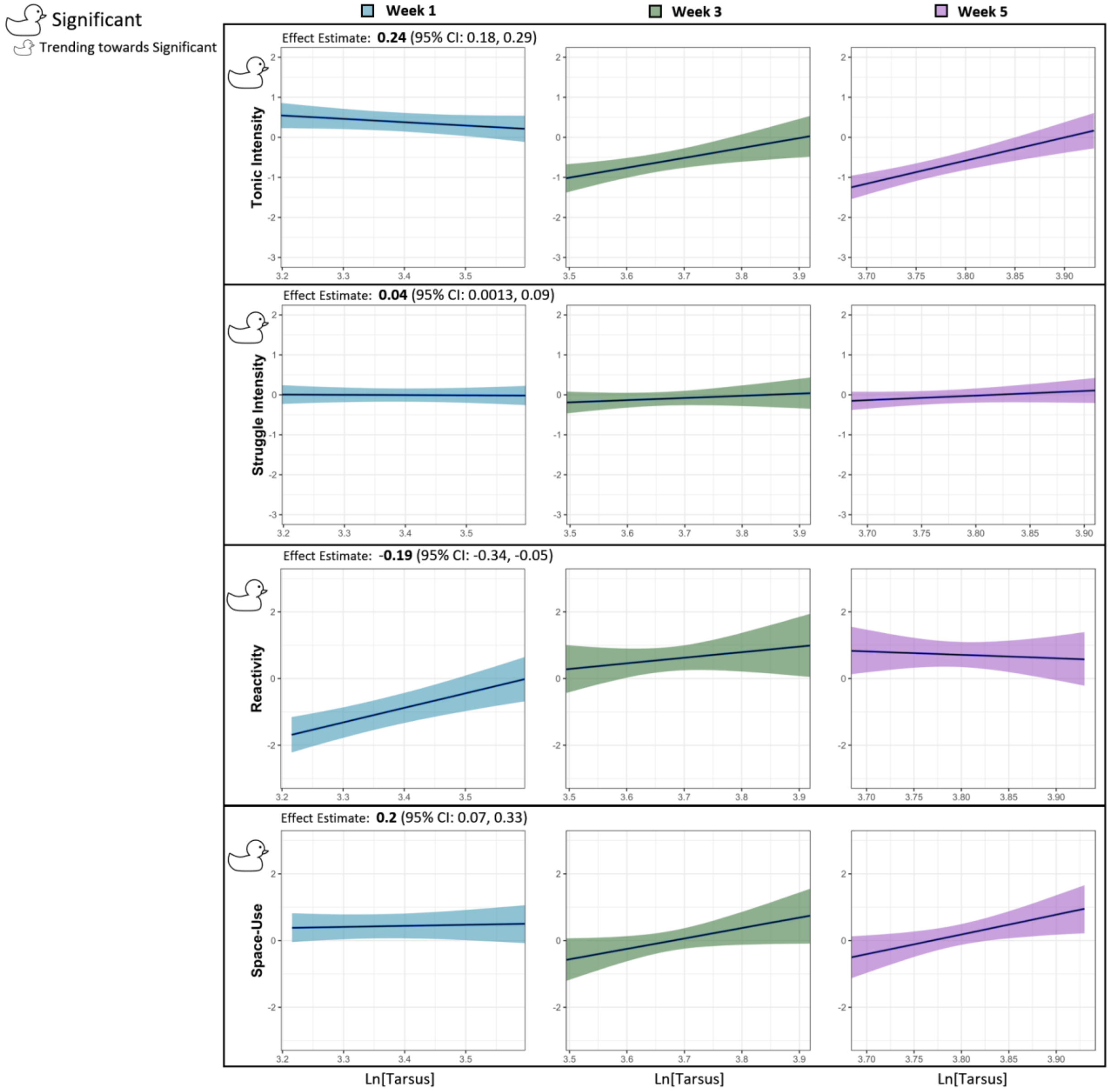


Figure 3.4: Graphical representation of change in the relationship between future body mass and behavior across time. We show linear estimates of the effect of behavior on future body mass when age is held constant at 7 days (bottom row, blue shading), 21 days (center row, green shading), and 35 days (top row, pink shading) for Tonic Intensity (left-most column), Struggle Intensity (left-center column), Reactivity (right-center column), and Space-use (right-most column). Shaded regions denote the 95% Credibility Interval for each effect estimate when age is held constant at a given point. We also provide the effect estimate for the [behavior]x[age] interaction term which informs how much the effect of behavior on future body mass changes and in which direction the relationship changes across time. Positive effect estimates indicate that the relationship becomes increasingly positive while negative effect estimates indicate that the relationship becomes increasingly negative as ducklings age. Significance of the interaction term is indicated by the duckling symbol as shown in the figure key. Note that the range of sizes changes at each time point as ducklings grow.

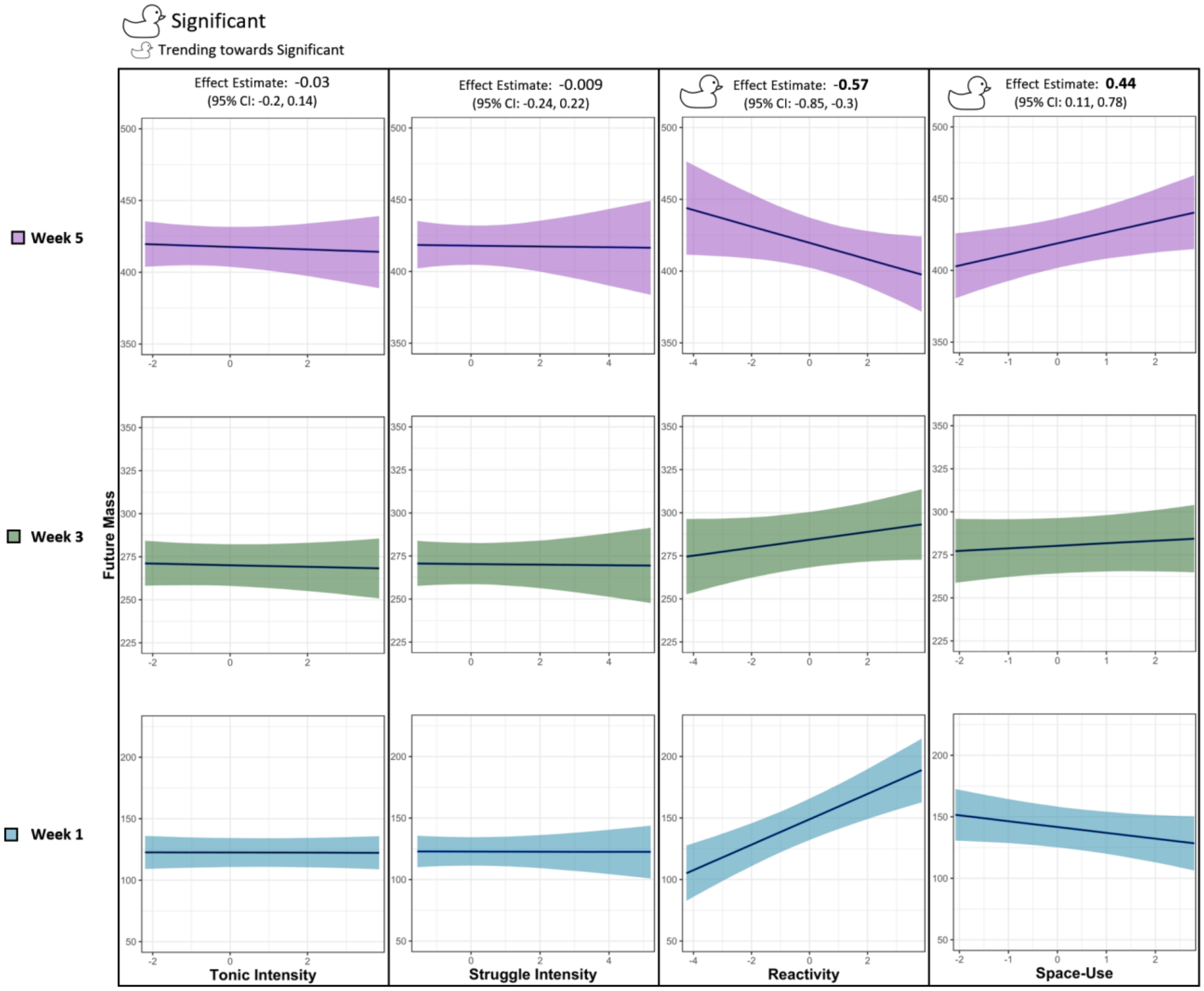


Figure 3.5: Graphical representation of change in the relationship between future tarsus length and behavior across time. We show linear estimates of the effect of behavior on future tarsus length when age is held constant at 7 days (bottom row, blue shading), 21 days (center row, green shading), and 35 days (top row, pink shading) for Tonic Intensity (left-most column), Struggle Intensity (left-center column), Reactivity (right-center column), and Space-use (right-most column). Shaded regions denote the 95% Credibility Interval for each effect estimate when age is held constant at a given point. We also provide the effect estimate for the [behavior]x[age] interaction term which informs how much the effect of behavior on future tarsus length changes and in which direction the relationship changes across time. Positive effect estimates indicate that the relationship becomes increasingly positive while negative effect estimates indicate that the relationship becomes increasingly negative as ducklings age. Significance of the interaction term is indicated by the duckling symbol as shown in the figure key. Note that the range of sizes changes at each time point as ducklings grow.

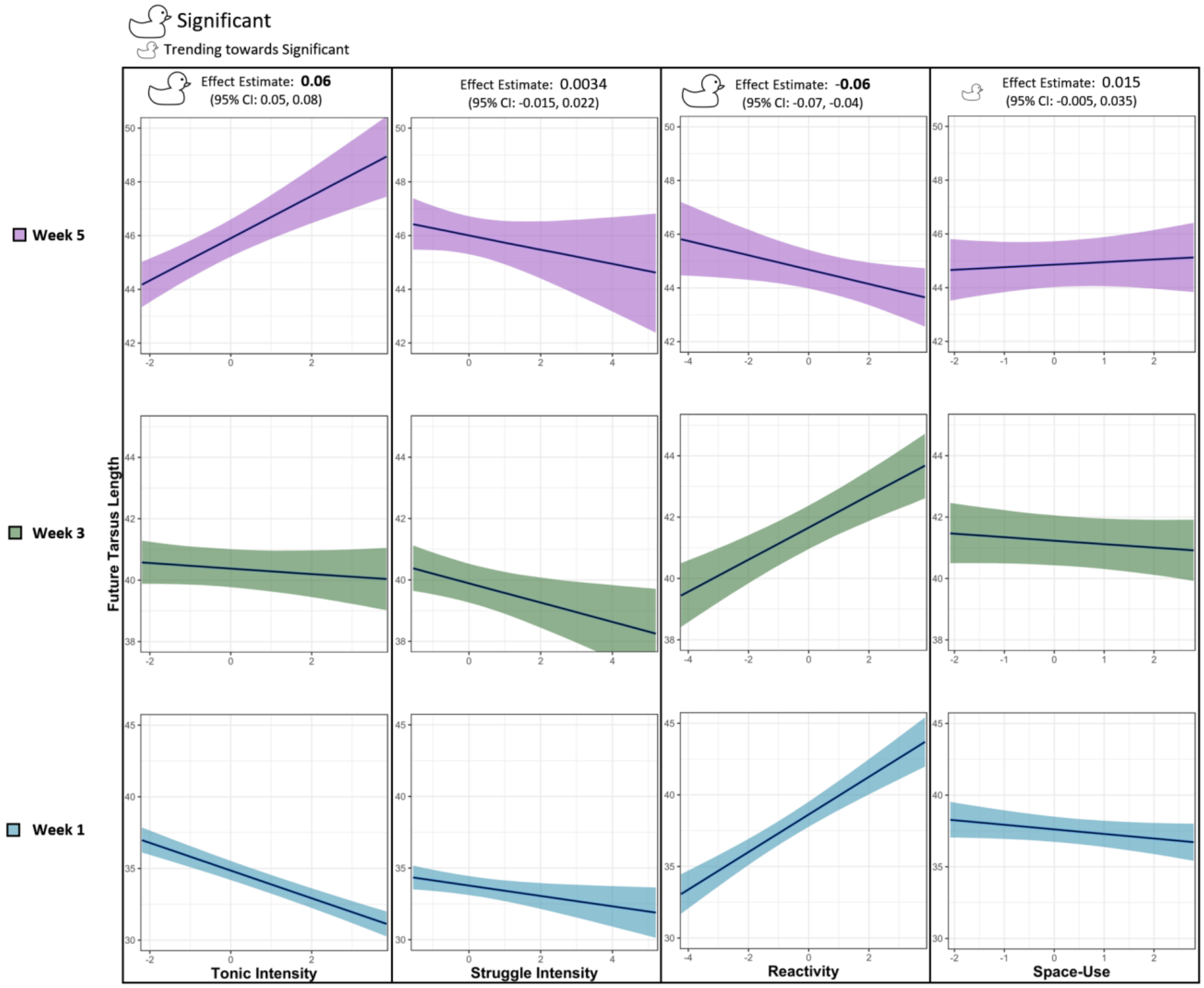
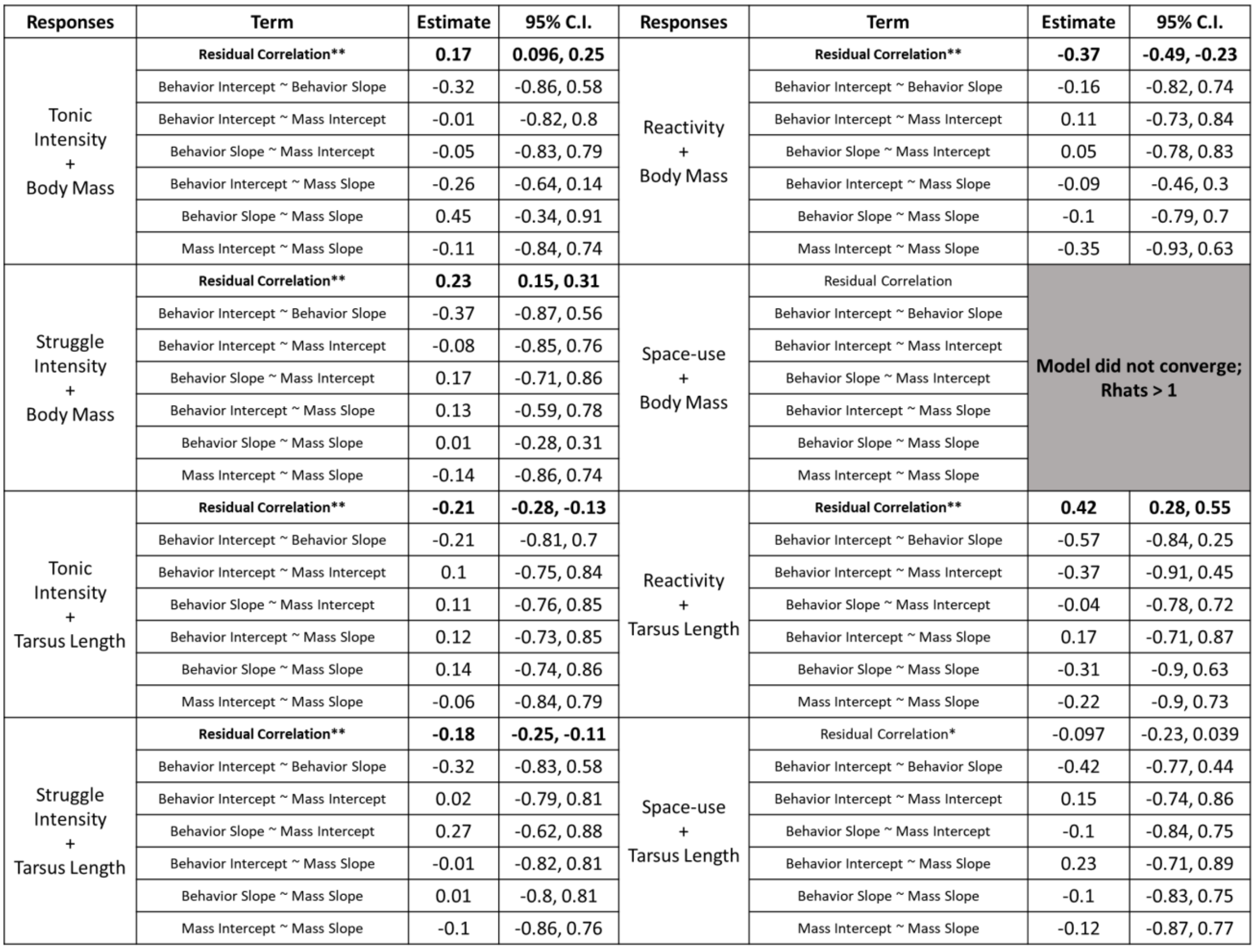


Table 3.4: Summary of important model estimates and their 95% Credibility Intervals for our multi-response Generalized Linear Mixed Models using *brms* in R. We show results from dyadic models with behavioral metrics and size metrics as the response variables. To reduce model fitting issues, we restricted each model to two response variables—one behavioral metric and one size metric for each possible combination—specifically examining within-individual correlations among intercepts and slopes as well as the residual correlation (average correlation while accounting for other model parameters) between the two response variables. In total, we ran eight models: one model each for Tonic Intensity, Struggle Intensity, Reactivity, and Space-use and their relationship with body mass and tarsus length. Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero). Effect estimates for our model with Response Intensity and Body Mass as response variables are not included because the model did not converge (Rhat scores greater than 1) even when parameters were adjusted. We found no significant correlations between intercepts and slopes across all our models and therefore found no evidence of feedback between state and behavior within-individuals in this study.



Responses	Term	Estimate	95% C.I.	Responses	Term	Estimate	95% C.I.
Tonic Intensity + Body Mass	**Residual Correlation****	**0.17**	**0.096, 0.25**	Reactivity + Body Mass	**Residual Correlation****	**-0.37**	**-0.49, -0.23**
	Behavior Intercept ~ Behavior Slope	-0.32	-0.86, 0.58		Behavior Intercept ~ Behavior Slope	-0.16	-0.82, 0.74
	Behavior Intercept ~ Mass Intercept	-0.01	-0.82, 0.8		Behavior Intercept ~ Mass Intercept	0.11	-0.73, 0.84
	Behavior Slope ~ Mass Intercept	-0.05	-0.83, 0.79		Behavior Slope ~ Mass Intercept	0.05	-0.78, 0.83
	Behavior Intercept ~ Mass Slope	-0.26	-0.64, 0.14		Behavior Intercept ~ Mass Slope	-0.09	-0.46, 0.3
	Behavior Slope ~ Mass Slope	0.45	-0.34, 0.91		Behavior Slope ~ Mass Slope	-0.1	-0.79, 0.7
	Mass Intercept ~ Mass Slope	-0.11	-0.84, 0.74		Mass Intercept ~ Mass Slope	-0.35	-0.93, 0.63
Struggle Intensity + Body Mass	**Residual Correlation****	**0.23**	**0.15, 0.31**	Space-use + Body Mass	Residual Correlation	**Model did not converge; Rhats > 1**	
	Behavior Intercept ~ Behavior Slope	-0.37	-0.87, 0.56		Behavior Intercept ~ Behavior Slope		
	Behavior Intercept ~ Mass Intercept	-0.08	-0.85, 0.76		Behavior Intercept ~ Mass Intercept		
	Behavior Slope ~ Mass Intercept	0.17	-0.71, 0.86		Behavior Slope ~ Mass Intercept		
	Behavior Intercept ~ Mass Slope	0.13	-0.59, 0.78		Behavior Intercept ~ Mass Slope		
	Behavior Slope ~ Mass Slope	0.01	-0.28, 0.31		Behavior Slope ~ Mass Slope		
	Mass Intercept ~ Mass Slope	-0.14	-0.86, 0.74		Mass Intercept ~ Mass Slope		
Tonic Intensity + Tarsus Length	**Residual Correlation****	**-0.21**	**-0.28, -0.13**	Reactivity + Tarsus Length	**Residual Correlation****	**0.42**	**0.28, 0.55**
	Behavior Intercept ~ Behavior Slope	-0.21	-0.81, 0.7		Behavior Intercept ~ Behavior Slope	-0.57	-0.84, 0.25
	Behavior Intercept ~ Mass Intercept	0.1	-0.75, 0.84		Behavior Intercept ~ Mass Intercept	-0.37	-0.91, 0.45
	Behavior Slope ~ Mass Intercept	0.11	-0.76, 0.85		Behavior Slope ~ Mass Intercept	-0.04	-0.78, 0.72
	Behavior Intercept ~ Mass Slope	0.12	-0.73, 0.85		Behavior Intercept ~ Mass Slope	0.17	-0.71, 0.87
	Behavior Slope ~ Mass Slope	0.14	-0.74, 0.86		Behavior Slope ~ Mass Slope	-0.31	-0.9, 0.63
	Mass Intercept ~ Mass Slope	-0.06	-0.84, 0.79		Mass Intercept ~ Mass Slope	-0.22	-0.9, 0.73
Struggle Intensity + Tarsus Length	**Residual Correlation****	**-0.18**	**-0.25, -0.11**	Space-use + Tarsus Length	Residual Correlation*	-0.097	-0.23, 0.039
	Behavior Intercept ~ Behavior Slope	-0.32	-0.83, 0.58		Behavior Intercept ~ Behavior Slope	-0.42	-0.77, 0.44
	Behavior Intercept ~ Mass Intercept	0.02	-0.79, 0.81		Behavior Intercept ~ Mass Intercept	0.15	-0.74, 0.86
	Behavior Slope ~ Mass Intercept	0.27	-0.62, 0.88		Behavior Slope ~ Mass Intercept	-0.1	-0.84, 0.75
	Behavior Intercept ~ Mass Slope	-0.01	-0.82, 0.81		Behavior Intercept ~ Mass Slope	0.23	-0.71, 0.89
	Behavior Slope ~ Mass Slope	0.01	-0.8, 0.81		Behavior Slope ~ Mass Slope	-0.1	-0.83, 0.75
	Mass Intercept ~ Mass Slope	-0.1	-0.86, 0.76		Mass Intercept ~ Mass Slope	-0.12	-0.87, 0.77

Table 3.5: Summary of significant results for Chapter 3. The ‘Effect of Size on Behavior’ column describes the significant effects of body mass and tarsus length (left most column) on each behavior (center column) in our behavioral response models while the ‘Effect of Behavior on Future Size’ column describes the significant effects of each behavior (center column) on body mass and tarsus length as measured in a future observation period (right most column). Significant effects are marked with a (+) or (-) to indicate directionality and the interaction with age is described.

	Effect of Size on Behavior		**Effect of Behavior on future Size**	
Body Mass	(+) at older ages	**Tonic Intensity**	No detectable effect	**Body Mass**
	(+) at older ages	**Struggle Intensity**	No detectable effect	
	(-) at older ages	**Reactivity**	(+) at young ages (-) at older ages	
	(+) at older ages	**Space-use**	(-) at young ages (+) at older ages	
Tarsus Length	(-) at young ages (+) at older ages	**Tonic Intensity**	(-) at young ages (+) at older ages	**Tarsus Length**
	(+) at older ages	**Struggle Intensity**	No detectable effect	
	(+) at young ages (-) at older ages	**Reactivity**	(+) at young ages (-) at older ages	
	(+) at older ages	**Space-use**	Non-significant effect	

Chapter 4

Experimental Manipulation of Developmental Environment Influences Behavior, Growth, and their Relationship in Captive-reared Wood Duck (*Aix sponsa*) Ducklings

Introduction

Individual differences in behavior that remain consistent across time and/or context (i.e., animal personality; Sih et al. 2004a&b; Réale et al. 2010a) can have far reaching ecological and evolutionary ramifications. For example, personality appears to be influential in dispersal (Cote et al. 2010), aggression and territory defense/acquisition (Amy et al. 2010), foraging (Bergvall et al. 2010), and reproductive success (Both et al. 2005). Understanding the developmental aspects of personality is essential in addressing how and why consistent individual differences in behavior are maintained within populations (Trillmich and Hudson 2011). This is of particular interest when constraints in behavioral expression can manifest as seemingly suboptimal decision-making within a single context (such as with pre-sexual cannibalism in fishing spiders; Johnson and Sih 2005; Foellmer and Khadka 2013). Furthermore, examining experiential influences on behavioral expression can provide essential insight into the proximate mechanisms that contribute to trait divergence, trait differentiation, and the maintenance of behavioral variation within populations (Stamps and Groothuis 2010a&b).

Although there is a genetic component to personality (van Oers et al. 2005; Dochtermann et al. 2015), environmental factors (as well as [gene]x[environment] interactions) clearly play a major role in shaping developmental trajectories and behavioral phenotype (Stamps and Groothuis 2010a&b). This is evident, for instance, during egg deposition and incubation in birds; maternal effects via yolk hormones (Ruuskanen and Laaksonen 2010) or small differences in incubation temperature (Hope et al. 2018) can have profound impacts on hatchling behavioral phenotype, physiology, and fitness. Post-hatch (or birth) factors—such as structural complexity of the physical environment (Liedtke et al. 2015; Xu et al. 2021), social environment (e.g., absence/presence of conspecifics or quantity/quality of rearing (Bölting and von Engelhardt 2017; Schausberger et al. 2017; Brandl et al. 2019), and predation risk (Edenbrow and Croft 2013)—shape and influence developmental trajectories as young individuals acquire and process information about their environment. Physical access to resources (e.g., food; McGhee and Travis 2011) or exposure to pathogens or parasites and environmental toxins can also impact growth and behavioral development (Klemme and Karvonen 2016; Grunst et al. 2018; Webber and Willis 2020). However, despite increased attention in recent years (Stamps and Groothuis 2010a&b; Cabrera et al. 2021), how personality develops and/or remains consistent across ontogeny and across life-history stages continues to remain poorly understood.

A possible relationship between endogenous state (e.g., physiology, energy reserves, or body size; McElreath and Strimling 2006; Careau et al. 2008; Biro and Stamps 2008; Houston 2010; Andersson and Höglund 2012) and behavior has often been invoked as an explanation for the maintenance of consistent individual differences within populations over time (Réale et al. 2010b; Wolf and Weissing 2010; Dingemanse and Wolf 2010). Growth and size are state-variables of particular importance to developing organisms due to a variety of life-history and fitness consequences (Rice et al. 1993; Mangel and Stamps 2001; reviewed by Dmitriew 2011). For young organisms, growth is nutritionally and energetically demanding. Slow growth can reduce (or be associated with reduced) resource acquisition, competitiveness, and reproductive success later in life (Mangel and Stamps 2001; van de Pol et al. 2006; Naguib et al. 2006; Quesnel et al. 2018) while rapid growth can impact longevity and physiology (e.g., immune function, mature function, and stress response), as well as increase the risk of predation (Mangel and Stamps 2001). The trade-off between growth and mortality (mostly due to predation) might drive behavioral differentiation during development and, therefore, influence the maintenance of consistent individual differences within a population (Stamps 2007). Allocation of resources and attention towards foraging (obviously necessary for growth) often comes at the cost of investment in anti-predator behaviors such as vigilance (Brown 1999; cognitive constraint on attention, Clark and Dukas 2003) and general avoidance of predation risk (e.g., refuge seeking) comes with a foraging opportunity cost (Lima and Dill 1990). As such, individuals that are less sensitive to potential predation risk (often referred to as 'bold', 'active', and/or 'exploratory') might experience higher rates of resource acquisition and growth relative to more risk-sensitive individuals. If proclivity to accept risk is positively correlated with size and/or growth across ontogeny, a positive feedback between growth and risky behaviors within-individuals could provide the mechanism for behavioral (and size) differentiation within a cohort (Sih et al. 2015), particularly when predation risk is high (see Chapter 3).

The presence and prevalence of active potential predators, the behavior of predators, and prey individuals' perceptions of predation risk all potentially influence behavioral expression of prey animals and likely play a role in shaping how individuals behave in the future with limited access to information (Lima and Dill 1990; Stamps and Krishnan 2017; Wirsing et al. 2021). In the wild, predation risk can dramatically vary across a heterogeneous landscape and this 'landscape of fear' can have significant implications for short term decision-making and survival during ontogeny (Laundré et al. 2010; Bleicher 2017; Gaynor et al. 2019) and so can have long lasting effects across life-history stages (Ituarte et al. 2014; Bennett and Murray 2015; Garcia et al. 2017). Predator-prey dynamics are also mediated by availability and distribution of food resources for prey organisms (e.g., optimal patch-use theory; Brown 1992; Hamilton 2010). Such variation at the micro-environmental level might present different individuals with distinguishably distinct developmental experiences across ontogeny thus driving differentiation, and possibly maintenance, of behavioral phenotypes within populations. To our knowledge, few studies have experimentally examined the impact of environment on personality development while longitudinally assessing behavior across ontogeny in a vertebrate system (but see Cabrera et al. 2021).

In the current study we longitudinally assess the impact of developmental environment on growth and behavioral developmental trajectories by experimentally manipulating foraging requirements and perception of predation risk in captive-reared Wood Ducks (WODU; *Aix sponsa*). In previous work, we established a relationship between size/growth and a suite of behaviors that appear to be representative of sensitivity to predation risk in a cohort of captive-reared WODU exposed to a standard developmental environment (Chapters 1-3). We found that these behaviors are consistently repeatable across ontogeny (Chapter 1) and increase in predictability across ontogeny suggesting canalization of behavioral expression as well as some plasticity very early in life (Chapter 2) corresponding to a period of high predation rates in wild WODU populations (Davis et al. 2007; Davis et al. 2009). However, we found no evidence of feedback between growth and behavior within-individuals as a factor driving behavioral and size differentiation in a common garden (Chapter 3). This last finding is not necessarily surprising as differences in experiential factors during ontogeny might be an essential component of behavior-state feedback and among-individual differentiation (Wolf and Weissing 2010; Stamps and Groothuis 2010a&b; Sih et al. 2015).

Given the importance of growth and predation for young organisms (particularly for WODU and other waterfowl in the wild) we conducted a 2x2 factorial experiment to examine the relative impact of increased perception of predation risk and increased requirements for food acquisition during ontogeny by applying a titration of developmental pressure across treatments (see Methods below). With this experimental approach, we address four main hypotheses:

First, we hypothesized that **simulated predation risk and increased foraging effort impact rates of growth across ontogeny**. Whether due to behavioral shifts (e.g., reduced foraging rates and increased refuge use), physiological responses, or a combination of the two, we predicted that our experimental treatment would reduce average growth rates of ducklings. This is commonly observed in numerous systems both in captivity and in the wild (Verdolin 2006). Furthermore, sustained stress response is known to increase metabolic requirements (van der Kooij 2020) which could redirect allocation of resources away from somatic growth and towards enabling escape and avoidance responses throughout development (Eyck et al. 2019).

Second, we hypothesized that **simulated predation risk and increased foraging effort impact overall (predation) risk sensitivity in a variety of standardized behavioral assays.** We predicted that birds exposed to our treatments would display greater sensitivity as a direct response to increased perception of overall risk (either via direct exposure to the predation treatment and/or accommodation of increased required effort to forage). The impact of predation pressure during ontogeny on consistent behavioral expression across contexts has been empirically demonstrated in a number of systems (e.g., Bell and Sih 2007). Additionally, as mentioned above, we established a relationship between average behavioral response and size in a previous cohort of captive-reared WODU (Chapter 3). If our treatment sufficiently impacts growth as described, it should also affect risk sensitivity as measured in our standardized testing contexts.

Last, we hypothesized that **simulated predation risk and increased foraging effort affect the relationship between size and behavior both within- and among-individuals**. If a trade-off between growth and mortality (Stamps 2007) is an influential factor in the relationship between size and risk sensitivity (discussed in Chapter 3 and above), we predicted that imposed pressure would serve to strengthen this relationship. Furthermore, we also predicted that imposed developmental pressure would encourage behavioral differentiation as individuals exposed to greater risk are forced to more directly deal with a growth-mortality trade-off. If behavior-state feedback is a driver of this differentiation (see above and Chapter 3), individuals should show strong within-individual correlations between behavior and size across ontogeny such that we see a 'fanning out' of behavioral and growth trajectories among individuals (as described in Sih et al. 2015).

Methods

Specimen collection, rearing protocol, and behavioral assay protocols for this study were similar to those of Chapters 1-3 (see General Methods above), although there were several smaller improvements and adjustments made to our protocols between seasons. Additionally, implementation of an experimental protocol required further rearing adjustments.

Specimen Collection and Rearing Protocol

In 2017, we collected 469 eggs (265 of which were viable) from four established, long-term field sites in Central Valley, California, and successfully hatched and reared 150 individuals. Specimen collection, incubation, and hatching followed the same protocol established in 2016 (Chapters 1-3). We collected eggs primarily from abandoned nests (a frequent occurrence at our field sites) and eggs that were left behind after nest exodus, but a subset of our sample was sourced from pre-incubated and unincubated clutches as well. After collection, we quickly measured eggs (mass in grams, egg length, and egg width), noted approximate stage of development, and transferred freshly collected eggs to an incubator at our aviary on campus at the University of California, Davis. We set the incubator (Rcom MARU Digital Incubator) to a standard 35.9 degrees Celsius (within the acceptable range for Wood Duck development; DuRant et al. 2010) and kept humidity set to 60%, programming egg trays to rotate every 60 minutes (simulating hen egg rotation in the wild and promoting healthy embryonic development). We also ensured the incubator shut off heat twice every day, allowing incubator temperature to drop to room temperature, for 30 minutes to simulate incubation breaks regularly taken twice per day by wild, incubating hens (*personal observations*). Every other day we temporarily removed eggs during the temperature reduction period to measure egg weight as embryos developed and check developmental status, permanently removing eggs with dead embryos. When eggs were internally pipped (a process by which birds break through the internal membrane of the egg to access an air pocket and take their first breath), we isolated eggs (to match hatchlings back to their egg) and moved them into a hatching unit (Rcom MARU Digital Hatcher) with temperature set to 36.2 degrees Celsius and humidity increased to 70-80% to improve ease of hatch. We followed the hatching process and noted rates of hatch (checking ~2-5 times per day). When individual ducklings hatched, we removed them from the incubator as soon as possible and processed them in hand, conducting their first behavioral assays (see below), collecting blood samples, measuring size (see below), and marking them for identification throughout the study. We used both color leg bands (regularly replacing them with appropriately sized bands as ducklings grew) and Passive Integrative Transponders (PIT tags) which we implanted subcutaneously in the scapular region. As discussed in prior chapters, PIT tag retention rates were high, but secondary identification methods ensured we did not misidentify individuals in the event of PIT tag rejection.

Immediately after hatch and initial processing (see below) we placed birds directly into outdoor enclosures (~3x6m with soil substrate and at least one artificial refuge) rather than using the traditional approach of housing young hatchlings in small, indoor brooding bins. We found that placing hatchlings inside a nest box situated on the ground within their enclosure and allowing them to leave on their own was effective in encouraging natural foraging initiation and increasing survival rates, even if hatchlings were not all introduced at the same time. We provided overhead ceramic heat elements in the corner of enclosures for broods under one week of age. When broods were approximately one week old (after we conducted the first Open Field Test assays; see below), we added a pool to their home enclosures to promote more natural swimming and grooming behavior; we did not provide pools for younger birds to prevent waterlogging and mortality due to drowning or rapid temperature loss (which can be common at very young ages in captive fowl that aren't able to easily waterproof their own feathers without maternal aid; Nye 1964). We linked feeding protocols to experimental treatment as described below.

Rather than feeding birds with an indiscriminate amount of food, as we did in 2016 (providing more food than necessary to every brood), we standardized the quantity of food (Mazuri waterfowl starter) provided to broods based on the estimated energetic demands of WODU ducklings for each stage of development. This allowed us to control for brood size in providing all ducklings access to the same relative amount of food (barring intra-specific, aggressive competition which we did not observe at any point among ducklings). In 2015 we closely monitored the growth of 10 WODU ducklings by collecting morphometrics (body mass in grams and structural size as described below) on a daily basis. This allowed us to calculate, with high resolution, the average rates of growth in grams per day across ontogeny. We then factored these data into a calculation with estimates of energetic requirements for adult North American waterfowl in captivity based on Resting Metabolic Rates (Miller and Eadie 2006; energetic requirement estimates were obtained across multiple species by regressing Resting Metabolic Rates on body mass) to produce an estimate of the necessary grams of food required per gram of growth per duckling per day. We ensured that our estimates were conservative so as to allow each individual the opportunity to have access to more than sufficient food resources. Our intent here was simply to account for variation in number of birds per brood across treatments. We completely replaced all food every other day; most broods did not consume all of the provided food, ensuring they had sufficient nutritional/energetic access.

Experimental Treatment

We implemented a 2x2 factorial experiment to assess the effects of exposure to increased perceived predation risk and increased foraging demands (centralized vs. distributed food) within the developmental environment across ontogeny (Figure 4.1). We assigned broods to a **Control treatment: Centralized food, low predation pressure** (n=39), **Treatment 1: Centralized food, high predation pressure** (n=31), **Treatment 2: Scattered food, low predation pressure** (n=27), and **Treatment 3: Both scattered food and high predation pressure** (n=29) groups in a fashion so as to promote a relative balance in number of individuals among treatment groups;

this was difficult considering the number of hatchlings produced and the lack of hatch synchrony in captivity across the reproductive season. The control groups had access to their standard allotment of food in a centralized location within the enclosure. To increase the amount of foraging effort required in foraging treatment groups (Treatments 2 and 3), we randomly distributed the standardized food allotment among 6 of 12 total foraging trays each filled with soil substrate. Foraging trays with and without food were shuffled around the enclosure every time we provided additional food thus encouraging birds to exert greater foraging effort to acquire the same relative amount of food. We ensured that foraging trays in foraging treatment enclosures were never any closer to refuge hides than the centralized, single foraging location in control groups.

For predator treatment groups, we began simulated predation events for each brood when the youngest bird of that brood was 4 days old (we waited to 4 days to ensure we were not increasing mortality by preventing hatchlings from establishing initial foraging behavior). We used human observers to simulate attempted predation events by chasing ducklings around their enclosure for 45-60 seconds before leaving. Due to the large variety of potential predators of WODU in the wild (particularly ducklings; see above), as well as the generally skittish responses of both wild and long-term captive WODU towards human presence and approach, we considered human observers to constitute a significant predator threat to the developing ducklings. Indeed, our anecdotal observations of highly intense duckling responses over the entire study corroborated this assertion and we had noted significant drops in weight of adult WODU (that had been held in captivity for over a year) in response to repeated capture by observers over just a few days (Figure 4.2). Our chase events were conducted a random number of times per day (from 1-5; determined with a random number generator) and each event was conducted at a randomly assigned time during the day between 0800h and 1700h (again using a random number generator). The random nature of these simulated attempted predation events ensured birds would be less capable of predicting these events. On days when we captured and removed broods from their enclosures for processing, we did not conduct any simulated predation events to reduce excess stress as birds typically spent 3-6 hours outside of their home enclosures on testing days. After the last assays on week 5 (Open Field Tests; see below), we ceased predation treatments.

In-hand Processing, Open Field Tests, and Home Enclosure Monitoring

At hatch and every week through week 5 (the most sensitive post-hatch ontogenetic period for WODU; see Chapters 1-3) we collected birds from either the hatcher or their outdoor enclosure, isolating them in small, opaque tubs to reduce stress and dampen any effects of vocalization or visual access to brood mates during processing. We randomly determined the sequence of measurement to control for potential sequence effects. While we had birds in hand, we recorded standard morphometrics (body mass in grams, tarsus length, culmen length, and culmen width in mm) and conducted two behavioral assays, the Struggle Test and Tonic Immobility (Table 4.1). Tonic Immobility is a behavioral response found in multiple species, possibly associated with anti-predator behavior post-capture (discussed in Chapter 1). The protocol for in-hand measurements followed that established in prior years and discussed in Chapters 1-3. While weighing birds we placed them, upside-down, in an opaque tube on a digital weighing scale. For a period of 30 seconds, we counted the number of times each bird kicked while in their weighing tube. Tube diameters were small enough to prevent birds from righting themselves, but large enough so as not to constrict their breathing. After collection of morphometrics, we then placed the bird with their back down on a standardized surface, gently pressing the bill into the breast while covering the eyes; this procedure induces Tonic Immobility in most Wood Ducks. Once the bird was motionless the observer removed their hand and we recorded the number of attempts (up to 5) it took to induce Tonic Immobility, a subjective score (0-3) of the bird's attentiveness and posture (‘alertness’) while in Tonic Immobility, and the bird's latency to break out of Tonic Immobility (which we considered to be an attempt to right itself) with a cap at 60 seconds.

On weeks 1, 3, and 5 we conducted modified Open Field Tests (OFT) as discussed in Chapters 1-3, with minor alterations (Table 4.1). We recorded all trials with an overhead camera, which also allowed us to remotely monitor birds with a live feed. We allowed the bird 2 minutes to emerge of its own volition, and then, whether the bird emerged or not, we lifted the entire acclimation chamber out of the arena with a pulley-system. The bird was given 5 minutes to move freely about the arena before an observer re-captured it and placed it back in its isolation tub. Behavioral metrics were transcribed from recorded video footage manually. As in 2016, we recorded the intensity of the birds response to the chamber removal (from 0-5 subjective score), activity based on lines crossed over a grid painted on the arena floor, 'anti-thigmotaxis' (proclivity to venture away from the arena walls and explore the center of the arena), avoidance of the acclimation chamber region of the arena, and intensity of response to the observer's approach at the conclusion of the assay (again on a scale from 0-5). In 2017 we did not record Rapid Head Movement (see Chapter 1) due to its high correlation with the responsivity scores which were logistically easier to record.

Statistical Analysis

Variable Reduction for Behavioral Variables

Following the analytical protocols we established in Chapters 1-3, we employed a Bayesian approach to our analysis (with an exception for modeling growth rates; see below), using the *brms* package (Bayesian Regression Models using Stan ver 2.13.0; Bürkner 2017) in R (ver 4.0.2). Again, note that this is a distinct dataset from the dataset used in prior chapters. To reduce our numerous variables to a subset of representative variables we conducted an Eigenvalue Decomposition, first using multi-response Generalized Linear Mixed Models (GLMMs) accounting for repeated measures (with identity as a 'random' effect) to extract the residual correlation coefficients among dyads of measured behaviors within each context (in-hand and OFT). This approach is similar to a traditional Principal Component Analysis but allows us to incorporate repeated measures accounting for potential concerns of pseudoreplication (see Chapter 1). It is also a reductionist approach that does not explicitly incorporate distributions around the correlation coefficient estimates, but exploratory analyses from Chapter 1 suggest this approach provides qualitatively similar insight as modelling each behavior individually, at least with our data for our purposes. We ran models as Gaussian with flat/uninformative priors, four chains, 1000 warmup iterations, and 6000-8000 total iterations. Using the *eigen* function in base R, we produced eigenvalues and eigenvectors from the resulting correlation matrices. For the following analyses, we used the first two components (each of which were near or above an eigenvalue of 1) for each behavioral context. We then centered and standardized each of our raw behavioral variables, multiplying the value for each behavior by its respective eigenvector and summing across behaviors to produce a single metric for each component.

It is important to note that there were some qualitative differences between eigenvector loadings in the 2017 OFT relative to the 2016 OFT (possibly, at least partially, due to the removal of the Rapid Head Movement variable in 2017). Activity, Startle Stimulus Revisitation, and Anti-Thigmotaxis loaded heavily on the first eigen component such that more active birds were more likely to revisit the location of origin for the startle stimulus and more likely to venture away from the walls of the arena. Both response scores and Startle Stimulus Revisitation loaded heavily on the second eigen component such that birds that responded intensely to the startle stimulus were more likely to avoid the location of origin for the startle stimulus and were more likely to respond intensely to an approaching observer and attempted capture. Exact loadings are provided in Table 4.2. Herein we refer to the first eigen component for our OFT as 'Activity & Space-use' and the second eigen component as 'Response Intensity'.

Generating Estimates of Instantaneous Growth Rates

Duckling growth tends to follow a sigmoidal curve across time. We first modelled body mass (a structural measure of size with important implications for locomotion potential) within-individuals across time using a Gompertz 3-point function in JMP (version 16). We then extracted the slopes of the tangents at each point of measurement to provide an estimate of 'instantaneous growth rate' for further analysis. We excluded tarsus length from analyses to simplify results and reduce redundancy (see Chapter 3).

Calculating Repeatability Estimates for Size, Growth, and Behavior Independently

To assess repeatability (Intraclass Correlation Coefficient, ICC), we conducted a series of single-response, varying intercept models. We used Generalized Additive Mixed Models

(GAMM with a spline for the effect of age) for body size and instantaneous growth rate and we used Generalized Linear Mixed Models (GLMM) for our four standardized behavioral variables. The first series included varying intercepts with individual identity and brood as 'random' effects, and age, sex, and treatment as fixed effects. We then calculated ICC (as the within-individual variance over the sum of within-individual variance and residual variance) by hand from model outputs, including calculations for the 95% Credibility Interval. With the next series of models, we also explicitly included a term for Individual by Treatment, allowing us to estimate within-individual variance components for each treatment group. Again, we present ICC estimates calculated by hand from model outputs and include 95% Credibility Intervals.

As in the previous chapters we wanted to account for potential confounding factors in our Open Field Test models, including wind intensity (a subjective score from 1-3), ambient temperature, and duration of time spent in the acclimation chamber (which slightly varied across birds due to logistical limitations). We only intended to include potentially impactful confounds to reduce likelihood of overfitting our models. To determine which of these variables were impactful for both of our OFT eigen components, we conducted a stepwise series of GLMMs and compared models using WAIC methodology with the *loo* package (ver 2.3.0) in R (ver 4.0.2). All three of these confounds were found to be potentially impactful for Response Intensity (eigen component 2) but none of them were found to be important for Activity & Space-use (eigen component 1); as such, we only included confounds as fixed effects for models with Response Intensity as a response variable.

Examining the Effects of Treatment on Size, Behavior, and the Size-Behavior Relationship

To specifically examine the impact of Treatment on average size and instantaneous growth rates, we ran single-response Generalized Additive Mixed Models including varying slopes and intercepts for individuals over time. Using an additive framework, fitting a spline to the effect of age over time, allowed us to account for the non-linear nature of size and growth rates across ontogeny. We also included brood as a 'random' effect and sex as a 'fixed' effect and ran models as Gaussian with weakly informative priors, four chains, 1000 warmup iterations, and 6000-8000 total iterations.

For each standardized behavior, we conducted varying slope and intercept, single-response GLMMs, this time including a three-way interaction term for the effect of body mass on behavior across time by treatment. We included identity and brood as 'random' effects, sex as a 'fixed' effect, and for the Response Intensity (OFT eigen component 2) model we included outside temperature, wind intensity, and acclimation duration. These models allowed us to assess the average effect of treatment on behavior directly, as well as the average effect of treatment on change in behavior over time, and the relationship between behavior and body mass over time. Again, we ran models as Gaussian with weakly informative priors, four chains, 1000 warmup iterations, and 6000-8000 total iterations.

To examine within-individual correlations among slopes and intercepts for size and behavior (essential for determining the presence of behavior-state feedback; see Chapter 3 and Sih et al. 2015), we conducted a series of multi-response models including both body mass and behavioral variables as responses in the same model. Due to the computational demand associated with running a single multi-response model including body mass and all behavioral variables (while explicitly modeling correlations among within-individual slopes and intercepts for each response), we ran multiple models separately for each behavior-state dyad and we were unable to efficiently run models specifying terms for variance components by treatment (likely due to small sample sizes). We included individual identity and brood as 'random' effects, with identity varying over time within-individuals. We also included sex and an interaction between age and treatment for each model. Again, for Response Intensity models we included temperature, wind intensity, and acclimation duration.

We ran these models as Gaussian with weakly informative priors, four chains, 2000 warmup iterations, and 8000 total iterations. Model specifications are summarized in Table 4.3.

Results

Repeatability of Behavior and Body Mass Across Ontogeny

To confirm consistent individual differences in behavior and size across ontogeny, we quantified repeatability using ICC estimates from our Bayesian GLMMs and GAMMs. We also compared repeatability across treatment groups to determine if recurring developmental exposure to potential predation pressure and/or increased foraging requirements impacted the consistency of individual differences.

Body mass was highly repeatable across ontogeny (Table 4.4). The repeatability of body mass within Treatment 2 (no predation pressure and scattered food) was higher than that of the Control group (no predation pressure and centralized food) based on non-overlapping credibility intervals (Figure 4.3). Though the other two treatment groups also seemed to have somewhat higher body mass repeatabilities, their credibility intervals overlapped that of the control group.

Both Tonic Intensity (in-hand eigen component 1) and Struggle Intensity (in-hand eigen component 2) were repeatable across ontogeny (Table 4.4). While Response Intensity (Open Field eigen component 2) was also repeatable, the Activity & Space-use metric (Open Field eigen component 1) was much less so (Table 4.4). We found only limited evidence for an effect of treatment on repeatability (subjective comparison using 95% Credibility Intervals; Figure 4.4). The repeatability of Struggle Intensity within Treatment 3 (predation pressure and scattered food) and Treatment 2 (no predation pressure and scattered food) appeared to be higher than that of Treatment 1 (predation pressure and centralized food) and the Control group (no predation pressure and centralized food). This pattern was similar for Tonic Intensity, but much less defined (i.e., greater overlap of credibility intervals across groups). For Activity & Space-use, Treatment 2 (no predation pressure and scattered food) was the only treatment group repeatability with a credibility interval that did not overlap zero. (Figure 4.4). The repeatability of Response Intensity within Treatment 3 (predation pressure and scattered food) was higher than that of the other three treatment groups (Figure 4.4).

Predation Treatments Reduce Body Mass and Growth Rates Across Ontogeny

We used Generalized Additive Mixed Models (GAMM) with body mass as the response variable to assess the average effect of each treatment on size and growth rates as ducklings aged. Both Treatment 1 (predation pressure and centralized food) and Treatment 3 (predation pressure and scattered food) significantly reduced body mass of ducklings across ontogeny (Table 4.5 and Figure 4.5). Body mass of Treatment 2 (no predation pressure and scattered food) ducklings, however, did not significantly differ from that of the Control group (Table 4.5 and Figure 4.5). The interaction estimates for Age and Treatment were not significant; this might suggest that Treatment did not have an effect on shape or "smoothness" of the growth curves. When body mass was modeled linearly, the interaction between Age and Treatment was significant; Treatments 1 and 3 (both predation treatments) had significantly lower growth rates (i.e., slope across time). Our GAMM with an estimate for instantaneous body mass growth rate as the response variable corroborated these findings; again, Treatments 1 and 3 showed significantly lower growth rates across ontogeny (Table 4.5 and Figure 4.6).

The Effect of Treatment on Behavior and the Relationship Between Behavior and Body Mass

Results for the relationships among behavior, body mass, and age (while controlling for a treatment effect) were qualitatively similar to those from Chapter 3 with a common garden data set (but see the Methods above for a discussion of differences in the Open Field Test between years; Tables 1.3 and 4.2), though the interaction between body mass and age was only close to significant for Struggle Intensity and our Activity & Space-use metric (Table 4.6). To test our hypotheses, we examined a 3-way interaction among body mass, age, and treatment for each behavioral variable. With these models we were able to estimate the effect of treatment on behavior directly, on the relationship between size and behavior, and on the relationship between size and behavior across development.

While Treatment 1 (predation pressure and centralized food) and Treatment 2 (no predation pressure and scattered food) did not have a significant effect on in-hand behaviors, Treatment 3 (predation pressure and scattered food) had a significantly negative impact on Tonic Intensity and Struggle Intensity (Table 4.6). Treatment 3 (predation pressure and scattered food) also significantly influenced the relationship between body mass and Struggle Intensity, but the effect of Treatment 3 was only close to significant for the relationship between body mass and Tonic Intensity (Table 4.6). While body mass alone was not significant for either in-hand behavioral metric, it was significantly positively correlated with Struggle Intensity and nearly significantly associated with Tonic Intensity among birds exposed to Treatment 3 (predation pressure and scattered food) across development (Table 4.6). Treatment did not have an effect on the relationship between age and in-hand behaviors or the relationship between body mass and in-hand behaviors across development (Table 4.6). Within-individuals, the intercept for Tonic Intensity was significantly negatively correlated with its slope; individuals that started with lower Tonic Intensity scores had larger rates of change in Tonic Intensity across development (Table 4.6).

The Activity & Space-use metric was not significantly impacted by any of our treatments, and treatment had no significant effect on the relationships among the Activity & Space-use metric, body mass, and age (Table 4.6). Similarly, treatment did not significantly affect Response Intensity directly or the relationship between body mass and Response Intensity (Table 4.6). Treatments 1 and 3 (both predation treatments) did, however, significantly reduce the slope of change in Response Intensity across development and the effect of Treatment 2 (no predation pressure and scattered food) on the change in Response Intensity with age was close to significant (Table 4.6 and Figure 4.7). Treatments 1 and 3 (both predation treatments) also significantly affected the relationship between body mass and Response Intensity across development (Table 4.6 and Figure 4.7). Again, the effect of Treatment 2 on the relationship between body mass and Response Intensity across development was close to, but not, significant (Table 4.6). We found that the two predation treatments led to no change in average Response Intensity across development and appeared to break down the relationship between body mass and Response Intensity (Figure 4.7). Finally, we also found a significant and negative correlation between the intercept and slope of Activity & Space-use within-individuals (Table 4.6).

Evidence for Within-individual Feedback Between Behavior and Body Mass when Individuals are Exposed to Differential Environments

We conducted a number of pair-wise multi-response models between behavioral variables and body mass to assess the potential for within-individual feedback between body mass and behavior across development (see Methods and Chapter 3). Residual correlations (while accounting for all modeled 'random' and 'fixed' effects including sex, age, treatment, brood, and individual) were significantly positive for Tonic Intensity and Struggle Intensity (Table 4.7), corroborating the significant (near significant in the case of Tonic Intensity) and positive effect of the interaction between body mass and age in our single-response models above and our results from Chapter 3. Residual correlations between body mass and behavior were close to, but not, significant for both Open Field behavioral metrics (Table 4.7).

We did not find any significant correlations among intercepts and slopes within-individuals for body mass and Tonic Intensity, body mass and Struggle Intensity, or body mass and Response Intensity. There were, however, significant correlations among intercepts and slopes within-individuals for our Activity & Space-use metric (Table 4.7; Figure 4.8). In this model we found a negative correlation between intercepts and slopes of the Activity & Space-use metric, a positive correlation between Activity & Space-use intercepts and body mass slopes, a negative correlation between Activity & Space-use slopes and body mass slopes, and a negative correlation between body mass intercepts and slopes (Table 4.7). Note that there was also a significant and negative correlation between body mass intercepts and slopes within-individuals in the Response Intensity model, but not the in-hand models (Table 4.7). We provide both a conceptual graphical representation and a data-driven graphical representation of these relationships in Figures 4.8 and 4.9. We provide a summary of significant results for Chapter 4 in Table 4.8.

Discussion

In this study we experimentally tested the influence of developmental environment on consistent behavioral expression, somatic growth, and their relationship across ontogeny. Because young WODU ducklings require immense nutrient/energy intake for growth and face extreme rates of predation in the wild, we focused our experiment on foraging effort (by manipulating distribution of food within enclosures) and perception of predation risk (by consistently simulating predator chase encounters throughout development) using a 2x2 factorial design. **<u>Our results partly supported our first hypothesis</u>**; both predation treatments significantly reduced body mass and growth rates of ducklings throughout their developmental period, while foraging treatment alone had no detectable effect (Figures 4.5 and 4.6). The foraging treatment however, appeared to increase the repeatability of body mass relative to the control group (Figure 4.3).

<u>We found limited evidence in support of our second and third hypotheses</u>. Increased foraging requirement seemed to broadly increase repeatability of in-hand behavioral metrics, while only the foraging treatment group (with no predation pressure) and the combined treatment group exhibited detectable levels of repeatability of Activity & Space-use and Response Intensity respectively (Figure 4.4). Predation pressure appeared to be a more important factor than foraging effort for the relationship between duckling body mass and Response Intensity across development (although treatments did not appear to have a direct effect on average expression of Open Field Test behavior). The combined treatment was required to influence average expression of in-hand behaviors and their relationship with body mass, which may support our *a* *priori* predictions if high in-hand ‘docility’ conveys a capture-escape advantage (see prior Chapters). The inclusion of variable developmental environments among individuals in our study population also seemed to lead to negative feedback between the Activity & Space-use metric and body mass across development within-individuals.

Predation Treatments Reduce Duckling Size and Growth Rates Across Development

Experimentally increasing predation pressure throughout ontogeny had a definitive, negative impact on duckling growth and size (Figures 4.5 and 4.6). There are multiple reasons that this treatment could have constrained growth. First, the lack of predictability and consistency of exposure to artificial predation events could have generally discouraged active and potentially ‘risky’ foraging behavior. In a wild context, foraging is inherently risky because it requires a substantial allocation of attention and energy (which is limited; Dukas 2004) away from vigilance and anti-predator behaviors. Additionally, food is not always accessible within the confines of refuge away from predators. Prey animals are often required to venture into highly exposed and open habitat to acquire essential resources and predators often select habitat with higher prey activity (Brown 1992; Brown 1999). Even when food was centrally located and easily accessible in this study, foraging ducklings were forced to spend time out in the open and away from refuge (see methods; Figure 4.1), likely leading to association of foraging in all treatments with potential predation risk. Thus, elevated levels of predation risk might have reduced average foraging effort among ducklings as they chose to either remain in refuge (at least during treatment hours) or allocate greater energy and effort towards vigilance and anti-predator behaviors. We were unable to directly quantify foraging behaviors (see below), and so we cannot directly confirm this effect of treatment on food intake other than through the reduction in body mass and growth rates.

Second, predation treatment might have influenced growth rates and size through impacts on physiology. Long term exposure to chronically induced stress has been shown to result in a reduction in body mass in several species (Le Maho et al. 1992). Short term responses to a predation event, in conjunction with increased epinephrine/norepinephrine and glucocorticoid production, results in rapid glucose mobilization as well as a suite of other energetically intensive physiological processes (Clinchy et al. 2013, Viblanc et al. 2018). Long term stress response often increases protein loss, disrupts various cellular processes, suppresses immune function, and suppresses growth both directly and indirectly (Siegel 1980). Direct assessment of the physiological response to predation pressure and its connection with consistent individual behaviors falls outside the scope of the current study. Interest in a connection between endocrinological processes and animal personality has grown in the past decade (e.g., Coppens et al. 2010; Baugh et al. 2013; Cockrem 2013), but few studies have examined this connection in a developmental context. Future research would be valuable to incorporate a deeper examination of stress-related hormonal response (possibly in adrenal response as well as glucocorticoid production) and how this connects to consistent individual differences in behavior as well as responses to predation risk, specifically across ontogeny.

Finally, simulated predation events involved a human observer actively chasing treatment ducklings for 45-60 seconds. These repeated chase events appeared to have a significant physical demand, thus potentially forcing ducklings to expend stored energy leading to reduced capacity for growth. We find this explanation to be unlikely, however; simulated predation events were short (45-60 seconds in duration for each event), only occurred 1-5 times per day (randomly determined each day), did not occur before 0800 hours or after 1700 hours each day (16 hours of the day were free of simulated predation events), and all ducklings had access to food *ad libitum*.

Regardless of the mechanism by which it occurs, significant reductions in growth rates can have fitness consequences both throughout development and later in life (Mangel and Stamps 2001). Somewhat surprisingly, however, we found that our foraging effort treatment alone had no detectable effect on growth and size (Figures 4.5 and 4.6). We were unable to impose stricter food limitation due to animal welfare and sample size concerns associated with our study system (see Methods). We attempted to experimentally increase foraging demands by randomly distributing food and presenting it in substrate, but it is possible that this was not enough of an increased pressure to result in detectable differences in size when birds had no exaggerated predation pressure. As such, we suggest that future research in other systems focus more directly on controlled limitation of food and its impact on development of behavior, growth, and their interaction.

Limited Evidence of Treatment Impact on Behavior and its Relationship with Size Across Development

Repeatability for in-hand behaviors was higher in this study than in our prior, common-garden study (Chapter 1; Figure 1.4 and Table 4.4). This might be due to an apparent increase in repeatability driven by both foraging treatments (though there was some overlap of credibility intervals among treatment groups; Figure 4.4). Note that the predation treatment for both in-hand behavioral metrics did not appear to differ from that of the control group. Additionally, the foraging treatment group (with no added predation pressure) was the only group with detectable Activity & Space-use repeatability. Why the foraging treatments might have increased the degree to which individual differences in response to handling, as well as activity and willingness to spend time in riskier portions of the OFT arena, remained consistent throughout the study is difficult to interpret. Perhaps something about the lack of predictability in the amount of effort required to forage (regardless of perception of predation risk) encouraged maintenance of behavioral differences that already existed. Meanwhile, the repeatability of Response Intensity within the combined treatment group appeared to be substantially higher than that of all other treatment groups (Figure 4.4), suggesting that the sustained pressure of both foraging and predation treatments encouraged maintenance of differences in response to startling stimuli within the Open Field Test.

Though certain treatments did seem to influence average behavioral expression and/or the relationship between behavior and body mass among individuals, their effects were not consistent across behaviors and behavioral testing contexts (Table 4.6). Only the added pressure of both an increase in required foraging effort and increased perception of predation risk appeared to be enough to illicit a significant response for in-hand behaviors. The combined treatment reduced both Tonic Intensity and Struggle Intensity on average while also increasing the correlation between body mass and Struggle Intensity (there was a similar trend for Tonic Intensity, but this effect was not significant). In prior Chapters (Ch1-3) we discuss the possibility of in-hand behaviors representing viable tactics for escaping predators post-capture, and with this consideration we can make biological sense of our findings. Low Tonic and Struggle Intensities could be representative of a "play dead" tactic in which the prey animal lulls the predator into a false sense of security, waiting for a lapse in the predator's attention to provide a window for escape. If ducklings perceive extremely high levels of predation risk (and therefore high likelihood of capture) and playing dead tends to be a more successful tactic than directly fighting the predator's grasp, this might explain why we saw a shift towards lower Tonic and Struggle intensity scores among our most intensive treatment individuals. Additionally, predator treatment also resulted in reduced growth rates and sizes on average, and in Chapter 3 (a common garden) we established a positive correlation between duckling size and Tonic/Struggle Intensity (at least among older ducklings). As such, the reduction in size might serve as the mechanism behind the effect of treatment on in-hand behaviors. We discussed above the potential that predator treatment resulted in an increased expenditure of energy which might have constrained ducklings from allocating additional energy to growth and potentially towards more energetically intensive behaviors (like increased Tonic and Struggle Intensity). Still, it is worth reiterating, that both the predator and the combined treatments had a similar effect on size and growth rates while only the combined treatment resulted in detectably significant differences for in-hand behaviors, so differences in body mass alone is not a sufficient explanation for these results.

Surprisingly, we found no effect of any treatment on our Activity & Space-use metric. Activity rates have been associated with size and predation pressure in a number of other systems (e.g., Biro and Stamps 2008). In Chapter 3 we found that Activity (in association with response intensity and rapid head movement) was negatively correlated with size, but variable reduction methods in our prior cohort separated scores of Activity into different metrics which were associated with size in opposing directions; in other words, there appeared to be individuals that displayed calm, exploratory activity and individuals that displayed more neurotic and frantic activity. Open Field Test variables in the current study did not load in exactly the same way as they did in our previous study (Tables 1.3 and 4.2), but Space-use behaviors in Chapter 3 were positively correlated with size. With this in mind, the lack of significant results for Activity & Space-use is difficult to interpret. It is possible that this result is an artifact of modeling or observer error (e.g., lack of sufficient sampling size), but an explanation might be found in the within-individual correlations we describe below (potential negative feedback between size and this particular behavioral metric).

In contrast, we did find that both predator and combined treatments influenced change in Response Intensity over time as well as change in the relationship between size and Response Intensity across development. In both cases, treatments appeared to break-down correlations that existed in the Control group (i.e., increase in Response Intensity with Age and decrease in correlation between size and Response Intensity with age; Figure 4.7). Treatment birds, on average, remained consistent in their Response Intensity throughout the study, while control birds (exposed to the least environmental pressure) varied in Response Intensity as they grew. In other words, when sufficient environmental pressure was experienced, both size and age did not influence whether or not birds responded intensely to the Open Field Test stimuli. This does make biological sense; when individuals are exposed to high predation risk, it might make sense to settle on a particular behavioral response and maintain that response as long as the pressure persists. Whether these behavioral differences are maintained later in life (into adulthood) and whether these differences can have fitness consequences in a wild context are important avenues for future research.

Evidence of Negative Feedback Between Activity & Space-use and Size Within-individuals

In contrast to our prior work with a common garden data-set (see Chapter 3) and all other behavioral metrics in this study, we found significant within-individual correlations among Activity & Space-use and body mass slopes and intercepts (Table 4.7). Though not all correlation estimates were significant, the direction of those that were provides some evidence of negative feedback between our Activity & Space-use metric and body mass across development within-individuals (following Sih et al. 2015). We present a conceptual graphic representing an interpretation of this finding in Figure 4.9. Individuals that started with lower scores in Activity & Space-use displayed a greater rate of change in this behavior across development. Individuals that started with lower body mass had greater rates of growth, providing some evidence for either compensatory growth on the part of those that began with a lower relative body mass or constraint/restraint on individuals that began with a higher body mass. There is extensive literature on compensatory growth in vertebrates (Hornick et al. 2000), but this often happens in a context of heavy restrictions to food intake during critical periods of growth; all of our birds did have access to food *ad libitum* and our food distribution treatment alone did not have any detectable influence on size and growth rates (see above).

Individuals that started with a higher Activity & Space-use score showed less positive change in this behavior across development and grew more rapidly than those that started with a lower score. Individuals that had a lower or negative change in Activity & Space-use across development also had a faster rate of growth. It makes sense that more active individuals in the OFT (who were also more willing to venture away from refuge in the constrained assay context) likely spent more effort foraging in their home enclosures. Possibly these individuals accepted greater risk to catch up to their larger counterparts in growth. These results are consistent with a dynamic of asset-protection within our study population whereby individuals with access to sufficient energy were less likely to take risks when predation pressure was high as they had 'more to lose', while individuals with lower energy reserves had 'more to gain' and displayed more risky behaviors (Clark 1994; McElreath et al. 2007; Sih et al. 2015).

The relationship between acceptance of risk and body mass within-individuals appears to have resulted in convergence of this behavior rather than differentiation, contrary to our initial expectation. This convergence might, at least in part, explain the low repeatability estimates for the Activity & Space-use metric as well as the lack of detectable effect of treatment on average Activity & Space-use in this study. However, partitioning variance by treatment revealed little difference in repeatability of this behavioral metric across treatment groups (the one exception being higher repeatability in the food treatment; Figure 4.4). Unfortunately, model fitting issues (due to a lack of sufficient sample sizes) prevented us from exploring the specific impact of treatment on within-individual intercept and slope correlations. As such, it is difficult to draw conclusions with regard to the effect that differential exposure to environmental pressure across ontogeny might have had on within-individual covariance between behavior and growth.

When considering our results from Chapter 3 (no evidence of within-individual correlations at all) it seems likely that the inclusion of exposure to varying developmental environments across individuals resulted in the emergence of these patterns for this behavioral metric. That said, Open Field Test behaviors loaded differently with our variable reduction method for this study than they did with our previous data set (Chapters 1-3), so comparison should be taken with a grain of salt. Additionally, we found that estimates for within-individual correlations between body mass intercepts and body mass slopes had a highly skewed posterior distribution and these effects were only significant in our OFT models (when we only had measurements on week 1, 3, and 5). Highly skewed posterior distributions in these Bayesian models suggest that there is a bit more uncertainty in the mean effect (that posterior chains did not mix particularly well). As such, this result should be interpreted with care. Still, the other significant correlations, including correlation between growth rates and behavioral intercepts/slopes (Table 4.7), showed more healthy posterior chains and more normal distributions.

These correlations provide evidence of negative feedback only if Activity & Space-use is indicative of, or related to, foraging activity and/or efficiency and is directly tied to body mass and growth. We cannot confirm this because we were unable to accurately collect individual-level foraging data in duckling home enclosures, and individuals did not reliably initiate foraging behavior in the Open Field Test even when presented with food (likely due to isolation from their brood and the stress of prior handling). We attempted to quantify latency to initiate foraging behavior upon release back into the home enclosure on testing days, but this did not allow us to gather individual-level data since WODU ducklings are heavily influenced by the actions of brood mates. We strongly suggest future research that aims to address feedback between growth and behavior focus on connecting these standardized behavioral assays to direct, individual level foraging behaviors (or quantify more direct aspects of foraging in a standardized fashion).

Concluding Remarks

We found that the developmental environment played an important a role in both the average expression of behavior and aspects of consistent individual differences in behavior; namely, by influencing repeatability and possibly inducing negative feedback between body mass and our Activity & Space-use metric. Our experimental treatments, nonetheless, did not universally affect all measured behaviors and acted on different aspects of behavior in different ways. While statistically controlling for predation risk and foraging treatments, we did still detect a relationship between average duckling size and behavior across development that mirrored our findings from Chapter 3 (in both our single-response and residual correlations from our multi-response models; Tables 4.6 and 4.7), providing strong evidence of reproducibility of our findings across two separate cohorts of Wood Duck juveniles. The definitive and negative effect of predation treatments on size and growth throughout the study therefore suggest that the influence of treatment on behavior that we did detect was at least partially mediated by impacts of treatment on growth.

To our knowledge, this study (paired with Chapter 3) represents one of the first empirical tests of the theoretical models introduced by Sih et al. (2015) regarding the potential for behavior-state feedback in driving either differentiation or convergence of behavioral types. We provide limited evidence of convergence across developmental time within-individuals while the population is exposed to differential pressures (i.e., simulated predation risk and increased foraging requirements) in a captive environment. Of course, there still remain gaps in our understanding of the underlying processes that drive the maintenance of individual differences in behavior. Future research that continues to examine the potential for behavior-state feedback within-individuals as a developmental driver of behavioral differentiation would be valuable.

Finally, it is important to reemphasize that Wood Ducks are social animals. Wood Ducks hatch into relatively large broods tended by a brooding mother, only fledging after roughly 4-8 weeks (Bellrose and Holm 1994). The behavioral type of the brood mother could influence duckling behavioral expression and development (as is the case in Japanese Quail; Pittet et al. 2014). Moreover, high rates of predation in the first few weeks post-hatch ensure a rapidly changing social environment for those ducklings lucky enough to survive, possibly having implications for consistent expression of behavior (i.e., removal of ‘keystone’ individuals; Modlmeier et al. 2014). Although we found no effect of brood on behavior or duckling size, we were only able to include 17 different broods in this study, limiting our ability to examine differences among broods. Furthermore, our behavioral assays involved isolated individuals. While this allowed us to control for the influence of other birds on focal individual behavior, the influence of brood mates can be critical in the wild and we observed strong brood attraction and response to brood mates when ducklings were not being tested. We suggest future study of these questions to more directly examine the influence of the brood mother and brood-mates on individual duckling behavioral expression both acutely and developmentally.

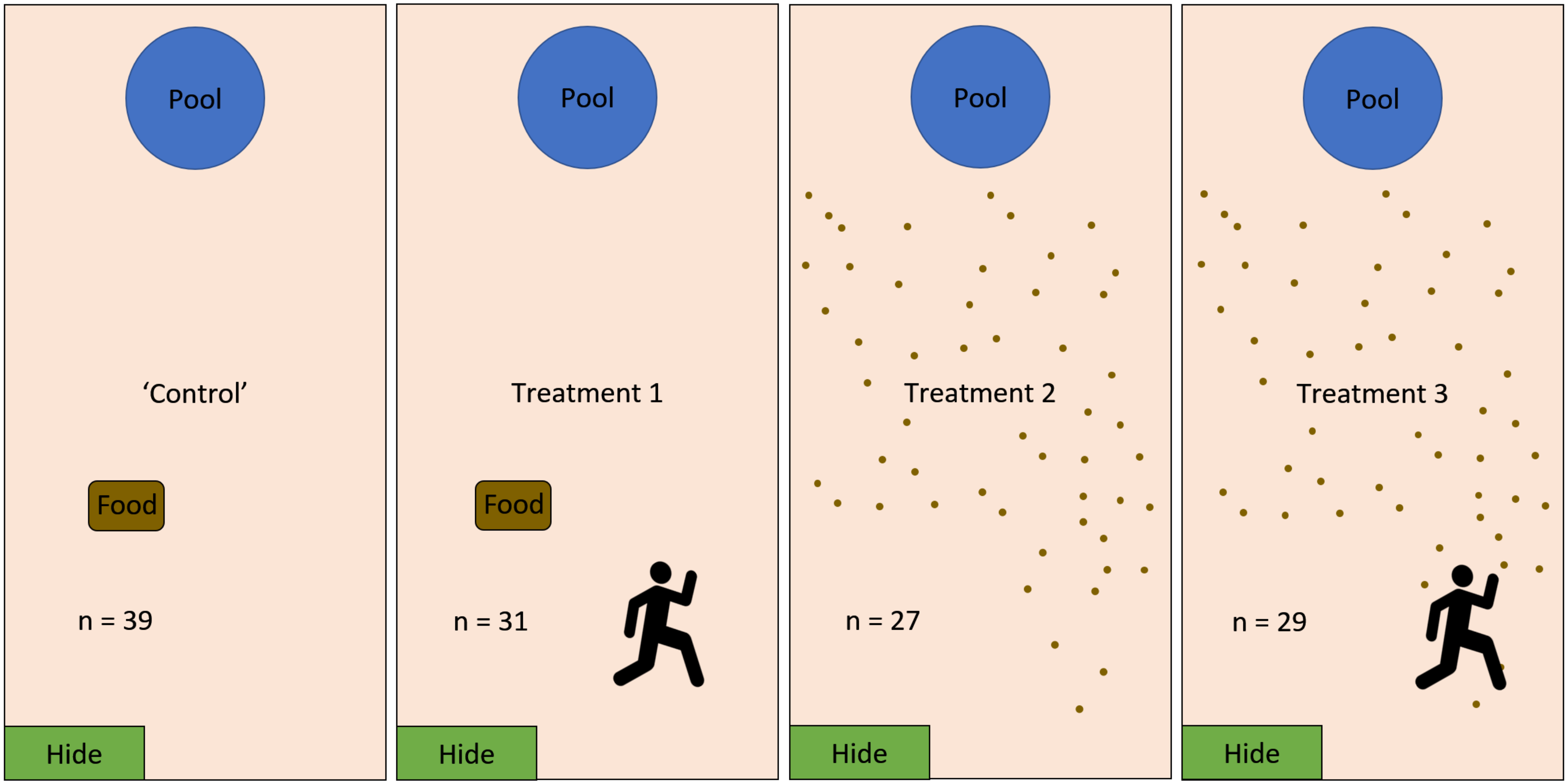
Table 4.1: Overview of behavioral variables measured as well as how they are associated with eigen and principal component variables (see Statistical Methods) and which behavioral assay they are associated with. Note that variables loaded differently in 2017 (Chapter 4) than in 2016 (Chapters 1-3) for the Open Field Test.

Assay Context	**Component Variable**	**Behavioral Variable**	**Description**
In-hand	**Tonic Intensity**	Tonic Attempts	Number of observer attempts to induce Tonic Immobility
		Tonic Alert	Score for the degree that the bird is alert while in Tonic Immobility
		Tonic Immobility	Latency to break out of Tonic Immobility
	Struggle Intensity	Tube Struggle	Number of kicks produced while constrained in an opaque weighing tube
Open Field Test	**Activity and Space-use**	Activity	Number of gridlines crossed during the 5-minute testing period
		Anti-Thigmotaxis	Proportion of observation intervals in which the focal duckling ventured away from the walls of the arena
	Response Intensity	Startle Stimulus Revisitation	Proportion of observation intervals in which the focal duckling was near the location of the startle stimulus
		Startle Response	Subjective score for intensity of response to lifting of the acclimation chamber
		Response to Observer	Subjective score for intensity of response to approach of observer at the conclusion of the assay

Table 4.2: Eigenvector loadings for each eigen decomposition (in-hand and Open Field Test analyses conducted separately). Eigenvector loadings (the values depicted under each component row) show how much each variable influenced our eigen components; we considered loadings to be important (i.e., heavily influence the eigen component) if their absolute value exceeded 0.3. Note that loadings were different for Open Field Test variables in 2017 than our prior analysis from Chapter 1; here, activity and both space-use metrics loaded heavily in the first component while the Response Intensity scores and Startle Stimulus Revisitation loaded heavily on the second.

In Hand Measures: Behaviors ~ (1|ID)			**OFT Measures: Behaviors ~ (1|ID)**		
	Component 1	**Component 2**		**Component 1**	**Component 2**
Tonic Alert	***0.597***	-0.0329	**Startle Stimulus Revisitation**	***0.468***	***-0.501***
Tonic Attempts	***0.49***	-0.1443	**Anti-Thigmotaxis**	***0.63***	-0.142
Tonic Immobility	***-0.624***	0.039	**Activity**	***0.573***	0.229
Struggle Test	0.116	***0.988***	**Startle Response**	0.188	***0.722***
			Response to Observer	0.146	***0.394***

Figure 4.1: We conducted a 2x2 factorial experiment to examine the effect of elevated predation risk (via human encounters) and increased foraging requirements (via scattered and randomized distribution of food) both independently and congruently. We also show here a graphical representation of the home enclosures in which our ducklings were housed for the duration of the study. Enclosures were roughly 3x6 meters with a hide on one end and a small pool at the other (see Methods for more details).



Centralized Food,
No Predation Pressure

Centralized Food,
High Predation Pressure

Scattered Food,
Low Predation risk

Scattered Food,
High Predation risk

Figure 4.2: Body mass measurements (in grams) of 12 captive-reared adult Wood Ducks (roughly one year of age) across one month. Birds were collected and measured four times (indicated by green stars). We observed notable declines in body mass measurements after 1-2 repeated observation periods. Body mass apparently recovered within two weeks.

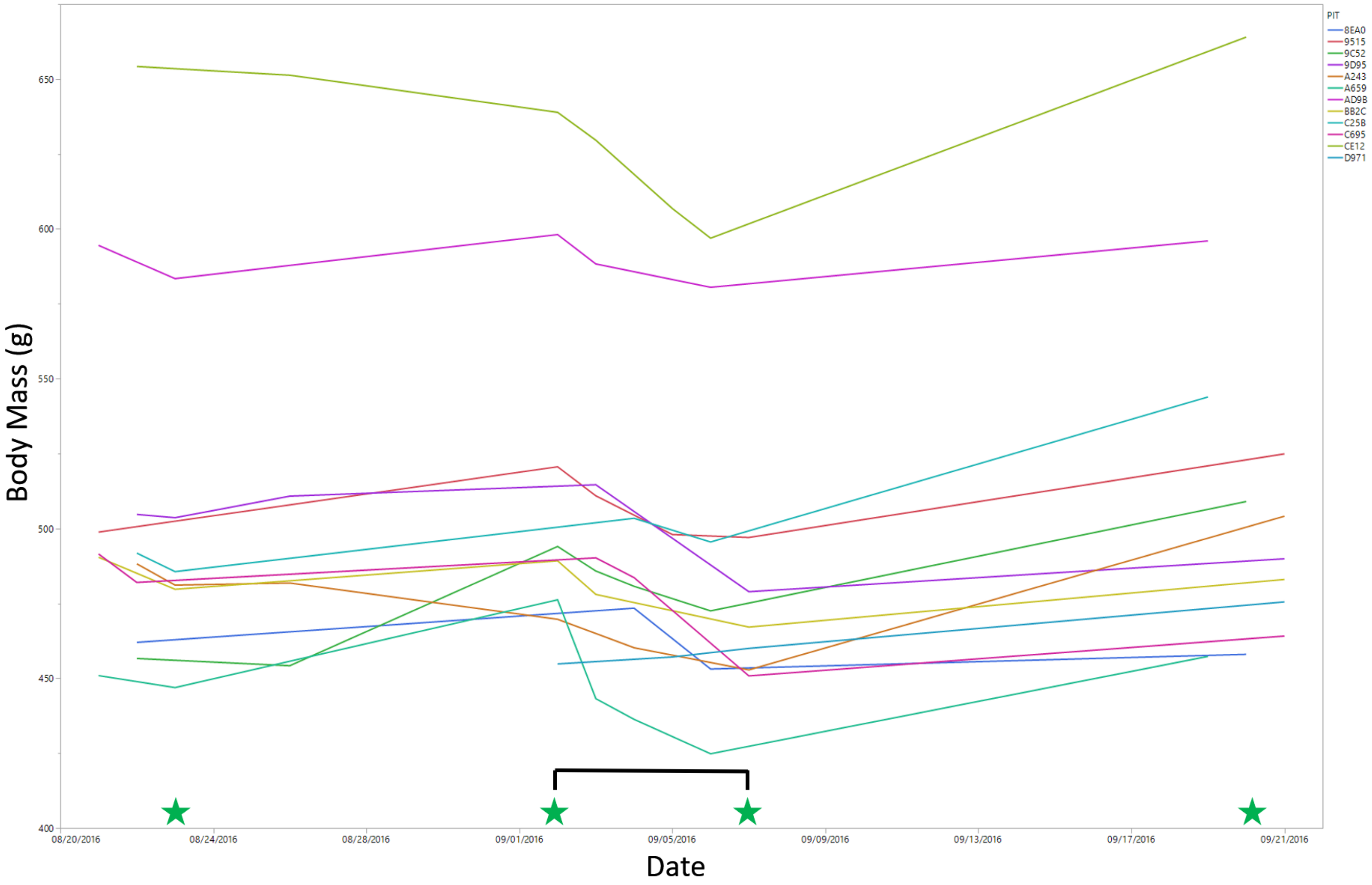
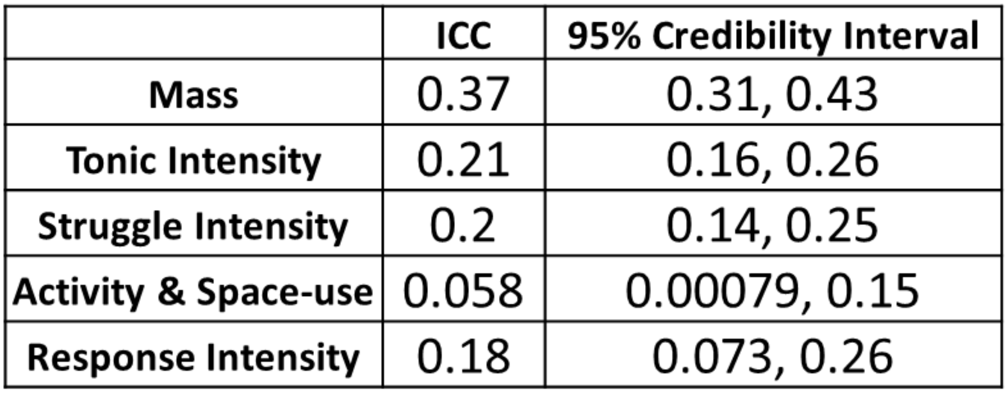


Table 4.3: Model specifications (simplified version of code used in the *brms* package for R) and brief description of the models' intended purpose.

Model specification	**Purpose**
Behavior ~ (1|Individual by Treatment) + (1|Brood) + Age + Treatment + Sex + Confounds [for OFT]	Random intercepts model with a grouped variance term to assess differences in repeatability among Treatment groups
Size ~ (1|Individual by Treatment) + (1|Brood) + s(Age) + Treatment + Sex	Random intercepts Generalized Additive Mixed Model with a grouped variance term to estimate repeatability among Treatment groups - s(Age) indicates a curvilinear specification of the effect of Age (modeled spline)
Size/Growth Rate ~ (1+Age|Individual) + (1|Brood) + s(Age)*Treatment + Sex	Random slopes and intercepts GAMM to estimate effect of Treatment on size and growth rate
Behavior ~ (1+Age|Individual) + (1|Brood) + Size*Age*Treatment + Sex + Confounds [for OFT]	Random intercepts and slopes model with a 3-way interaction to assess effect of Treatment on Behavior directly, on the relationship between Behavior and Size, and on the relationship between Behavior and size across development
Size + Behavior ~ (1+Age|Individual + (1|Brood) + Age*Treatment + Sex + Confounds [for OFT]	Multi-response random slopes and intercepts model to estimate within-individual correlations among slopes and intercepts – examine evidence of feedback between size and behavior

Table 4.4: Intraclass Correlation Coefficient estimates from our varying intercepts models. For this analysis we calculated ICC by hand (within-individual variance over the sum of within-individual variance and residual variance) and determined 95% Credibility Intervals from the posterior distributions of each variance component using the same calculation.



	ICC	**95% Credibility Interval**
Mass	0.37	0.31, 0.43
Tonic Intensity	0.21	0.16, 0.26
Struggle Intensity	0.2	0.14, 0.25
Activity & Space-use	0.058	0.00079, 0.15
Response Intensity	0.18	0.073, 0.26

Figure 4.3: Bar graph showing the qualitative differences in Intraclass Correlation Coefficient (y-axis) by Treatment for body mass across ontogeny. In a varying intercepts Generalized Linear Mixed Model, we included a term for Individual by Treatment, explicitly modeling variance components by Treatment. Again, we calculated ICC by hand (within-individual variance over the sum of within-individual variance and residual variance), this time for each Treatment group, and determined 95% Credibility Intervals from the posterior distributions of each variance component using the same calculation.

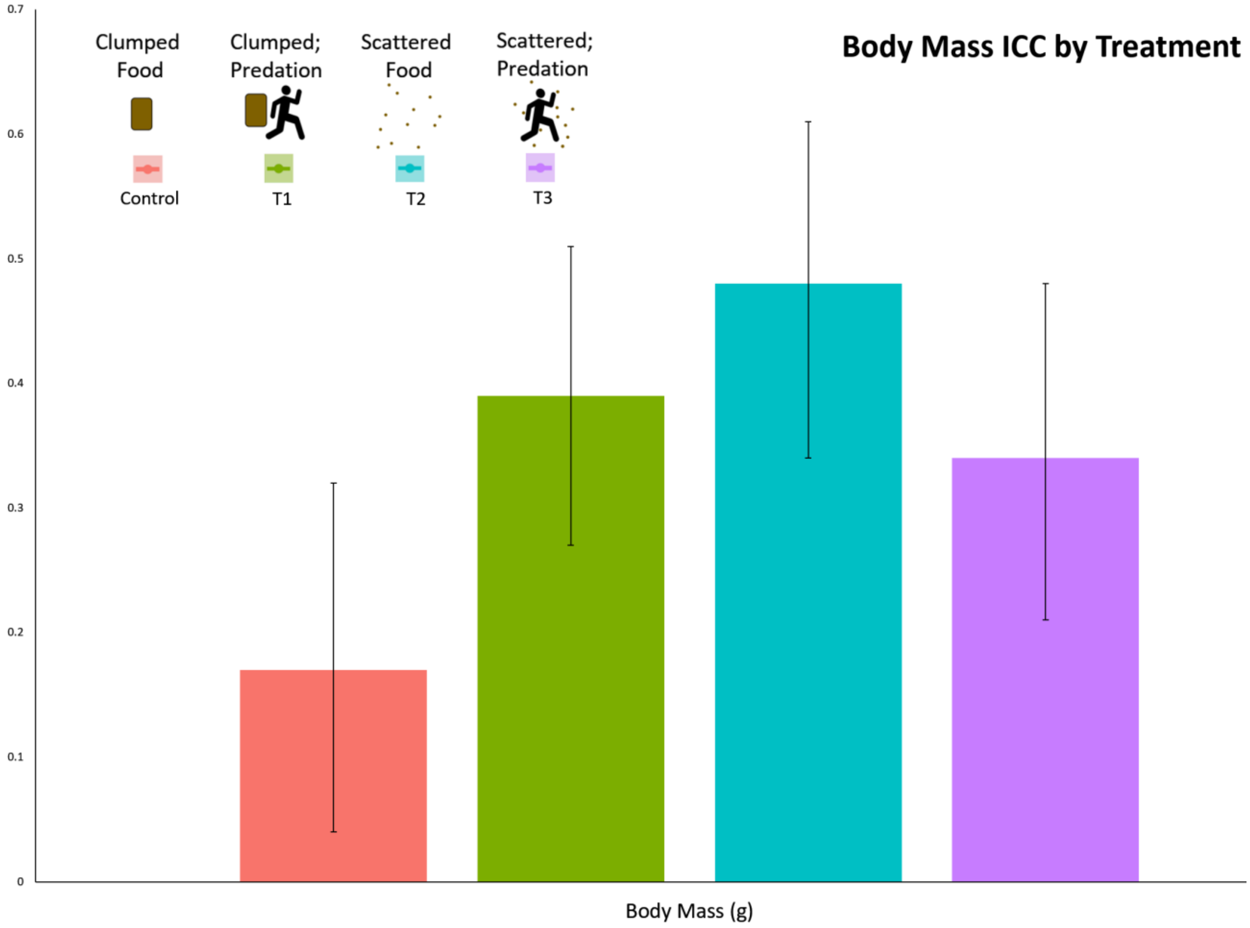


Figure 4.4: Bar graph showing the qualitative differences in Intraclass Correlation Coefficient by Treatment for each behavioral metric across ontogeny. Using varying intercepts Generalized Linear Mixed Models, we included a term for Individual by Treatment, explicitly modeling variance components by Treatment. Again, we calculated ICC by hand (within-individual variance over the sum of within-individual variance and residual variance), this time for each Treatment group, and determined 95% Credibility Intervals from the posterior distributions of each variance component using the same calculation.

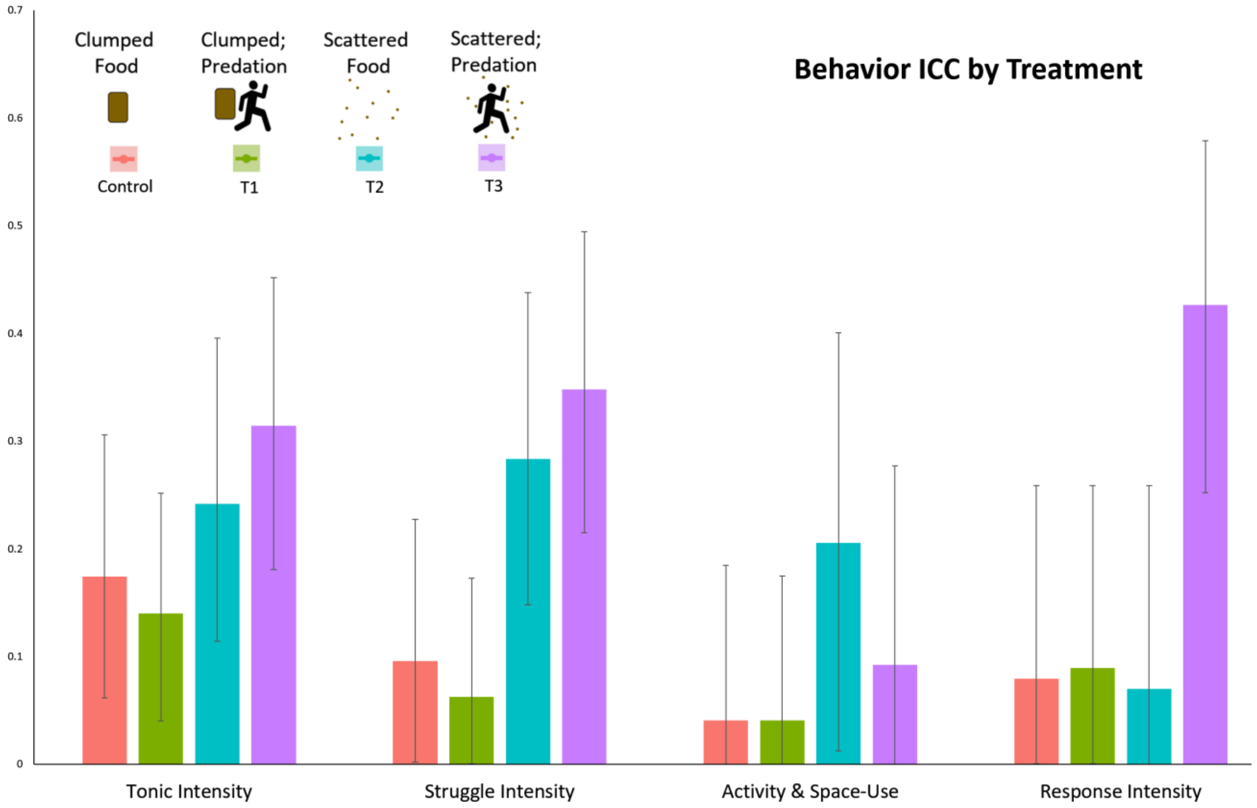


Table 4.5: Summary of important model estimates and their 95% Credibility Intervals for our body mass and growth rate Generalized Additive Mixed Models run using *brms* in R. Treatment effect estimates are shown in relation to the Control group (i.e., significant Treatment effects were significantly different from the Control). Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero).

Response	Effect	Estimate	95% C.I.
Body Mass	Within-individuals: Intercept ~ Slope	-0.65	-0.99, 0.41
	Sex**	**-2.47**	**-4.76, -0.18**
	Treatment 1 (Predation)**	**-21.13**	**-30.06, -11.89**
	Treatment 2 (Food)	3.62	-6.14, 13.37
	Treatment 3 (Predation+Food)**	**-20.37**	**-29.95, -10.51**
Instantaneous Growth Rate (Body Mass)	**Within-individuals: Intercept ~ Slope****	**-0.64**	**-0.78, -0.45**
	Sex*	0.29	-0.049, 0.63
	Treatment 1 (Predation)**	**-0.97**	**-1.64, -0.28**
	Treatment 2 (Food)	0.46	-0.25, 1.2
	Treatment 3 (Predation+Food)**	**-1.01**	**-1.73, -0.27**

Figure 4.5: Plot of differences in body mass among treatment groups across development. We derived figures from our Generalized Additive Mixed Model output. As in other figures, significance is indicated by the duckling symbol as shown in the figure key.

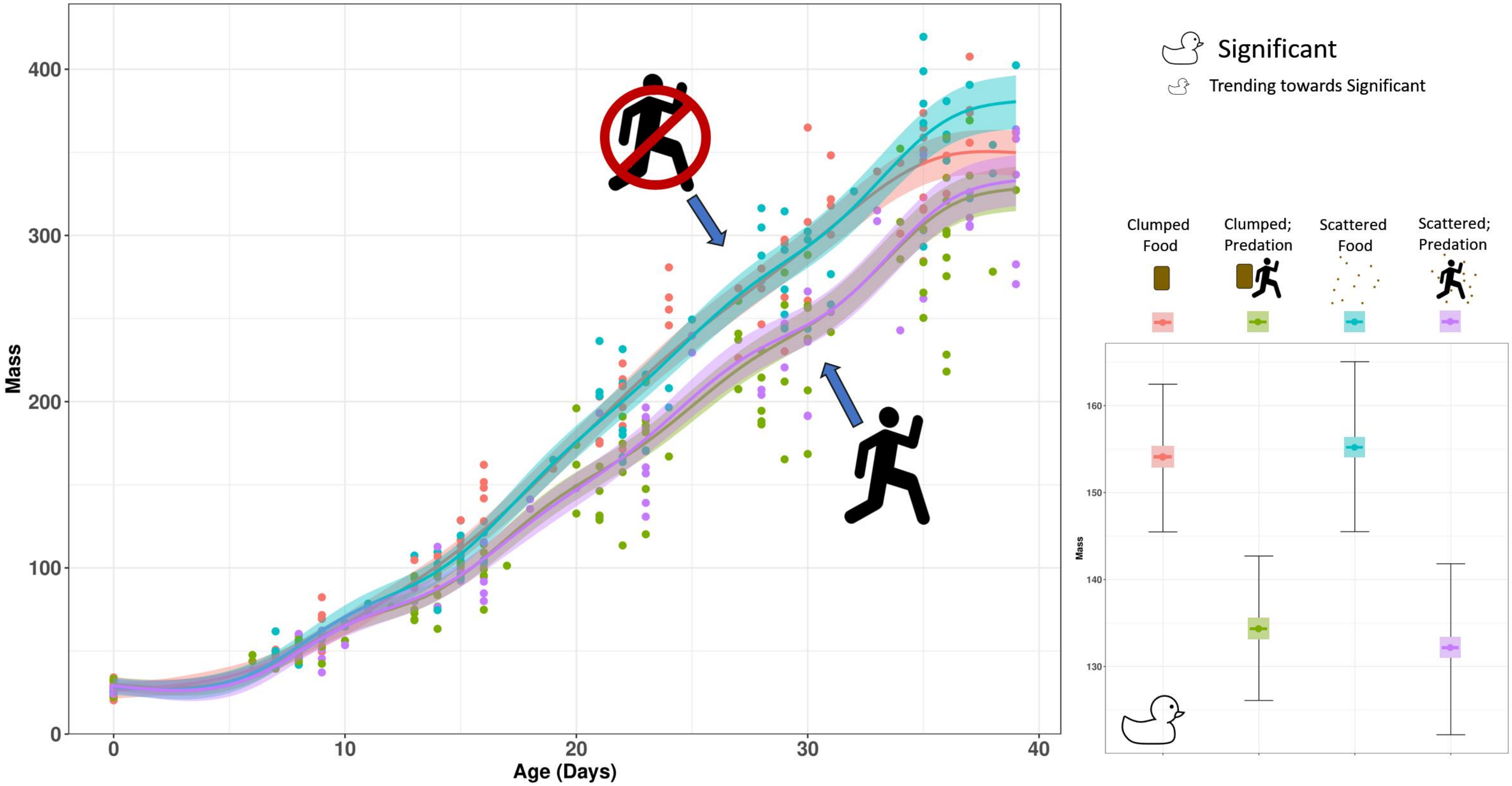


Figure 4.6: Plot of differences in instantaneous rate of body mass growth among treatment groups across development (i.e., the derivative of the change in body mass over time; see Methods). We drew figures from our Generalized Additive Mixed Model output. As in other figures, significance is indicated by the duckling symbol as shown in the figure key.

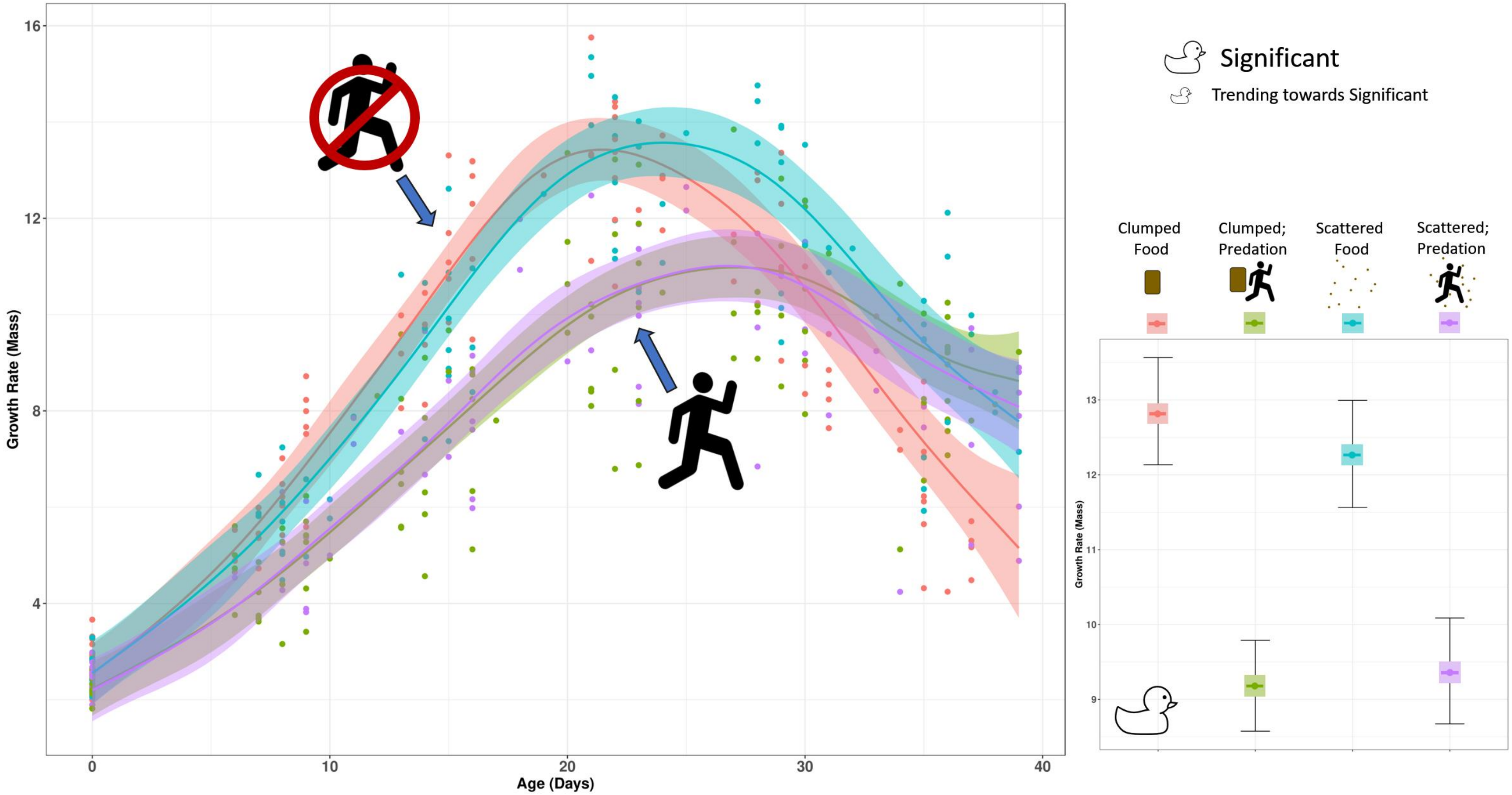
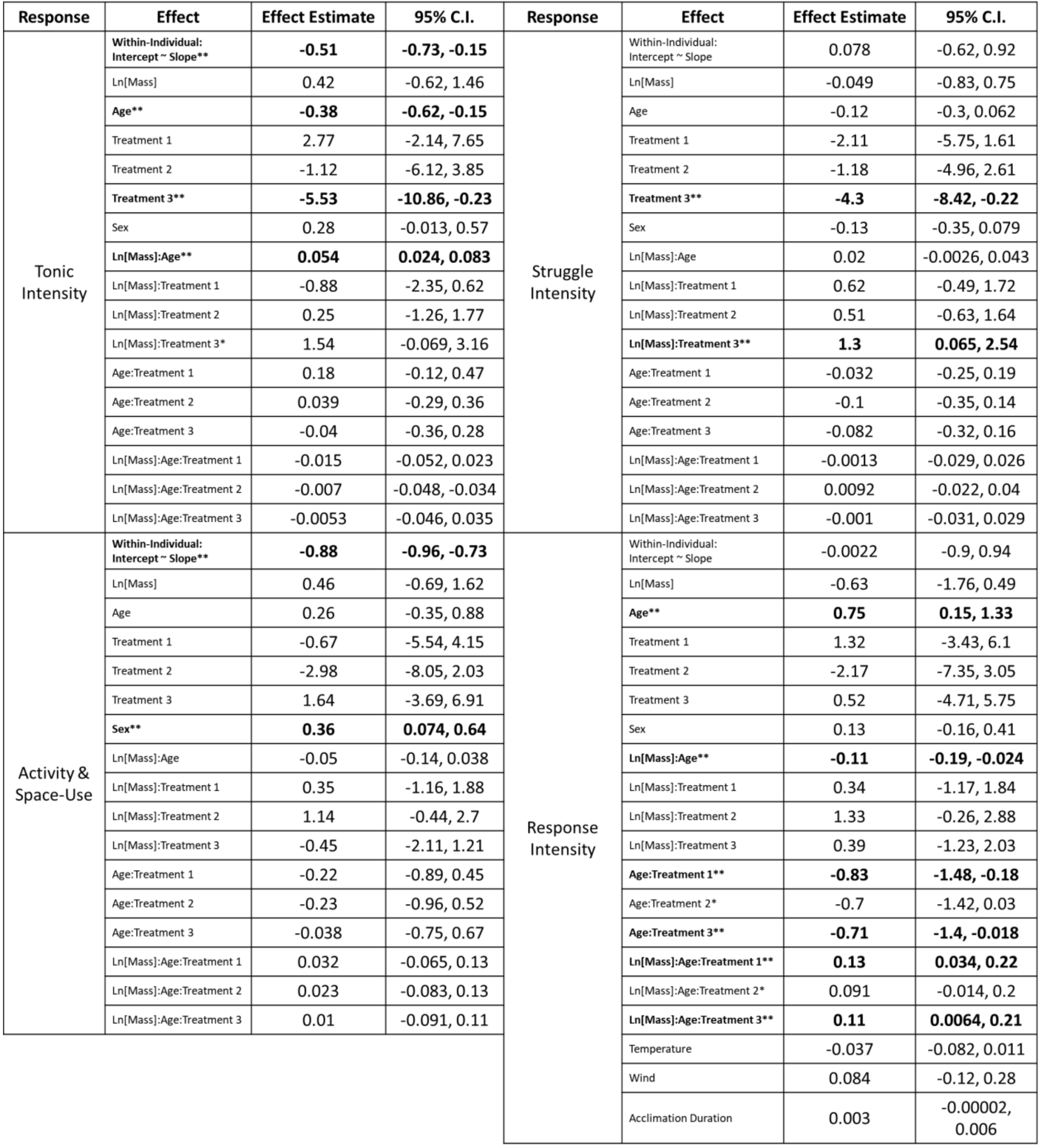


Table 4.6: Summary of important model estimates and their 95% Credibility Intervals for our behavior response Generalized Linear Mixed Models run using *brms* in R. We show here results from individual models for Tonic Intensity, Struggle Intensity, Activity & Space-use, and Response Intensity. These models included a three-way interaction term between body mass, age, and treatment. Treatment effect estimates are shown in relation to the Control group (i.e., significant Treatment effects were significantly different from the Control). Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero).



Response	Effect	Effect Estimate	95% C.I.	Response	Effect	Effect Estimate	95% C.I.
Tonic Intensity	**Within-Individual: Intercept ~ Slope****	**-0.51**	**-0.73, -0.15**	Struggle Intensity	Within-Individual: Intercept ~ Slope	0.078	-0.62, 0.92
	Ln[Mass]	0.42	-0.62, 1.46		Ln[Mass]	-0.049	-0.83, 0.75
	Age**	**-0.38**	**-0.62, -0.15**		Age	-0.12	-0.3, 0.062
	Treatment 1	2.77	-2.14, 7.65		Treatment 1	-2.11	-5.75, 1.61
	Treatment 2	-1.12	-6.12, 3.85		Treatment 2	-1.18	-4.96, 2.61
	Treatment 3**	**-5.53**	**-10.86, -0.23**		**Treatment 3****	**-4.3**	**-8.42, -0.22**
	Sex	0.28	-0.013, 0.57		Sex	-0.13	-0.35, 0.079
	Ln[Mass]:Age**	**0.054**	**0.024, 0.083**		Ln[Mass]:Age	0.02	-0.0026, 0.043
	Ln[Mass]:Treatment 1	-0.88	-2.35, 0.62		Ln[Mass]:Treatment 1	0.62	-0.49, 1.72
	Ln[Mass]:Treatment 2	0.25	-1.26, 1.77		Ln[Mass]:Treatment 2	0.51	-0.63, 1.64
	Ln[Mass]:Treatment 3*	1.54	-0.069, 3.16		**Ln[Mass]:Treatment 3****	**1.3**	**0.065, 2.54**
	Age:Treatment 1	0.18	-0.12, 0.47		Age:Treatment 1	-0.032	-0.25, 0.19
	Age:Treatment 2	0.039	-0.29, 0.36		Age:Treatment 2	-0.1	-0.35, 0.14
	Age:Treatment 3	-0.04	-0.36, 0.28		Age:Treatment 3	-0.082	-0.32, 0.16
	Ln[Mass]:Age:Treatment 1	-0.015	-0.052, 0.023		Ln[Mass]:Age:Treatment 1	-0.0013	-0.029, 0.026
	Ln[Mass]:Age:Treatment 2	-0.007	-0.048, -0.034		Ln[Mass]:Age:Treatment 2	0.0092	-0.022, 0.04
	Ln[Mass]:Age:Treatment 3	-0.0053	-0.046, 0.035		Ln[Mass]:Age:Treatment 3	-0.001	-0.031, 0.029
Activity & Space-Use	**Within-Individual: Intercept ~ Slope****	**-0.88**	**-0.96, -0.73**	Response Intensity	Within-Individual: Intercept ~ Slope	-0.0022	-0.9, 0.94
	Ln[Mass]	0.46	-0.69, 1.62		Ln[Mass]	-0.63	-1.76, 0.49
	Age	0.26	-0.35, 0.88		**Age****	**0.75**	**0.15, 1.33**
	Treatment 1	-0.67	-5.54, 4.15		Treatment 1	1.32	-3.43, 6.1
	Treatment 2	-2.98	-8.05, 2.03		Treatment 2	-2.17	-7.35, 3.05
	Treatment 3	1.64	-3.69, 6.91		Treatment 3	0.52	-4.71, 5.75
	Sex**	**0.36**	**0.074, 0.64**		Sex	0.13	-0.16, 0.41
	Ln[Mass]:Age	-0.05	-0.14, 0.038		**Ln[Mass]:Age****	**-0.11**	**-0.19, -0.024**
	Ln[Mass]:Treatment 1	0.35	-1.16, 1.88		Ln[Mass]:Treatment 1	0.34	-1.17, 1.84
	Ln[Mass]:Treatment 2	1.14	-0.44, 2.7		Ln[Mass]:Treatment 2	1.33	-0.26, 2.88
	Ln[Mass]:Treatment 3	-0.45	-2.11, 1.21		Ln[Mass]:Treatment 3	0.39	-1.23, 2.03
	Age:Treatment 1	-0.22	-0.89, 0.45		**Age:Treatment 1****	**-0.83**	**-1.48, -0.18**
	Age:Treatment 2	-0.23	-0.96, 0.52		Age:Treatment 2*	-0.7	-1.42, 0.03
	Age:Treatment 3	-0.038	-0.75, 0.67		**Age:Treatment 3****	**-0.71**	**-1.4, -0.018**
	Ln[Mass]:Age:Treatment 1	0.032	-0.065, 0.13		**Ln[Mass]:Age:Treatment 1****	**0.13**	**0.034, 0.22**
	Ln[Mass]:Age:Treatment 2	0.023	-0.083, 0.13		Ln[Mass]:Age:Treatment 2*	0.091	-0.014, 0.2
	Ln[Mass]:Age:Treatment 3	0.01	-0.091, 0.11		**Ln[Mass]:Age:Treatment 3****	**0.11**	**0.0064, 0.21**
					Temperature	-0.037	-0.082, 0.011
					Wind	0.084	-0.12, 0.28
					Acclimation Duration	0.003	-0.00002, 0.006

Figure 4.7: Plots showing the effect of Treatment on the relationship between body mass and Response Intensity across development. We show linear estimates of the effect of body mass on behavior when age is held constant at 7 days (left column, blue shading), 21 days (center column, green shading), and 35 days (right column, pink shading) for Control (top-most row), Treatment 1 (top-middle row; clumped food and predation treatment), Treatment 2 (bottom-middle row; scattered food but no additional predation treatment), and Treatment 3 (bottom-most row; both scattered food and predation treatment). Shaded regions denote the 95% Credibility Interval for each effect estimate when age is held constant at a given point. Significance of the interaction term is indicated by the duckling symbol as shown in the figure key and treatment effect estimates are shown in relation to the Control group (i.e., significant Treatment effects were significantly different from the Control). Note that the range of sizes changes at each time point as ducklings grow.

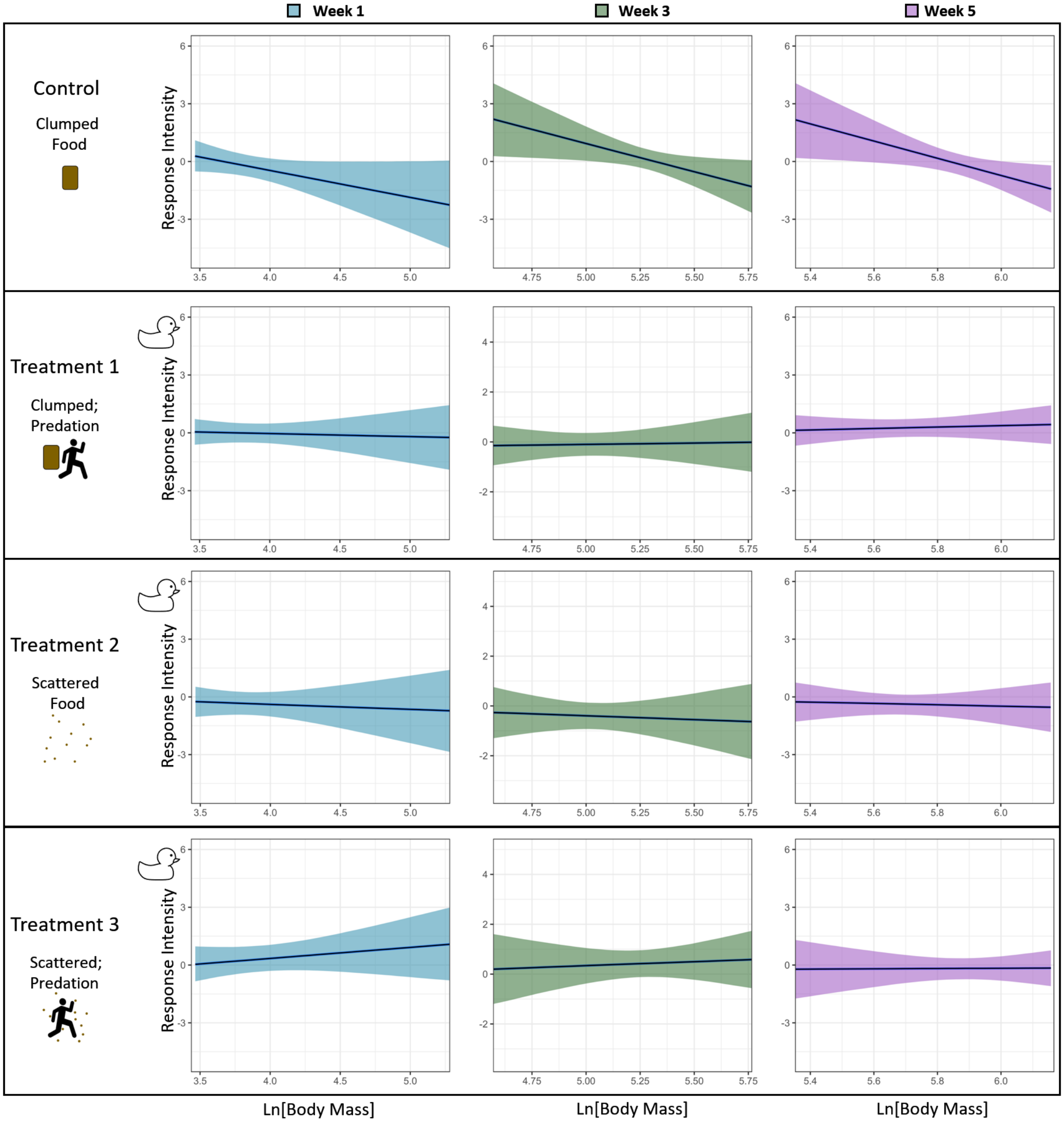


Table 4.7: Summary of important model estimates and their 95% Credibility Intervals for our multi-response Generalized Linear Mixed Models run using *brms* in R. We show here results from dyadic models with behavioral metrics and body mass as the response variables. To reduce model fitting issues, we restricted each model to two response variables, specifically examining within-individual correlations among intercepts and slopes as well as the residual correlation (average correlation while accounting for other model parameters) between the two response variables. Effect estimates are considered significant if the Credibility Interval does not overlap zero; effect significance is denoted by double asterisks and bold font. Effect estimates that are labeled with a single asterisk are considered close to significant (i.e., Credibility Intervals do overlap zero but a bulk of the posterior distribution lies on either side of zero). We found significant correlations between intercepts and slopes across for o Activity & Space-use model indicating negative feedback between this behavioral metric and body mass within-individuals across development.

Responses	Term	Estimate	95% C.I.
Tonic Intensity + Body Mass	**Residual Correlation****	**0.25**	**0.17, 0.32**
	Behavior Intercept ~ Behavior Slope	-0.42	-0.69, 0.021
	Behavior Intercept ~ Mass Intercept	-0.12	-0.85, 0.74
	Behavior Slope ~ Mass Intercept	0.12	-0.74, 0.85
	Behavior Intercept ~ Mass Slope*	0.29	-0.0013, 0.56
	Behavior Slope ~ Mass Slope	-0.13	-0.43, 0.17
	Mass Intercept ~ Mass Slope	-0.2	-0.88, 0.72
Struggle Intensity + Body Mass	**Residual Correlation****	**0.12**	**0.045, 0.2**
	Behavior Intercept ~ Behavior Slope	0.063	-0.53, 0.76
	Behavior Intercept ~ Mass Intercept	0.0067	-0.79, 0.81
	Behavior Slope ~ Mass Intercept	-0.068	-0.84, 0.78
	Behavior Intercept ~ Mass Slope	-0.12	-0.47, 0.23
	Behavior Slope ~ Mass Slope	0.33	-0.17, 0.77
	Mass Intercept ~ Mass Slope	-0.17	-0.88, 0.72
Activity/Space-use + Body Mass	Residual Correlation*	-0.1	-0.24, 0.036
	Behavior Intercept ~ Behavior Slope**	**-0.83**	**-0.95, -0.43**
	Behavior Intercept ~ Mass Intercept	-0.26	-0.84, 0.47
	Behavior Slope ~ Mass Intercept	0.1	-0.59, 0.76
	Behavior Intercept ~ Mass Slope**	**0.46**	**0.16, 0.8**
	Behavior Slope ~ Mass Slope**	**-0.4**	**-0.75, -0.068**
	Mass Intercept ~ Mass Slope**	**-0.7**	**-0.96, -0.023**
Response Intensity + Body Mass	Residual Correlation*	-0.11	-0.23, 0.024
	Behavior Intercept ~ Behavior Slope	-0.032	-0.77, 0.78
	Behavior Intercept ~ Mass Intercept	0.05	-0.66, 0.74
	Behavior Slope ~ Mass Intercept	-0.11	-0.82, 0.69
	Behavior Intercept ~ Mass Slope	-0.093	-0.61, 0.38
	Behavior Slope ~ Mass Slope	0.19	-0.57, 0.83
	Mass Intercept ~ Mass Slope**	**-0.73**	**-0.97, -0.1**

Figure 4.8: Spaghetti plots depicting the variety of individual trajectories in body mass (top) and our Activity & Space-use behavioral metric (bottom). Plots were generated using outputs from our multi-response GLMM with body mass and Activity & Space-use as the response variables. Each colored line represents a single individual. The bold central line represents the average while the light gray shaded region outlines the 95% Credibility Interval. Output was derived from our multi-response model examining within-individual correlations among intercepts and slopes for behavior and body mass.

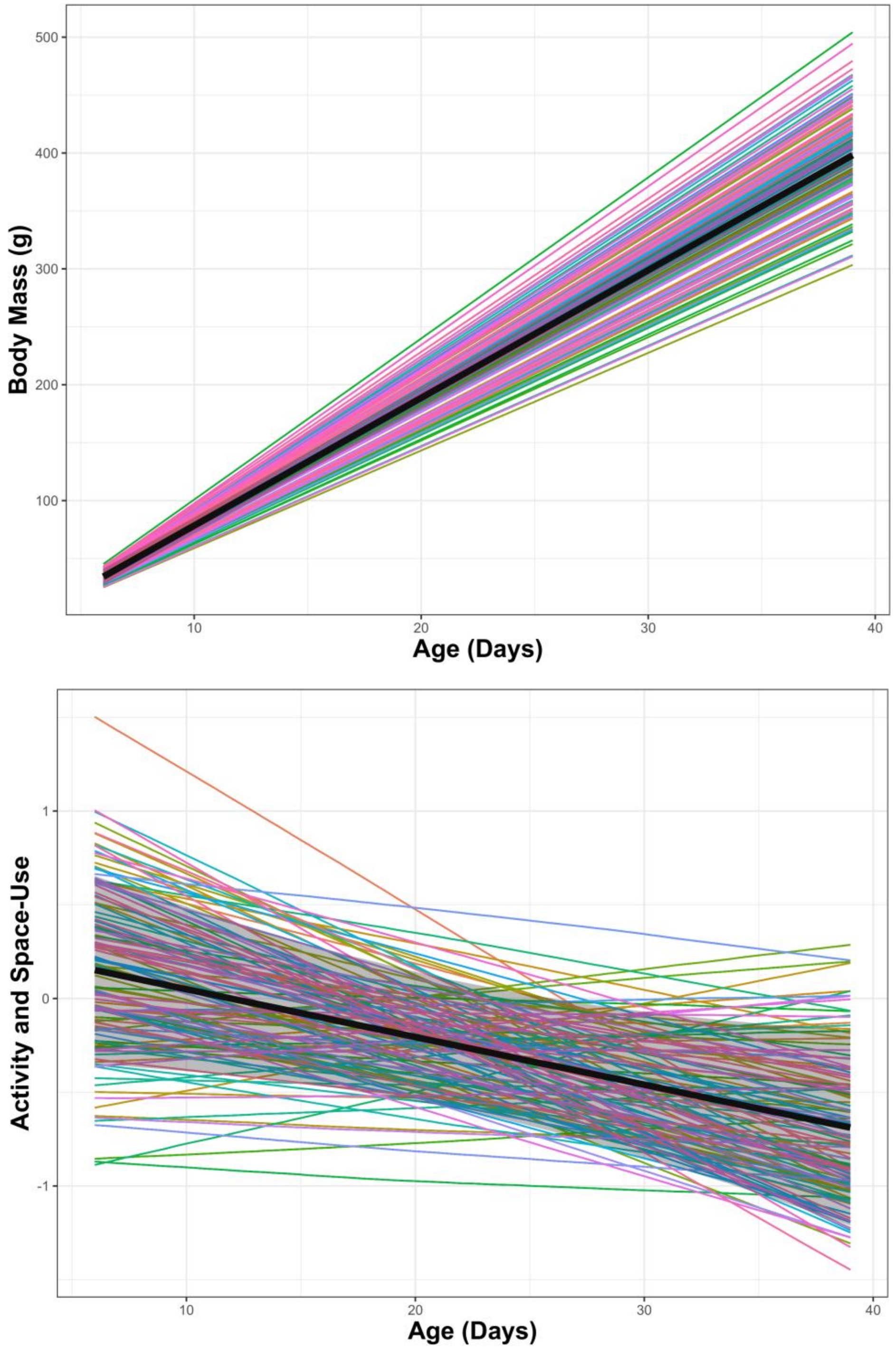


Figure 4.9: Conceptual plots depicting results from our multi-response Generalized Linear Mixed Model examining correlations among slopes and intercepts for Activity & Space-use and body mass within-individuals. Significant correlations are denoted with double asterisks. We simplify our findings here by showing two hypothetical individuals at the extremes of what our results suggest in relation to each other.

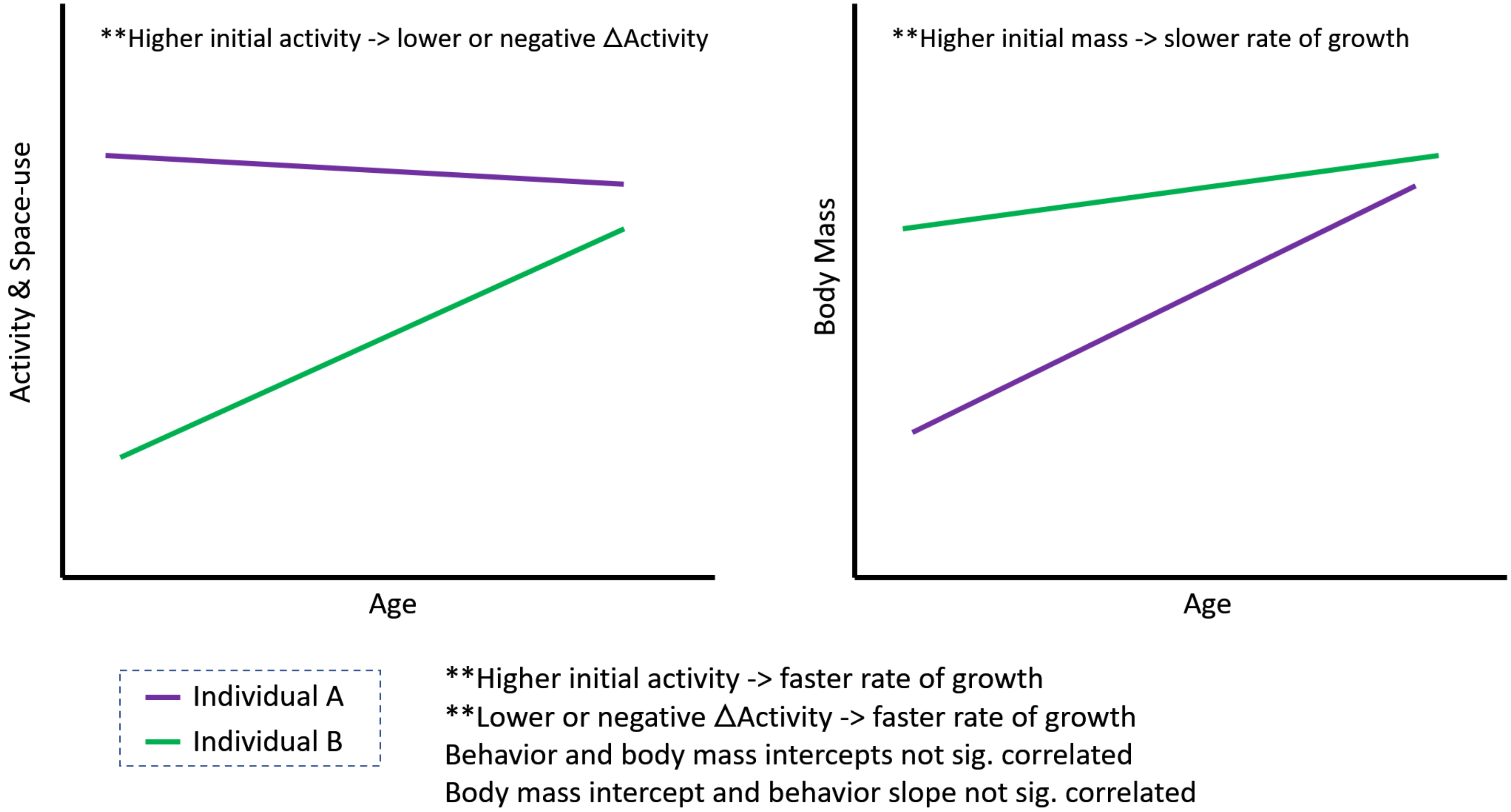
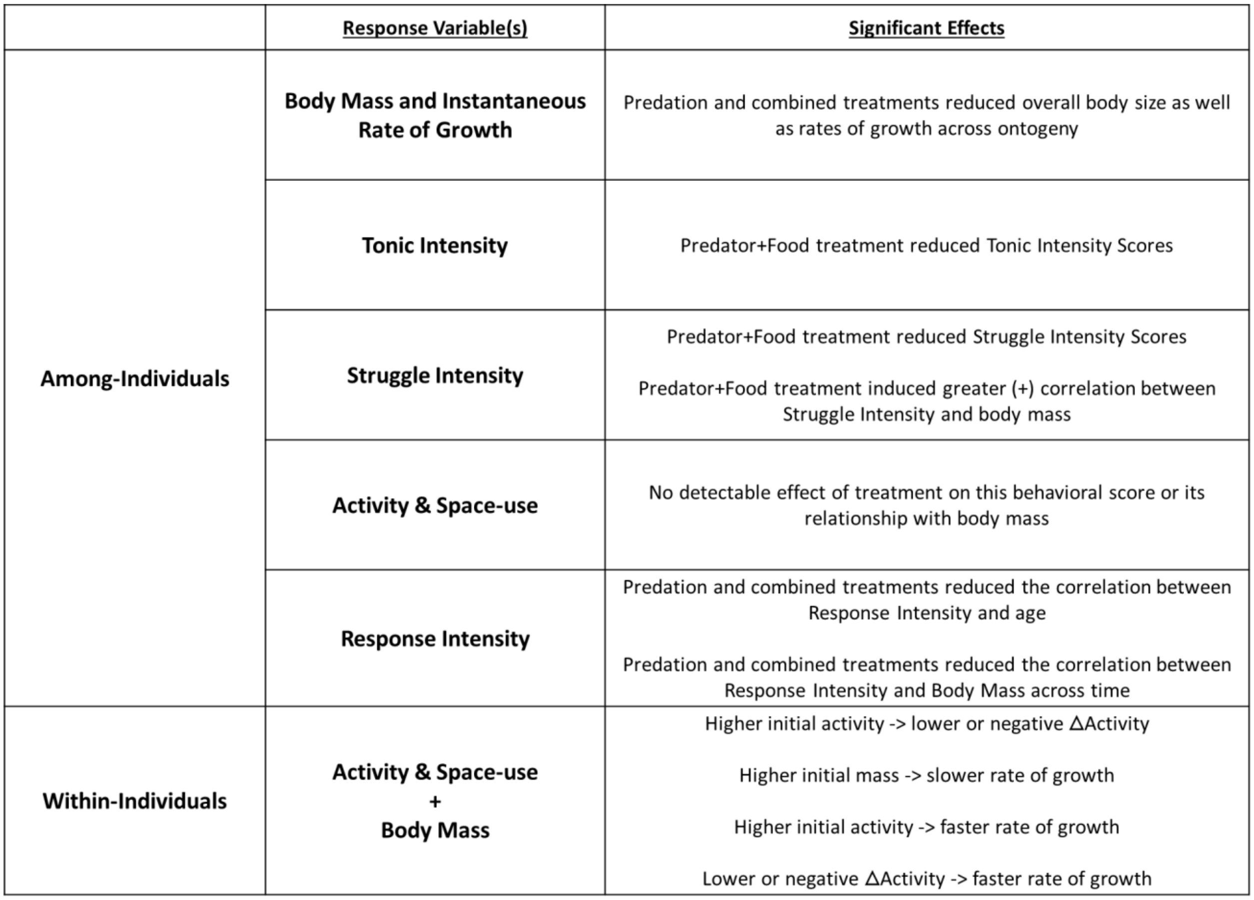


Table 4.8: Summary of significant results for Chapter 4.



	Response Variable(s)	Significant Effects
Among-Individuals	**Body Mass and Instantaneous Rate of Growth**	Predation and combined treatments reduced overall body size as well as rates of growth across ontogeny
	Tonic Intensity	Predator+Food treatment reduced Tonic Intensity Scores
	Struggle Intensity	Predator+Food treatment reduced Struggle Intensity Scores Predator+Food treatment induced greater (+) correlation between Struggle Intensity and body mass
	Activity & Space-use	No detectable effect of treatment on this behavioral score or its relationship with body mass
	Response Intensity	Predation and combined treatments reduced the correlation between Response Intensity and age Predation and combined treatments reduced the correlation between Response Intensity and Body Mass across time
Within-Individuals	**Activity & Space-use + Body Mass**	Higher initial activity -> lower or negative ∆Activity Higher initial mass -> slower rate of growth Higher initial activity -> faster rate of growth Lower or negative ∆Activity -> faster rate of growth

Dissertation Impact and Concluding Remarks

Empirical research on behavioral development, while extremely important in understanding why and how consistent individual behavioral variation is maintained within populations, poses many challenges. Longitudinal studies (that include more than two observations per individual) addressing development of consistent individual differences in behavior, in particular, are rare due to the logistical complications involved with collecting repeated measures of young animals both in the wild and in the lab. Of the studies that do exist, few incorporate longitudinal data within the early-life developmental period (prior to sexual maturity), and even fewer appear to examine development in precocial vertebrates (Cabrera et al. 2021). This dissertation represents a series of studies that longitudinally assess behavior and somatic growth in captive-reared animals, contributing to the still-growing personality development literature. Namely, our findings provide support for a theoretical Bayesian model predicting differences in developmental trajectories even in a common garden (Stamps and Krishnan 2014). We also provide evidence of canalization of certain behaviors associated with sensitivity to predation risk. Because so few studies address canalization of personality traits, we strongly encourage future work that examines personality development to explicitly quantify changes in within-individual variance and repeatability of behaviors across ontogeny (e.g., Kok et al. 2019). Animal personality theory also states that a close relationship between behavior and state might explain the maintenance or reinforcement of consistent individual differences in behavior (Dingemanse and Wolf 2010; Wolf and Weissing 2010). We provide, herein, empirical support for the state-dependence (in this case size and growth) of consistent individual differences in behavior as well as evidence that developmental environment can influence said state-dependence over developmental time. We also provide some support for negative feedback between behavior and state within-individuals leading to convergence in behavioral expression (Sih et al. 2015).

The findings we disseminate in this dissertation also have implications for our understanding of Wood Duck, and general waterfowl, ecology. In waterfowl systems, duckling size (which is typically correlated with egg size; Krist 2011) is often correlated with recruitment success (Dawson and Clark 1996; Pelayo and Clark 2003). This pattern also appears to be the case with the wild populations of Wood Ducks we specifically sourced our eggs from (Wells et al. *In prep*). In other words, larger ducklings are more likely to survive and return to breeding territories the following reproductive season. A link between size and sensitivity to predation risk under controlled conditions suggests that there is a real fitness consequence of these consistent individual differences in behavior, thus providing greater insight into the selective pressures these young birds face and how they appear to shape life-history dynamics. That there is considerable variation in duckling growth curves and sizes even when food is provided *ad libitum* is illuminating in-and-of itself (but note that variable incubation histories of our eggs may have had a carry-over effect through post-hatch development; DuRant et al. 2010). Of course, our results do not allow us to reasonably assert directionality in the relationship between size and the behaviors we measured; none-the-less, the behavior-size dynamic we report (assuming the patterns in our captive-reared birds do represent actual patterns in the wild) likely impacts duckling success depending on ecological context and can therefore have larger scale life-history consequences.

In this light, our research also provides a springboard for evaluation of behavioral types in the context of captive-rearing and release program efficacy. Though we do not discuss it much in this dissertation, we released a majority of our captive-reared birds back into their source populations once they began to molt in their primary flight feathers. Currently, our program (albeit with a species of least conservation concern and access to quality resources upon release) boasts a roughly 30% success rate (i.e., ~30% of released hens have returned to the site of release for following reproductive seasons; *preliminary analysis of unpublished data*). Through monitoring of female reproductive behavior in our wild populations (using the RFID technology we discuss in the General Methods and a robust nest-box program), future work in our lab aims to examine the reproductive and survival consequences of the developmental behavior and growth trajectories we quantified (Scheck et al. *In progress*). Many conservation projects rely on captive-release programs but, to our knowledge, do not employ individual behavioral assessment, nor strive to encourage variation in behavioral types among their animals (Stamps and Swaisgood 2007; Sinn et al. 2014). Though we acknowledge the many difficulties associated with the captive-release of endangered species, we posit that a holistic understanding of individual behavior, how it varies, and how it might impact both survival and reproduction post-release would serve to improve conservation outcomes for species that require this type of directed attention (Swaisgood 2007).

Finally, we would like to revisit and emphasize the fact that our study system represents a precocial reproductive strategy in which young ducklings leave their nest within approximately 24 hours of hatch, fully capable of moving and feeding themselves. This reproductive strategy contrasts with altricial systems in which hatchlings (or nestlings) require significant and direct parental provisioning from either the mother, the father, cooperative siblings, or all of the above. In the context of our study, this distinction is important for a number of reasons. Most studies of personality development in vertebrates (particularly among birds) have been done with model altricial systems. In their recent review of personality development empirical research, Cabrera et al. (2021) document a number of studies in Great Tits (*Parus major*), Eurasian Blue Tits (*Cyanistes caeruleus*), and Zebra Finches (*Taeniopygia castanotis*). While these systems provide a number of advantages in assessing various aspects of personality (such as a great amount of resources, research effort across multiple labs and regions, and a well-developed understanding of their biology), they are not fully representative of birds as a whole. Of the bird-centric developmental studies, the only precocial systems Cabrera et al. (2021) reviewed were that of domestic chickens. We also note a few behavioral development studies in Japanese Quail (Pittet et al. 2014; Pittet et al. 2019). This gap in empirical effort provided us the opportunity to examine personality development in a wild precocial system (a captive study with wild-type Wood Ducks).

However, organismal representation is not the only reason we endeavored to address developmental questions in this system. As we mention above, a majority of early neural development in precocial species occurs prior to hatch; presumably this is linked to the ability of young hatchlings to move and forage on their own soon after hatching. While Wood Duck hens brood their young for 4-8 weeks, brooding in this system consists mostly of guiding broods to foraging areas and providing refuge from harsh environmental stressors at a young age when ducklings are particularly vulnerable to shifts in temperature (Bellrose and Holm 1994). Hens are known to leave their brood while flushing for a variety of reasons and are generally considered to be fairly skittish. We are not aware of any observations of Wood Duck hens physically defending their brood from potential predators (unlike some other duck species). All of these ecological observations suggest that the behavior exhibited by young Wood Ducks is directly influential for their own long-term fitness with little moderation by their brood mother (at least relative to altricial and even some other precocial systems). As such, our studies provide insight into the development of behavior in a system in which juvenile survival is directly dependent on their own ability to avoid/prevent, or at least reduce the risk of predation while also consuming enough food to meet growth requirements. Both of these pressures might not only influence duckling survival, but also might have carry-over effects into later life stages, potentially influencing reproductive behavior and success as well as adult survival. **We propose that precocial systems serve as a model for assessing personality development in vertebrates as it allows empiricists a simpler framework to assess the direct fitness consequences of early life behavioral expression.**

Statement of Ethical Animal Care and Use

All animals included in this study were collected and housed following scientific collection and use requirements in accordance with the State of California Department of Fish and Wildlife (Scientific Collecting Permit #SC-009565) and the U.S. Fish and Wildlife Service (Federal Fish and Wildlife Permits #MB73393B-0 & #MB789345-0 and U.S. Geological Survey Federal Bird Banding Permit #10562). Captive care of our animals followed the Institutional Animal Care and Use Committee (IACUC) standards at the University of California, Davis between the years of 2015 and 2018 (IACUC Protocol #18633). We prioritized animal welfare (including physical and behavioral health following our understanding of general Wood Duck biology) while developing our study protocols and ensured that every bird was provided the best possible environmental conditions. Individuals were housed in social and physical conditions that were built to replicate natural conditions as much as possible, while removing actual threats of predation and providing a buffer from extreme temperatures. Birds were monitored daily, and individuals were swiftly removed from conditions in which they presented clear signs of extreme stress. Captive Wood Ducks were also given regular visual examinations by our attending veterinarian Dr. Rhonda Oates. Rather than euthanizing individuals at the conclusion of this study, we opted to release a majority of them back into their populations of origin after extensive disease and physical health screening under the supervision of our attending veterinarian and with appropriate state and federal permitting (California SCP #SC-009565 and Federal Fish and Wildlife Permit #MB789345-0). To date, a subset of our study animals (28) remain in captivity for ongoing, longitudinal research.

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References Cited

Adkins-Regan, E. (2012). Hormonal organization and activation: Evolutionary implications and questions. *General and Comparative Endocrinology*, *176*(3), 279–285. https://doi.org/10.1016/j.ygcen.2011.12.040

Amy, M., Sprau, P., De Goede, P., & Naguib, M. (2010). Effects of personality on territory defence in communication networks: A playback experiment with radio-tagged great tits. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 3685–3692. https://doi.org/10.1098/rspb.2010.0598

Anderson, V. R., & Alisauskas, R. T. (2001). Egg size, body size, locomotion, and feeding performance in captive King Eider ducklings. *The Condor*, *103*(1), 195–199. https://doi.org/10.1650/0010-5422(2001)103[0195:ESBSLA]2.0.CO;2

Andersson, M. Å., & Höglund, E. (2012). Linking personality to larval energy reserves in rainbow trout (*Oncorhynchus mykiss*). *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0049247

Arendt, J. D. (1997). Adaptive intrinsic growth rates: An integration across taxa. *The Quarterly Review of Biology*, *72*(2), 149–177.

Baugh, A. T., van Oers, K., Naguib, M., & Hau, M. (2013). Initial reactivity and magnitude of the acute stress response associated with personality in wild great tits (*Parus major*). *General and Comparative Endocrinology*, *189*, 96–104. https://doi.org/10.1016/j.ygcen.2013.04.030

Beatty, W. W. (1984). Hormonal Organization of Sex Differences in Play Fighting and Spatial Behavior. *Progress in Brain Research*, *61*(C), 315–330. https://doi.org/10.1016/S0079-6123(08)64444-1

Beecher, M. D., & Brenowitz, E. A. (2005). Functional aspects of song learning in songbirds. *Trends in Ecology and Evolution*, *20*(3), 143–149. https://doi.org/10.1016/j.tree.2005.01.004

Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in three-spined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, *10*(9), 828–834. https://doi.org/10.1111/j.1461-0248.2007.01081.x

Bellrose, F.C, & Holm, D.J. (1994). Ecology and management of the Wood Duck. *Stackpole Books*.

Bennett, A. M., & Murray, D. L. (2015). Carryover effects of phenotypic plasticity: Embryonic environment and larval response to predation risk in wood frogs (*Lithobates sylvaticus*) and northern leopard frogs (*Lithobates pipiens*). *Canadian Journal of Zoology*, *93*(11), 867–877. https://doi.org/10.1139/cjz-2015-0129

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology and Evolution*, *25*(9), 504–511. https://doi.org/10.1016/j.tree.2010.06.012

Bergvall, U. A., Schäpers, A., Kjellander, P., & Weiss, A. (2010). Personality and foraging decisions in fallow deer, *Dama dama*. *Animal Behaviour*, *81*(1), 1–12. https://doi.org/10.1016/j.anbehav.2010.09.018

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology and Evolution*, *23*(7), 361–368. https://doi.org/10.1016/j.tree.2008.04.003

Björkman, C., & Larsson, S. (1991). Pine sawfly defence and variation in host plant resin acids: a trade-off with growth. *Ecological Entomology*, *16*(3), 283–289. https://doi.org/10.1111/j.1365-2311.1991.tb00219.x

Bleicher, S. S. (2017). The landscape of fear conceptual framework: Definition and review of current applications and misuses. *PeerJ*. https://doi.org/10.7717/peerj.3772

Bobyn, M. L., & Brooks, R. J. (1994). Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). *Journal of Zoology*, *233*(2), 233–257. https://doi.org/10.1111/j.1469-7998.1994.tb08586.x

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Bölting, S., & von Engelhardt, N. (2017). Effects of the social environment during adolescence on the development of social behaviour, hormones and morphology in male zebra finches (*Taeniopygia guttata*). *Frontiers in Zoology*, *14*(5), 1–18. https://doi.org/10.1186/s12983-017-0190-4

Both, C., Dingemanse, N. J., Drent, P. J., & Tinbergen, J. M. (2005). Pairs of extreme avian personalities have highest reproductive success. *Journal of Animal Ecology*, *74*(4), 667–674. https://doi.org/10.1111/j.1365-2656.2005.00962.x

Brandl, H. B., Farine, D. R., Funghi, C., Schuett, W., & Griffith, S. C. (2019). Early-life social environment predicts social network position in wild zebra finches. *Proceedings of the Royal Society B: Biological Sciences*, *286*. https://doi.org/10.1098/rspb.2018.2579

Britton, N. F. (1989). Aggregation and the competitive exclusion principle. *Journal of Theoretical Biology*, *136*(1), 57–66. https://doi.org/10.1016/S0022-5193(89)80189-4

Brown, J. S. (1992). Patch use under predation risk: I. Models and predictions. *Annales Zoologici Fennici*, *29*(4), 301–309.

Brown, J. S. (1999). Vigilance, patch use and habitat selection: Foraging under predation risk. *Evolutionary Ecology Research*, *1*(1), 49–71.

Brown, J. S., Laundré, J. W., & Gurung, M. (1999). The ecology of fear: Optimal foraging, game theory, and trophic interactions. *Journal of Mammalogy*, *80*(2), 385–399. https://doi.org/10.2307/1383287

Bürkner, P. C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*. https://doi.org/10.18637/jss.v080.i01

Cabrera, D., Nilsson, J. R., & Griffen, B. D. (2021). The development of animal personality across ontogeny: a cross-species review. *Animal Behaviour*, *173*, 137–144. https://doi.org/10.1016/j.anbehav.2021.01.003

Campbell, D. L. M., Dickson, E. J., & Lee, C. (2019). Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. *PeerJ*, *2019*(11), 1–19. https://doi.org/10.7717/peerj.8122

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos*, *117*(5), 641–653. Retrieved from https://www.jstor.org/stable/40235351

Careau, V., Bininda-Emonds, O. R. P., Thomas, D. W., Réale, D., & Humphries, M. M. (2009). Exploration strategies map along fast-slow metabolic and life-history continua in muroid rodents. *Functional Ecology*, *23*(1), 150–156. https://doi.org/10.1111/j.1365-2435.2008.01468.x

Careau, V., Buttemer, W. A., & Buchanan, K. L. (2014). Early-developmental stress, repeatability, and canalization in a suite of physiological and behavioral traits in female zebra finches. *Integrative and Comparative Biology*, *54*(4), 539–554. https://doi.org/10.1093/icb/icu095

Carere, C., & Locurto, C. (2011). Interaction between animal personality and animal cognition. *Current Zoology*, *57*(4), 491–498. https://doi.org/10.1093/czoolo/57.4.491

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: What are behavioural ecologists measuring? *Biological Reviews*, *88*, 465–475. https://doi.org/10.1111/brv.12007

Chapple, D. G., Simmonds, S. M., & Wong, B. B. M. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology and Evolution*, *27*(1), 57–62. https://doi.org/10.1016/j.tree.2011.09.010

Charnov, E. L. (1976). Optimal foraging: the marginal value theorem. *Theoretical Population Biology*, *9*(2), 129–136. https://doi.org/10.1016/0040-5809(76)90040-X

Clark, C. W. (1994). Antipredator behavior and the asset-protection principle. *Behavioral Ecology*, *5*(2), 159–170. https://doi.org/10.1093/beheco/5.2.159

Clark, C. W., & Dukas, R. (2003). The behavioral ecology of a cognitive constraint: Limited attention. *Behavioral Ecology*, *14*(2), 151–156. https://doi.org/10.1093/beheco/14.2.151

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, *27*, 56–65. https://doi.org/10.1111/1365-2435.12007

Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology*, *181*(1), 45–58. https://doi.org/10.1016/j.ygcen.2012.11.025

Coppens, C. M., De Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 4021–4028. https://doi.org/10.1098/rstb.2010.0217

Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *365*, 4065–4076. https://doi.org/10.1098/rstb.2010.0176

Dahl, G. J., & Allord, T. E. (1997). History of Wetlands in the Conterminous United States. Retrieved from http://water.usgs.gov/nwsum/WSP2425/history.html

Daisley, J. N., Bromundt, V., Möstl, E., & Kotrschal, K. (2005). Enhanced yolk testosterone influences behavioral phenotype independent of sex in Japanese quail chicks *Coturnix japonica*. *Hormones and Behavior*, *47*, 185–194. https://doi.org/10.1016/j.yhbeh.2004.09.006

Davis, J. B., Cox, R. R., Kaminski, R. M., & Leopold, B. D. (2007). Survival of Wood Duck Ducklings and Broods in Mississippi and Alabama. *Journal of Wildlife Management*, *71*(2), 507–517. https://doi.org/10.2193/2005-720

Davis, J. B., Leopold, B. D., Kaminski, R. M., & Cox, R. R. (2009). Wood duck duckling mortality and habitat implications in floodplain systems. *Wetlands*, *29*(2), 607–614. https://doi.org/10.1672/08-136.1

Dawson, R. D., & Clark, R. G. (1996). Effects of variation in egg size and hatching date on survival of Lesser Scaup *Aythya affinis* ducklings. *Ibis*, (138), 693–699.

Dearborn, D. C. (1999). Brown-headed Cowbird nestling vocalizations and risk of nest predation, *116*(2), 448–457.

Delval, I., Fernández-Bolaños, M., & Izar, P. (2020). A longitudinal assessment of behavioral development in wild capuchins: Personality is not established in the first 3 years. *American Journal of Primatology*, *82*(11), 1–15. https://doi.org/10.1002/ajp.23116

Descamps, S., Boutin, S., Berteaux, D., McAdam, A. G., & Gaillard, J.-M. (2008). Cohort effects in red squirrels: the influence of density, food abundance and temperature on future survival and reproductive success. *Journal of Animal Ecology*, *77*, 305–314. https://doi.org/10.1111/j.1365-2656.2007.01340.x

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology and Evolution*, *25*(2), 81–89. https://doi.org/10.1016/j.tree.2009.07.013

Dingemanse, N. J., & Wolf, M. (2010). Recent models for adaptive personality differences: A review. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3947–3958. https://doi.org/10.1098/rstb.2010.0221

Dingemanse, N. J., & Wright, J. (2020). Criteria for acceptable studies of animal personality and behavioural syndromes. *Ethology*, *126*, 865–869. https://doi.org/10.1111/eth.13082

DiNuzzo, E. R., & Griffen, B. D. (2020). The effects of animal personality on the ideal free distribution. *Proceedings of the Royal Society B: Biological Sciences*, *287*, 1–9. https://doi.org/10.1098/rspb.2020.1095

DiRienzo, N., Pruitt, J. N., & Hedrick, A. V. (2013). The combined behavioural tendencies of predator and prey mediate the outcome of their interaction. *Animal Behaviour*, *86*, 317–322. https://doi.org/10.1016/j.anbehav.2013.05.020

Dmitriew, C. M. (2011). The evolution of growth trajectories: What limits growth rate? *Biological Reviews*, *86*, 97–116. https://doi.org/10.1111/j.1469-185X.2010.00136.x

Dochtermann, N. A., Schwab, T., & Sih, A. (2014). The contribution of additive genetic variation to personality variation: Heritability of personality. *Proceedings of the Royal Society B: Biological Sciences*, *282*. https://doi.org/10.1098/rspb.2014.2201

dos Santos, M. B., de Oliveira, M. C. L. M., Verrastro, L., & Tozetti, A. M. (2010). Playing dead to stay alive: Death-feigning in *Liolaemus occipitalis* (Squamata: Liolaemidae). *Biota Neotropica*, *10*(4), 361–364. https://doi.org/10.1590/s1676-06032010000400043

Dougherty, L. R., & Guillette, L. M. (2018). Linking personality and cognition: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*. https://doi.org/10.1098/rstb.2017.0282

Dunham, R. A., Cameron, A. D., & Petty, J. A. (1999). The effect of growth rate on the strength properties of sawn beams of silver birch (*Betula pendula Roth*). *Scandinavian Journal of Forest Research*, *14*(1), 18–26. https://doi.org/10.1080/02827589908540805

DuRant, S. E., Hepp, G. R., Moore, I. T., Hopkins, B. C., & Hopkins, W. A. (2010). Slight differences in incubation temperature affect early growth and stress endocrinology of Wood Duck (*Aix sponsa*) ducklings. *Journal of Experimental Biology*, *213*, 45–51. https://doi.org/10.1242/jeb.034488

DuRant, S. E., Hopkins, W. A., Wilson, A. F., & Hepp, G. R. (2012). Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird. *Functional Ecology*, *26*, 416–422. https://doi.org/10.1111/j.1365-2435.2011.01945.x

DuRant, S. E., Hopkins, W. A., Hepp, G. R., & Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews*, *88*, 499–509. https://doi.org/10.1111/brv.12015

Düttmann, H., Bergmann, H.H., & Engländer, W. (1998). Development of behavior. In J.M. Starck & R.E. Ricklefs (Eds.): Avian growth and development: evolution within the altricial-precocial spectrum (pp. 223-246). *Oxford University Press*.

Dzubin, A., & Cooch, E. (1992). Measurement of Geese: General Field Methods. *California Waterfowl Association*.

Edenbrow, M., & Croft, D. P. (2013). Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. *Oikos*, *122*, 667–681. https://doi.org/10.1111/j.1600-0706.2012.20556.x

Eyck, H. J. F., Buchanan, K. L., Crino, O. L., & Jessop, T. S. (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews*, *94*, 1143–1160. https://doi.org/10.1111/brv.12496

Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L. (2016). Host behaviour-parasite feedback: An essential link between animal behaviour and disease ecology. *Proceedings of the Royal Society B: Biological Sciences*, *283*. https://doi.org/10.1098/rspb.2015.3078

Ferren, W. R., Fiedler, P. L., & Leidy, R. A. (1996). Wetlands of California, Part I: History of wetland habitat classification. *California Botanical Society*, *43*(1), 105–124. Retrieved from https://www.jstor.org/stable/41425128

Flatt, T. (2005). The evolutionary genetics of canalization. *Quarterly Review of Biology*, *80*(3), 287–316. https://doi.org/10.1086/703580

Foellmer, M. W., & Khadka, K. K. (2013). Does personality explain variation in the probability of sexual cannibalism in the orb-web spider Argiope aurantia? *Behaviour*, *150*, 1731–1746. https://doi.org/10.1163/1568539X-00003117

Gallup, G. G. (1977). Tonic Immobility: the role of fear and predation. *The Psychological Record*, *27*(1), 41–61. https://doi.org/10.1007/bf03394432

Garcia, T. S., Urbina, J. C., Bredeweg, E. M., & Ferrari, M. C. O. (2017). Embryonic learning and developmental carry-over effects in an invasive anuran. *Oecologia*, *184*, 623–631. https://doi.org/10.1007/s00442-017-3905-5

Gaynor, K. M., Brown, J. S., Middleton, A. D., Power, M. E., & Brashares, J. S. (2019). Landscapes of fear: Spatial patterns of risk perception and response. *Trends in Ecology and Evolution*, *34*(4), 355–368. https://doi.org/10.1016/j.tree.2019.01.004

Gilbert, S. F. (2012). Ecological developmental biology: Environmental signals for normal animal development. *Evolution and Development*, *14*(1), 20–28. https://doi.org/10.1111/j.1525-142X.2011.00519.x

Gill, F.B. (2006). Ornithology, Third Edition. *Macmillan*.

Gottlieb, G. (1991a). Experiential canalization of behavioral development: Theory. *Developmental Psychology*, *27*(1), 4–13. https://doi.org/10.1037/0012-1649.27.1.35

Gottlieb, G. (1991b). Experiential canalization of behavioral development: Results. *Developmental Psychology*, *27*(1), 35–39. https://doi.org/10.1037/0012-1649.27.1.35

Greene, H.W. (1988). Antipredator mechanisms in reptiles. *Biology of the reptilia, 16(1),* 1-152.

Gregory, T. R., & Wood, C. M. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology*, *72*(3), 286–295. https://doi.org/10.1086/316673

Groothuis, T. G. G., Müller, W., von Engelhardt, N., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews*, *29*, 329–352. https://doi.org/10.1016/j.neubiorev.2004.12.002

Grunst, A. S., Grunst, M. L., Thys, B., Raap, T., Daem, N., Pinxten, R., & Eens, M. (2018). Variation in personality traits across a metal pollution gradient in a free-living songbird. *Science of the Total Environment*, *630*, 668–678. https://doi.org/10.1016/j.scitotenv.2018.02.191

Haage, M., Maran, T., Bergvall, U. A., Elmhagen, B., & Angerbjörn, A. (2017). The influence of spatiotemporal conditions and personality on survival in reintroductions–evolutionary implications. *Oecologia*, *183*, 45–56. https://doi.org/10.1007/s00442-016-3740-0

Hall, M. L., van Asten, T., Katsis, A. C., Dingemanse, N. J., Magrath, M. J. L., & Mulder, R. A. (2015). Animal personality and pace-of-life syndromes: Do fast-exploring fairy-wrens die young? *Frontiers in Ecology and Evolution*, *3*, 1–14. https://doi.org/10.3389/fevo.2015.00028

Hamilton, I.M. (2010). Foraging Theory. In D. Westneat & C.W. Fox (Eds.): Evolutionary behavioral ecology. *Oxford University Press*.

Hepp, G.R., & Bellrose, F.C. (2013). Wood Duck (*Aix sponsa*), version 2.0. *The Birds of North America. Ithaca (NY): Cornell Lab of Ornithology*.

Hepp, G., DuRant, S. E., & Hopkins, W. A. (2015). Influence of incubation temperature on offspring phenotype and fitness in birds. In D. C. Deeming & S. J. Reynolds (Eds.): Nests, Eggs, and Incubation (pp. 171–178). *Oxford University Press*. https://doi.org/10.1093/acprof

Hogan, R. (2005). In defense of personality measurement: New wine for old whiners. *Human Performance*, *18*(4), 1–13. https://doi.org/10.1207/s15327043hup1804

Hohtola, E., & Visser, H. (1998). Development of locomotion and endothermy in altricial and precocial birds. In J.M. Starck & R.E. Ricklefs (Eds.): Avian growth and development: evolution within the altricial-precocial spectrum (pp. 157-173). *Oxford University Press*.

Hope, S. F., Kennamer, R. A., Moore, I. T., & Hopkins, W. A. (2018). Incubation temperature influences the behavioral traits of a young precocial bird. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*, 191–202. https://doi.org/10.1002/jez.2176

Hopkins, B. C., DuRant, S. E., Hepp, G. R., & Hopkins, W. A. (2011). Incubation temperature influences locomotor performance in young Wood Ducks (*Aix sponsa*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *315*, 274–279. https://doi.org/10.1002/jez.673

Hornick, J. L., van Eenaeme, C., Gérard, O., Dufrasne, I., & Istasse, L. (2000). Mechanisms of reduced and compensatory growth. *Domestic Animal Endocrinology*, *19*, 121–132. https://doi.org/10.1016/S0739-7240(00)00072-2

Houpt, T. A., Cassell, J. A., Hood, A., DenBleyker, M., Janowitz, I., Mueller, K., Ortega, B., & Smith, J. C. (2010). Repeated exposure attenuates the behavioral response of rats to static high magnetic fields. *Physiology and Behavior*, *99*, 500–508. https://doi.org/10.1016/j.physbeh.2009.12.024

Houston, A. I. (2010). Evolutionary models of metabolism, behaviour and personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3969–3975. https://doi.org/10.1098/rstb.2010.0161

Ituarte, R. B., Vázquez, M. G., González-Sagrario, M. de los Á., & Spivak, E. D. (2014). Carryover effects of predation risk on postembryonic life-history stages in a freshwater shrimp. *Zoology*, *117*, 139–145. https://doi.org/10.1016/j.zool.2013.09.004

Jäger, H. Y., Han, C. S., & Dingemanse, N. J. (2019). Social experiences shape behavioral individuality and within-individual stability. *Behavioral Ecology*, *30*(4), 1012–1019. https://doi.org/10.1093/beheco/arz042

Johnson, J. C., & Sih, A. (2005). Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): A role for behavioral syndromes. *Behavioral Ecology and Sociobiology*, *58*, 390–396. https://doi.org/10.1007/s00265-005-0943-5

Johnsson, J. I., Jönsson, E., & Björnsson, B. T. (1996). Dominance, nutritional state, and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). *Hormones and Behavior*, *30*, 13–21. https://doi.org/10.1006/hbeh.1996.0003

Jones, R. B. (1986). The tonic immobility reaction of the domestic fowl: A review. *World's Poultry Science Journal*, *42*, 82–96. https://doi.org/10.1079/WPS19860008

Klemme, I., & Karvonen, A. (2016). Learned parasite avoidance is driven by host personality and resistance to infection in a fish-trematode interaction. *Proceedings of the Royal Society B: Biological Sciences*, *283*. https://doi.org/10.1098/rspb.2016.1148

Kohler, S. A., Parker, M. O., & Ford, A. T. (2018). Shape and size of the arenas affect amphipod behaviours: Implications for ecotoxicology. *PeerJ*, 1–20. https://doi.org/10.7717/peerj.5271

Kok, E. M. A., Burant, J. B., Dekinga, A., Manche, P., Saintonge, D., Piersma, T., & Mathot, K. J. (2019). Within-individual canalization contributes to age-related increases in trait repeatability: A longitudinal experiment in red knots. *American Naturalist*, *194*(4), 455–469. https://doi.org/10.1086/704593

Kozlovsky, D., Branch, C., Freas, C. A., & Pravosudov, V. V. (2014). Elevation-related differences in novel environment exploration and social dominance in food-caching mountain chickadees. *Behavioral Ecology and Sociobiology*, *68*, 1871–1881. https://doi.org/10.1007/s00265-014-1796-6

Krause, J., James, R., & Croft, D. P. (2010). Personality in the context of social networks. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 4099–4106. https://doi.org/10.1098/rstb.2010.0216

Krist, M. (2011). Egg size and offspring quality: A meta-analysis in birds. *Biological Reviews*, *86*, 692–716. https://doi.org/10.1111/j.1469-185X.2010.00166.x

Laundré, J. W., Hernández, L., & Ripple, W. J. (2010). The landscape of fear: Ecological implications of being afraid. *The Open Ecology Journal*, *3*, 1–7. https://doi.org/10.2174/1874213001003030001

Le Maho, Y., Karmann, H., Briot, D., Handrich, Y., Robin, J. P., Mioskowski, E., Cherel, Y., & Farni, J. (1992). Stress in birds due to routine handling and a technique to avoid it. *American Journal of Physiology*, *263*(4), R775–R781. https://doi.org/10.1152/ajpregu.1992.263.4.r775

Lehtonen, J., & Jaatinen, K. (2016). Safety in numbers: the dilution effect and other drivers of group life in the face of danger. *Behavioral Ecology and Sociobiology*, *70*, 449–458. https://doi.org/10.1007/s00265-016-2075-5

Liedtke, J., Redekop, D., Schneider, J. M., & Schuett, W. (2015). Early environmental conditions shape personality types in a jumping spider. *Frontiers in Ecology and Evolution*, *3*, 1–14. https://doi.org/10.3389/fevo.2015.00134

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*, 619–640. https://doi.org/10.1139/z90-092

Luttbeg, B., & Sih, A. (2010). Risk, resources and state-dependent adaptive behavioural syndromes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3977–3990. https://doi.org/10.1098/rstb.2010.0207

Lyon, B. E., & Eadie, J. M. (2008). Conspecific Brood Parasitism in Birds: A Life-History Perspective. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 343–363. https://doi.org/10.1146/annurev.ecolsys.39.110707.173354

Mangel, M., & Stamps, J. (2001). Trade-offs between growth and mortality and the maintenance of individual variation. *Evolutionary Ecology Research*, *3*, 583–593.

McElreath, R., & Strimling, P. (2006). How noisy information and individual asymmetries can make "personality" an adaptation: a simple model. *Animal Behaviour*, *72*, 1135–1139. https://doi.org/10.1016/j.anbehav.2006.04.001

McElreath, R., Luttbeg, B., Fogarty, S. P., Brodin, T., & Sih, A. (2007). Evolution of animal personalities. *Nature*, *450*, 5–6. https://doi.org/10.1038/nature06326

McGhee, K. E., & Travis, J. (2011). Early food and social environment affect certain behaviours but not female choice or male dominance in bluefin killifish. *Animal Behaviour*, *82*, 139–147. https://doi.org/10.1016/j.anbehav.2011.04.009

Miyatake, T., Katayama, K., Takeda, Y., Nakashima, A., Sugita, A., & Mizumoto, M. (2004). Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. *Proceedings of the Royal Society B: Biological Sciences*, *271*, 2293–2296. https://doi.org/10.1098/rspb.2004.2858

Modlmeier, A. P., Keiser, C. N., Watters, J. V., Sih, A., & Pruitt, J. N. (2014). The keystone individual concept: An ecological and evolutionary overview. *Animal Behaviour*, *89*, 53–62. https://doi.org/10.1016/j.anbehav.2013.12.020

Moiron, M., Araya-Ajoy, Y. G., Mathot, K. J., Mouchet, A., & Dingemanse, N. J. (2019). Functional relations between body mass and risk-taking behavior in wild great tits. *Behavioral Ecology*, *30*(3), 617–623. https://doi.org/10.1093/beheco/ary199

Montaña, C. G., Layman, C. A., & Winemiller, K. O. (2011). Gape size influences seasonal patterns of piscivore diets in three Neotropical rivers. *Neotropical Ichthyology*, *9*(3), 647–655. https://doi.org/10.1590/S1679-62252011005000028

Moore, J. (1999). Population density, social pathology, and behavioral ecology. *Primates*, *40*(1), 1–22. https://doi.org/10.1007/BF02557698

Morandini, V., & Ferrer, M. (2019). Nutritional condition determines behavioral response of nestling Black-browed albatrosses to a shy-bold continuum test. *Ethology Ecology and Evolution*, *31*(3), 266–276. https://doi.org/10.1080/03949370.2019.1592229

Mutzel, A., Dingemanse, N. J., Araya-Ajoy, Y. G., & Kempenaers, B. (2013). Parental provisioning behaviour plays a key role in linking personality with reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, *280*. https://doi.org/10.1098/rspb.2013.1019

Naguib, M., Nemitz, A., & Gil, D. (2006). Maternal developmental stress reduces reproductive success of female offspring in zebra finches. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 1901–1905. https://doi.org/10.1098/rspb.2006.3526

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*, 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Nettle, D., & Bateson, M. (2015). Adaptive developmental plasticity: What is it, how can we recognize it and when can it evolve? *Proceedings of the Royal Society B: Biological Sciences*, *282*. https://doi.org/10.1098/rspb.2015.1005

Nicieza, A. G., & Metcalfe, N. B. (1997). Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. *Ecology*, *78*(8), 2385–2400. https://doi.org/10.1890/0012-9658(1997)078[2385:GCIJAS]2.0.CO;2

Nicolaus, M., Mathot, K. J., Araya-Ajoy, Y. G., Mutzel, A., Wijmenga, J. J., Kempenaers, B., & Dingemanse, N. J. (2015). Does coping style predict optimization? An experimental test in a wild passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, *282*. https://doi.org/10.1098/rspb.2014.2405

Nonacs, P., & Blumstein, D.T. (2010). Predation Risk and Behavioral Life History. In D. Westneat & C.W. Fox (Eds.): Evolutionary behavioral ecology). *Oxford University Press*.

Nowlin, W. H., Drenner, R. W., Guckenberger, K. R., Lauden, M. A., Alonso, G. T., Fennell, J. E., & Smith, J. L. (2006). Gape limitation, prey size refuges and the top-down impacts of piscivorous largemouth bass in shallow pond ecosystems. *Hydrobiologia*, *563*, 357–369. https://doi.org/10.1007/s10750-006-0024-4

Nye, P. A. (1964). Heat loss in wet ducklings and chicks. *Ibis*, *106*(2), 189–197. https://doi.org/10.1111/j.1474-919X.1964.tb03695.x

Pearish, S., Bensky, M. K., & Bell, A. M. (2019). Social environment determines the effect of boldness and activity on survival. *Ethology*, *125*, 855–862. https://doi.org/10.1111/eth.12939

Pelayo, J. T., & Clark, R. G. (2003). Consequences of egg size for offspring survival: A cross-fostering experiment in Ruddy Ducks (*Oxyura jamaicensis*). *The Auk*, *120*(2), 384–393. https://doi.org/10.2307/4090190

Pittet, F., Houdelier, C., Le Bot, O., Leterrier, C., & Lumineau, S. (2014). Fearfulness affects quail maternal care and subsequent offspring development. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0102800

Pittet, F., Tyson, C., Herrington, J. A., Houdelier, C., & Lumineau, S. (2019). Postnatal care generates phenotypic behavioural correlations in the Japanese Quail. *Behavioral Ecology and Sociobiology*, *73*(9). https://doi.org/10.1007/s00265-019-2735-3

Prange, H. D., & Schmidt-Nielsen, K. (1970). The metabolic cost of swimming in ducks. *Journal of Experimental Biology*, *53*, 763–777. https://doi.org/10.1242/jeb.200.3.531

Quesnel, L., King, W. J., Coulson, G., & Festa-Bianchet, M. (2018). Tall young females get ahead: size-specific fecundity in wild kangaroos suggests a steep trade-off with growth. *Oecologia*, *186*, 59–71. https://doi.org/10.1007/s00442-017-4003-4

Ramamonjisoa, N., Rakotonoely, H., & Natuhara, Y. (2018). Defense investments and growth responses under different predation risks and gape-limitation predation threats in a tadpole prey. *Behavioral Ecology and Sociobiology*, *72*. https://doi.org/10.1007/s00265-018-2559-6

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*, 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Réale, D., Dingemanse, N. J., Kazem, A. J. N., & Wright, J. (2010a). Evolutionary and ecological approaches to the study of personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3937–3946. https://doi.org/10.1098/rstb.2010.0222

Réale, D., Garant, D., Humphries, M. M. M., Bergeron, P., Careau, V., & Montiglio, P. O. P.-O. (2010b). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 4051–4063. https://doi.org/10.1098/rstb.2010.0208

Rice, J. A., Miller, T. J., Rose, K. A., Crowder, L. B., Marschall, E. A., Trebitz, A. S., & DeAngelis, D. L. (1993). Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 133–142. https://doi.org/10.1139/f93-015

Royle, N. J., Lindström, J., & Metcalfe, N. B. (2005). A poor start in life negatively affects dominance status in adulthood independent of body size in green swordtails *Xiphophorus helleri*. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 1917–1922. https://doi.org/10.1098/rspb.2005.3190

Ruuskanen, S., & Laaksonen, T. (2010). Yolk hormones have sex-specific long-term effects on behavior in the pied flycatcher (*Ficedula hypoleuca*). *Hormones and Behavior*, *57*, 119–127. https://doi.org/10.1016/j.yhbeh.2009.09.017

Salazar, M. O. L., Planas-Sitjà, I., Sempo, G., & Deneubourg, J. L. (2018). Individual thigmotactic preference affects the fleeing behavior of the American cockroach (Blattodea: Blattidae). *Journal of Insect Science*, *18*(1), 1–7. https://doi.org/10.1093/jisesa/iex108

Sargeant, A. B., & Eberhardt, L. E. (1975). Death feigning by ducks in response to predation by red foxes (*Vulpes fulva*). *The American Midland Naturalist*, *94*(1), 108–119. Retrieved from https://www.jstor.org/stable/2424542

Schausberger, P., Gratzer, M., & Strodl, M. A. (2017). Early social isolation impairs development, mate choice and grouping behaviour of predatory mites. *Animal Behaviour*, *127*, 15–21. https://doi.org/10.1016/j.anbehav.2017.02.024

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments*, (96), 1–9. https://doi.org/10.3791/52434

Semel, B., & Sherman, P. W. (1995). Alternative placement strategies for Wood Duck nest boxes. *Wildlife Society Bulletin*, *23*(3), 463–471.

Shams, S., Chatterjee, D., & Gerlai, R. (2015). Chronic social isolation affects thigmotaxis and whole-brain serotonin levels in adult zebrafish. *Behavioural Brain Research*, *292*, 283–287. https://doi.org/10.1016/j.bbr.2015.05.061

Siegel, H. S. (1980). Physiological stress in birds. *BioScience*, *30*(8), 529–534. https://doi.org/10.2307/1307973

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004a). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology*, *79*(3), 241–277. https://doi.org/10.1086/703580

Sih, A., Bell, A., & Johnson, J. C. (2004b). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution*, *19*(7), 372–378. https://doi.org/10.1016/j.tree.2004.04.009

Sih, A., Bell, A.M., & Johnson, J.C. (2010). Behavioral Syndromes. In D. Westneat & C.W. Fox (Eds.): Evolutionary behavioral ecology (pp. 516-530). *Oxford University Press*.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012a). Ecological implications of behavioural syndromes. *Ecology Letters*, *15*(3), 278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x

Sih, A., Giudice, M. Del, & Del Giudice, M. (2012b). Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*, 2762–2772. https://doi.org/10.1098/rstb.2012.0216

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P. O., Wolf, M., & Dingemanse, N. J. (2015). Animal personality and state-behaviour feedbacks: A review and guide for empiricists. *Trends in Ecology and Evolution*, *30*(1), 50–60. https://doi.org/10.1016/j.tree.2014.11.004

Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behavioural Brain Research*, *61*, 59–64. https://doi.org/10.1016/0166-4328(94)90008-6

Sinn, D. L., Cawthen, L., Jones, S. M., Pukk, C., & Jones, M. E. (2014). Boldness towards novelty and translocation success in captive-raised, orphaned Tasmanian devils. *Zoo Biology*, *33*, 36–48. https://doi.org/10.1002/zoo.21108

Stamps, J. A. (2007). Growth-mortality tradeoffs and “personality traits” in animals. *Ecology Letters*, *10*, 355–363. https://doi.org/10.1111/j.1461-0248.2007.01034.x

Stamps, J. A., & Swaisgood, R. R. (2007). Someplace like home: Experience, habitat selection and conservation biology. *Applied Animal Behaviour Science*, *102*, 392–409. https://doi.org/10.1016/j.applanim.2006.05.038

Stamps, J. A., & Groothuis, T. G. G. (2010a). Developmental perspectives on personality: Implications for ecological and evolutionary studies of individual differences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 4029–4041. https://doi.org/10.1098/rstb.2010.0218

Stamps, J., & Groothuis, T. G. G. (2010b). The development of animal personality: Relevance, concepts and perspectives. *Biological Reviews*, *85*, 301–325. https://doi.org/10.1111/j.1469-185X.2009.00103.x

Stamps, J. A., & Krishnan, V. V. (2014). Individual differences in the potential and realized developmental plasticity of personality traits. *Frontiers in Ecology and Evolution*, *2*, 1–15. https://doi.org/10.3389/fevo.2014.00069

Stamps, J. A., & Krishnan, V. V. (2017). Age-dependent changes in behavioural plasticity: insights from Bayesian models of development. *Animal Behaviour*, *126*, 53–67. https://doi.org/10.1016/j.anbehav.2017.01.013

Stankowich, T., & Blumstein, D. T. (2005). Fear in animals: A meta-analysis and review of risk assessment. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 2627–2634. https://doi.org/10.1098/rspb.2005.3251

Starck, J.M., & Ricklefs, R.E. (Eds.). (1998). Avian growth and development: evolution within the altricial-precocial spectrum (No. 8). *Oxford University Press*.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology*, *3*(3), 259–268. Retrieved from https://www.jstor.org/stable/2389364

Swaisgood, R. R. (2007). Current status and future directions of applied behavioral research for animal welfare and conservation. *Applied Animal Behaviour Science*, *102*, 139–162. https://doi.org/10.1016/j.applanim.2006.05.027

Taborsky, B., Arnold, C., Junker, J., & Tschopp, A. (2012). The early social environment affects social competence in a cooperative breeder. *Animal Behaviour, 83*, 1067–1074. https://doi.org/10.1016/j.anbehav.2012.01.037

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift Für Tierpsychologie*, *20*, 410–433. https://doi.org/10.1111/j.1439-0310.1963.tb01161.x

Tonkins, B. M., Tyers, A. M., & Cooke, G. M. (2015). Cuttlefish in captivity: An investigation into housing and husbandry for improving welfare. *Applied Animal Behaviour Science*, *168*, 77–83. https://doi.org/10.1016/j.applanim.2015.04.004

Travis, J. (1984). Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. *Ecology*, *65*(4), 1155–1160. https://doi.org/10.2307/1938323

Treit, D., & Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology, Biochemistry and Behavior*, *31*, 959–962. https://doi.org/10.1016/0091-3057(88)90413-3

Trillmich, F., & Hudson, R. (2011). The emergence of personality in animals: The need for a developmental approach. *Developmental Psychobiology*, *53*, 505–509. https://doi.org/10.1002/dev.20573

van de Pol, M., Bruinzeel, L. W., Heg, D., Van Der Jeugd, H. P., & Verhulst, S. (2006). A silver spoon for a golden future: Long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). *Journal of Animal Ecology*, *75*, 616–626. https://doi.org/10.1111/j.1365-2656.2006.01079.x

van der Kooij, M. A. (2020). The impact of chronic stress on energy metabolism. *Molecular and Cellular Neuroscience*, *107*. https://doi.org/10.1016/j.mcn.2020.103525

van Oers, K., De Jong, G., Van Noordwijk, A. J., Kempenaers, B., & Drent, P. J. (2005). Contribution of genetics to the study of animal personalities: A review of case studies. *Behaviour*, *142*, 1185–1206. https://doi.org/10.1163/156853905774539364

van Oers, K., Kohn, G. M., Hinde, C. A., & Naguib, M. (2015). Parental food provisioning is related to nestling stress response in wild great tit nestlings: implications for the development of personality. *Frontiers in Zoology*, *12*. https://doi.org/10.1186/1742-9994-12-S1-S10

Verdolin, J. L. (2006). Meta-analysis of foraging and predation risk trade-offs in terrestrial systems. *Behavioral Ecology and Sociobiology*, *60*, 457–464. https://doi.org/10.1007/s00265-006-0172-6

Vetter, S. G., Brandstätter, C., Macheiner, M., Suchentrunk, F., Gerritsmann, H., & Bieber, C. (2016). Shy is sometimes better: Personality and juvenile body mass affect adult reproductive success in wild boars, *Sus scrofa*. *Animal Behaviour*, *115*, 193–205. https://doi.org/10.1016/j.anbehav.2016.03.026

Viblanc, V. A., Schull, Q., Cornioley, T., Stier, A., Ménard, J. J., Groscolas, R., & Robin, J. P. (2018). An integrative appraisal of the hormonal and metabolic changes induced by acute stress using king penguins as a model. *General and Comparative Endocrinology*, *269*, 1–10. https://doi.org/10.1016/j.ygcen.2017.08.024

Waddington, C. H. (1942). Canalization of Development and the Inheritance of Acquired Characters. *Nature*.

Wauters, L. A., Mazzamuto, M. V., Santicchia, F., Martinoli, A., Preatoni, D. G., Lurz, P. W. W., Bertolino, S., & Romeo, C. (2021). Personality traits, sex and food abundance shape space use in an arboreal mammal. *Oecologia*, *196*, 65–76. https://doi.org/10.1007/s00442-021-04901-2

Webber, Q. M. R., & Willis, C. K. R. (2020). Personality affects dynamics of an experimental pathogen in little brown bats: Behaviour and pathogen dynamics in bats. *Royal Society Open Science*, *7*. https://doi.org/10.1098/rsos.200770

Westneat, D., & Fox, C.W. (Eds.). (2010). Evolutionary behavioral ecology. *Oxford University Press*.

Williams, T. D. (2008). Individual variation in endocrine systems: Moving beyond the "tyranny of the Golden Mean." *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*, 1687–1698. https://doi.org/10.1098/rstb.2007.0003

Wirsing, A. J., Heithaus, M. R., Brown, J. S., Kotler, B. P., & Schmitz, O. J. (2021). The context dependence of non-consumptive predator effects. *Ecology Letters*, *24*(1), 113–129. https://doi.org/10.1111/ele.13614

Wolf, M., van Doorn, G., Leimar, O., & Weissing, F.J. (2007). Wolf et al. *reply*. *Nature, 450*, E5-E6. https://doi.org/10.1038/nature06327

Wolf, M., van Doorn, G.S., Leimar, O., & Weissing, F.J. (2008). Do animal personalities emerge. *Nature, 451,* E9-E10.

Wolf, M., & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 3959–3968. https://doi.org/10.1098/rstb.2010.0215

Xu, W., Yao, Q., Zhang, W., Zhang, F., Li, H., Xu, R., Li, C. & Zhang, B. (2021). Environmental complexity during early life shapes average behavior in adulthood. *Behavioral Ecology*, *32*(1), 105–113. https://doi.org/10.1093/beheco/araa108