

INSULIN-LIKE GROWTH FACTOR 1 AND THE
HORMONAL MEDIATION OF SIBLING RIVALRY IN
EASTERN BLUEBIRDS (*SIALIA SIALIS*)

By

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Abstract: In altricial animals, young are completely dependent on parents for provisioning. Because this provisioning is limited, the ability to outcompete siblings to receive food items and parental attention has clear fitness benefits. This ability may be mediated by hormones, particularly those that influence growth. In this study, I analyzed the effects of the hormone insulin-like growth factor 1 (IGF-1) on growth and sibling rivalry in eastern bluebirds (*Sialia sialis*). In order to determine whether this hormone is adaptively upregulated in response to high competition environments, I performed brood size manipulations and examined the effect of these manipulations on serum IGF-1 levels, morphometrics, and behavior. Additionally, I injected young bluebirds with exogenous IGF-1 to study its impacts on growth and sibling competition. I found that serum IGF-1 levels were not related to brood size, but fledging was later in larger broods. I also found a trend toward longer wings in larger broods. IGF-1 was positively related to mass late in the nestling period. Behaviorally, I found that larger nestlings were able to spend more time at the front of the nest box near the opening than their smaller siblings, however time spent at the front of the nest was not directly related to feeding rate, nor was there a direct relationship between IGF-1 and time spent at the front of the box. In the injection experiment, male nestlings had lower IGF-1 levels late in the nestling period than females. Consequently, IGF-1 was significantly increased by injection in males but not females. However, injection did not cause an increase in growth in either sex. The evidence collected here suggests that while IGF-1 may offer certain benefits to nestlings in terms of sibling competition, it is not upregulated to adapt to this competition.

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CHAPTER I

INTRODUCTION

Sibling rivalry and competition within broods are particularly important in species in which siblings compete for limited parental provisioning. This competition is seen across multiple taxa, to varying degrees. In extreme cases, this rivalry culminates in siblicide (Tarlow et. al 2000, Wahaj et. al 2007). An individual's level of success at securing provisioning from parents is integrally related to the likelihood that the individual will survive to sexual maturity. Consequently, investment in traits that promote success in sibling rivalry can have profound impacts on fitness. While many studies have dealt with the consequences of sibling rivalry (e.g., Bebbington et al. 2016, Both et al. 1999, Roulin et al. 2000, Tarlow et al. 2000), the physiological mechanisms by which individuals adapt to competitive brood environments are not well understood.

Postnatal behavior, such as begging, could be hormonally mediated either by endogenous or maternal hormones (Kitaysky et al. 2001, Schwabl 1996, Smiseth et al. 2011). Certain hormones, especially testosterone and corticosterone enhance begging behavior in birds, and levels of these hormones also affect survival and postnatal growth. However, the direction of the effects on survival and growth are inconsistent across species and may be context dependent (Smiseth et al. 2011). Most studies on the effects of hormones on begging behavior have quantified only these two hormones, and few

other hormones have been studied in this context (Smiseth et al. 2011). Growth rate, which is also hormonally mediated, could influence the outcome of sibling competition by providing larger individuals with a mechanical advantage in scramble competitions. The adaptive regulation of hormones involved in mediating growth rates could therefore provide individual siblings with an advantage in highly competitive environments.

Larger nestlings receive more food from parents and may have an increased probability of successfully fledging and recruiting (Both et al. 1999), so rapid growth early in life may be beneficial to nestlings in high competition environments (Kozłowski and Ricklefs 2011, Weibe and Slagsvold 2012). As a result, hormones involved in growth or securing food could have profound impacts on survival in early life. Insulin-like growth factor 1 (IGF-1) is a key mediator of postnatal growth in birds and in vertebrates in general (Lodjak et al. 2014, Lupa et al. 2001). Consequently, its upregulation could allow individuals within a brood to compete more effectively with siblings by promoting growth and subsequent competitive ability (Oddie 2000).

IGF-1 is a growth-stimulating hormone released primarily from the liver. It is necessary for growth and development across taxa, both in the embryonic stage and postnatally. Mice lacking functional IGF-1 and IGF-1 receptors grow to only 30% of normal weight (Baker et al. 1993). Postnatally, IGF-1 acts as a mediator of the effects of growth hormone (GH), but it acts independently of GH prenatally (Baker et al. 1993). As a result of IGF-1 signaling, cells are protected from apoptosis through multiple pathways. The primary pathway involves the binding of IGF-1 to its receptor and the subsequent activation of phosphatidylinositol 3-kinase (PI3-ki), which leads to Akt/protein kinase B (PKB) activation and resultant phosphorylation of BCL-2 proteins, which are involved in

apoptosis (Peruzzi et al. 1999). Additionally, the MAPK/Ras-Raf-Erk and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways are activated, leading to cell proliferation (Arnaldez et al. 2013).

While IGF-1 may provide benefits in terms of growth, high levels of IGF-1 have been associated with reduced lifespan (Lewin et al. 2016, Lodjak et al. 2017), and in the absence of siblings, individuals may down-regulate investment in IGF-1 mediated postnatal growth. There is some evidence to support this idea. Lodjak (2014) found that IGF-1 is negatively correlated with growth rate in Great Tit (*Parus major*) broods that had been reduced in size. This is somewhat counterintuitive, but Lodjak speculated that this is indicative of an adaptive downregulation of IGF-1 in larger nestlings that no longer need to grow rapidly. In support of this, IGF-1 levels naturally decrease as nestlings near fledging (Lodjak et al. 2017). IGF-1 allows young to invest simultaneously in both structural growth and mass gain (Lodjak et al. 2018), and as such, is directly involved in mediating the life history strategies of various species (Lodjak et al 2017). Research into the role of IGF-1 in mediating life-history strategies within and among species would be beneficial to our understanding of evolution and behavior, particularly in the context of sibling rivalry.

In this study, I analyzed the role of IGF-1 in modulating the ability of eastern bluebird (*Sialia sialis*) nestlings to effectively compete with siblings and receive food from parents. Specifically, I examined whether endogenous and exogenous IGF-1 are linked to rivalry related traits such as size and begging behavior. I had two hypotheses regarding the role of IGF-1 in eastern bluebirds. First, elevation of endogenous IGF-1 levels should stimulate faster nestling growth rates and be favored in larger broods.

Previous studies have found positive links between IGF-1 levels and growth rate (Lodjak et al. 2014, Lupu et al. 2001). I predicted that endogenous IGF-1 levels would be positively correlated with growth rates. I also expected that larger nestlings would receive more food items than their smaller siblings, and would maintain a better position in the nest (i.e., top of “pile” close to box opening), thus providing an indirect link between IGF-1 and competitive success. To test this hypothesis, I experimentally manipulated brood size to create single nestling broods, broods of two, enlarged broods, and control broods. I also provided each nest with supplemental food to reduce energetic constraints on IGF-1 mediated growth. I predicted that IGF-1 would be higher in nestlings raised in broods of two than in nestlings raised in broods of one. Furthermore, I predicted that nestlings raised in enlarged broods would have higher IGF-1 levels than nestlings raised in smaller broods.

My second hypothesis was that exogenous IGF-1 should increase nestling growth rates and that nestlings with elevated IGF-1 relative to siblings would grow larger and concurrently receive more food from parents. Because exogenous IGF-1 may induce the same elevated growth rate characteristic of naturally elevated IGF-1 (Lodjak et al. 2014), the same competitive advantages I expected to be conferred by endogenous IGF-1 (e.g. the ability to maintain a good position in the nest box and outcompete siblings for parental provisioning) should also be present in nestlings injected with recombinant IGF-1.

CHAPTER II

METHODOLOGY

Study Species

Eastern bluebirds nest in nest boxes and naturally occurring cavities (Stanback et al. 2019). They are socially monogamous, and a breeding pair may rear multiple clutches in a breeding season, each consisting of 3-6 eggs (Ambardar 2016, Gowaty and Plissner 1998, Pinkowski 1975, Soley et al. 2011). The number of clutches laid in a breeding season varies among populations, with the study population around Stillwater, OK (36°06'56.57"N and 97°03'35.15"W) typically laying a maximum of 3 clutches in a breeding season (Burtka and Grindstaff 2013). Hatching is fairly synchronous in this species, with the first and last eggs in a clutch hatching within 6-36 hours of each other (Soley et al. 2011). Hatching occurs roughly 14 days after incubation by the female begins (Burtka and Grindstaff 2013). Fledging occurs between days 16 and 22 post-hatch (Pinkowski 1975), though it can occur earlier if nests are disturbed. During the nestling period, both parents care for and feed nestlings (Burtka and Grindstaff 2013).

Monitoring, Video Analysis, and Blood Sample Collection

Seven nest box trails (147 boxes) near Stillwater, OK, were monitored for nest building activity by checking each box 1-2 times weekly from mid-March-August in 2018 and 2019. When nests were complete, boxes were monitored daily to determine the date on which the first egg was laid. Eleven days after the last egg of a clutch was laid, boxes were again monitored daily for hatching. When the date on which the last egg of a clutch was laid was uncertain, daily monitoring began on the earliest day that hatching could occur. The day on which the majority of the nestlings in a brood hatched was considered “day 0” of the nestling period. Adults were banded with United States Geological Survey (USGS) aluminum bands and a unique combination of colored plastic bands for identification. During the second field season of the study, a small subset of nests (n=13) were monitored for fledging to determine the effect of brood size and IGF-1 levels on age at fledging.

All nestlings were marked with non-toxic markers on the top of the head to facilitate differentiation among individuals within a brood. Nestlings were banded 11 to 14 days after hatch, but additional marking was necessary for identification in videos. In a subset of broods (n=27), the Pi Noir infrared camera for Raspberry Pi, in conjunction with an infrared LED bulb powered by a 1.5-volt alkaline battery, was used to film nestlings in the low light conditions inside nest boxes. Filming occurred on day 11 or 12 post-hatch and lasted for 3 hours. Notably, time of day, weather, season, and other factors were not consistent between filming events. Therefore, analysis of this data involved comparisons within nests rather than between nests, as described in the statistical analysis section below. The use of nest box cameras does not have a significant effect on behavior (Seress et al. 2017). The number of times each nestling was fed by a parent during the 3-

hr. recording period was quantified, as well as the amount of time each nestling spent at the front of the nest and the number of successful and unsuccessful begging attempts each nestling made. Because nestlings occasionally beg when no parent is in the nest box, I recorded whether or not a parent was present when a begging attempt was made. All video analysis was accomplished using BORIS behavioral observation software (Friard and Gamba 2016).

Blood samples (~25-100 uL) were obtained on days 5 and 14 post-hatch for measurement of hormone levels. To accomplish this, the brachial vein of each nestling was punctured with a 26.5-gauge needle, and blood was collected in heparinized microcapillary tubes. The time of day that each nest box was sampled was recorded, as well as the time elapsed between removing a nestling from the nest and completing sampling for that nestling. Body measurements were taken on days 5, 11, and 14. On days 11 and 14, mass, wing length, and tarsus length were measured. On day 5, only mass was recorded. Mass was measured to the nearest 0.1 grams with a digital pocket balance. Tarsus measurements were recorded to the nearest 0.01 mm using digital calipers, and wing length was measured to the nearest 0.1 mm using a wing rule. Nestling sex was determined by plumage coloration on day 14 (Gowaty and Plissner 2015). Blood samples were transported to the lab within three hours of sampling. In the field, blood was kept in microcentrifuge tubes placed in a cooler with ice packs. Plasma was separated from whole blood by centrifuging samples at 5000 rpm for 7 minutes. Plasma was stored at -80°C for no more than 18 months.

Brood Size Manipulation Experiment

Single nestling broods (n=13) were created by relocating nestlings to enlarged broods, which consisted of 5-8 nestlings (see experimental timeline, Figure 1A). Broods of 2 nestlings (n=15) were created in the same way. The total number of enlarged broods was 35. The size of control broods (n=7) was not manipulated, but entire four or five nestling broods were cross fostered between nests hatched within one day of each other to control for any effects of cross fostering. Nestlings were cross fostered within 36 hr. of hatching and were kept warm in transit using a warm water bag or rice bag when the temperature required it (Lodjak et. al 2014). All broods were provided with mealworms (*Tenebrio molitor*) to reduce any differences in provisioning between large and small broods. Approximately 15 mealworms (~ 1.217 g) per individual in the box, including parents, were placed in a small plastic cup inside the box three times weekly (Perryman 2017).

Because bluebird broods generally have substantially more than two nestlings (See *Study Species section*) (Grindstaff et al. 2012, Pinkowski 1975), I did not expect a significant difference in *per capita* provisioning between nests of one and two offspring, especially when the nests were provided with mealworms, as they were during this experiment. Bluebirds in the local population have been shown to increase provisioning to compensate for brood enlargement, with the number of nest visits per nestling by parents not significantly impacted by brood size (Ambardar 2016).

IGF-1 Injection Experiment

IGF-1 injection was carried out according to the protocol developed by Lodjak et al. (2017). IGF-1 doses consisted of 8.5 μg of human IGF-1 (Peprtech) suspended in 50 μL of a 15% gelatin solution and injected subcutaneously near the sternum. Dosages were adapted for the mean body weight of four-day old bluebirds, as reported by Pinkowski (1975). In 20 otherwise unmanipulated broods, one-half of the nestlings were selected at random and injected once a day for seven days, starting on the third day after hatching (Figure 1B). In nests consisting of odd numbers of nestlings, the last nestling was assigned either to the IGF-1 injected or control group in alternate nests. Control nestlings not injected with IGF-1 received a vehicle-only injection. Each nestling was injected with the same amount of IGF-1 on each day of injection. Because the nestlings gained body mass during this period, the dose of IGF-1 per gram of body weight decreased each day. This mimics natural cycles of IGF-1 during postnatal growth (Lodjak et al 2017). Blood samples were taken as described above and used to confirm that IGF-1 had been elevated by the administration of exogenous hormone. Body measurements were taken on days 5, 11 and 14 as described. Due to the predation of some broods and logistical constraints, not all broods were included at all 3 time points. The sample size on day 5 was 20 broods, and the sample size for days 11 and 14 was 13 broods.

Radioimmunoassay

IGF-1 levels in plasma samples were quantified using a radioimmunoassay (RIA) following methods in Echterkamp (1990). Plasma samples (12.5 μL) were acid-ethanol extracted and incubated at 4°C for 16 hours to remove IGF-1 binding proteins and then neutralized with 12.5 μL of 0.855 M Tris (Echterkamp et al. 1990, Spicer et al. 1988). Pooled extracted samples of bluebird plasma subjected to RIA yielded curves parallel to

chicken IGF-1 standards (Figure 2). All samples were assayed in duplicate. All samples from the injection experiment were run in one assay. Recombinant chicken IGF-1 (Kingfisher) was used to generate duplicate standards for standard curves for each assay. Additionally, a recovery of mass validation step resulted in approximately 100% recovery of recombinant IGF-1 added to extracted bluebird plasma. Samples were run over the course of six assays with an inter-assay CV of 14.4% and an average intra-assay CV of 10.4%.

Statistical Analysis

All statistical analyses were accomplished using SAS 9.4 (SAS Institute, Inc.). Normal distribution of hormone concentrations was confirmed graphically. To test for effects of manipulated brood size or IGF-1 injection on growth and IGF-1 levels, I used general linear mixed models with nest identity (i.e. an alphanumeric identifier indicating nest location and a numeric modifier to differentiate between different nests built in the same nest box throughout each season) as a random effect. The assay each sample was run in was included as a fixed effect. The amount of time between blood sampling and removing a nestling from the nest, as well as time of day, was not significant and therefore excluded from the model (Sample collection time: $F_{1,149}=1.66$, $p=0.200$, Time of day: $F_{1,65.1}=0.18$, $p=0.676$). For the injection experiment, sex differences in IGF-1 concentrations were analyzed using t-tests, while this was accomplished with a general linear mixed model for the brood size manipulation experiment in order to include the assay each sample was run in as a fixed effect. I also used general linear mixed models with nest as a random effect to test for relationships between IGF-1 levels, begging behavior, position in the nest, feeding rate and body mass. For these analyses, data were

standardized for each nest by centering mass, time spent at the front of the nest box, IGF-1 levels, begging events, and feedings by the means for each nest box. This was necessary because nest boxes varied substantially in terms of the frequency of begging and feeding events and sibling rivalry occurs within, rather than between, broods. By centering data by the nest mean, I was able to more effectively evaluate the relationships within broods between hormone levels, growth parameters, and sibling rivalry. For the nests monitored for fledge date, I used a general linear model to test for a relationship between brood size and age at fledging.

CHAPTER III

RESULTS

Brood Size Manipulation Experiment

IGF-1 levels did not differ significantly among brood sizes on either day 5 ($F_{3,112}=0.11$, $p=0.955$) or day 14 ($F_{3,88.3}=0.20$, $p=0.897$). Broods of 1 did not have significantly lower IGF-1 levels than either broods of 2 or control broods ($F_{1,22}=1.46$, $p=0.240$; $F_{1,22}=0.54$, $p=0.470$). Wing length tended to be positively correlated with brood size on day 14 ($F_{3,74}=2.72$, $p=0.0507$; Figure 3) but not day 11 ($F_{3,71.3}=1.17$, $p=0.323$). Mass gain between days 5 and 14 ($F_{3,63.5}=2.06$, $p=0.114$) and tarsus length on day 14 ($F_{3,79.3}=0.30$, $p=0.825$) were not affected by brood size. Irrespective of brood size, IGF-1 levels measured on day 14 were positively correlated with nestling mass on day 14 ($F_{1,177}=4.18$, $p=0.042$, figure 4). Day 5 IGF-1 was not significantly related to mass on either day 5 or 11 (day 5: $F_{1,255}=3.52$, $p=0.062$, day 11: $F_{1,191}=0.60$, $p=0.438$), though there was a positive trend toward increased weight with higher IGF-1 on day 5. Day 5 IGF-1 levels did, however, positively correlate with a nestling's change in mass between day 5 and day 11 ($F_{1,199}=5.41$, $p=0.021$). Wing length on day 14 was not significantly related to IGF-1 titer on day 14 ($F_{1,174}=1.42$, $p=0.235$), but IGF-1 levels on day 14 and tarsus length on day 14 tended to be positively correlated ($F_{1,197}=3.69$, $p=0.0561$). Age at

fledging increased with brood size ($F_{1,11}=12.10$, $p=0.005$; Figure 5). IGF-1 levels were not significantly different between male and female nestlings (day 5: $F_{1,182}=0.06$, $p=0.808$, day 14: $F_{1,195}=2.77$, $p=0.097$). There was, however, a trend toward higher IGF-1 levels in males on day 14.

Injection Experiment

Injection with IGF-1 from day 3 to day 11 post-hatch significantly elevated day 14 IGF-1 levels in males ($F_{1,24}=5.41$, $p=0.029$) but not females ($F_{1,17}=1.03$, $p=0.33$, Figure 6). This sex difference appeared to be driven by higher overall IGF-1 levels in females at this time point, resulting in a relatively smaller change in total IGF-1 due to injection. On day 14, females had significantly higher IGF-1 levels than males, counter to the trend seen in the brood size manipulation experiment. ($t_{43}=2.56$, $p=0.014$). IGF-1 injection did not affect the change in mass between days 3 and 14 for either sex (females: $F_{1,18}=0.86$, $p=0.36$; males: $F_{1,23}=0.36$, $p=0.55$; Figure 6). Wing length on days 11 and 14 was not affected by injection ($F_{1,25}=0.06$, $p=0.810$; $F_{1,24.9}=0.24$, $p=0.629$), nor was tarsus length ($F_{1,21.5}=0.00$, $p=0.957$; $F_{1,23.4}=0.04$, $p=0.839$). Injection did not result in significantly greater nestling mass on day 5, 11, or 14 ($F_{1,38.2}=0.44$, $p=0.511$; $F_{1,25.4}=0.280$, $p=0.603$; $F_{1,25.8}=1.23$, $p=0.278$).

Behavioral Analysis

To increase sample size, behavioral videos from broods used in the injection and brood size manipulation experiment were pooled. IGF-1 levels were not related to feeding rate ($F_{1,96}=0.32$, $p=0.574$, Figure 7) or begging behavior ($F_{1,96}=0.33$, $p=0.565$) of eastern bluebird nestlings. Feeding rate was not significantly related to day 11 or day 14

mass ($F_{1,128}=0.70$, $p=0.404$; $F_{1,103}=1.52$, $p=0.221$); however, nestlings that were heavier on day 14 spent more time in the optimal position at the opening of the nest box ($F_{1,84}=8.33$, $p=0.005$). Despite the positive correlation between mass and IGF-1 levels on day 14, IGF-1 levels and time spent at the front of the nest were not significantly correlated ($F_{1,89}=2.74$, $p=0.101$). Also, even though there was a relationship between nestling body size and nest position, time spent at the front of the nest did not significantly correlate with the number of feedings a nestling received in the three-hour filming period ($F_{1,117}=2.86$, $p=0.094$), though there was a positive trend toward increased feedings with larger amounts of time spent at the front of the nest.

CHAPTER IV

DISCUSSION

In this study, I analyzed the effects of IGF-1 on growth and sibling competition. I hypothesized that elevation of endogenous IGF-1 levels would stimulate faster nestling growth rates and be favored in larger broods. While endogenous IGF-1 levels were associated with faster mass gain, brood size manipulation did not impact these levels. I also predicted that larger nestlings would receive more food from parents and spend more time near the front of the nest. In fact, heavier nestlings did spend more time near the front of the nest, but they did not receive significantly more food. My second hypothesis was that exogenous IGF-1 in the form of daily injections should increase nestling growth rates and that nestlings with elevated IGF-1 relative to siblings would grow larger than their siblings. This hypothesis also was not supported, with injected nestlings not significantly differing from siblings in terms of mass, tarsus length, or wing length.

I found that while IGF-1 is related to mass gain late in the nestling period, it does not appear to be upregulated in response to elevated sibling competition. IGF-1 has been shown to decrease with dietary restriction (Dunn et al. 1997), so reduced *per capita* provisioning could prevent its upregulation. Eastern bluebirds in our population have

been shown to increase provisioning efforts in response to artificial increases in brood size (Ambardar 2016), but many of my manipulations increased brood size beyond what occurs naturally, potentially exceeding the ability of parents to compensate. Parents might have been unable to completely compensate for brood enlargement through increased provisioning, despite supplemental feeding, and thus IGF-1 levels were not significantly elevated.

Conversely, artificially increasing brood size tended to increase wing length. While this trend was not statistically significant, it may be biologically significant. The sample size for reduced broods was much smaller than that of enlarged broods, due to the logistics of cross-fostering. Most likely, the increase in wing length is indicative of an allocation of resources toward fledging-related traits to facilitate leaving a stressful or dangerous brood environment, as wing length has been shown to be the best morphometric predictor of fledge date (Kouba et al. 2015). However, this does not result in early fledging in enlarged broods, which in fact fledged later than smaller broods in this study. Relative wing length has been shown to be a good predictor of flight ability (Jones et al. 2019) and a tradeoff between wing length and ability to reach an ideal fledging weight has been established in Nazca Boobies (*Sula granti*) (Maness and Anderson 2013). In the same study, both wing length and fledging mass were positive indicators of survival. This indicates that the stress of a larger brood environment may trigger a shift in resource allocation towards wing growth, which may aid in survival during the vulnerable post-fledging period by increasing flight ability (Jones et al. 2019). This would result in improved predator avoidance and consequent improved fitness, as predation is a key cause of death during this period (Naef–Daenzer and Gruebler 2016).

While I did not find a significant correlation between day 14 mass and brood size, other studies have found that weight at fledging is depressed in enlarged broods (Ward and Bird 1992). The supplemental food provided to nestlings in this experiment may have negated the effects of brood enlargement on mass during the late nestling period.

Potentially, wing growth is less energetically costly than mass gain, and as such can serve as a compensatory mechanism to mitigate the decreased fledging mass associated with large broods.

I also found that exogenous IGF-1 injections did not increase growth in eastern bluebird nestlings. Previously, this injection protocol has been shown to cause an increase in both body mass and tarsus length in Great Tits (Lodjak et al. 2017). Because IGF-1 injections only significantly increased IGF-1 levels in males in this study, an increased dose of IGF-1 may be required to significantly increase growth rates of bluebirds. Alternatively, smaller, more frequent doses may have been more effective. A study of growth hormone deficient mice found that single, large doses of IGF-1 did not increase growth rate and caused hypoglycemia, whereas smaller doses administered multiple times per day resulted in elevated weight gain (Woodall et al. 1991). The gelatin used as the vehicle in my injections increases the half-life of the injection (Lodjak et al. 2017), but this may not have been sufficient to negate the aforementioned effects. Cannulation studies on captive animals may be useful for determining the effect of exogenous IGF-1 released over a longer timeframe. Another multi-year study found that injection of IGF-1 with this protocol in house sparrow (*Passer domesticus*) nestlings caused an increase in growth only during certain years, indicating that exogenous IGF-1 may only increase growth under specific conditions (Sirman 2019). The human IGF-1 used in the injection

experiment is not identical to the endogenous IGF-1 of eastern bluebirds. While the amino acid sequence of eastern bluebird IGF-1 is not available, the sequence published by the human IGF-1 manufacturer (Peprotech) shares 90% of its 70 amino acids with that of chickens (*Gallus gallus*), and the sequence tends to be very conserved within birds (Genbank, Peprotech). The human IGF-1 injected should be readily detectable by the RIA used to measure it, because the antibodies used were developed against human IGF-1.

The difference in day 14 IGF-1 levels between males and females in the injection experiment could have arisen in two ways. First, it could reflect a sex difference in the life-history strategies of eastern bluebird nestlings. This possibility is lent some credibility by previous work on the local bluebird population, which found that female nestlings had greater telomere attrition late in the nestling period than did males (Ambardar 2016). Additionally, other work has found that telomere length is inversely related to IGF-1 concentration in young Franklin's Gulls (*Leucophaeus pipixcan*), and that exogenous IGF-1 is associated with shorter telomeres late in the nestling period under certain conditions (Sirman 2019). Because IGF-1 is inversely related to lifespan (Lewin et al. 2017, Lodjak et al. 2017), it is possible that it is a mediator of pace-of-life via a trade-off between growth and telomere integrity. Second, the difference in IGF-1 levels between males and females in the injection experiment could have been driven by sex-specific responses to handling stress. Given that there was a trend toward higher IGF-1 levels in males in the brood size manipulation experiment, in which nestlings were not handled as frequently, this possibility seems more likely. Under certain circumstances, males may release more corticosterone in response to handling than females (Kalinichev

et al. 2002), and in birds, IGF-1 has been shown to decrease in response to handling stress (Toth et al. 2018). Males may decrease their IGF-1 levels in response to frequent handling more than their female counterparts do. Alternatively, males may have increased their IGF-1 in response to increased competition in the brood size manipulation experiment.

Begging rate was not related to IGF-1 titer. Most bluebird nestlings begged each time a parent entered the nest, unless they had recently received one or more consecutive food items and subsequently become satiated, or they were mechanically prevented from doing so by an adjacent sibling impeding their movement. Consequently, IGF-1 may not play an important role in mediating begging behavior in bluebirds.

Larger nestlings were more easily able to attain and maintain the position at the front of the nest box. While IGF-1 titer did not significantly predict the ability to attain and maintain the position at the front of the nest box, time at the front of the nest box was positively related to mass, which suggests that IGF-1 is indirectly related to a nestling's ability to maintain a favorable nest position. Neither IGF-1 titer nor nest position were significantly related to feeding rates over the three-hour filming period, but larger nestlings maintained better nest positions than their siblings, and this indicates that over a longer time period, greater mass and IGF-1 concentration might result in higher feeding rates, particularly given that the relationship between time spent at the front of the nest box and feeding rate approached significance. Alternatively, it is possible that eastern bluebird parents are more egalitarian in their feeding strategies than some species and do not differentially feed nestlings based on size or nest position, resulting in the lack of

increased feedings received by nestlings at the front of the nest. However, it seems unlikely that the competition for favorable nest positions observed here would be favored unless there is a biologically significant tendency toward asymmetrical feeding. In other words, there would be little reason to compete for a position in the nest that has no advantage associated with it. Future work with this species should determine whether male and female parents differentially discriminate between nestlings based on size or nest position, as is seen in Tree Swallows (*Tachycineta bicolor*) (Leonard and Horn 1996).

Sibling competition in eastern bluebirds is not well studied, and this study sheds light on the interaction between growth, hormones, and behavior in this species. Potentially, this knowledge could be applied to our understanding of the evolution of life-history strategies and post-hatching behavior in diverse avian taxa. Further work is required to understand the relative costs and benefits of IGF-1 and other hormones in the context of competition. Particularly, work on species with heavily asymmetrical competitions, such as species that exhibit siblicide, could be very informative. Similar work with brood parasites, particularly invasive ones, could be very useful from an ecological perspective. In both of these cases, the ability to outcompete siblings has exaggerated fitness consequences, often to the point that survival is contingent upon competitive dominance. A species adapted for brood parasitism, for example could be expected to prioritize rapid growth. Work in these areas could add to our understanding of the evolution of competition and its hormonal drivers.

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APPENDICES



A.



B.

Figure 1. Experimental timelines for brood size manipulation experiment (A.) and injection experiment (B.)

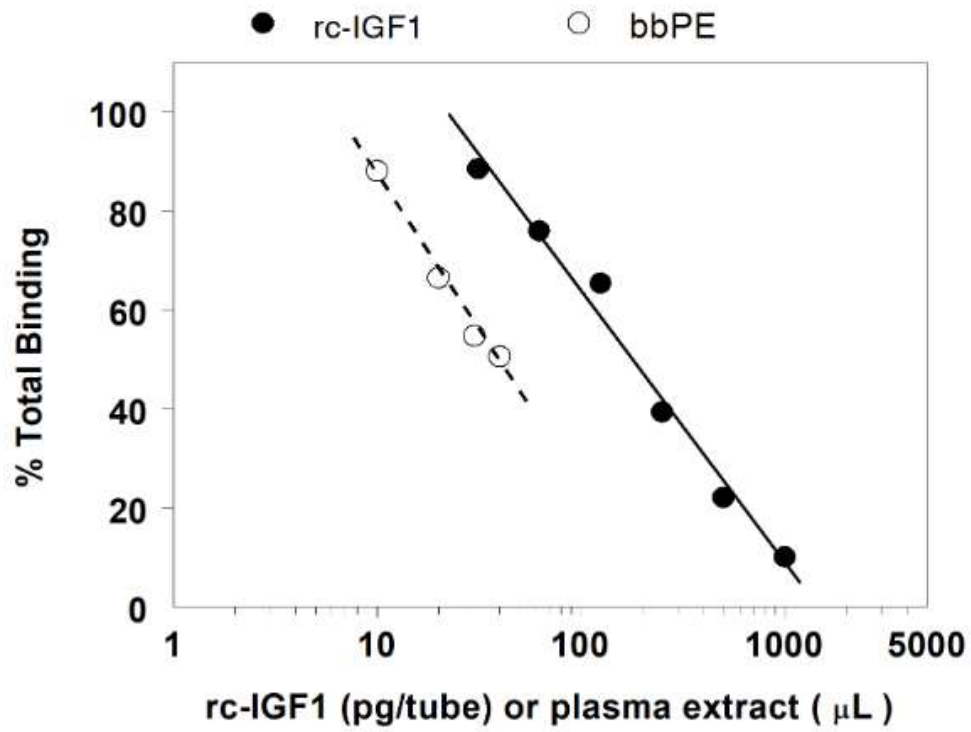


Figure 2. Parallelism between recombinant chicken IGF-1(rc-IGF1) standard curve and acid-ethanol-extracted Eastern Bluebird plasma (bbPE). rc-IGF1: slope=-63.99, $r^2=0.994$; bbPE: slope=-55.09, $r^2=0.992$

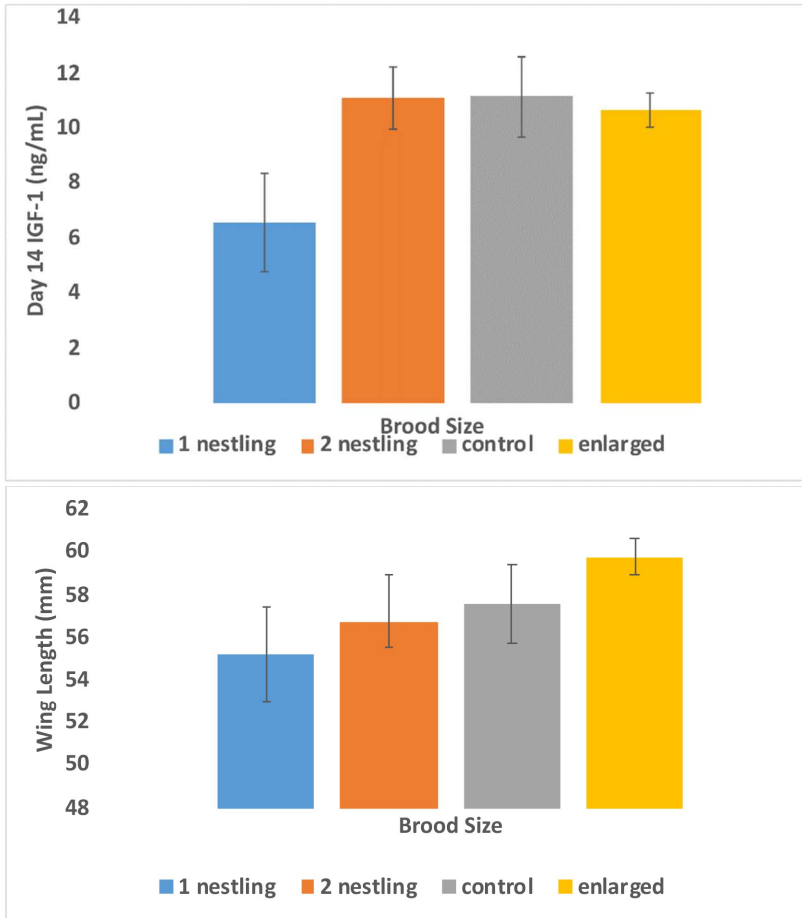


Figure 3. Day 14 IGF-1 levels did not differ significantly by brood size, but wing length increased with brood size (error bars= standard error).

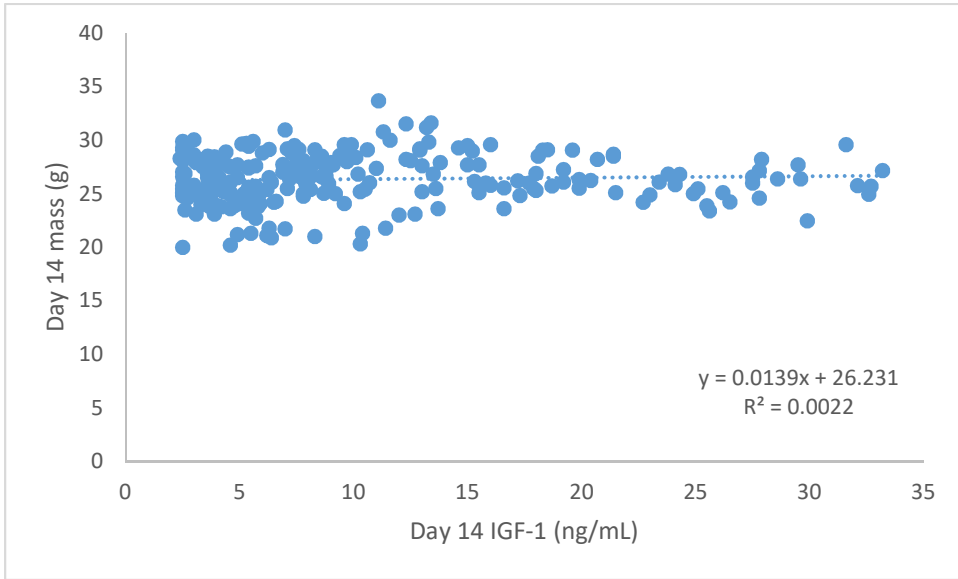


Figure 4. Day 14 mass increased significantly with day 14 IGF-1 titer.

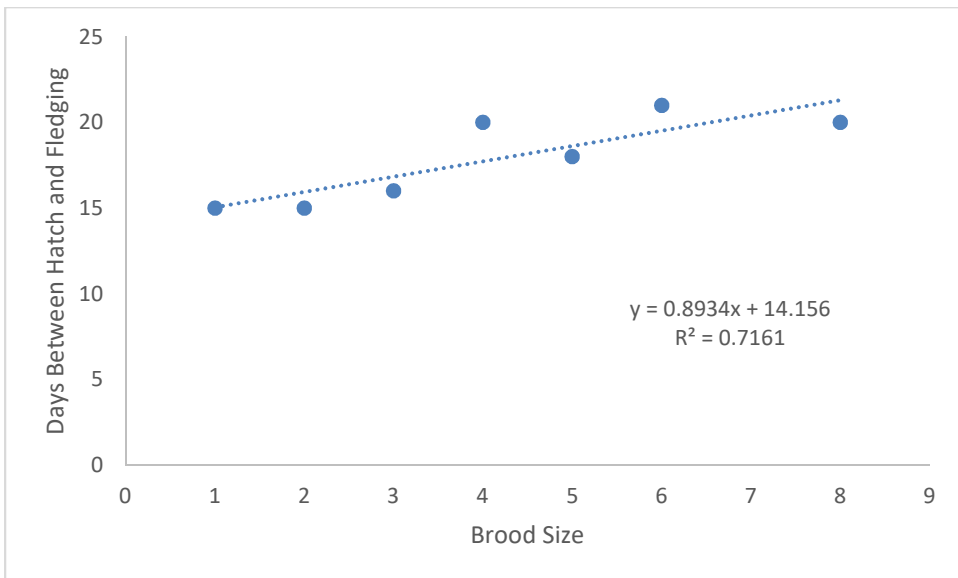


Figure 5. Average age at fledging increased with brood size.

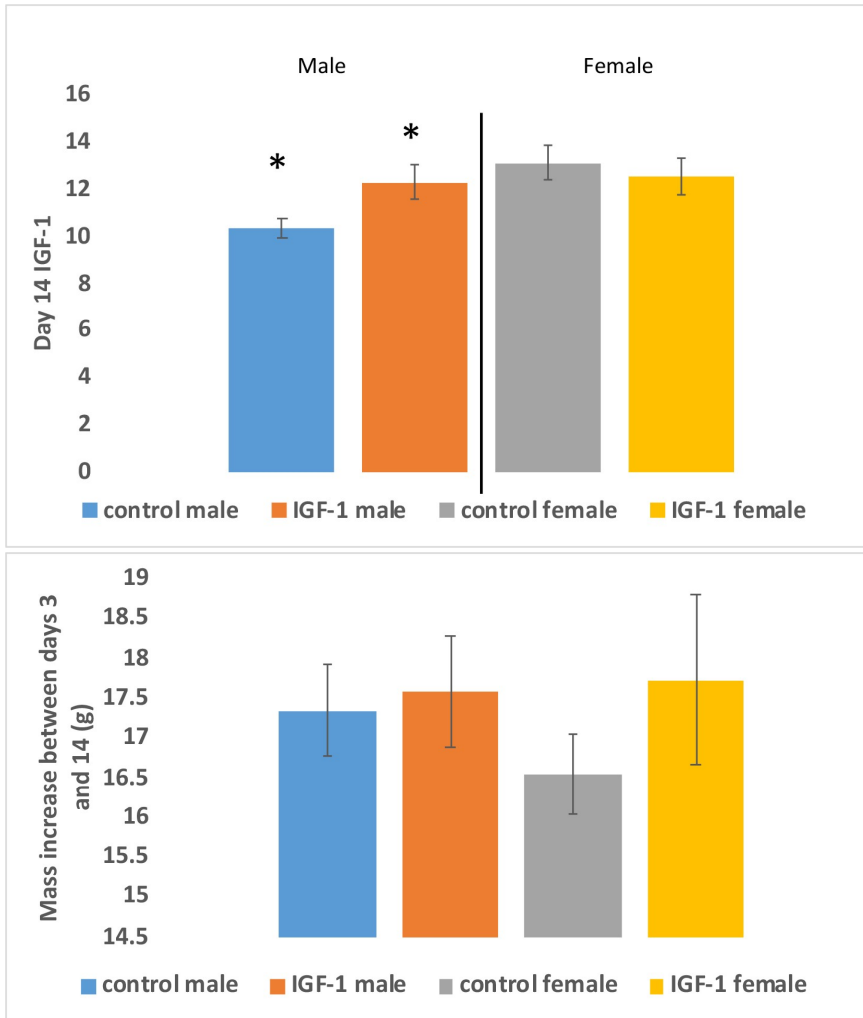


Figure 6. Day 14 IGF-1 levels were elevated by injection in males but not females. IGF-1 injection did not significantly increase growth rate in either sex (Error bars= standard error) Asterisks (*) indicate significant difference within a sex.

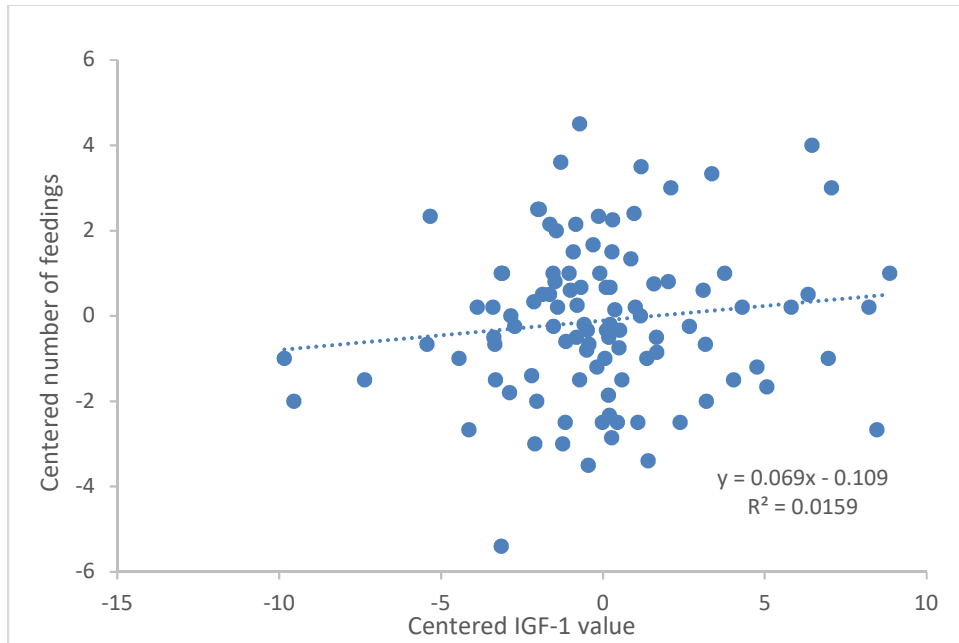


Figure 7. IGF-1 levels and number of feedings (both centered around the average for each nest) were not significantly correlated.

VITA

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