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Evolution and development of wing form, body size and flight in large- and small-bodied fruit bats (Artibeus jamaicensis and Carollia perspiciillata)

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THE EVOLUTION AND DEVELOPMENT OF WING FORM, BODY SIZE AND FLIGHT IN LARGE- AND SMALL-BODIED FRUIT BATS (*ARTIBEUS JAMAICENSIS* AND *CAROLLIA PERSPICIILLATA*)

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has been approved as meeting the requirement for the Degree of Doctor of Philosophy in the College of Natural and Health Sciences in the School of Biological Sciences, Program of Biological Education

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ABSTRACT

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Differences in developmental patterns important to diversification are produced through heritable variation of the onset/offset and timing of juvenile growth. As the size and shape of an organism changes during ontogeny, morphological, and behavioral components must adjust to accommodate proper function. This study explored the ontogenetic pathways of two closely related Phyllostomids differing in flight ability, body size, life history strategies, and developmental state at birth. We hypothesized that *Artibeus jamaicensis* and *Carollia perspicillata* will show ontogenetic differences that account for the diversification of morphological, body size, and behavioral patterns. Comparisons between the two species' flight development, growth rates, and morphometrics were made from day 1 to adult size (AJ $n = 45$, CP $n = 25$). Forearm length, mass, wing area, and wingspan were measured on a daily basis. Flight behavior was compared with juveniles being dropped from a 1 meter high roost from day 1 post-partum. Logistic growth equations were used to compare growth rates of all measured parameters and *t*-tests ($p < 0.001$) showed significant differences between the species of all measured variables. Muscle development in the pectoralis major was

significantly different with *A. jamaicensis* having significantly more slow-twitch fibers. There were significant differences between the day of first flap (*t*-test, $p = 0.01$) and flight (*t*-test, $p < 0.0001$) with *C. perspicillata* achieving flight at 22 days and *A. jamaicensis* achieving flight 33 days post-partum. *C. perspicillata* was shown to be significantly more maneuverable than *A. jamaicensis*. Our data suggest that growth trends are significantly different with the more altricial *A. jamaicensis* developing faster than the more precocial *C. perspicillata*. Ontogenetic comparisons are important proxies when determining evolutionary diversification of closely related species. Data can be combined with phylogenetic information, providing possible mechanisms as to what factors could have influenced the divergence of closely related species.

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CHAPTER I

INTRODUCTION

Organisms evolve over time producing many different species that coexist and are closely related, exploiting different niches. Species vary both phenotypically and genotypically and a large portion of these variations are inherited as an integral part of evolution. Developmental processes are connected to evolution by the changes that occur in organisms during embryogenesis through adulthood through inherited variation. The form these developing organisms achieve is the result of two processes; evolutionary steps from a common ancestor (Phylogeny) and the developmental process from an egg (Ontogeny). Phylogeny pertains to relationships, specifically the relationship of organisms, which is categorized by convergence and divergence from a common ancestor throughout their evolutionary history (King & Stansfield, 1985). In this sense phylogentic trees emphasize changes that occur in relatedness and not specifically an ancestor. The steps of divergence or convergence can be seen in many instances throughout the developmental pathway of an organism.

Garstang (1922) expressed that "ontogeny does not recapitulate phylogeny, it creates it." Ontogeny has been linked to phylogeny in taxonomic analysis of relationships between organisms. Nelson (1973) recommended that an ontogenetic series provides a scientific and organized means of ordering organisms, with more widespread characteristics being more primitive and more specialized characteristics being more advanced. Ontogeny and phylogeny are two aspects that are key to evolutionary

processes with phylogeny reflecting changes in ontogeny. To understand the divergence of the multitude of animals, it is imperative that these two relationships are understood. Darwin (1859) introduced the idea that over many thousands of generations and comprising millions of years, body parts and processes can change, producing different forms that are adapted to specific circumstances.

Changes in the rules of development result in phylogenetic changes in the evolutionary line and are represented by changes in the morphology of the developing organism. Morphology refers to the shape and the pattern of specific structures which represents developmental changes throughout evolutionary history. Evolutionary changes can be understood by looking at small genetic changes that can cause variation in form (Huxley, 1942). Many of the traits that change are not often noticeable in adults, however, they can be observed during developmental growth phases.

Development of form is regulated by the turning off and on of regulatory genes throughout the developmental period. Specifically, changes in regulatory genes and the DNA that contains the instructions for development can have an effect on the size and shape of structures within an organism, leading to diversity of forms (Baguna & Garcia-Fernandez, 2003). Genes influence evolutionary change through duplications, mutations, and reordering of protein sequences. Observations have shown that the majority of the differences among closely related species are from simple changes that occur, such as developmental timing and in many cases this can be observed during ontogeny (McKinney & McNamara, 1991).

Alberch, Gould, Oster, and Wake (1979) described ontogeny as developmental patterns that are the outcome of differential events that alter morphological form. This

suggests that we can make inferences regarding closely related individuals' evolutionary trajectories based on changes that occur during developmental periods. The majority of these observations can be classified and measured using specific measurements of change in size and shape, as in the morphometric analysis accomplished using allometry and heterochrony (Haeckel, 1887; Huxley, 1942; McKinney & McNamara, 1991). Heterochrony is a concept that pertains to how developmental processes are arranged and organized and not necessarily a developmental mechanism (Hall, 1990).

De Beer (1930, 1958) stated that the change in developmental timing of an organism relative to an ancestor's is an important developmental step, linking ontogeny to phylogenetic changes. Allometry describes trait changes relative to other traits while heterochrony addresses trait change relative to time, specifically the change in timing or rate of a developmental event (McKinney & McNamara, 1991). Observations of growth and the outcome of comparing allometric and heterochronic patterns allow for specific links between ontogeny and phylogeny to be made.

With this in mind, growth is ordered, and follows temporal patterns like rate, duration, and offset and onset (Raff, 1996). These patterns are categorized using heterochonic analysis in a variety of ways. An organism may have whole body developmental timing differences or differences may be just a specific part of the organism. These changes are associated with trends, such as an organism beginning growth earlier or later in which the onset of development is shifted. Ontogeny may be prolonged with offset of development occurring at a later time period. The rate of development can change without any shifts in onset or offset of growth, with development being either accelerated or retarded. These patterns are fundamental in

development and the evolutionary history of organism. Evolutionarily, during ontogeny it is essential to show that one or more of these patterns has been altered. Heterochrony may affect the entire organism (Raff & Wray, 1989) or be local, affecting specific tissues or organs within an organism. When there is a change in form due to growth differences, an organism will show a change in size and in shape.

Size and shape change can be determined by using allometry (Gould, 1977). Allometry can be especially important in determining morphological changes that occur based solely on trait comparison. Thompson (1961) emphasized that there are important relationships between an organism's form or shape and its biological function. This can provide information on the association between changes in size and shape that happen over ontogenetic time periods and the behavioral outcomes of these specific changes. Allometric associations occur when there is significant change in the trait along with size differences. Importantly, allometric comparisons can be compared between individuals both intra- and interspecifically.

When age is known, heterochrony also allows direct comparison between a trait and age, termed longitudinal sampling. This can then be used to compare rates of growth between species and with an ancestor, however, in practice, heterochrony can be used in a comparative aspect, in regards to changes that occur among taxa that are closely related (Smith, 2003).

Gould (1977) broke heterochrony into two major parts, paedomorphosis and peramorphosis. Paedomorphosis pertains to the organism reaching maturity with juvenile features still existing. Progenesis occurs when an organism grows at the same rate, however, they stop growing while in the juvenile form. Neoteny occurs when the

organism has specific body parts that grow at a slower rate, maintaining juvenile form when maturity occurs. Lastly, predisplacement, is when the organism starts growing at a later period of time reaching maturity with juvenile characteristics.

Peramorphosis consists of growth that goes beyond that of the ancestor prior to reaching maturity. First there is hypermorphosis, occurring when the organism grows for a longer period of time prior to reaching maturity, becoming larger in size. Acceleration occurs when an organisms grows at a faster rate than the ancestor, again obtaining a larger size. Lastly, predisplacemnt occurs when the organism starts growth sooner than the ancestral organism.

For heterochrony to be truly effective the trait needs to be compared directly to age. This will give information on specific timing of the developmental changes that occur during ontogeny.

In addition to the analysis of growth patterns, analysis of life history traits is also an important factor when looking at changes in body size and shape. Life history strategies can include many aspects of an organism including: size at birth, age at weaning, size and age at maturity, number of offspring, growth patterns and length of generations. Many animals are born either in a precocial or altricial state or somewhere along this developmental spectrum. This strategy has been found to influence growth parameters and timing (Ricklefs, 1973).

Like morphology, the behavior of an organism changes as it goes from a juvenile state to adulthood. These changes can be at discrete points of time or occur gradually as the organism ages. An organism's behavior as with morphology can be affected by an organism's specific growth patterns. Changes in timing and rates of growth will dictate

when an organism is capable of performing certain behaviors. This shows that the final outcome of the changes in size and shape will determine the specific changes in behavior and the way the organism interacts within its ecosystem.

Allometry and heterochrony can be an effective way to decipher information regarding bat evolution, which is widely unknown and somewhat controversial. The fossil evidence has provided little help in-regards to bat evolution, with a few exceptions (Caple, Balda, & Willis, 1983; Thewissen & Babcock, 1992). Based on the lack of evolutionary history in-regards to the fossil record, phylogenetic studies have tried to link bats together based on phenotypes and genotypes (Jones & Teeling, 2006).

Allometric and heterochronic analysis can prove to be an important tool in understanding the evolutionary pathway that bats have followed by comparing ontogenetic patterns and the ecological implications of coexistence of closely related species. These observations can be studied both behaviorally, such as flight abilities and foraging patterns, and morphologically, looking at changes that occur to the overall body and wing structure during development.

Bat flight morphology is adapted to the mode of flight and foraging techniques specific for each individual bat species (Norberg, 1981, 1987, 1990; Norberg & Rayner, 1987). For example, open-foragers can have longer wings while bats that forage in dense vegetation have short wings.

In order for bats to be successful as they fly, they must expend energy, using muscles for wing movement. This movement will generate lift and thrust which is required for flight (Norberg, 1990; Pennycuick, 1975). The power expended by the bat depends on their body size and wing shape. The size and shape of wings can be

described in general by specific measurements such as wing loading and aspect ratio. Wing loading is the relationship between body mass and wing surface area, while aspect ratio is the relationship of the wingspan squared and the width of the wing, with wing surface area generally being used for this comparison (Norberg & Rayner, 1987). Wing loading and aspect ratio will vary in bat species depending on body size and wing morphology and has also been linked to specific flight abilities and patterns. Narrow wings with small areas have high wing loading and low aspect ratio. These bats, however, must fly fast to gain the appropriate lift. Most fast flying bats in order to reduce energy costs will have short wings. Bats with long wings and slow flight have low wing loading and high aspect ratio. This allows for sustained flight while reducing energy needs, such as in migration (Pennycuick, 1969).

Information as described above is available regarding flight and wing morphometrics in adult bats, less is known about bat ontogeny and its implication on evolution. Growth of the juvenile wing, however, has been observed in some species of bats. Juvenile evening bats, *Nycticeius* humeralis, show an accelerated growth of the forearm and digit V between days 0 and 35 (Jones, 1967). *Rhinolophus ferrumequinum*, a horseshoe bat, shows a pattern of wing span and surface area increase between days 0 and 30 (Hughes, Ransome, & Jones, 1989). The little brown bat, *Myotis lucifugus*, also showed an accelerated growth of the wing span and surface area between days 0 and 30 (Powers, Kandarian, & Kunz, 1991). Powers et al. (1991) also correlated wing loading with flight ability and showed a strong correlation (r-squared = 0.85) between wing loading and age. Taft and Handley (1991) quantified basic growth characteristics and wing morphology of *Artibeus jamaicensis* on Barro Colorado Island, Panama. Chaverri

and Kunz (2006) found that tent-making bats (*Artibeus watsoni*) sustained flight after 35 days and at 100% of adult forearm length and 80% of adult weight. Swift (2001) found that juvenile Natterer's bats (*Myotis nattereri*) achieved flight at 20 days. Elangovan, Priya, Raghuram, & Marimuthu, (2007) found that short-nosed fruit bats (*Cynopterus sphinx*) sustained flight at 55 days of age. Research on the ontogeny of flight has been quantified mainly with small insectivorous bats. These bats are fast flying, have small wings and are highly maneuverable. They also hunt primarily in open, less dense habitats for small flying insects. There is a need to expand to different bat species that use different flight and feeding behaviors as well as comparing two closely related species that encompass different life history traits, to determine evolutionary trajectories. There is also a lack of comparative empirical data supporting the ideas of heterochrony and allometry as important factors in the diversification of the order Chiroptera.

The frugivorous bats *Artibeus jamaicensis* and *Carollia perspicillata* are in the same family yet fall within different subfamilies, with Carollia being the more ancestral form, phylogenetically (Baker, Hoofer, Porter, & Van Den Bussche, 2003). They have different body sizes and overall different wing structure that leads to their foraging habitats, either in dense vegetation in the jungle understory as with *Carollia perspicillata* or within or near the canopy as with *Artibeus jamaicensis* (Cloutier & Thomas, 1992; Fleming, 1988; Ortega & Castro-Arellano, 2001). Both species have large, wide wings which enables them to fly slower and carry large loads to a roost site (Cloutier & Thomas, 1992; Fleming, 1988; Ortega & Castro-Arellano, 2001). Adult wing loading measurements show that both species have medium to high wing loading and lower aspect ratio which corresponds to medium flight speed within vegetation with the ability

of short hovering bouts as with *C. perspicillata* (Altringham, 1996; Norberg, 1990). The observed differences in size, shape and foraging behaviors between these two bat species make the measurements of their ontogeny of flight and development of great evolutionary interest.

Using allometric scaling and heterochronic rates, information regarding specific traits can be compared between these closely related animals. Heterochrony can also help in determining the differences if any in growth rates and overall developmental trajectories these bats follow. The differences in growth rates can bring insight to how two closely related species may have diverged from a common ancestor and be of different body size and shape.

Due to the interest in bat development and evolution of morphological diversity within this order, there were two main objectives to this study. Objective 1: Observe and quantify the variation in growth patterns and rates of the wings, body size, and muscle development between *A. jamaicensis* and *C. perspicillata*. Objective 2: Quantify how growth rates affect the flight development and performance of both bat species which in turn can have ecological implications.

H1: Differences in ontogenetic pathways lead to distinct variation in adult form.

Prediction*: Artibeus jamaicensis* and *Carollia perspicillata* will show ontogenetic differences that account for the diversification of morphological, body size, and behavioral patterns.

H2: Divergent development between species leads to differing rates of flight development.

Prediction: The more precocial *C. perspicillata* will achieve flight earlier than *A. jamaicensis* due to the fact that more energy can be applied to flight development rather than overall body development.

H3: Divergent development between species leads to differing flight agility and maneuverability.

Prediction: *C. perspicillata* will have wing morphology traits that allow for more maneuverable flight than *A. jamaicensis,* based on adult flight ability and foraging habits.

Delimitations

This research was limited to a captive colony of both *A. jamaicensis* and *C. perspicillata* that was housed in the animal facility at the University of Northern Colorado. Ontogenetic observations were limited to the amount of babies that were conceived by the adult bats within the colony. All methods were approved by the Institutional Animal Care and Use Committee (IACUC) committee at the University of Northern Colorado and research methods stay within the approved protocol.

CHAPTER II

REVIEW OF LITERATURE

Artibeus jamaicensis and Carollia perspicillata

Artibeus jamaicensis is a member of the order Chiroptera, suborder Microchiroptera, family Phyllostomidae, subfamily Stenodermatinae, tribe Stenodermatini, and genus *Artibeus* (Baker, Hood, & Honeycutt,1989; Baker et al., 2003). The subfamily Stenodermatinae, according to Baker et al. (2003) is the most recently evolved subfamily in the family Phyllostomidae (Figure 60 in Appendix A). This subfamily contains the highest biodiversity and species numbers of all the subfamilies with 62 species and 20 genera. The tribe Stenodermatini contains at least 50 species and 19 genera (Baker et al., 2003).

Artibeus jamaicensis is widely distributed, ranging from central Mexico to northern Argentina being widely distributed throughout the Amazon basin. They are commonly found as well on the islands of the Caribbean (Ortega & Castro-Arellano, 2001). *A. jamaicensis* is a habitat generalist, being found in wide range of habitats from humid tropical to dry tropical rainforest locations (Morrison, 1979). They are also fruit generalists, eating a wide variety of fruits which are usually located in or near the canopy (Gardner, 1977).

Artibeus jamaicensis has two estrous cycles annually and produces two to four young per year with single births being more common (Heithaus, Fleming, & Opler, 1975). Normal gestation is 3.5 to 4 months in normal environmental conditions (Taft &

Handley, 1991). Young are carried by the mother; however, the mother leaves the juvenile at the roost site later in the developmental period (Ortega & Castro-Arellano, 2001).

Carollia perspicillata is a member of the order Chiroptera, suborder Microchiroptera, family Phyllostomidae, subfamily Carolliinae, and genus *Carollia* (Baker et al., 1989; Baker et al., 2003). Subfamily *Carollia* contains only on genera containing the *Carollia* species (Baker et al., 2003). This subfamily evolutionarily evolved prior to the subfamily Stenodermatinea making the genus *Carollia* more ancestral to that of the genus *Artibeus* (Figure 60 in Appendix A).

Carollia perspicillata are a wide spread species, ranging from south central Mexico to southern Brazil throughout the Amazon Basin (Cloutier & Thomas, 1992). They are found in humid tropical to dry tropical forests (Fleming, 1988). They forage near the ground in the dense understory and has been found to be a fruit and flower generalist with nectar and pollen consumption occurring during the dry season and low fruit years (Fleming, 1988).

Carollia perspicillata have two estrous cycles per year and usually produce 1 young per pregnancy (Fleming, Hooper, & Wilson, 1972). Newborns are born in a precocial state with eyes and ears open as well as dense fur covering the entire body (Kleiman & Davis, 1979). Adults have been found to fly with the juveniles until they are ready to wean (Porter, 1978).

The family Phyllostomidae constitutes a large proportion of extant bats with 53 total genera and 141 total species (Wetterer, Rockman, & Simmons, 2000) with a high degree of morphological variation. This family has been found to be monophyletic, with

a wide degree of morphological variation occurring from evolutionary events that have occurred since their divergence from a common ancestor (Baker et al., 2003).

The Jamaican fruit bat, *Artibeus jamaicensis,* and the short-tailed fruit bat, *Carollia perspicillata,* are fruit bats that coexist in similar habitats and were used for this study (Lopez & Vaughan, 2007). Both species have been found to flourish in captivity (Cretekos et al., 2005; Taft & Handley, 1991). *Artibeus jamaicensis* is born in a more altricial state, with less fur covering the body and smaller body size when compared to adults, making it more dependent on its mother for thermoregulation and care (Kleiman & Davis, 1979). Females become sexually mature at 8 months and males at 12 months (Keast & Handley, 1991). *A. jamaicensis* is a large bodied, heavy (30-50g) fruit bat that has fast flight speeds and higher wing loading. This allows for long distant flight as well as maneuverability amongst the vegetation while carrying heavy loads such as figs (Kalko, Handley, & Handley, 1996; Norberg & Rayner, 1987; Taft & Handley, 1991).

Carollia perspicillata are born in a more precocial state, with its body being completely furred and a larger body size overall when compared with adult *Carollia perspicillata*. This allows for better thermoregulation and energy budgeting for other aspects of growth (Kleiman & Davis, 1979). It has been shown that some females reach sexual maturity by 8-9 months with all females becoming sexually mature by 1 year (Porter, 1979a). Males become sexually mature between one and two years of age (Taddei, 1976). They are a smaller (15-25g) bat and are rapid fliers that are highly maneuverable, with high wing loading (Fleming, 1988; Heithaus & Fleming, 1978).

Ontogenetic Implications of Bat Ecology and Co-existence

Gould (1977) stated that different kinds of heterochrony may be associated with different life history trajectories. Many of these developmental heterochronies have led to different traits that allow for animals to coexist, benefiting from the use of different regions within the same habitat. Closely related animals with different body types and morphological features can inhabit the same locations while foraging or exploiting different locations within that habitat (Tokehsi, 1999).

All of these principles can be informative of the evolutionary trajectory that certain animals have taken and how they differ from closely related taxa. The observation of ontogeny, specifically morphological aspects, can tell much about the evolutionary development that an animal has followed (Gould, 1977). Ecomorphological studies have found that the correlation between morphology and the behavioral ecology of an organism is profound (Leisler & Winkler, 1985). Studies have also found that the relationship of the mechanical ability and physical form of an organism is a component of the organism's community and the way they exploit resources (Reilly, 1994). Morphology is what determines an organism's limits to performance as well as behavior. In an example, a bird cannot fly faster or eat prey that is bigger than its body is capable of carrying

Niche determination can be shown by the overall differences in body size and morphologies that relate to how a species exploits resources. Lack (1944) hypothesized that size differences are a result of natural selection and a way to avoid interspecific competition. Competition between species in the past has brought about the separation of niches that we currently see leading to a lack of strong competition.

A good example of this is the shape of bat wings. Morphology has been found to have a significant effect on how bats function in their environment (Norberg & Rayner, 1987). Bat wings are a means of locomotion, and provide for a wide diversity of foraging types and flight abilities. Many studies have looked at the morphological make-up of bats and have correlated this to the resource partitioning that is found among many bat communities (Aguirre, Herrel, Van Damme, & Matthysen, 2002; Findley, 1993; Kalko, 1998; Van Cakenberghe, Herrel, & Aguirre, 2002). Norberg (1994) found that organismal size and shape are associated with foraging style and behavior. Aldridge and Rautenbach (1987) found that the habitat that a bat will forage in can be predicted based on their body size, echolocation style, and wing design. With this they predicted that bats with similar wing shape, echolocation style and body size can occupy similar habitats and foraging areas. This has been show to occur based on ecomorphological studies, revealing coexistence of morphologically similar bat species (Arita, 1997; Arlettaz, 1999). With this in mind many biologists have stated that morphology can reflect ecology (Aldridge & Rautenbach, 1987; Findley & Wilson, 1982). However, one must remain cautious. Differences have been found in foraging habitat and niche structure between bats that are close in morphological aspects (Arlettaz, 1997; Saunders & Barclay, 1992).

Bat flight morphology is adapted to the mode of flight and foraging techniques specific for each bat species (Norberg, 1981, 1987, 1990; Norberg & Rayner, 1987). For example, open-foragers have longer wings while bats that forage in dense vegetation have short wings. The power expended by the bat during foraging correlates directly with their body size and wing shape. The size and shape of wings can be described in

general by wing loading and aspect ratio (Norberg & Rayner, 1987). Wing loading and aspect ratio will vary in bat species depending on morphology, including body size and their flying requirements.

Observations have also shown that habitat use and wing morphology is highly correlated (Aldridge & Rautenbach, 1987; Anthony & Kunz, 1977; Crome & Richards, 1988; Findley & Wilson, 1982; Hodgkison, Balding, Zubaid, & Kunz, 2004; Jennings, Parsons, Barlow, & Gannon, 2004; Richmond, Banack, & Grant, 1998; Saunders & Barclay, 1992; Sevcik, 2003). Flight ability provides valuable information about ecological aspects of bats, such as, where they will forage and the habitats that they will be found in (Bininda-Emonds & Russell, 1994; Bullen & McKenzie, 2001; Fenton, 1972; Findley & Black, 1983; Kalko et al., 1996; Kingston, Jones, Zubaid, & Kunz, 2000; Kunz, 1974; McKenzie, Gunnell, & Williams, 1995; Vaughan, 1970). Findley (1976) studied five different bat communities and found that the bats within each community were morphologically similar, reflecting the ecology of the bat and its surrounding habitat. Morphology can explain some of these natural history aspects, such as why bats inhabit certain habitats over others, with most information coming from body size and wing structure.

Intraspecific variation can lead to ecological implications. Werner and Gilliam (1984) found that it is common for organisms of the same species to exploit different niches in the course of growth and development. This change from one niche to another during development is referred to as the ontogenetic niche shift. The ontogenetic shift has been found in some cases to be fast, i.e., metamorphosis in amphibians and insects and in other cases slow taking a gradual pattern, i.e., switching of food choice by fishes

(Werner, 1988). The ontogenetic niche shift has been attributed to the different energy needs and physiological and morphological size limitations. Organisms that proceed through large changes in body size during development often show these niche shifts during ontogeny. These ontogenetic shifts are often seen as shifts in diet as well as habitat use which in many cases can create complex webs of interactions in a community (Mittelbach, 1986; Werner & Gilliam, 1984). Ontogenetic niche shifts reduce competition intraspecifically for specific resources, thus increasing the organism's overall fitness (Werner & Gilliam, 1984). They can also reduce predation risks and maximize growth by going through dietary shifts (Olson, 1996; Shelton, Davies, King, & Timmons, 1979). This was shown in Claessen and Diekmann (2002) where ontogenetic niche shifting when pertaining to an organism's life history has the ability to give rise to evolutionary divergence from a common ancestor.

Lahti and Beck (2008) found that variation during ontogeny in the insectivorous lizards (*Phrynosoma douglasii*) is associated with prey size and type. The juveniles do not have mouths as large as adults and they lack the needed muscle strength, limiting bite force due to a smaller overall body size (Herrel, Joachim, LaFramboise, & Daood, 2006). Due to this result there is a niche difference between the juvenile and adult lizards with the juveniles on average consuming smaller, more soft-bodied insects than the adults.

The diversity of wing morphologies within the bats is great and has lead to the bats being able to inhabit many different types of habitats. The differences are highly correlated to the type of foraging strategy of the bat (Altringham, 1996; Lacki, Amelon, & Baker, 2007; Morrison, 1978). These habitats range from wide open areas to highly cluttered locations and everything in-between. Wings will vary based on the size

compared to the body size, known as wing loading. Also wings can be either short or long and either wide or skinny, known as aspect ratio. Hand-wing length and arm-wing length are also important to the flight ability of the bat (Norberg, 1990; Norberg & Rayner, 1987). Studies have been conducted to investigate the affect that wing morphology has on the flight speed and ability of bats (Boonman, Parsons, & Jones, 2003; Bullen & McKenzie, 2002; Elangovan, Raghuram, Priya, & Marimuthu, 2004; Fullard, Koehler, Surlykke, & McKenzie, 1991; Norberg, 1990; Norberg & Rayner, 1987; Rayner & Aldridge, 1985; Wainwright, 1994). As previously mentioned flight behavior and wing morphology is highly correlated and has been found to be related with the habitat used (Aldridge & Rautenbach, 1987; Crome & Richards, 1988; Hodgkison et al., 2004; Saunders & Barclay, 1992). Flight performance specifically maneuverability is determined by the wing shape, wing size, wing camber ability, wing tip shape and the overall size of the bat (Aldridge & Rautenbach, 1987; Kalcounis & Brigham, 1995; Norberg & Rayner, 1987; Stockwell, 2001).

Stockwell (2001) found that maneuverability was different among a group of Phyllostomid bats. In the study, the key difference between the bats was size and the ability to camber their wings. She found that the smaller bats were more maneuverable. The smaller bats also had a greater ability to camber their wings. There was as significant difference in length of the third and fifth digits with the smaller bats having longer digits, allowing for higher camber ability.

The larger bats cannot generate the lift needed to support their body weight at slow flight speeds. This becomes a problem at the slow flight speeds that are necessary for maneuverable turns. As the wing loading increases the ability of the bat to perform

tight maneuverable turns decreases (Aldridge, 1986, 1987; Aldridge & Brigham, 1988). Wing camber has been found to be important in slow flight as it allows for the wings to maximize lift during slow turning flight without stalling (Norberg, 1972, 1990; Vaughan, 1970).

Implications of maneuverability can have importance when it comes to the development of flight. Even though the juveniles are flying at a certain age this does not mean that they can fly like an adult (Adams, 1996, 1997; Hamilton & Barclay, 1998; Polis, 1984; Sleep & Brigham, 2003). Adams (1996, 1997) found through netting in different clutter types that there is indeed a separation in habitat use. He found that juveniles *Myotis lucifugus* were foraging in less cluttered habitats than the adults. As the juveniles age and their wings, body size and muscles become more adult-like their flight abilities should become more adult-like as well.

Adult *A. Jamaicensis* forage in the canopy where vegetation is not as thick where *C. perspicillata* forage in the understory, in thicker vegetation requiring more maneuverable flight. Each species have wing morphologies and maneuverability skills that are specific for the habitats that they occupy. This allows for these two species to coexist and not cause problems of habitat overlap. Resource partitioning then becomes the key to survival of each species in its own specific habitat.

The Evolution and Development of Wing Form and Body Size

The evolution of taxonomically and ecologically similar species produces assemblages of organisms that may overlap substantially in niche breadth. In such instances, continued coexistence among species may be reliant upon spatial or temporal resource partitioning. Morphological differences allow for this niche diversification, with

the majority of these differences being among closely related species that are within the same taxa. The evolution of many traits within taxa has been found to occur and be observable during ontogeny (McKinney & McNamara, 1991). To understand the timing of these trait differences, such as size and body shape, heterochronic and allometric comparisons are used.

Heterochrony can be as all encompassing as "global" heterochrony with whole-body changes or may be associated with dissociated heterochrony, referring to development that affects specific organs or locations of the organism (McKinney & McNamara, 1991). As described by Alberch et al. (1979) and Gould (1977), heterochrony can be broken down into two main categories, peramorphic or "overdeveloped", allowing for an organisms to achieve a larger size with sexual maturity being prolonged in many cases. Secondly, paedomorphic or "underdeveloped", the overall size of the organism being smaller and sexual maturity arising earlier in development, usually while the organism is still in a juvenile form.

Peramorphic events occur when the traits of descendants develop beyond that of the ancestral traits. By beyond, I mean that the organism may grow for an overall longer period of time (hypermorphosis), in a sense extending the juvenile growth period, or the organism may grow at a faster pace (acceleration), and finally certain traits may begin to develop or grow sooner in time than the specific traits of the ancestor (predisplacement) (Gould, 1977).

Paedomorphic events occur when a descendent retains juvenile ancestral traits. In paedomorphosis an organism may stop growing at an earlier period during development (progenesis), retaining juvenile traits. An organism may growth at a slower rate

(neotony) with juvenile traits being retained as well as sexual maturity occurring earlier, or the organism may start their growth at a later time period (postdisplacement). When developmental timing (rate) is involved, an organism can either be categorized as having accelerated growth or neotenic growth. Differences in the timing of growth in the organism can change, with growth either beginning early, as in predisplacement, or late as in postdisplacement. An organism could also grow for a longer period of time, as in hypermorphosis, or have growth truncated as in progenesis.

These six developmental processes comprise the possible changes an organism can pass through during development. In the process of the evolution of size, be it an increase or decrease, more than one heterochronic event may occur, such as an accelerated growth rate as well as hypermorphosis, with growth lasting for a longer period of time prior to sexual maturation, as in many cases of sexual dimorphism (Clutton-Brock & Harvey, 1983). All of these processes, no matter if they are acting alone or in concert with another; will have a direct effect on the outcome of the size and shape of an organism.

Peramorphic Heterochrony

As mentioned previously, peramorphic heterochrony is a method that results in increased body size when compared to the ancestor. Accelerated growth rate and hypermorphism have been accounted for in many species that are sexually dimorphic, with the males usually being the larger of the two sexes. Jarman (1983) found that large land herbivores in general were hypermorphic, meaning that they achieved their large size by growing for a longer period of time prior to sexual maturity. He also found that heterochrony could account for the sexual dimorphism that is found, with the males

having an accelerated growth rate when compared to the females. This allows the males to grow to larger sizes and surpass the female size due to the overall faster growth.

The same pattern has since been attributed to many species that show sexual dimorphism. O'Higgins and Dryden (1993) found that male chimpanzees and gorillas grow at an accelerated rate when compared with the females, which they attribute to the observed sexual dimorphism in size.

In addition to sexual dimorphism many species show peramorphic growth in both sexes. Kangaroo rats (*Dipodomys* spp.) have local accelerated growth (Hafner & Hafner, 1988). They contain enlarged auditory bullae within the skull in addition the tail of these rodents has been found to be longer than normal, however, they contain fewer number of tail vertebrae when compared with the kangaroo mouse (*Microdipodops spp*). These differences in tail length have been found to occur by the accelerated growth of the tail vertebrae in the kangaroo rats (Hafner & Hafner, 1988).

Lessa and Patton (1989) have shown that the pocket gopher (genus *Thomomys*) has attained a larger overall size due to hypermorphosis. This rodent has evolved a size that is bigger than the general size attained by related rodents. This has been shown to occur with the pocket gopher extending its juvenile growth period, therefore extending the onset of sexual maturity, allowing for an overall larger body size.

Additionally, MacFadden (1986) investigated the evolution of horses and found that there was an overall increase in body size, with the ancestral horses being much smaller than modern horses. This increase in body size is correlated with hypermorphosis or a longer growth period. He also found evidence of earlier Miocene horses attaining sexual maturity early than later horses.

Ralph and Fancy (1996) found that the beak differences of *Hemignathus* species is due to accelerated growth. The birds that acquiring nectar from flowers that had longer or deeper perianth had evolved to have longer beaks, with the development of the beaks being accelerated. The beak growth acceleration has produced the long curving bills that are around twice the length of the head.

Paedomorphic Heterochrony

As mentioned previously, paedomorphic heterochrony results in smaller body sizes of adult organisms, while reaching sexual maturity with some juvenile characteristics. Progenesis, by definition means growth stops earlier in the developmental period with accelerated gonad development with sexual maturity developing at an earlier period (Klingenberg, 1998). Progenesis is also thought to be a mechanism for shortening generation time.

Many parasites that require a host for survival and reproduction have followed developmental strategies that are progenetic. In the case of these parasite types, they have a need to attain sexual maturity at an early stage, with many needing this to occur in their secondary intermediate host. The parasite *Neochasmus spp.* have been found to be progenetic, attaining the ability to produce eggs in their intermediate hosts while still early in ontogeny, with many juvenile characteristics still present (McLaughlin, Marcogliese, & Kelly, 2006).

Progenesis has been observed in amphibians, specifically, newts and salamanders. Many have been shown to apply progenesis with the retention of the larval feeding apparatus while having gonad development that becomes functional early in life. Within these newts and salamanders, *Triturus alpestris,* the alpine newt, a species that inhabit

temporary ponds are found to be progenetic (Denoel & Joly, 2000) with their developmental rate being normal, however, due to the need to reproduce prior to the disappearance of water, they have accelerated sexually maturity (progenesis) while many aspects of their development are still in the juvenile state.

Struck (2006) found that many polychaetes, annelid worms, in the family Dinophilidae lack morphological structures that are in the larger polychaete's mature forms, which are in different families. The most common are the parapodia (head appendages). It has also been found that the progenetic polychaetes are also much smaller in size than the nonprogenetic polychaetes. Westheide (1987) hypothesized that the reason for these polychaetes reaching sexual maturity while still in juvenile form has to do with their basic ecology. There is competition for colonization of the marine interstitial space (area between the sand grains) during the juvenile stage, increasing their protection from predators. Therefore, becoming sexually mature faster and having a smaller body size allows for these polychaetes to successfully inhabit and colonize these areas permanently, giving them an ecological advantage.

In flightless birds, such as the ostrich and the emu, the reduction of the wing and the overall pectoral apparatus size has been shown to be a result of both progenesis and neoteny, with the birds reaching sexual maturity with their wings still in a juvenile state (Cubo & Casinos, 1997; James & Olson, 1983). One of the original assessment of progenesis in flightless birds was by Strickland and Melville (1848) refering to the extinct dodo (*Raphus cucullatus*). They found that the wings of the dodo were too short for flight and their plumage was too loose as with the ostrich for proper lift to occur. There are many aspects of the wings that are progenetic, such as the feathers not having

the barbs neccesary for a strong, aerodynamic feather capable of flight, and shorter wing bones of the pectoral apparatus (Livezey, 1995).

In neotony, the rate of development is reduced, which results in the retaining of juvenile features as an adult (Alberch et al., 1979). Shea (1983) found that the skull shape of the pygmy chimpanzee (*Pan paniscus*) is the result of neotony, with the overall growth rate being slower than the common chimpanzee (*Pan troglodytes*). The skull of *P. paniscus* resembles the skull of the juvenile *P. troglodytes* in both size and shape. Interestingly, Shea also mentions that if the skull of *P. troglodytes* was the more modern of the two and was being compared with the skull of *P. paniscus* there would be a mirror image of the results with an accelerated growth of the skull to reach the current size and shape.

One of the most studied examples of neotony and hypomorphosis is in the Mexican axolotl, *Ambystoma mexicanum*. Shaffer (1984) found that the Mexican axolotl had retained the external gills of their juvenile form while an adult. Looking at the growth of these animals he found that they both grow at a slower rate and have also truncated growth at a much earlier period than the ancestor (*Ambystoma tigrinum*). This has led to the permanent and complete retention of larval morphology.

An additional example of hypomorphosis, or the earlier offset of growth when compared with the ancestor is again found in the amphibians. The hellbender (*Cryptobranchus allegheniensis*) follows the developmental rate pathway of the Asian giant salamander (*Andrias japonicas*), a member of a sister taxon, nearly through metamorphosis, however, development is terminated before the gill structures have completely changed (Kuwabara et al., 1989).

As shown, changes in body size, or in specific anatomical structures, has occurred commonly throughout vertebrate and invertebrate evolution, in many cases such evolutionary outcomes are the construct of heterochronic shifts in ontogeny between ancestors and decedents. As mentioned, Shea (1983) found that larger gorillas grew faster than chimpanzees rather than growing for a longer period of time and Ishikawa and Namikawa (1987) showed that larger shrews grow for a longer period of time, showing prolonged growth phase in the juvenile stage.

Heterochrony can be quantified and observed using growth curves. Growth curves are helpful in identifying what specific type of heterochronic event is being used and also a tool for comparing two or more organisms or species. Each species will have a specific growth pattern that is inherited. Ricklefs (1973) argued that growth rates of species are determined within specific limits by adult body size and the precocity of development. These growth rate patterns in many cases follow a sigmoid curve pattern that have been worked out mathematically, such as the von Bertanlanffy's, Gompertz's, and logistic models (Zullinger, Ricklefs, Reddord, & Mace, 1984). Growth curve analysis provides important information such as the overall growth rate, asymptotic growth (maximum size), and the point of inflection (age at maximum growth) (Kaufmann, 1981). Growth curves show the overall growth and maturity of the individual or population. The analysis of these growth patterns allow for the direct comparison between individuals in a population or between individual species. The use of longitudinal data (collecting information from the same individual throughout the growth period) provides for increased confidence in the interpretation of the data.

Changes in growth (that can be observed using growth curves) allow for increased coexistence due to behavioral changes that accompany the morphological changes. With these changes there are different niches occupied based on the organism's abilities. One way of analyzing these changes in conjunction with heterochrony is allometry.

Allometric Comparisons

Allometry is defined as the comparative shape change during growth, in other words it can be seen as the change in shape of an organism with its change in size. Allometry describes the shape change that occurs in a particular structure or body location during growth, in-comparison to another feature, usually body size, with mass being the usual feature compared. For biological purposes, allometry can be compared using the following equation:

$$Y = aM^b$$

For easier interpretation which converts the sigmoidal growth curves to linear format, one may transform the equation to a logarithmic version:

$$\text{Log } Y = \text{Log } a + b \log M.$$

As mentioned, this will give a straight line with the intercept log *a*, the slope *b*, and M referring to the mass and Y refers to the shape variable (Gould, 1966). In interpreting this equation, the slope b can be referred to as the scaling coefficient or exponent. When the slope equals one, there is no relative change in shape with the change in size, the growth patterns are said to be isometric, however, if the slope is greater than one, positive allometry occurs meaning that shape change happens at a faster rate relative to size changes during growth. Finally, a slope less than one represents negative allometry, meaning the relative change in shape is less than the relative change in size.

One important aspect of allometry and evolutionary change in size or shape of an organism is that the organism is likely going to show an overall change in some aspects of behavior. This can include ontogenetic behaviors that are affected by the many changes that occur in timing and rate during the developmental period. If a species has prolonged development then there could be behavioral changes or shifts in relation to the ancestral form. Heterochronic and allometric shifts therefore apply not only to morphological changes but with behavioral changes as well. These behavioral changes have the ability to create major impacts on the organism as well as the species as a whole. With more and more research being performed in terms of the morphology and ontogeny of organisms, it is becoming clear that the changes occurring in shape, size and growth rate are going to have an influence on the overall outcome of behavior within these organisms that has influenced the evolutionary trajectories and aided in the overall divergence of organisms from a common ancestor.

Evolution of Bats

The evolution of bats is something that could benefit from comparative ontogenetic studies. Bat evolution is widely unknown and somewhat controversial, especially in regards to the origin and development of flight. Based on the lack of evolutionary history, phylogenetic studies have tried to link bats together based on phenotypes and genotypes (Baker et al., 2003; Jones & Teeling, 2006; Wetterer et al., 2000). Echolocation and flight are the key evolutionary designs that have allowed bats to be successful nocturnal, aerial hunters. Flight has given bats the ability to exploit a variety of foraging niches that cannot be accessed by other mammals (Norberg 1976, 1985; Norberg & Rayner 1987). The evolution of the bat wing and the ability to use

them for flight has given bats the opportunity to exploit these new habitats and ecosystems.

The fossil evidence has provided little help in-regards to bat evolution, with a few exceptions (Caple et al, 1983; Thewissen & Babcock, 1992). Until recently the oldest known bat fossil was *Icaronycteris index*, dating back to the early Eocene, roughly 52 million years ago, and is thought to have had the ability to echolocate (Jepson, 1966, 1970). However, recently a newly discovery species of Eocene bat, *Onychonycteris finneyi*, roughly 52.5 million years old, appears to lacks the ability to echolocate (Simmons, Seymour, Habersetzer, & Gunnell, 2008). This has stemmed the debate whether flight or echolocation evolved first (Simmons et al., 2008; Speakman, 2008). Both of the fossil bats had the ability to fly, with wing morphological specializations such as: wing membrane formation, elongation of the fingers and forearm, as well as an aerodynamic body makeup. This evidence advocates for a reasonably rapid evolution of these traits (Simmons & Geisler, 1998).

Due to the lack of fossil evidence, research has advanced into the molecular mechanisms, regarding the formation and elongation of the bat wing. New insights into regulatory proteins such as bone morphogenetic proteins (bmp), Prx1 and Hoxd13 expression have shown possibilities for the development of the wing and elongation of the fingers by altering their expression when the specific genes are turned on and off (Chen, Cretekos, Rasweiler, & Behringer, 2005; Cretekos et al., 2008; Sears, Behringer, Rasweiler, & Niswander, 2006; Sears, 2008; Weatherbee, Behringer, Rasweiler, & Niswander, 2006; Weatherbee, 2008). The inhibition of bmp by Fgf8 and Gremlin expression, which stops cell death, allows for the interdigital webbing to keep growing,

forming the wing membrane on the bat hands, however, bmp is not inhibited during foot formation, ultimately forming feet without webbing (Sears et al., 2006; Sears, 2008; Weatherbee et al., 2006; Weatherbee, 2008).

Morphological and developmental patterns can add to the evolutionary process of bats, as shown by these recent molecular advances. The developmental stages a bat goes through from birth to volancy can shed light on the stages necessary for flight and successful foraging, which is key to understanding the evolutionary steps leading up to bat flight (Adams, 1989, 1992a, 1992b, 1998, 2008).

The evolution and ontogeny of bat flight development including, morphological examination, has also been studied in detail in few bat species, including: *Nycticeius humeralis* (Jones, 1967); *Rhinolophus ferrumequinum* (Hughes et al., 1989); *Myotis lucifugus* (Adams 1992a,b, 1996, 1997, 1998; Kunz & Anthony, 1982; O'Farrell & Studier, 1973; Powers et al., 1991); *Pipistrellus pipistrellus* (Boyd & Myhill, 1987; Hughes, Rayner, & Jones, 1995); *Myotis nattereri* (Swift, 2001); *Artibeus watsoni* (Chaverri & Kunz, 2006); *Cynopterus sphinx* (Elangovan et al., 2007), *Megaderma lyra* (Rajan & Marimuthu, 1999), *Miniopterus schreibrsi* (Serra-Cobo, 1987), *Tadarida brasiliensis* (Allen, Richarson, McCracken, & Kunz, 2010), *Pipistrellus subflavus* (Hoying & Kunz, 1998), *Rhinolophus hipposideros* (Reiter, 2004), *Plecotus auritus* (Mclean & Speakman, 2000), Rousettus leschenaultia (Elangovan, Raghuram, Priya, & Marimuthu, 2002), *Myotis thysanodes* (O'Farrell & Studier, 1973), *Nyctalus lasiopterus schreber* (Maeda, 1973), *Hipposideros cineraceus* (Jin, Lin, Sun, Liu, & Feng, 2010), *Phyllostomus hastatus* (Stern & Kunz, 1998), *Myotis macrodactylus* (Liu, Jin, Metzner, & Feng, 2009), *Eptesicus fuscus* (Hood, Bloss, & Kunz, 2002) with the majority being

insectivorous bats. All these studies include intraspecific comparison with many examining the effect of environmental factors on growth. Most do not include flight mechanics as well as the behavior changes that occur during flight development.

As mentioned, flight mechanics and development in bats is not well understood, with comparative studies conducted on closely related bat species lacking. Comparisons between closely related species such as members of the same family in areas such as flight development can provide insight into the mechanics and developmental differences of flight between these species. These studies will add insight into the developmental changes that occurred to allow for the evolution of the wide range of body sizes seen in extant bat species.

Key to these comparisons are morphological comparisons made using mass, forearm length, wing area, wingspan, arm-wing, hand-wing and development of the flight muscles. These traits can be tracked and analyzed by plotting growth rates which will show the overall developmental timing and comparisons can be made between the two species. Growth curves have been used for comparisons in many types of animals and plants including: rodent (*Oryzomys albigularis*) (Moscarella, Benado, & Aguilera, 2001), Sheep (Topal, Ozdemir, Aksakal, Yildiz, & Dogru, 2004), Turtles (Frazer & Ehrhart, 1985), Indian barn-owls (Nagarajan, Thiyagesan, & Natarajan, 2002), and bats (Kunz & Anthony, 1982). Additionally, allometry can provide important evolutionary developments trends that have occurred during the evolution of bats. As mentioned, Zullinger et al. (1984) formulated growth curve equations that have been used, and provide valuable information about mammalian growth. There are three main growth equations that are used and compared:

von Bertalanffy equation (Ricker, 1979),

$$M(t) = A\{1\text{-}1/3e^{-K(t-1)}\}^3,$$

Gompertz equation,

$$M(t) = A*e^{-e\text{-}K(t-I)},$$

Logistic equation,

$$M(t) = A\{e^{-K(t-I)}+1\}^{-1}.$$

When used for analyzing growth and development of bats, the logistic growth equation has been the best fit equation in most cases (Boyd & Myhill, 1987; De Fanis & Jones, 1995; Jin et al., 2010; Liu et al., 2009; Reiter, 2004; Stern & Kunz, 1998). These growth curves have been useful in providing accurate growth rates, asymptotic mass and time of the initial growth period during the ontogenetic period of mammals.

Bat Development

Newborn bats develop rapidly and must develop the skills necessary for survival. For young bats to forage successfully they must master both echolocation and flight. However, flight is an expensive mode of transportation so there is a large selective pressure to minimize the energetic costs associated with flight (Norberg, 1990). Based on energy constraints, size has a direct affect on flight behavior, morphology and ecology (Arita & Fenton, 1997; Norberg, 1990). In order to allow for the most economical flight, bats must refine their flight apparatus during development, which includes the development of the appropriate wing morphology (Jones, 1967; O'Farrell & Studier, 1973). Wing morphology includes the skeletal structure as well as the musculature that is needed for flight.

The growth and development of the wing is a process unique to bats. This includes the divergence from a hand-plate to a hand-wing (Adams, 1989, 1992a, 1992b). The development of flight remains to be one of the most important aspects of bat ontogeny and evolution. Wings have evolved in many different shapes and sizes; however, they function in similar ways (Norberg, 1990). Development of adult-like wing formation, motor programming, and flight muscles in synchrony are key to achieving normal flight success (Yokoyama & Uchida, 1979a, 1979b). In many species of insectivorous bats, the mother weans the pup at approximately two weeks (Powers et al., 1991; Tuttle & Stevenson, 1982), leaving the pup at a point where flight is still developing and maneuverability is far from adult-like.

Flight ontogeny generally occurs in two primary stages. The first stage is when the juvenile bat first becomes airborne, limited to straight flight and flapping or fluttering (Pearson, Koford, & Pearson, 1952; Powers et al., 1991). The second stage is when the bat can truly fly, maintaining flight with the ability to maneuver (Davis, 1969a; Pearson et al., 1952). As the juvenile bat is progressing through these stages, wing and body size are in a constant state of change. These changes are important for flight development and overall success of the juvenile bats.

There is a need to expand to different bats that have followed a different evolutionary tract, using different flight and feeding behaviors. Many of these bats have been found to co- habitat; sharing both day and night roost sites (Fleming, 1988). However, evolutionarily they have followed a different tract both morphologically and ecologically, having different body sizes and wing structures that have led to a difference in foraging ecology (Fleming, 1988; Kalko et al., 1996). Larger bat species are generally

found foraging in more open areas whereas the smaller more maneuverable species are found in more cluttered dense habitats (Aldridge & Rautenbach, 1987; Fenton, 1990; Kalcounis & Brigham, 1995). There is also a difference in life history traits, such as, state at birth (precocial or altricial), gestation time, maternal care and so forth. Bats that are born in a more precocial state have been found to develop at a slower rate than those that are born in a more altricial state (Orr, 1970). Understanding the changes in these bats' wing morphology, flight development, behaviors and muscle physiology is important in revealing evolutionary links that could add to the already lacking knowledge of bat evolution.

Wing Morphology

Within bat species there is an array of adult skeletal and wing characteristics that aid in flight adaptations (Vaughan, 1959). Much has been documented about these adult adaptations, however, little is known about wing morphogenesis (Smith & Starrett, 1979). Wing morphology affects the flight performance and behavior of bats and can be a good predictor of function (Elangovan et al., 2002; Fenton & Kunz, 1977; Mclean & Speakman, 2000; Norberg, Brooke, & Trewhella, 2000; Wainwright, 1994). Wing shape and flight behavior has been shown to correlate with flight theory, determining the mode of flight amongst bat species (Findley, Studier, & Wilson, 1972; Norberg, 1972, 1981, 1986, 1990, 1994; Norberg, 1983; Norberg, Kunz, Steffensen, Winter, & Von Helversen, 1993; Rayner, 1987). Observations have also shown that habitat use and wing morphology is highly correlated (Aldridge & Rautenbach, 1987; Anthony & Kunz, 1977; Crome & Richards, 1988; Findley & Wilson, 1982; Hodgkison et al., 2004; Jennings et al., 2004; Richmond et al., 1998; Saunders & Barclay, 1992; Sevcik, 2003). Flight ability

provides valuable information about ecological aspects of bats, such as, where they will forage and the habitats that they will be found in (Bininda-Emonds & Russell, 1994; Bullen & McKenzie, 2001; Fenton, 1972; Kunz, 1974; Findley & Black, 1983; Kalko et al., 1996; Kingston et al., 2000; McKenzie et al., 1995; Vaughan, 1970). Morphology can explain some of these natural history aspects, such as why bats inhabit certain niches over others, with most information coming from body size and wing structure.

The size of the wing is essential in determining the lift and the shape of the wing; in turn this will determine the bats ability to generate thrust and maneuverability (Birch, 1997; Norberg, 1994). Flight behavior and ability can be determined by the size of the wingspan, wing surface area and the overall mass of the bat (Norberg & Rayner, 1987). Wing loading, a scale for animals that fly, compares the body weight that is supported by a flight surface or airfoil (Aldridge, 1986; Farney & Fleharty, 1969; Hughes & Rayner, 1991; Norberg & Rayner, 1987; Poole, 1936; Vaughan, 1959). Wing loading can be described as body weight divided by wing area and is a determinant in the flight speed of the bat. Lower wing loading permits slow flight and high wing loading permits fast flight. Additionally, aspect ratio is wingspan squared divided by wing area and is an indicator of wing width and also helps in determining the bats maneuverability. Low aspect ratio wings means the bats can fly at slower speeds permitting more maneuverable flight as high aspect ratio permits less maneuverable, open space flight (Norberg & Rayner, 1987).

To provide more detail on wing shape, Norberg and Rayner (1987) produced three indices used to describe the wing-tip shape. First is the wing-tip length ratio, which is the ratio of the length of the arm-wing to the length of the hand-wing. The arm-wing is

the area of the wing extending from the body to the wrist and extending down the fifth digit. The hand-wing is the part of the wing distal to the wrist and the fifth digit. Next is the tip area ratio, which is the ratio of the arm-wing area to the hand-wing area. Finally, the tip shape index. This is a measure of the wing-tip shape. A high tip index indicates a rounded wingtip and a low index indicates pointed wingtips. Bats with more elongated, round wing tips have the ability to fly slow and even hover with the distal end of the wing generating the majority of the force (Findley et al., 1972).

Wing camber is an additional aspect of a wing that assists in flight type and ability (Stockwell, 2001). Camber is the ability of the wing to curve in a concave pattern with the edges being lower than the middle. Morphological aspects of a wing contribute to the ability of a wing to have high or low camber ability. Bats use their dactylopatagium and phalanges of the third and fifth finger to produce camber. The area of the dactylopatagium located between the second and third digit (dactylopatagium minor) is lowered, increasing the camber of the wing. Stockwell (2001) found that bats with wider dactylopatagium between digit two and three and longer third and fifth digits have the ability to camber their wings more, producing more lift and allowing for the bat to fly slower and be more maneuverable without stalling.

Morphology has been looked at in the development of many bat species. The majority of information on bat morphological development pertains to changes in wing loading, aspect ratio and forearm length. Jones (1967) found that juvenile *Nycticeius humeralis* had increased growth of the forearm and digit five. McManus and Nellis (1972) showed an overall increase in wing loading as *Artibeus jamaicensis* body weight increased. De Fanis and Jones (1995) found in *Plecotus auritus* that wing loading

decreased, aspect ratio increased, and tip area increased over time. Powers et al. (1991) found that *Myotis lucifugus* increased wing area, wing span, and aspect ratio with decreased wing loading as the juveniles grew. Elangovan et al. (2007) found that *Cynopterus sphinx* had an increase in wingspan, wing area and aspect ratio and a decrease in wing loading.

It has been found in insectivorous bats that there are large developmental changes within the wings, growing substantially faster than overall body size. Necessary changes occur during the period of flight development with the juvenile becoming closer to the body size and wing shape of the adult bats (Kunz, Wrazen, & Burnett, 1998). Adams (1998, 2008) found that during growth, juvenile *Myotis lucifugus* wing shape did not change, with the changes occurring only in wing size, showing that there is importance in one aspect of the growth staying constant (wing shape) while the change is occurring in the wing size. Adams (1998, 2008) also hypothesized that the growth of the wing structure (i.e., bones) is regulated by soft tissue growth. As the bats use their wings the wing membrane and muscles dictate the overall growth of the hard tissue development.

Morphological changes that occur during development are key to understanding the flight behaviors and the evolution of flight. As bone length, wing area, wingspan and body mass change, so does a bat's abilities. Detailed, quantified observations of wing morphology are imperative when studying flight development.

Flight Behavior

Flight has brought about many foraging and habitat opportunities for bats. This has allowed evolutionarily for bats, to specialize their hunting and foraging efforts to suit their foraging needs. Differences in wings can correlate with many different forms of

flight, including: slow, fast, hovering, maneuverable, and the ability to carry heavy loads (Norberg & Winter, 2006; Pennycuick, 1975; Rhodes, 1995). Flight entails flapping of the wings and has been shown to be a demanding mode of transportation (Hartman, 1963; Norberg, 1990).

The development of flight involves the coordination of many physiological and behavioral aspects. As a bat develops, its mass increases, the wing size increases and the coordination of the nervous system with the muscles become mature (Norberg, 1990; Yokoyama & Uchida, 1979a). The bones also begin to harden and become strengthened to the point of supporting the wing (Adams, 1992a, 1992b, 1998). As the bat develops, it precedes through a series of developmental flight behaviors. At first the bat has no flight ability and falls with no wing movement, as time goes on they begin to move their wings in a fluttering motion and eventually they achieve horizontal flight (Elangovan et al., 2007; Powers et al., 1991). During this period the hand-wing is changing rapidly, producing the proper thrust during the down stroke (Norberg, 1976; Powers et al., 1991). Changes of the wing allow for more advanced flight abilities and maneuverability (Kalcounis & Brigham, 1991; Norberg & Rayner, 1987). Before a juvenile can forage or fly successfully it would be advantageous to have developed wings that have wing loading, aspect ratio and tip ratio similar to the adult form. Powers et al. (1991) found that *Myotis lucifugus* achieved adult like wing loading at 15 days and adult aspect ratio values at 22 days, both being adult like prior to sustained flight. De Fanis and Jones (1995) found that *Plecotus auritus* juvenile achieved adult like wing loading, aspect ratio and tip ratio near or prior to the time of flight. This pattern has been seen in many species of bats including: *Nycticeius humeralis* (Jones, 1967), *Myotis lucifugus* (Buchler,

1980), *Pipistrellus pipistrellus* (Hughes et al., 1995).In addition to morphological changes, Powers et al. (1991) found that there was no significant difference in flapping rate as the juveniles matured toward flight, suggesting that there is no relationship between the ability to flap wings, and flight behavior.

Powers et al. (1991) using juvenile *Myotis lucifugus* analyzed flight behavior from day one to determine the stages of flop (no wing movement), flutter (falling straight down with wing movement), flap (achieving some horizontal movement), and flight (sustained flight). A time line of flop, flutter, flap, and flight was analyzed and comparisons were made to determine when the juveniles progressed from one stage to the next. This was then correlated with wing morphology.

Muscle Development

Muscles of locomotion in mammals are composed of up to three different fiber types, belonging to motor units that have distinct functional properties resulting in varying performance capabilities. Muscle fiber types are therefore characterized by the differences in their functional and structural properties (Pette & Staron, 2000). There are many classification paradigms that are based on the properties of myosin ATPase which can be broken down into type I, type IIa and type IIb motor units (Brooke & Kaiser, 1970; Guth & Samaha, 1969, 1970). Type I fibers are considered slow while type IIa are fast-contracting fatigue resistant and typeIIb are fast-contracting fatigue sensitive (Burke, Levine, Tsairis, & Zajac, 1973; Edstrom & Kugelberg, 1968). All three of these fibers can be linked to the activity of metabolic enzymes. Slow type I fibers have an oxidative response, meaning they are highly aerobic with up to 38 ATP being produced in the mitochondria during the break down of glucose. This provides for fatigue resistant

muscle properties. Fast fibers can be glycolytic and anaerobic, termed fast glycolytic (FG), producing only 2 ATP within the cytoplasm during metabolic activity. This provides for short-term explosive muscle use. Fast fibers can also be glycolytic and oxidative, termed fast oxidative glycolytic (FOG) (Nemeht & Pette, 1984). This gives the muscle the potential to be fatigue resistant by using the oxidative aerobic pathways and the potential to use the anaerobic pathway during short powerful activity. Muscle fibers have been found to be dynamic with the possibility of altering phenotypic properties under certain conditions such as: increased or decreased neuromuscular activity, changes in hormone levels, and aging. The changes in fiber isoforms have been found to follow specific trends, from fast to slow or slow to fast (Pette & Staron, 2000). These changes have been related to the gradual changes in the energy cost of force production (Bottinelli, Canepari, Reggiani, & Stienen, 1994b). Changes that occur with the ATP phosphorylation potentials of the fast and slow fibers have also been linked to this transition (Conjard, Peuker, & Pette, 1998). These transition states are dependent on the function of the muscle throughout ontogeny as well as the overall job it performs in the adult organism.

Flight muscles are extremely important to bats for both producing the appropriate power for flight as well as creating the force for maneuverability. The pectoralis muscles are used for forward motion, specifically performing the up and downstroke motion of flight (Hermanson & Altenbach, 1981, 1985; Vaughan, 1970). The muscles of the arm including muscles of the shoulder and forearm, such as the deltoid musculature, are creating the power for maneuverability during flight (Powers et al., 1991). Flight muscles in the adult bat have been classified as “unitypic or bitypic” (Armstrong, 1977;

Foehring & Hermanson, 1984; George & Jyoti, 1955; Hermanson & Foehring, 1988; Hermanson, LaFramboise, & Daood, 1991; Strickler, 1980). Unitypic means the muscle is composed of all one fiber type with a specialized contraction pattern. Bitypic consists of two different fiber types with specialized contraction rates and patterns. All adult insectivorous bats that have been studied to this date have been found to have "unitypic" musculature in their flight muscles, consisting entirely of fast oxidative fiber types that have fast contraction ability and have high oxidative capabilities, including: *Myotis lucifugus* (Armstrong, Ianuzzo, & Kunz, 1977; Brigham, Ianuzzo, Hamilton, & Fenton, 1990; Hermanson et al., 1991) and *Tadarida brasiliensis* (Foehring & Hermanson, 1988). A "bitypic" muscle composition, consisting of both fast and slow fiber types, has been found in an adult phyllostomid, *Artibeus jamaicensis* (Hermanson & Foehring, 1988). Ohtsu and Uchida (1979) found that adult *Miniopterus fuliginosus* and *Rhinolophus ferrumequinum nippon* had bitypic pectoralis muscle patterns with *Rhinolophus ferrumequinum nippon* having both fast-twitch isoforms IIa and IIb along with slow-twitch type I fibers. With the finding of different motifs of fiber types in different species of bats this may suggest that fiber type may be dependent on aspects such as flight style, body size, foraging patterns which are greatly linked with evolutionary adaptations (Bullen & McKenzie, 2004).

The ontogeny of the flight muscles have been studied predominantly in the insectivorous bat *Myotis lucifugus* (Kunz & Anthony, 1982; Powers et al., 1991; Schutt, Cobb, Petrie, & Hermanson, 1994) with the focus being on the pectoralis muscle. It has been found that the muscles develop rapidly, so that the juvenile will have the neuromuscular control needed to be successful at flying and foraging at the time of

weaning. At the time of weaning *M. lucifugus* pectoralis and acromiodeltoideus, a muscles of the forearm, are in a homogeneous pattern, which is comparable to that of an adult with the muscles being "unitypic." The muscle fibers consist of fast oxidative fibers that are fatigue resistant which are important for sustained flight (Powers et al., 1991; Schutt et al., 1994). This, however, is not the case during the early postnatal period of *M. lucifugus.* Schutt et al. (1994) and Powers et al., (1991) both found that in bats less than a week old, type I fibers are present deep within the pectoralis muscle however after the first week these fibers are absent. Powers et al. (1991) also found that in both the pectoralis and the acromiodeltoideus the overall cross-sectional area increased significantly during the juveniles first 15 days.

Currently the information we have about muscle ontogeny comes from only a few species of bat which are classified as insectivorous bats. There is a lack of knowledge from bats that have different flight habits and ecological backgrounds. As previously mentioned, Hermanson and Foehring (1988) found two fiber types in the pectoralis muscle of adult *Artibeus jamaicensis.* It is not understood or know what the developmental patterns are of these bats are and what the evolutionary implications of specific types of muscle fibers may have on specific flight styles and abilities.

CHAPTER III

METHODS AND MATERIALS

Species and Housing

Study species for my project were two species of New World fruit bats *Artibeus jamaicensis* and *Carollia perspicillata*. *Artibeus jamaicensis* and *Carollia perspicillata* were housed together in two rooms (A and B) connected by an opening in the wall, allowing for access to both rooms. The bat rooms were located in the University of Northern Colorado animal care facility. The bats were able to hang and roost anywhere in the room as the walls had a rough surface providing for support. Room A, which is the entrance room, contained an anteroom 1.32 M X 2.13 M X 2.2 M with a second door that entered into the bat facility. The anteroom allowed for a person to enter the room safely without the risk of bats escaping. This room was used for observations and animal handling (i.e., making sure the mother had the baby secure before released back into the main colony). The dimensions of room A, in meters, were 4.14 X 2.13 X 2.72. Three baskets, two of which were metal mesh and one which was wood wicker, hung from the ceiling in room A.

Room B was the larger of the two rooms, measuring 5.46 X 2.29 X 2.72 meters and also had three baskets for roosting, two of which were wood wicker and one metal mesh. The opening connecting the rooms was 2.3 meters wide X 2 meters high.

The colony contained both male and female bats which allowed for reproduction to occur. Pups when born were housed in the same location with the mother being the

care giver. The light cycle was on a 12 hour light/dark cycle with the dark cycle being during the day, allowing for the bats to be active during the daytime hours. The colony was fed daily, with food consisting of fruit and processed monkey chow for added nutrients (Harlan Global, 25% protein primate diet). The fruit was cut into pieces small enough for the bats to be able to carry to a roosting site for consumption. In addition, larger pieces of fruit were hung in different locations around the rooms, allowing for increased enrichment activity. The monkey chow was soaked in water for thirty minutes and blended in a food processer to near liquid form in which additional ingredients included: corn syrup for added sugar, powdered milk for extra calcium and dry Jell-O gelatin (usually strawberry or raspberry) for added flavor were added. Clean water was provided daily in shallow dishes that were located on the floor away from the walls, allowing for the bats to skim the surface and drink while in flight. Rooms were spot cleaned daily, which included removal of leftover food and any obvious messes. Every two weeks the rooms were cleaned completely, including scrubbing of the walls and floors.

Adult bats were monitored visually on a daily basis to determine when pups were born, providing for an accurate date of birth. Pups with attached umbilical cords and placenta were recorded as being one day or less than one day old (Figure 1) (Kunz, Adams, & Hood, 2009). The day new pups were located they were taken with their mother to the lab for morphometric measurements and flight tests. Measurements and flight tests were performed daily for 100 days at which time the juvenile had reached adult size and adult flight ability. Each juvenile was fitted with a numbered split-band

wing band (at the point when the mother started leaving it in the roost site), which was used for future identification (Porzana Ltd., East Sussex, UK).

Figure 1. One day old *Artibeus jamaicensis.* The photo shows the umbilical cord and placenta still attached. Photo by Jason Shaw.

Ontogenetic Implications of Bat Ecology and Co-existence

Flight Development and Behavior

Flight development was measured using the technique described by Powers et al. (1991). Flight tests were performed within a flight chamber that was 5 x 3 x 2.5 meters within a darkened room. The flight chamber contained a foam pad (2 X 1.5 meters) marked with concentric circles extending from the drop rod out every 20 centimeters extending to a total length of 200 centimeters used to measure flight ability (Figure 2).

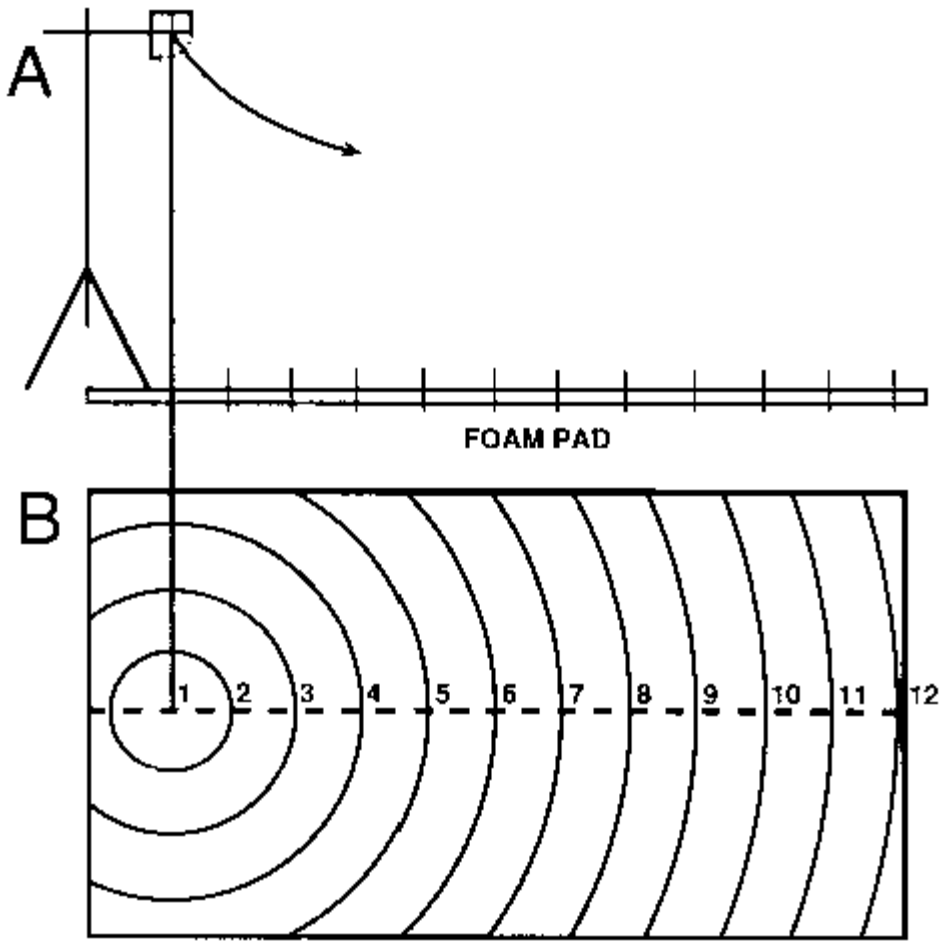

Figure 2. Schematic of the flight development apparatus. (A) Side view illustration, giving an example of the stand and pad set-up. (B) Top view of pad set-up, illustrating the concentric circles used in flight development. (From Powers et al., 1991).

Flight attempts were organized into 4 developmental categories: flop (falling from the rod to the floor with minimal wing movement), flutter (falling from the rod with wing movement without achieving horizontal movement), flap (falling from the rod, achieving some horizontal movement, within the 200 centimeter pad) and flight (falling from the rod achieving horizontal movement that went beyond the 200 centimeter mark) (Powers et al., 1991). Wing movement was determined by using a Sony Handicam camcorder (Sony, Inc.) which provided for accurate classification into the flop or flutter stages. If

the bat showed forward movement, distance measurements (in centimeters) were recorded using a measuring tape from the base of the launch rod to the nearest body part, usually a foot. In the case where the bat flew into the side or back of the flight chamber the trial was not recorded and the bat was flown again, however, flight attempt were limited to three per day per bat.

Flight Maneuverability

I performed maneuverability tests at the time juveniles were capable of flight. Flight maneuverability was assessed using an obstacle course similar to the one used by Stockwell (2001) (Figure 3). The obstacle course consisted of six rows of dowels (each 0.6cm in width) suspended from half inch metal conduit poles that were 2.75 m long extending the width of the flight chamber. The dowels hung from the conduit by string which allowed for the poles to freely move. The dowel spacing was adjustable allowing for different settings which depended on the wingspan of each individual bat. The dowels were spaced at three different settings for each bat, the spacing order was randomly decided using a random number generator in Excel (Microsoft). Dowels were placed equidistantly at full wingspan, three-fourth wingspan and half wingspan. The spacing between individual dowels was scaled to the wingspan of individual bats as a way to normalize the spacing for variation in body size. The dowel end was fitted with a small eyehook and a 0.5cm neodymium magnet attached to the eyehook and placed in the center of a metal mesh cylinder 15 cm in diameter by 19 cm high. Bat agility was quantified by counting the number of dowels stuck to the mesh cages after the first pass through the obstacle course. As the bats flew through the course the dowels that were hit were displaced with the magnets sticking to the metal cylinder allowing for quantification

of agility. Each bat was given three flight trials at each of the three dowel spacing and each bat was flown in the course every five days starting with the first day of flight, continuing until there was no significant difference in maneuverability between the juvenile and the adults. All data were recorded on data sheets for later analysis (Table 8 in Appendix B).

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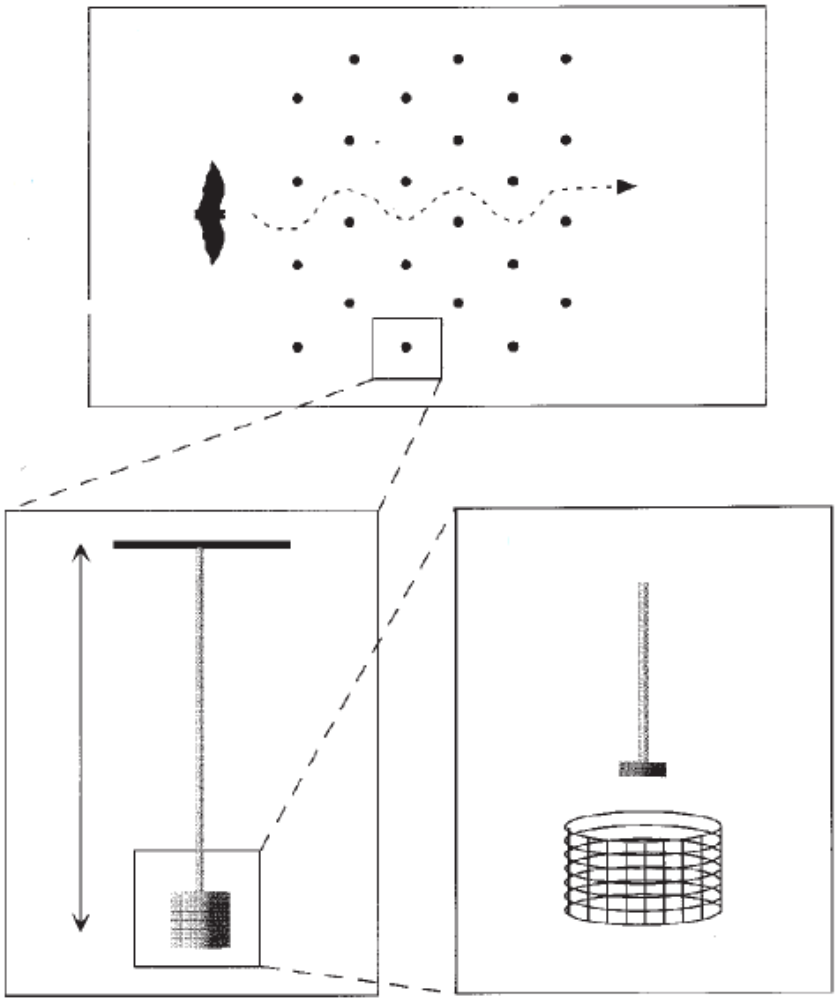

Figure 3. Schematic of the maneuverability course. Six of offset dowels (.6cm) hang from crossbars. Each dowel has a magnet on the end. The dowel hangs in a metal mesh cylinder. When hit, the dowel swings and the magnet sticks to the mesh cylinder . (From Stockwell, 2001).

Many juveniles early on could not maneuver the course, these trials were not recorded, and recording starting when the juvenile was capable of completing the maneuverability course. To determine if the juveniles were learning or memorizing the maneuverability course, five juveniles from each species were not allowed to fly in the course until twenty days post first-flight then identical flight tests were performed.

The Evolution and Development of Wing Form and Body Size

Morphometrics

I took morphological measurements daily, beginning with the day of parturition throughout the developmental period until the juvenile reached adult size. Each individual had a data sheet that included species, date, age, mass, forearm length, wing area, wing loading, and wingspan (Table 9 in Appendix C). Juveniles were weighed daily to the hundredth of a gram using an Acculab VI-1mg pan scale (Sartorius Group, Goettingen, Germany). The juveniles were weighed either by placement directly on the scale if they remained immobile; when they became mobile they were wrapped in a holding bag whose weight was zeroed prior to weighing.

Forearm measurements, from the elbow to the wrist, were taken using a Mitutoyo digital caliper (Mitutoyo USA), measured to the hundredth of a millimeter. The right forearm was always measured three times and then averaged. I used a digital camera (Olympus Camedia C-5000 Zoom; Olympus Corporation of the Americas, Inc.; Center Valley, PA) to take photos of the wings from a distance of 1 meter above the wing with the camera angle of 90° to the subject. The bat was placed ventrum down on graph paper having 5mm square grids for calibrations with the right wing outstretched. I loaded the

pictures onto a computer and edited them using adobe Photoshop (Adobe master suite, PS4) which included: cropping and filling the wing with a single color for use in dimension analysis. Wing surface area measurements, which included the entire wing membrane from the body distal, were used, excluding the thumb. When preparing wing membranes for separate arm-wing and hand-wing measurements, the wing was divided along the fifth digit, with the arm-wing extending from the body to the fifth digit and the hand-wing extending from the 5th digit to the wing-tip.

The surface area and wing-tip analysis of the wing were determined using a computer program, Sigmascan Pro 5.0.0 (SPSS, Inc., Chicago). I used the Sigmascan program to determined wing area by calibrating each wing with its specific length to the millimeter, which was determined from the graph paper in the picture. I determined wing length by measuring the distance from the shoulder to the wing-tip which was used for whole-wing surface area measurements. For the length of the arm-wing, I measured from the shoulder to the fifth digit. For hand-wing length I measured from the fifth digit to the wing-tip (Figure 4). Surface areas were measured in millimeters squared. I calculated wing tip length ratio from the formula: $T_l = l_{hw}/l_{aw}$ with hw referring to hand-wing and aw referring to arm-wing. Wing tip area ratio was calculated using the formula: $T_S = S_{hw}/S_{aw.}$ Lastly, the wing tip shape index was calculated using the formula: $T_i = T_S/(T_l - T_S)$ with l referring to wing tip length ratio and S referring to wing tip area ratio (after Norberg, 1990).

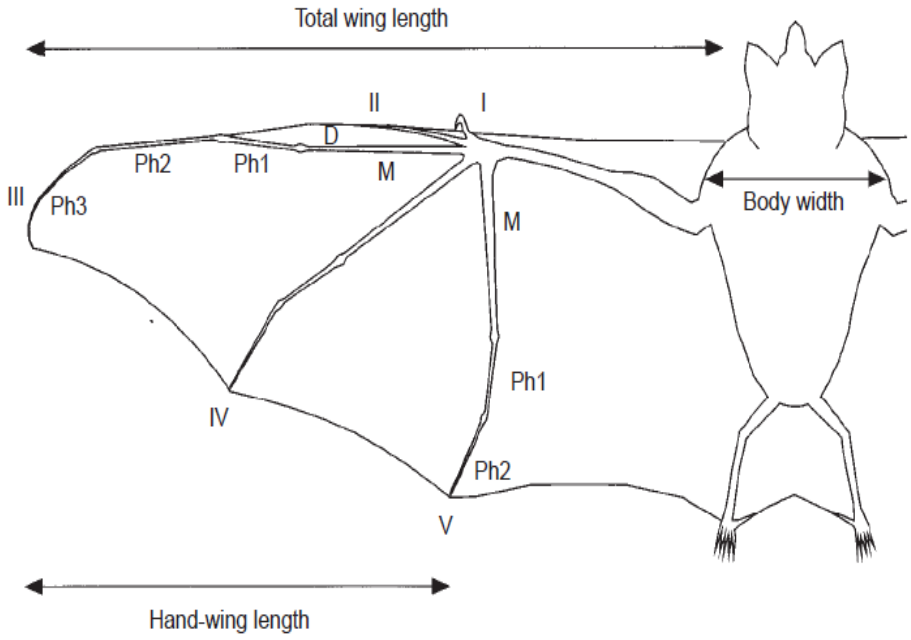

Figure 4. Diagram of an outstretched bat wing. Wing illustration shows the digits, hand-wing and arm-wing and the total wing length. (From Stockwell, 2001).

Bone Development

Bone ossification measurements and clear and staining protocol followed the procedures in Adams (1992a, 1992b) and Kunz et al. (2009). In brief, juveniles were processed soon after death or preserved in jars containing 100% ethyl alcohol until used. I skinned the bats completely including the wings and all internal organs, the tongue, and the eyes were removed. The bats were then cleared and stained for cartilage and bone after being fully submerged in distilled water for 24 hours. For cartilaginous tissue staining the bats were then soaked in alcian blue (8GX ICN Biomedicals, Inc. catalog number 152624) for 24 hours. Following the alcian blue stain, the specimens were rehydrated in a series of ethyl alcohol baths consisting of the following percentages, in order, each for 24 hours: 100%, 100%, 75%, 40%, and 25%. After the specimens were

rehydrated in the alcohol they were transferred to distilled water to be washed for 24 hours. Following the wash the specimens were placed in trypsin (Fisher, catalog number 9002-07-7), which is an enzyme that digests protein, for 24 hours for muscle degradation. The muscles should be clear or close to clear after this stage with bones and cartilage being visible. The specimens were then washed in distilled water for 24 hours. After the washing stage the specimen was soaked in alizarin red (S ICN Biomedicals, Inc. catalog number 100375) for 24 hours. Alizarin red is a stain specific to bone. Following this stage the specimen is cleared. Clearing consists of the bat being soaked in glycerol (ICN Biomedicals, Inc. catalog number 151194) and KOH at different concentrations. The first 24 hours the specimens were soaked in 1:3 glycerol/KOH then transferred to 1:1 glycerol/KOH for 24 hours and finally soaked in 3:1 glycerol/KOH for 24 hours. The specimens were then allowed to soak in 100% glycerol for 24 hours, and transferred to a jar and covered in 100% glycerol for storage. To accurately measure bone and cartilage development, specimens were placed under a dissecting scope (Olympus SZX 12; Olympus Corporation of the Americas, Inc.; Center Valley, PA) and photos were taken of the fourth digit epiphyseal growth plate using the built in camera.

I measured the fourth digit total epiphyseal gap at the metacarpal-phalangeal epiphyseal growth plate to the nearest 0.1 millimeter using Sigmascan (SPSS, Inc., Chicago) (Kunz et al., 2009; Kunz & Robson, 1995). In addition to using clear and stained specimens, live specimens were used to increase the sample size for measurement data collected on the fourth digit epiphyseal gap measurements. The right wing was stretched across the light on the stage of a dissecting microscope (Leica Zoom 2000, Leica Microsystems, Wetzlar, Germany) for transillumintion and Mitutoyo digital

calipers (Mitutoyo USA) were used to measure the gap to the nearest 0.1 millimeter. These measurements were taken on the first day a of each flight stage.

Muscle Development

Three bats in each flight group (flop, flutter, flap, and flight) as well as three adults were euthanized by an overdose of isoflurane. The right and left pectoralis major and the right and left acromiodeltoideus were immediately dissected and weighed (Vaughan, 1959). The samples were mounted on a cork with Tissuetek, then immediately frozen for 30 seconds in isopentane (also called methylbutane or 2-methylbutane) that had been cooled in liquid nitrogen to the point that it was in a slightly jelly form. Samples were then placed in a -70°C freezer (Thermo Scientific, Waltham, MA) until sectioned. The muscle samples were sectioned by Colorado histoprep (Fort Collins, CO) at a thickness of 8 microns using a cryostat then the cold tissue sections were mounted on cold slides. The slides were then allowed to adjust to room temperature (no longer than one hour). When the slides were at room temperature immunohistochemistry procedures were applied. Circles were drawn around the samples with a grease pen (Newcomer supplies). Phosphate buffered saline (PBS) blocking solution, at 7.2 pH, was applied for ten minutes. After ten minutes the blocking solution was removed using kimwipes. Two primary antibodies were applied to separate slides, consisting of, mouse myosin heavy chain antibody for fast twitch muscle fibers, MHCf (Vector Labs, VP-M665) diluted at 1:30 with PBS and mouse myosin heavy chain antibody for slow muscle fibers, MHCs (Vector Labs, VP-M667) diluted to 1:80 with PBS. It is important that all the tissue is covered by the antibody solution. The primary antibodies remained on the slide overnight in a humid chamber in the refrigerator. After

24 hours the primary antibodies were removed. The slides were then rinsed in PBS for ten minutes then the buffer was removed. The muscle fiber types were detected using an anti-mouse secondary antibody (Super Sensitive Multilink, BioGenix, catalog number HK340-9KT; a biotinylated secondary antibody that contains multiple antibodies to mouse, rabbit, guinea pig and rat antibodies) which binds to the mouse primary antibody, was applied for one hour in a humid chamber. After one hour, the secondary antibody was removed and the tissue was rinsed for ten minutes with PBS. Horseraddish peroxidase (HRP) was applied for ten minutes then rinsed. DAP was then applied for 6 minutes in the dark and rinsed. HRP binds to the secondary antibody and contains an enzyme that digests the DAP turning the specific locations that the primary antibody bound to brown which allowed for muscle fibers to be analyzed. Hematoxylin and eosin counterstains were applied making the fiber membranes visible. The IHC and final hematoxylin and eosin counterstains were outsourced to Colorado Histoprep (Fort Collins, CO). Negative control samples were exposed to all conditions except primary antibodies. In all cases, negative controls displayed an absence of signal.

Muscle fiber type and quantity were determined for each slide. This was done using an Olympus CX41 microscope with an Insight 2 Spot Image Sample (Diagnostic Instruments, Inc.) camera. Spot (version 4.0.4) software was used to acquire digital photos of the muscle tissue. Photographs were taken of each sample at 40x, 100x, 200x, and 400x. The muscle fibers were either classified as fast or slow twitch fibers. For each flight stage the percentage of fast and slow twitch muscle fibers were calculated. A digital grid (Grid Cell Counter version 0.9.9.8 beta, HeracleSoftware, Pitesti, Romania) was placed over each photo of the 200x magnification for fiber counting. The grid

consisted of 48 cells, each 1”X1.” Grid cells were randomly chosen using the Microsoft Excel 2007 randbetween function. Ten grid cells were chosen for each picture and all fast or slow twitch fibers were counted in each of the grid cells. If the cell had less the 75% of the area covered in fibers or if a number appeared more than once they were not used and an additional random number was obtained. All counted fibers were brown in color allowing for easy identification. Each flight stage had 1,000 individual fibers counted using pictures from the 3 individual bats. The percent fast and slow muscle fibers were then calculated.

Muscle fiber diameter and area were measured using the cross-sections of the muscle fibers at 200x. Fast and slow fibers were measured for each flight stage using slides from each of the three bats. Individual muscle fibers were isolated using Photoshop (Adobe Master Suite, PS4). A 100 micron bar was added to each picture for calibration purposes. The pictures were then analyzed using the Sigmascan (SPSS, Inc., Chicago) program obtaining accurate fiber surface area. The surface areas were then compared across flight stages. A total of 200 fibers were analyzed from each flight stage.

Validity and Reliability

All instruments that were used were valid and reliable. All instruments were calibrated and have certificates of calibration allowing for assessment of reliability and were tested regularly for consistency and accuracy of measurement.

Statistical Analysis of Data

All data was analyzed using SAS 9.1 unless stated otherwise. All tests used an alpha level of 0.05 for significance. Variation between age groups and flight stages of individual morphological measurements was analyzed using student’ t-test. Student’s

t-tests were used to determine differences between juveniles at each age with the adults to determine when the juvenile morphologies become adult like. ANOVA was used to look for overall variation using multiple morphometirc measurements between bats at each individual age group. PCA was used to determine which variables were interrelated, the most and when the juveniles and adult variables overlapped. Allometric comparisons were performed on log-transformed data using linear regression. To compare differences between species and slopes of the regression lines ANCOVA tests were performed. Postnatal growth rates were analyzed with non-linear regression and logistic growth models with comparisons of slope to determine any significant differences in growth rates between the two species. The logistic growth model has been shown to be the most accurate model for use with bat (Kunz & Robson, 1995); however, the von Bertalanffy and Gompertz models (Zullinger et al., 1984) were also run for comparisons. The Marquart-Levenburg algorithm (Marquart, 1963) in SPSS 19 (SPSS, Inc., Chicago) to obtain grow parameters from the growth equations. Flight developmental stages were compared between species using Student's *t*-test comparing the mean day of first achieving a specific flight stage. Maneuverability tests were compared using two-factor ANOVA, with the age groups as the factors and the dowel spacing as the variables. This compared the number of dowel hits at specific spacing against the amount of dowels hit at specific spacing by adults and gave an overall significance taking into account all the hits at each dowel spacing at once. Contrasts were used within the two-way ANOVA to determine significance between age groups at individual dowel spacing. Student's *t*-test was used to determine differences in muscle fiber area and percentage between species using contrasts to determine specific flight stage differences. Comparisons of gap lengths

and muscle fiber area were performed using Student's *t*-test on adjusted data that was adjusted for body size. Gap length data was divided into the total length of the fourth metacarpal and first phalange. Muscle fiber surface area was divided into wing surface area. Adjusting the data allowed for comparisons that did not include mass as a variable.

CHAPTER IV

RESULTS

In this study I collected morphometric measurements and flight development data from 45 *Artibeus jamaicensis* and 25 *Carollia perspicillata* that were born between August 2006 and January 2011. I did not find sexual dimorphism in all morphometric measurements ($p < 0.05$, Student's *t*-test) in either species, therefore, all data for males and females were pooled. All results are based on 45 *A. jamaicensis* and 25 *C. perspicillata* unless otherwise noted.

Artibeus jamaicensis and *Carollia perspicillata* are considered precocial, however, there were obvious differences at birth between species. *A. jamaicensis* were born with less fur than *C. perspicillata*, with *C. perspicillata* being completely covered in a dark, near black pelage. *A. jamaicensis* had thin fur covering the body and in some areas the skin was exposed to the elements. At birth, newborn young of both species had their eyes open. The pups positioned themselves diagonally along the ventral surface of the mother's ventrum with their mouth attached to a nipple and their feet near the underside of the mother's plagiopatagium. Individuals of *A. jamaicensis* were found to be significantly less developed than *C. perspicillata* when compared using percent of adult in the following morphological areas: forearm ($t = 5.49$, $p < 0.0001$), mass ($t = 2.8$, $p = 0.008$), wing surface area ($t = 0.485$, $p < 0.0001$), arm-wing area ($t = 12.36$, $p < 0.0001$), hand-wing area ($t = 7.00$, $p < 0.0001$), wing length ($t = 8.02$, $p < 0.0001$), arm-wing length ($t = 7.81$, p 0.0001), and hand-wing length ($t = 3.99$, $p = 0.0003$). Wingspan

was the only morphological trait measured that did not show a significant difference between the percentage of adult wingspan and wingspan of the pups at birth ($t = 0.78$, $p = 0.442$).

Ontogenetic Implications of Bat ecology and Co-existence

Flight Development

I found that the development of flight in *A. jamaicensis* and *C. perspicillata* began with flop (falling from with little movement of wings) and proceeded through the developmental stages of flutter (falling with wing flapping and no horizontal movement), flap (some horizontal movement) and flight (flying beyond 200 cm), similar to that found in Powers et al. (1991) (Figures 5 and 6). *A. jamaicensis* and *C. perspicillata* achieved the flop and flutter stages at similar time periods while achieving the flap and flight stages at significantly different time periods (Table 1). *A. jamaicensis* and *C. perspicillata* also remained in the flop and flutter stage a similar amount of time whereas the time they remained in the flap stage was significantly different (Table 2). *C. perspicillata* and *A. jamaicensis* pups both exhibited flop behavior on the first day post-partum when dropped from the 1.5 meter rod wherein they fell to the ground exhibiting minimal wing movement. *A. jamaicensis* remained in the flop stage on average of 1.64 ± 0.82 days ($n = 45$) while *C. perspicillata* was found to remain in the flop stage on average of 1.55 ± 0.99 days ($n = 25$), the time period that *A. jamaicensis* and *C. perspicillata* remained in the flop stage did not show significant differences between species ($t = 0.401$, $p = 0.699$). Transition to the flutter stage (dropping with wing movement and not obtaining horizontal flight) occurred on average in *A. jamaicensis* 2.5 ± 0.82 days post-partum, whereas *C. perspicillata* first began fluttering on

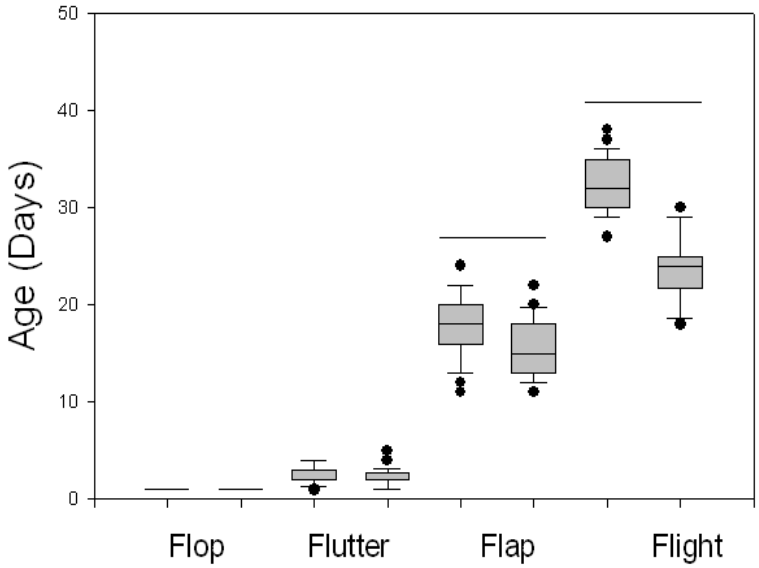

Figure 5. Mean (± *SD*) age at first observation of each flight development category. Left boxes coincide with *Artibeus jamaicensis* and right boxes coincide with *Carollia perspicillata.* * = P-value of 0.01, ** = p-value of 0.0001 (Students t-test).

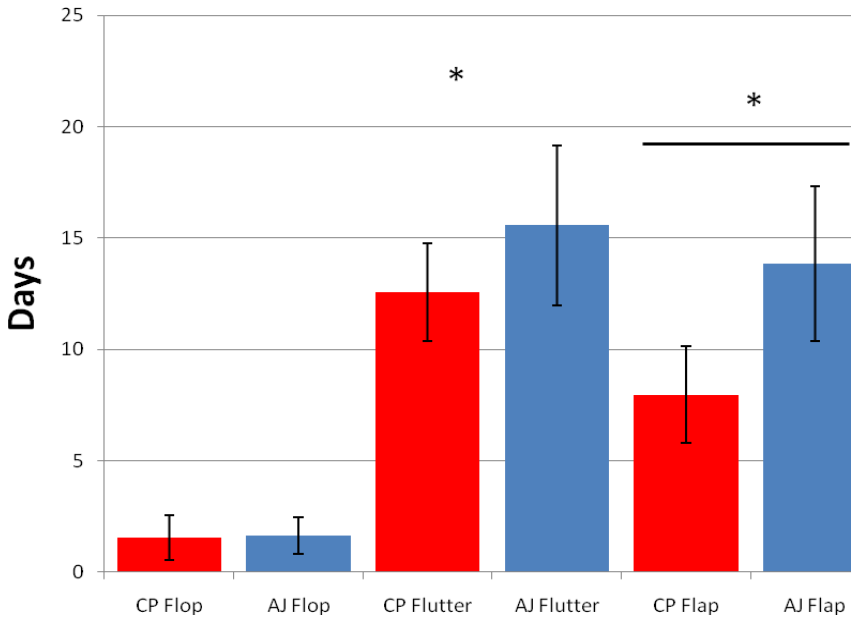

Figure 6. Mean (± *SD*) days spent within each developmental flight category. Blue bars coincide with *Artibeus jamaicensis* and red bars coincide with *Carollia perspicillata.* * = *p*-value ≤ 0.001.

Table 1

Average Day A. jamaicensis and C. perspicillata Achieve Each Flight Stage

Flight Stage	Species	Mean ± *SD**	Significance
Flop	*Artibeus jamaicensis* (AJ)	1	
	Carollia perspicillata (CP)	1	
Flutter	*Artibeus jamaicensis* (AJ)	2.5 ± 0.82	$t = 1.91$, $p = 0.06$
	Carollia perspicillata (CP)	2.18 ± 0.9	
Flap	*Artibeus jamaicensis* (AJ)	17.84 ± 2.99	$t = 2.65$, $p = 0.01$
	Carollia perspicillata (CP)	15.82 ± 2.92	
Flight	*Artibeus jamaicensis* (AJ)	32.45 ± 2.75	$t = 11.72$, $p < 0.0001$
	Carollia perspicillata (CP)	23.62 ± 3.30	

Note: The flop stage was not compared as day 1 was the only day that this could begin on. Significance was found using Student's *t*-test.

* *SD* represents one standard deviation from the mean.

Table 2

Average Length (Days) A. jamaicensis and C. perspicillata Remained Within Each Flight Development Stage

Flight Stage	Species	Mean ± *SD**	Significance
Flop	*Artibeus jamaicensis* (AJ)	1	
	Carollia perspicillata (CP)	1	
Flutter	*Artibeus jamaicensis* (AJ)	2.5 ± 0.82	$t = 1.91$, $p = 0.06$
	Carollia perspicillata (CP)	2.18 ± 0.9	
Flap	*Artibeus jamaicensis* (AJ)	17.84 ± 2.99	$t = 2.65$, $p = 0.01$
	Carollia perspicillata (CP)	15.82 ± 2.92	
Flight	*Artibeus jamaicensis* (AJ)	32.45 ± 2.75	$t = 11.72$, $p < 0.0001$
	Carollia perspicillata (CP)	23.62 ± 3.30	

Note: Significance was found using Student's *t*-test.

* *SD* represents one standard deviation from the mean.

average at 2.18 ± 0.9 days of age. The first day the pups fluttered was not significantly different between the two species (t = 1.91, p = 0.06). In both species some pups were found to flutter within 24 hours post-partum, skipping the flop stage entirely. *A. jamaicensis* remained in the flutter stage on average of 15.57 ± 3.59 days while *C. perspicillata* remained in the flutter stage on average of 12.56 ± 2.19 days which was a significantly shorter time period than for *A. jamaicensis* (t = 3.95, p = 0.0002). Juvenile *A. jamaicensis* achieved short horizontal flights, flap stage (flights of less than 200cm), at 17.84 ± 2.99 days, while *C. perspicillata* first began short flights (flap stage) at 15.82 ± 2.92 days with the first day of obtaining the flap stage being significantly different between the two species (t =2.65, p = 0.01). *A. jamaicensis* remained within the flap stage on average of 13.85 ± 3.56 days with *C. perspicillata* at 7.96 ± 2.17 days which was a significant difference in the time period the two species remained in the flap stage (t =7.89, p < 0.0001). The first day of flight occurred for *A. jamaicensis* at 32.45 ± 2.75 days with *C. perspicillata* achieving flight at 23.62 ± 3.30 days. *C. perspicillata* achieved flight significantly sooner than *A. jamaicensis* (t = 11.72, p < 0.0001).

Fight Ability and Maneuverability

On the first day that juveniles were capable of sustained flight, I quantified flight agility in *A. jamaicensis* (n = 20) and *C. perspicillata* (n = 15) using a maneuverability course as described in the methods section with dowl rods spaced at a distance indicated by the wingspan of each juvenile bat (Stockwell, 2001). Importantly, these juveniles were not all of the same age, but of the same flight stage.

I found that both species had difficulty maneuvering through the course at all dowel rod spacing based on individual wingspans. Juveniles could not complete the

course, falling to the ground near the entrance. Beginning at 5 days post first-flight, bats were able to successfully complete the maneuverability course with trials continuing until 60 days post first-flight. Two-way ANOVA, taking into account full, 75%, and 50% wingspan spacing showed that *A. jamaicensis* juveniles were able to successfully maneuver the course with adult-like flight ability at 45 days post first-flight [$F(5, 114) = 2.47$; $p = 0.088$] (Figure 7). Adults contacted on average 0.74 dowel rods spaced at full wingspan, 2.53 contacts at 75% wingspan spacing and 5.42 dowels at 50% wingspan spacing.

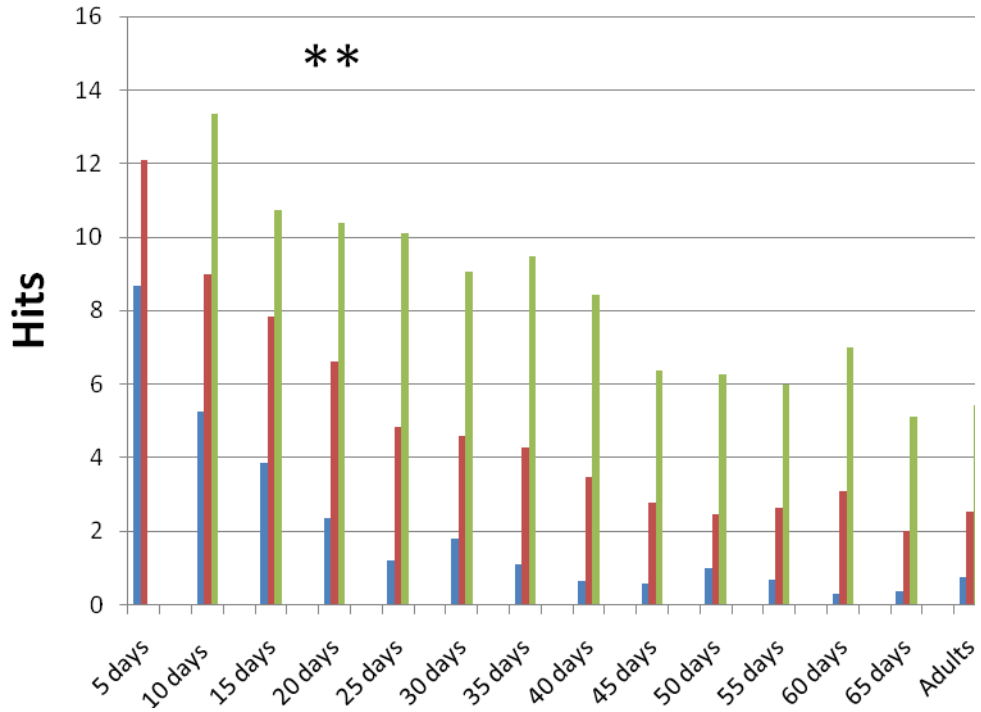

Figure 7. Summary of dowel rods contacted by *A. jamaicensis*. At each age group the blue bars represent the mean number of hits at full wingspan spacing, the red bars represent hits at 75% wingspan spacing and the green bars represent hits at 50% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.01$).

Contrasts used in conjunction with the two-way ANOVA dissected the flight ability of juveniles at the individual dowel rod spacing, showing that as spacing became more compact more dowel rods were contacted. *A. jamaicensis* juveniles were able to successfully maneuver through the full wingspan dowel spacing course at adult-like agility at 35 days post first-flight [$F(5, 114) = 1.05$; $p = 0.3072$] (Figure 8). *A. jamaicensis* juveniles were successful at maneuvering through the 75% wingspan dowel spacing at adult-like agility at 45 days post first-flight [$F(5, 114) = 0.72$; $p = 0.3986$] (Figure 9). At the most compact dowel spacing of 50% wingspan, *A. jamaicensis* juveniles were capable of maneuvering with adult-like agility 65 days post first-flight [$F(5, 114) = 0.61$; $p = 0.4359$] (Figure 10).

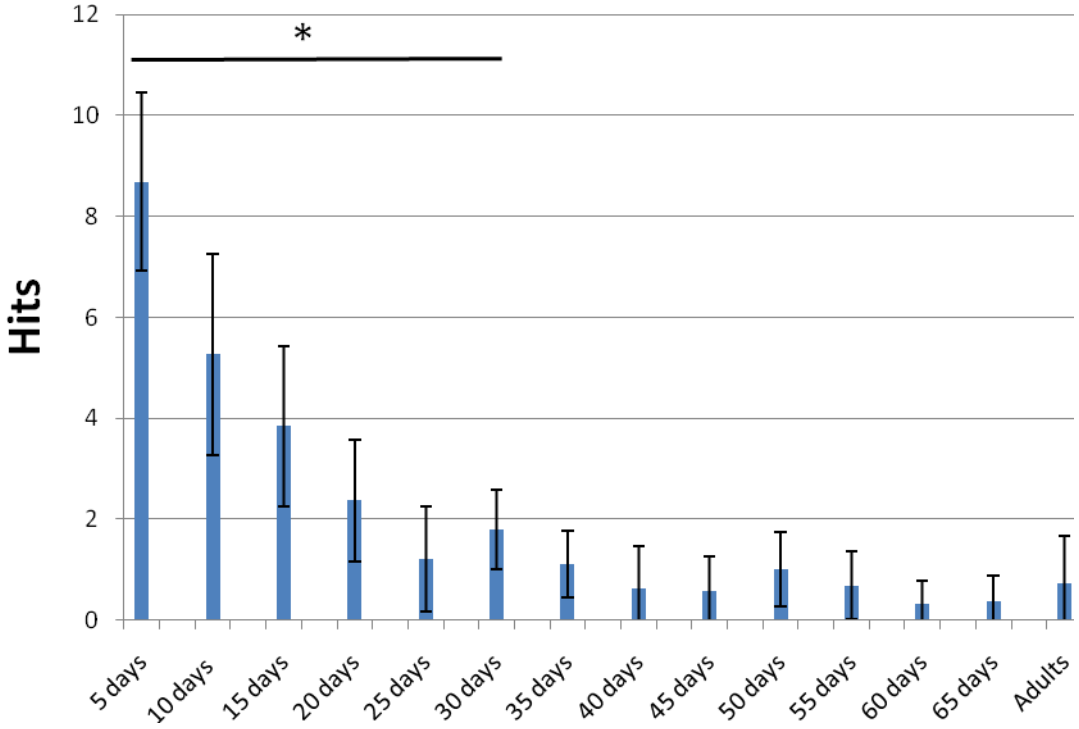

Figure 8. Dowels contacted by *A. jamaicensis* at full wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.01$; mean ± *SD*).

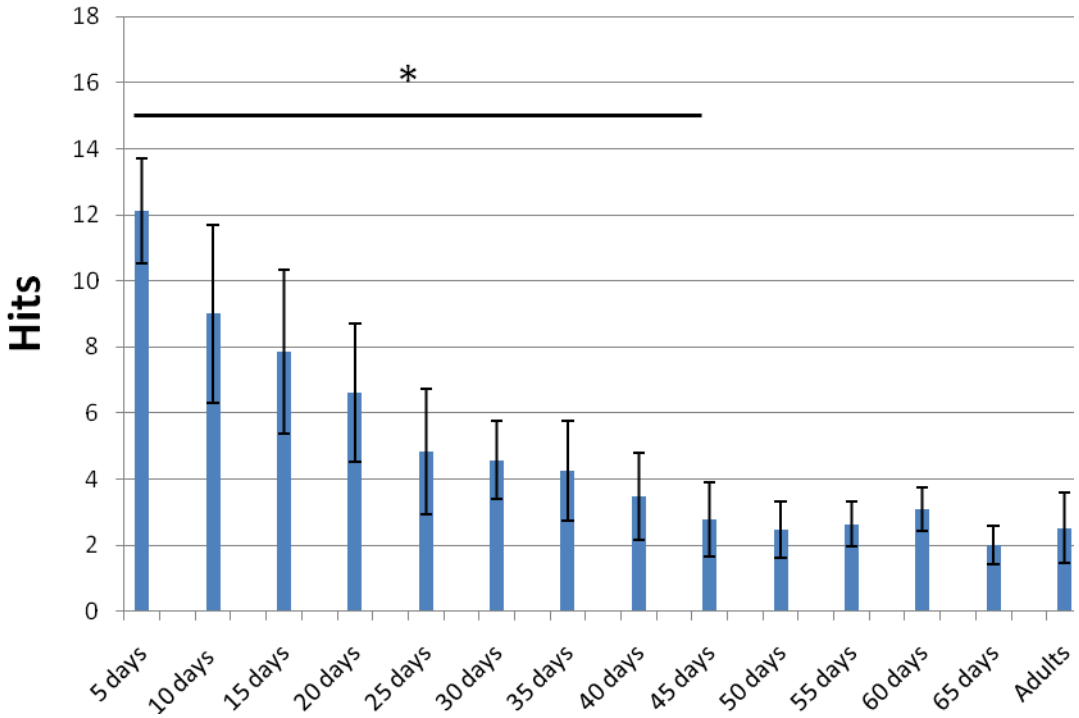

Figure 9. Dowels contacted by *A. jamaicensis* at 75% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p \leq 0.01$; mean ± *SD*).

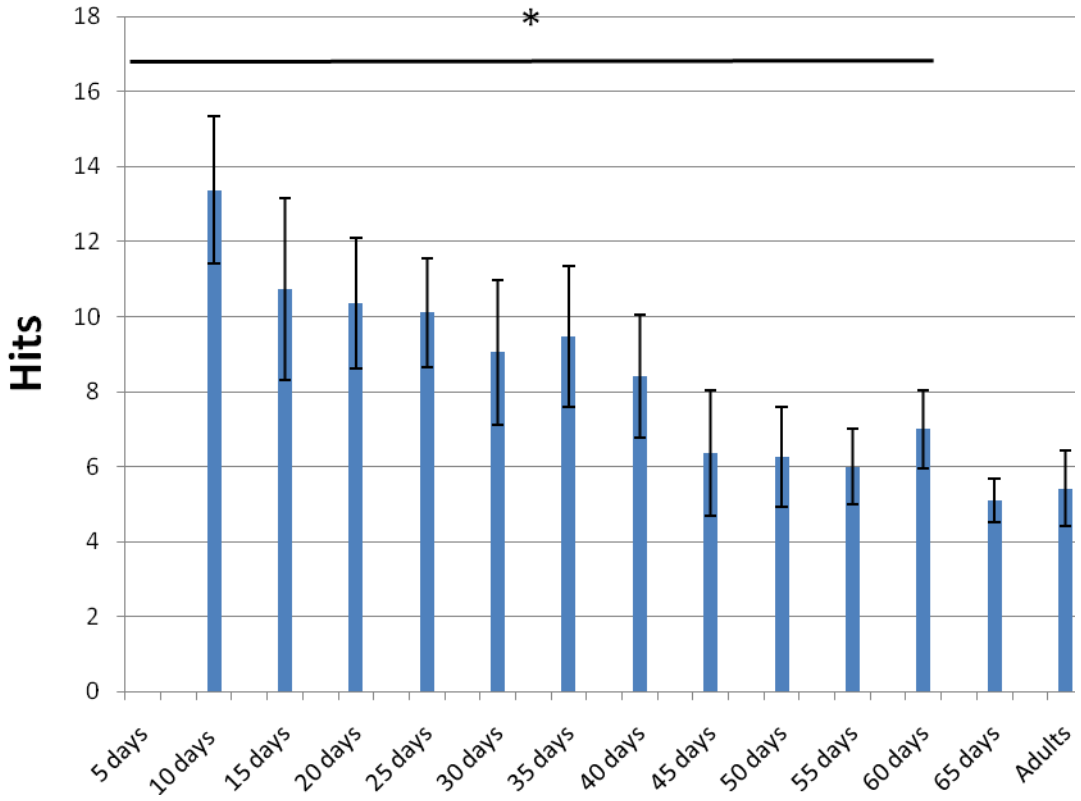

Figure 10. Dowels contacted by *A. jamaicensis* at 50% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.05$; mean ± *SD*).

Two-way ANOVA divulged that *C. perspicillata* juveniles were overall able to successfully maneuver through the dowel course at 40 days post first-flight with adult-like agility [$F(5, 84) = 1.65$; $p = 0.1979$] (Figure 11). Contrasts in conjunction with two-way ANOVA showed that *C. perspicillata* juveniles like *A. jamaicensis* had increased difficulty maneuvering through the dowel course as the spacing decreased. *C. perspicillata* were able to successfully maneuver the course at full wingspan dowel spacing with adult-like agility at 30 days post first-flight agility [$F(5, 84) = 1.90$; $p = 0.1721$] (Figure 12). *C. perspicillata* juveniles were found to be capable of successfully

maneuvering the course with the dowels set at 75% wingspan with adult-like agility at 40 days post first-flight [$F(5, 84) = 3.31$; $p = 0.0723$] (Figure 13). Adult-like ability for juvenile *C. perspicillata* maneuvering through the dowel course set at 50% wingspan was found to occur at 50 days post first-flight [$F(5, 84) = 0.32$; $p = 0.6587$] (Figure 14).

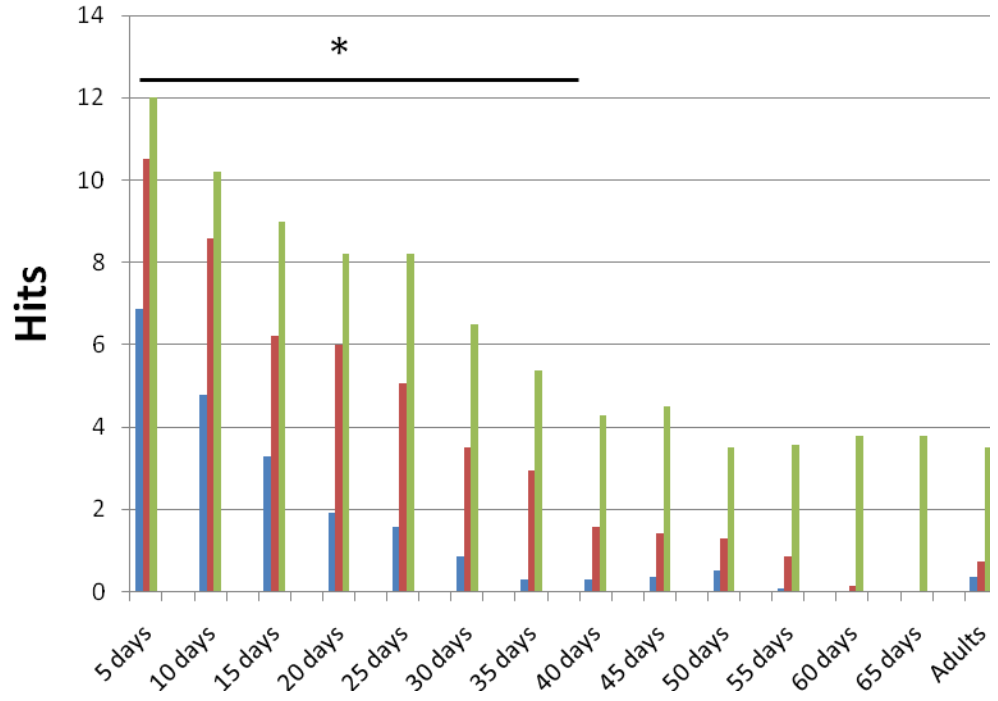

Figure 11. Summary of dowel rods contacted by *C. perspicillata*. At each age group the blue bars represent the mean number of hits at full wingspan spacing, the red bars represent hits at 75% wingspan spacing and the green bars represent hits at 50% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.01$).

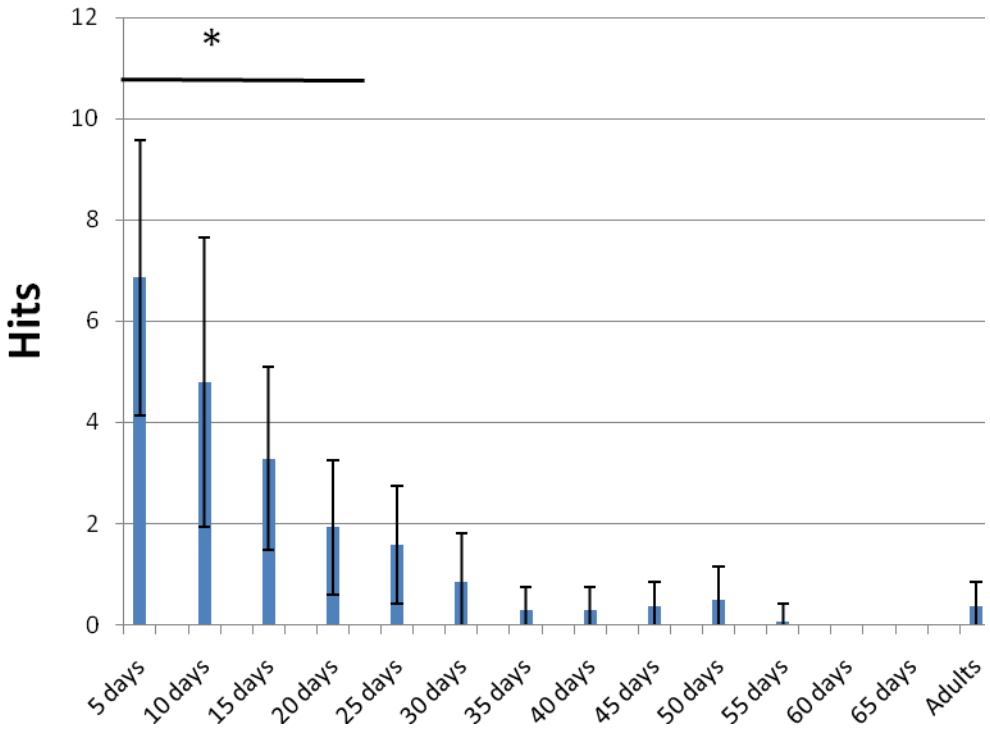

Figure 12. Dowels contacted by *C. perspicillata* at full wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.01$; mean ± *SD*).

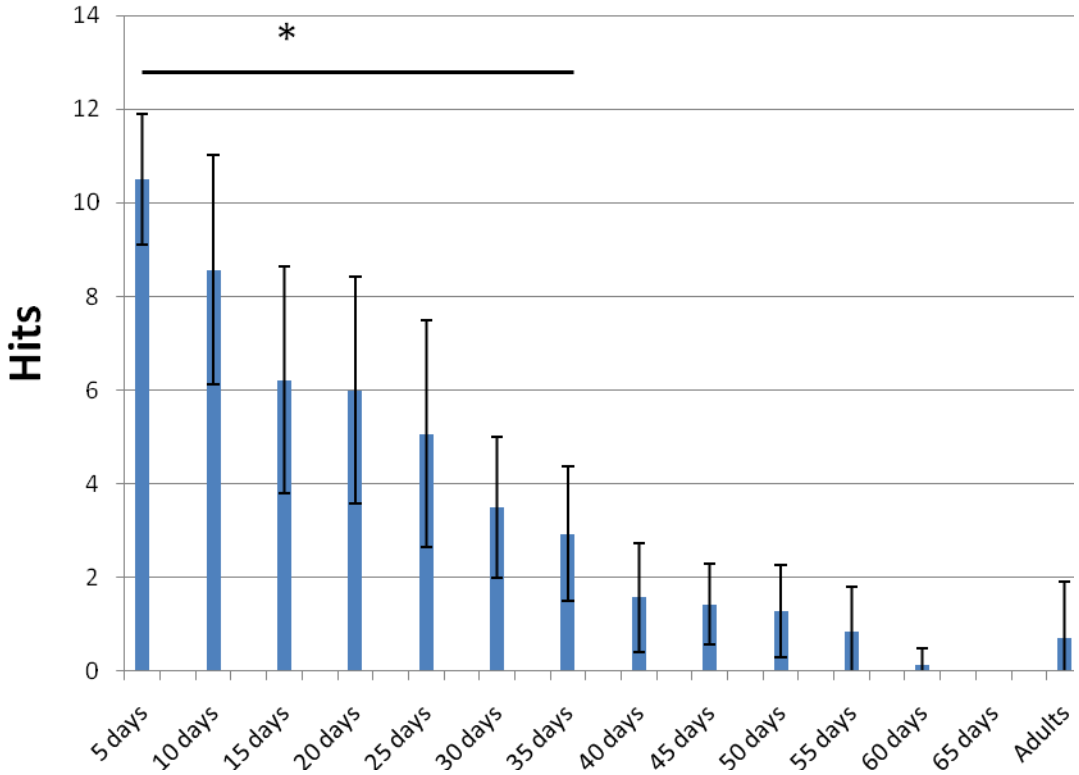

Figure 13. Dowels contacted by *C. perspicillata* at 75% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.001$; mean ± *SD*).

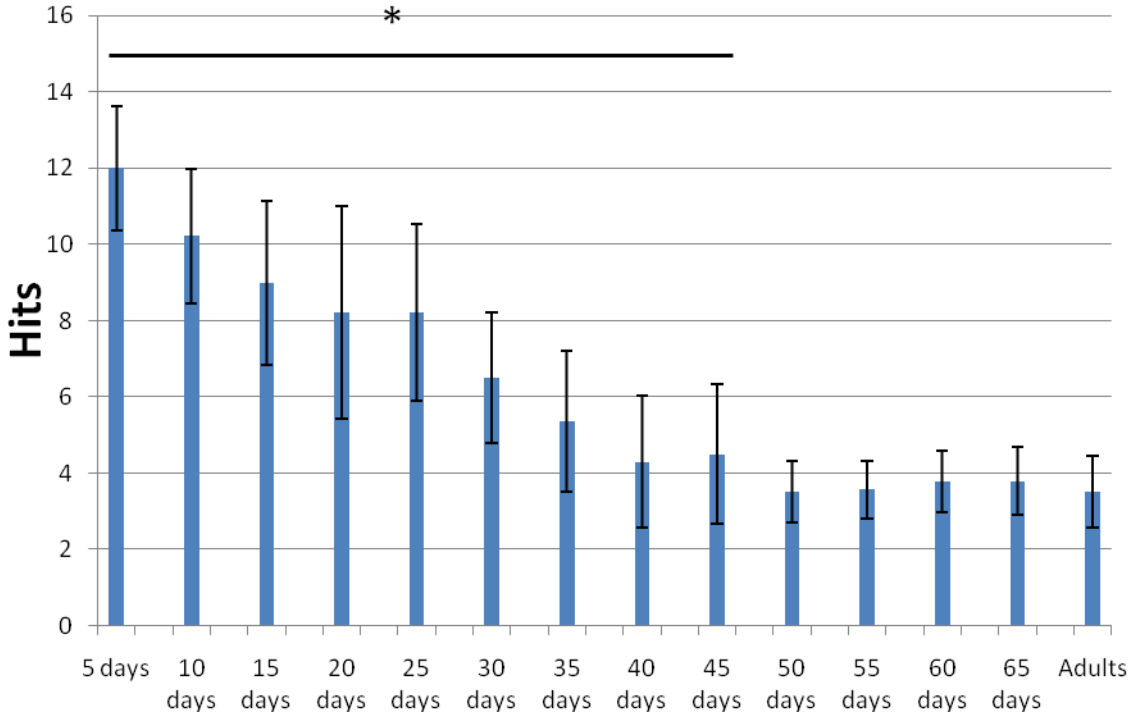

Figure 14. Dowels contacted by *C. perspicillata* at 50% wingspan spacing. The line at the top represents ages where flight ability was significantly different from adults (*, $p < 0.01$; mean ± *SD*).

When comparing agility, taking into account all dowel rod spacing *A. jamaicensis* and *C. perspicillata* had overall similar abilities for the first 20 days post first-flight with a significant difference first occurring at 25 days post first-flight [$F(5, 93) = 4.18$, $p = 0.0182$] and remaining significantly different through adult-like ability. Species-specific differences were found regarding maneuverability at individual wingspan spacing of the dowel rods. *C. perspicillata* at 10 days post first-flight was significantly more maneuverable than *A. jamaicensis* at 50% wingspan dowel spacing [$F(5, 93) = 3.66$, $p = 0.0295$], remaining significantly different through adult ability (Figure 15). *C. perspicillata* were found to be significantly more maneuverable than *A. jamaicensis* at

75% wingspan dowel spacing starting at 30 days post first-flight [$F(5, 93) = 4.81$, $p = 0.0308$] and remained different up to adult ability (Figure 16). When comparing full wingspan dowel spacing *C. perspicillata* was significantly more maneuverable than *A. jamaicensis* at 55 days post first-flight [$F(5, 93) = 3.97$, $p = 0.0493$], however, at 60 days through adult ability, maneuverability was similar ($p > 0.05$) (Figure 17).

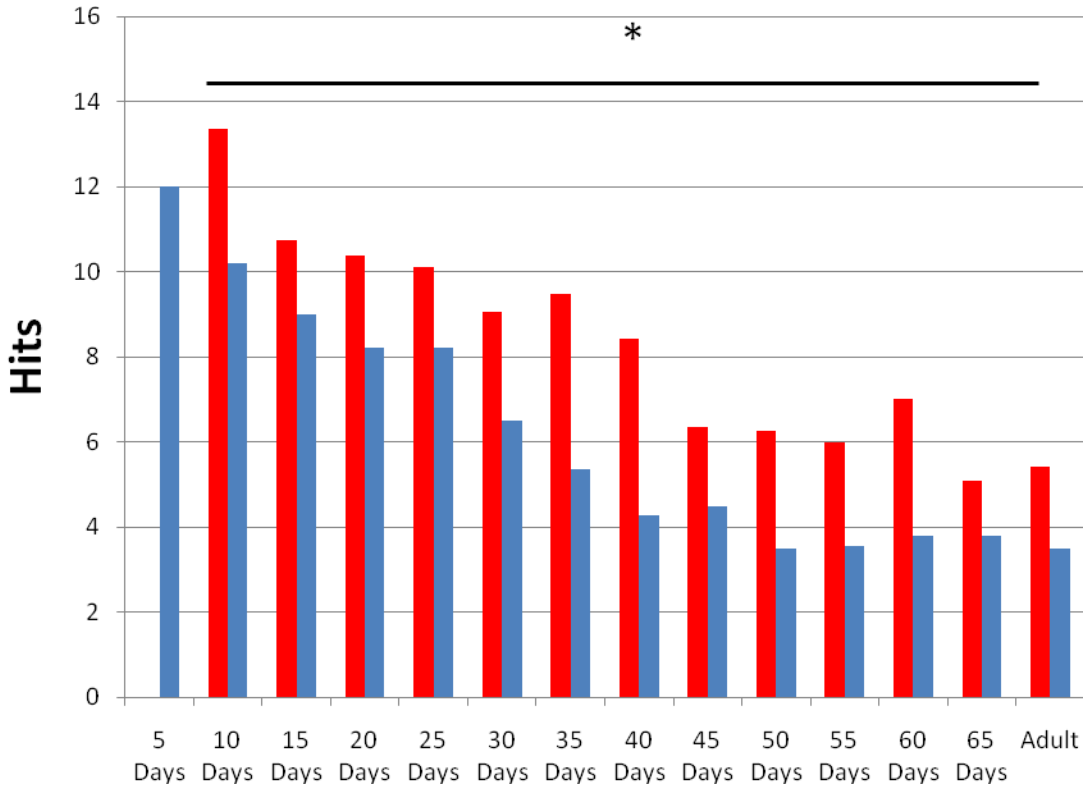

Figure 15. Dowels contacted at 50% wingspan spacing. *Carollia perspicillata*, blue bars and *Artibeus jamaicensis*, red bars. The line at the top represents ages where flight ability was significantly different between species (*, $p < 0.01$; mean ± *SD*).

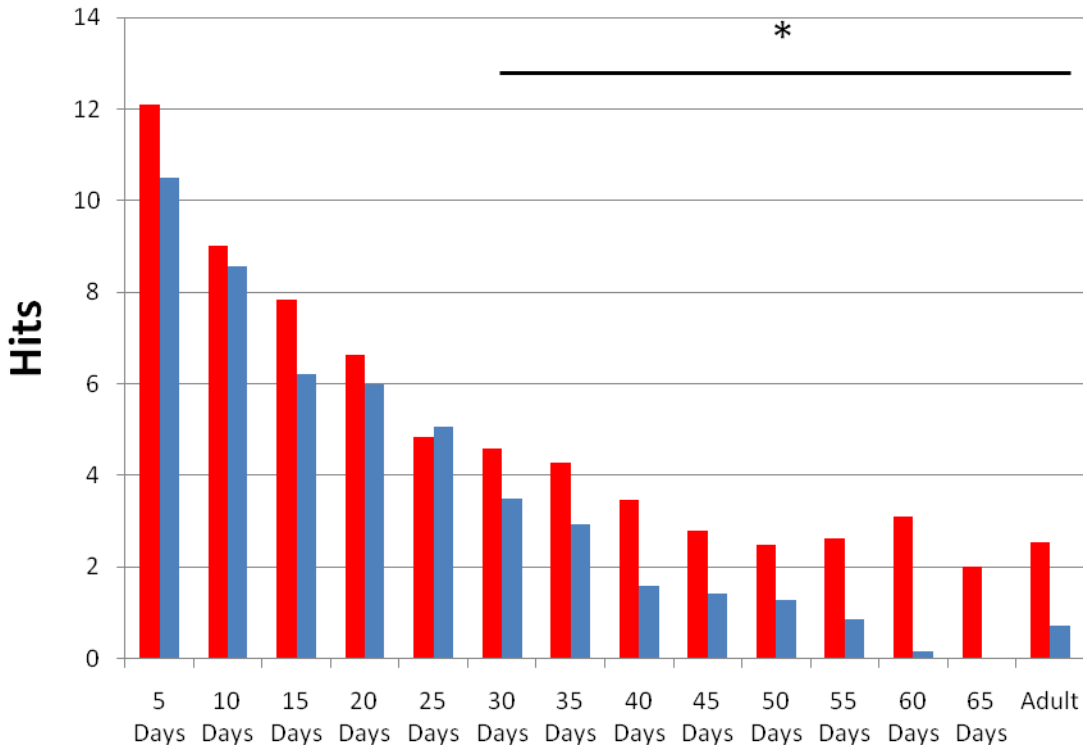

Figure 16. Dowels contacted at 75% wingspan spacing. *Carollia perspicillata*, blue bars and *Artibeus jamaicensis*, red bars. The line at the top represents ages where flight ability was significantly different between species (*, $p < 0.01$; mean ± *SD*).

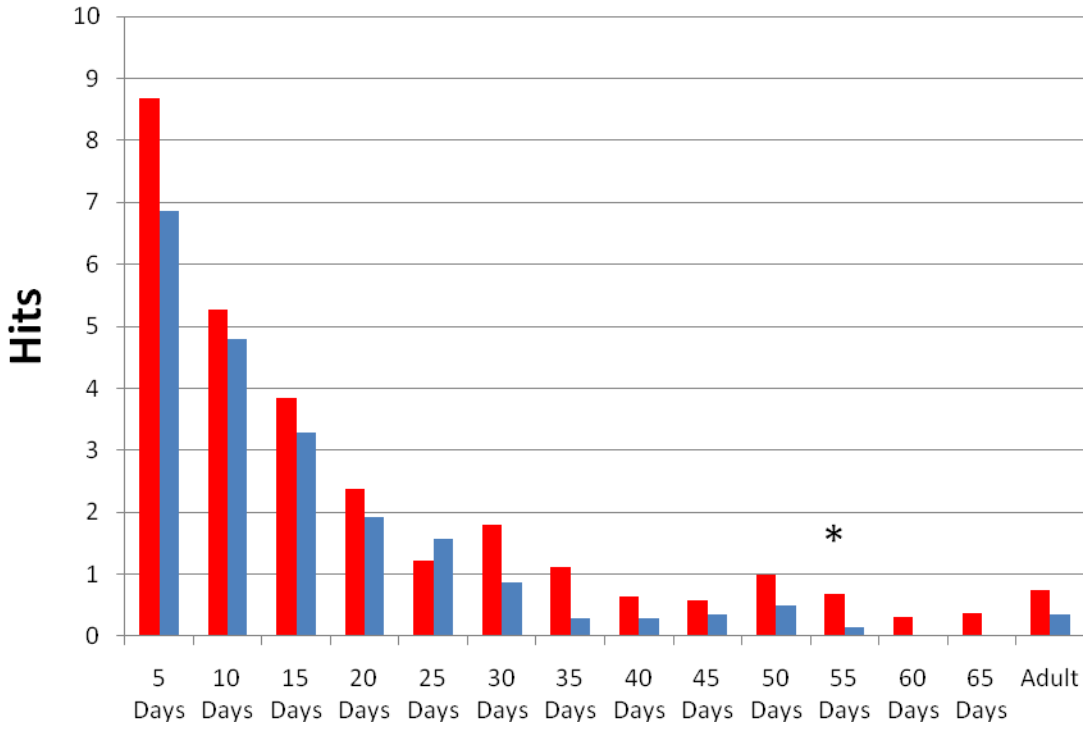

Figure 17. Dowels contacted at full wingspan spacing. *Carollia perspicillata*, blue bars and *Artibeus jamaicensis*, red bars at full wingspan dowel spacing (*, $p < 0.01$; mean ± *SD*).

The Evolution and Development of Wing Form and Body

Morphological Growth Patterns

Growth was measured and analyzed from day one through day 100 post-partum for both *A. jamaicensis* and *C. perspicillata*. Empirical growth curves were calculated using the logistic ($M(t) = A\{e^{-K(t-I)}+1\}^{-1}$), Gompertz ($M(t) = A*e^{-e-K(t-I)}$) and von Bertalanffy ($M(t) = A\{1-1/3e^{-K(t-1)}\}^{3}$) equations (Ricker, 1979) for mass, forearm, wing surface area, wingspan, arm-wing area, hand-wing area, wing length, arm-wing length, and hand-wing length. From each growth equation, growth asymptote (A), growth rate

constant (K), and the point of growth inflection (I, age at maximal growth) were calculated. The logistic equation provided the best fit for both species. This was determined by the smallest coefficient of variation for A, K, and I of the three equations (Table 3).

Mass

The mean ± *SD* mass of one day old *A. jamaicensis* was 10.89 g ± 0.99 g which is 27% of adult mass. Mean *C. perspicillata* mass for one day olds was 4.29 g ± 0.041 g, corresponding to 30% of adult mass. There was a significant difference in the percentage of adult mass of newborn pups between species ($t = 2.8$; $p = 0.008$) with *C. perspicillata* body mass being at a higher percentage of adult mass. The mass of both species increased linearly for the first 50 days with mass increasing at a slower rate thereafter (Figure 18). *A. jamaicensis* reached 90% of adult mass at 68 days post-partum while 100% of adult mass was achieved at 88 days of age.

When taking into account flight development, *A. jamaicensis* achieved flight while at 59% of adult mass, being able to maneuver like an adult at 77 days of age, roughly 93% of adult mass. In contrast *C. perspicillata* attained 90% of adult mass at 56 days post-partum with 100% of adult mass achieved at 60 days post-partum. *C. perspicillata* achieved flight while at 56% of adult mass and was able to maneuver like an adult at 62 days with a mass corresponding to 97% of that of an adult.

Growth parameter obtained from the logistic growth equation showed that *A. jamaicensis* had and an asymptotic mass (A) of 42.892 g, which falls within the mean (± *SD*) of adult mass. The growth rate constant (K) for *A. jamaicensis* was 0.053, while the point of

Table 3

Growth Parameter Comparisons of Artibeus jamaicensis *and* Carollia perspicillata *Derived from the Logistic, Gompertz, and von Bertalanffy Growth Models*

		Species			
		Artibeus jamaicensis		*Carollia perspicillata*	
Model	Parameter	Estimate ± SE	Coefficient of Variation (%)	Estimate ± SE	Coefficient of Variation (%)
Logistic					
Forearm	Growth Asymptote (A)	58.191 (0.095)	0.163	44.414 (0.184)	0.414
	Growth Constant (K)	0.0760 (0.001)	1.316	0.063 (0.002)	3.175
	Growth Inflection Point (I)	0.9760 (0.129)	13.217	0.127 (0.32)	251.969
Mass	Growth Asymptote (A)	42.892 (0.483)	1.126	16.607 (0.13	0.783
	Growth Constant (K)	0.0530 (0.001)	1.887	0.042 (0.001)	2.381
	Growth Inflection Point (I)	26.184 (0.650)	2.482	19.495 (0.393)	2.06
Wing Area	Growth Asymptote (A)	7334.175 (24.737)	0.337	4562.780 (43.953)	0.963
	Growth Constant (K)	0000.070 (00.001)	1.429	0.067 (0.002)	2.985
	Growth Inflection Point (I)	0016.658 (00.158)	0.948	17.510 (0.454)	2.593
Wingspan	Growth Asymptote (A)	442.253 (24.737)	0.393	345.096 (1.064)	0.308
	Growth Constant (K)	0.076 (0.001)	1.316	0.062 (0.001)	1.613
	Growth Inflection Point (I)	3.996 (5.831)	5.831	6.030 (0.178)	2.952
Arm-wing Area	Growth Asymptote (A)	4215.511 (17.298)	0.410	2299.455 (23.14)	1.006
	Growth Constant (K)	0.068 (0.001)	1.471	0.066 (0.483)	4.545
	Growth Inflection Point (I)	14.925 (0.196)	1.313	13.464 (0.483)	3.587
Hand-wing area	Growth Asymptote (A)	3118.180 (18.629)	0.597	2246.500 (33.524)	21.353
	Growth Constant (K)	0.075 (0.002)	2.667	0.070 (0.003)	4.286
	Growth Inflection Point (I)	18.784 (0.273)	1.453	21.353 (0.686)	3.213

Table 3 (continued)

Model	Parameter	Species: *Artibeus jamaicensis* Estimate ± SE	*Artibeus jamaicensis* Coefficient of Variation (%)	*Carollia perspicillata* Estimate ± SE	*Carollia perspicillata* Coefficient of Variation (%)
Wing Length	Growth Asymptote (A)	145.514 (0.385)	0.265	108.689 (0.616)	0.567
	Growth Constant (K)	0.062 (0.001)	1.613	0.058 (0.002)	3.448
	Growth Inflection Point (I)	5.530 (0.151)	3.333	3.760 (0.346)	9.202
Arm-wing Length	Growth Asymptote (A)	58.728 (0.135)	0.229	46.105 (0.535)	1.160
	Growth Constant (K)	0.060 (0.001)	1.667	0.045 (0.003)	6.667
	Growth Inflection Point (I)	0.443 (0.169)	38.149	0.152 (0.061)	40.132
Hand-wing Length	Growth Asymptote (A)	86.776 (0.421)	0.485	63.316 (0.305)	0.482
	Growth Constant (K)	0.075 (0.001)	1.333	0.071 (0.002)	2.817
	Growth Inflection Point (I)	7.458 (0.254)	3.406	6.333 (0.271)	4.279
Gompertz					
Forearm	Growth Asymptote (A)	58.577 (0.137)	0.234	44.851 (0.196)	0.437
	Growth Constant (K)	0.064 (0.001)	1.563	0.052 (0.001)	1.923
	Growth Inflection Point (I)	-4.140 (0.210)	-5.072	-5.979 (0393)	-6.573
Mass	Growth Asymptote (A)	46.544 (0.867)	1.862	17.371 (0.196)	1.117
	Growth Constant (K)	0.027 (0.001)	3.704	0.037 (0.001)	2.701
	Growth Inflection Point (I)	15.915 (0.751)	4.719	10.588 (0.369)	3.489
Wing Area	Growth Asymptote (A)	7549.236 (41.1930)	0.546	4703.086 (48.46)	1.030
	Growth Constant (K)	0.050 (0.001)	2.000	0.048 (0.002)	4.167
	Growth Inflection Point (I)	9.417 (0.188)	1.990	10.116 (0.347)	3.340
Wingspan	Growth Asymptote (A)	447.624 (2.481)	0.554	348.840 (1.692)	0.485
	Growth Constant (K)	0.050 (0.001)	2.000	0.060 (0.002)	3.333
	Growth Inflection Point (I)	-2.820 (0.367)	-13.014	0.140 (0.288)	205.714

Table 3 (continued)

Model	Parameter	Species: *Artibeus jamaicensis* Estimate ± SE	*Artibeus jamaicensis* Coefficient of Variation (%)	*Carollia perspicillata* Estimate ± SE	*Carollia perspicillata* Coefficient of Variation (%)
Arm-wing Area	Growth Asymptote (A)	4333.173 (21.208)	0.487	2356.347 (22.467)	0.953
	Growth Constant (K)	0.048 (0.001)	2.083	0.051 (0.002)	3.922
	Growth Inflection Point (I)	7.524 (0.172)	2.286	6.542 (0.352)	5.381
Hand-wing area	Growth Asymptote (A)	3213.777 (29.796)	0.927	2328.985 (44.00)	1.889
	Growth Constant (K)	0.053 (0.002)	3.774	0.048 (0.003)	6.250
	Growth Inflection Point (I)	11.778 (0.309)	2.623	13.886 (0.613)	4.415
Wing Length	Growth Asymptote (A)	147.765 (0.558)	0.398	110.188 (0.618)	0.561
	Growth Constant (K)	0.046 (0.001)	2.174	0.050 (0.002)	4.000
	Growth Inflection Point (I)	-2.819 (0.215)	-7.627	-2.853 (0.366)	-12.829
Arm-wing Length	Growth Asymptote (A)	59.302 (0.172)	0.290	46.722 (0.572)	1.224
	Growth Constant (K)	0.050 (0.001)	2.000	0.037 (0.002)	5.405
	Growth Inflection Point (I)	-6.028 (0.247)	-4.097	-9.857 (0.930)	-9.435
Hand-wing Length	Growth Asymptote (A)	88.526 (0.553)	0.625	64.184 (0.322)	0.502
	Growth Constant (K)	0.044 (0.001)	2.273	0.058 (0.002)	3.448
	Growth Inflection Point (I)	-0.300 (0.298)	-99.333	0.350 (0.286)	81.714
Von Bertalanffy					
Forearm	Growth Asymptote (A)	58.742 (0.157)	0.267	45.032 (0.203)	0.451
	Growth Constant (K)	0.060 (0.001)	1.667	0.049 (0.001)	2.041
	Growth Inflection Point (I)	-6.089 (0.264)	-4.336	-8.335 (0.438)	-5.255
Mass	Growth Asymptote (A)	48.826 (1.128)	2.310	17.795 (0.239)	1.343
	Growth Constant (K)	0.022 (0.001)	4.545	0.032 (0.001)	3.125
	Growth Inflection Point (I)	10.482 (0.705)	6.726	6.269 (0.359)	5.727

Table 3 (continued)

		Species			
		Artibeus jamaicensis		*Carollia perspicillata*	
Model	Parameter	Estimate ± SE	Coefficient of Variation (%)	Estimate ± SE	Coefficient of Variation (%)
Wing Area	Growth Asymptote (A)	7660.699 (55.848)	0.729	4778.655 (53.199)	1.113
	Growth Constant (K)	0.043 (0.001)	2.326	0.042 (0.001)	2.381
	Growth Inflection Point (I)	6.050 (0.221)	3.653	6.676 (0.327)	4.898
Wingspan	Growth Asymptote (A)	449.970 (2.832)	0.629	350.488 (1.990)	0.568
	Growth Constant (K)	0.046 (0.002)	4.348	0.055 (0.002)	3.633
	Growth Inflection Point (I)	-5.546 (0.442)	-7.969	-2.241 (0.357)	-15.930
Arm-wing Area	Growth Asymptote (A)	4393.450 (26.379)	0.600	2385.775 (23.170)	0.971
	Growth Constant (K)	0.042 (0.001)	2.381	0.045 (0.002)	4.444
	Growth Inflection Point (I)	4.146 (0.195)	4.703	3.445 (0.339)	9.840
Hand-wing area	Growth Asymptote (A)	3265.212 (38.244)	1.171	2376.048 (51.843)	2.182
	Growth Constant (K)	0.045 (0.002)	4.444	0.041 (0.003)	7.317
	Growth Inflection Point (I)	8.434 (0.335)	3.972	10.269 (0.587)	5.716
Wing Length	Growth Asymptote (A)	148.761 (0.635)	0.427	110.848 (0.636)	0.574
	Growth Constant (K)	0.042 (0.001)	2.381	0.046 (0.001)	2.174
	Growth Inflection Point (I)	-5.820 (0.273)	-4.691	-5.526 (0.392)	-7.094
Arm-wing Length	Growth Asymptote (A)	59.541 (0.195)	0.328	47.004 (0.601)	1.279
	Growth Constant (K)	0.046 (0.001)	2.174	0.034 (0.002)	5.882
	Growth Inflection Point (I)	-8.524 (0.297)	-3.484	-13.147 (1.090)	-8.291
Hand-wing Length	Growth Asymptote (A)	89.335 (0.631)	0.706	64.574 (0.343)	0.531
	Growth Constant (K)	0.040 (0.001)	2.500	0.053 (0.001)	1.887
	Growth Inflection Point (I)	-3.593 (0.358)	-9.964	-2.115 (0.318)	-15.035

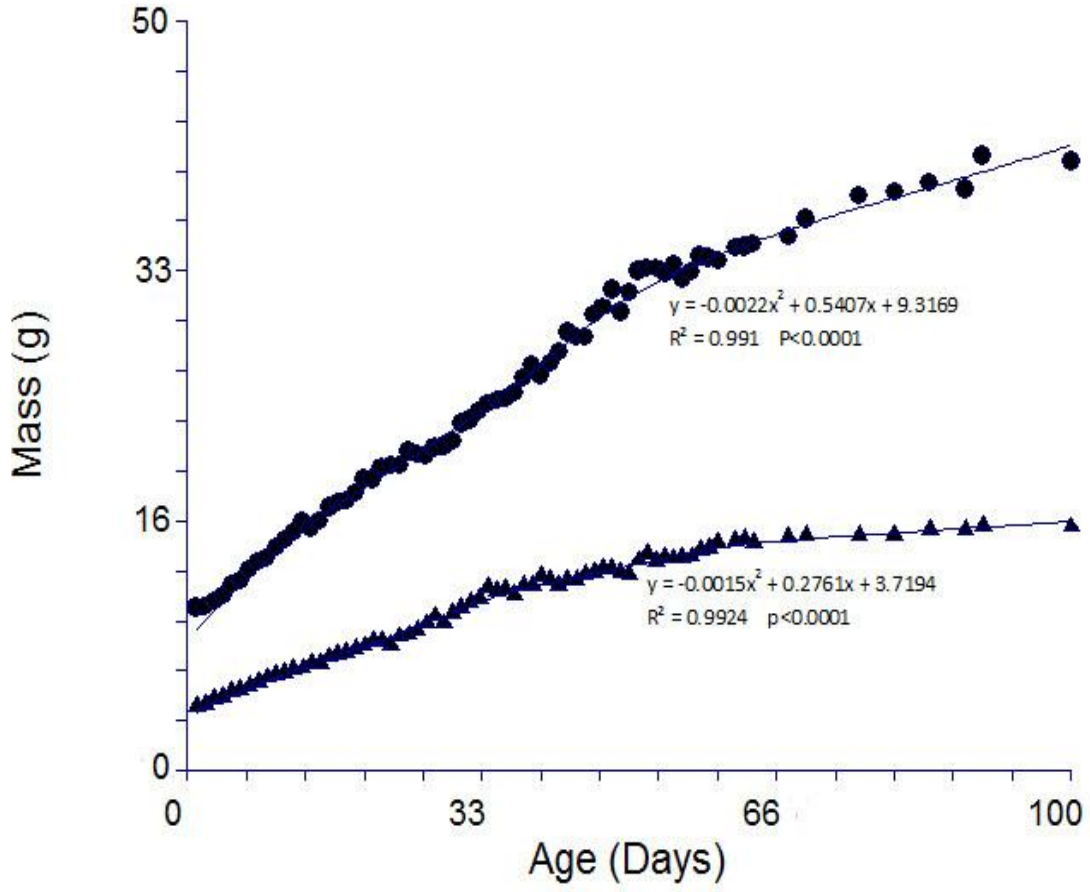

Figure 18. Daily mean mass for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

growth inflection (I) indicated *A. jamaicensis* had the most growth occurring at 26.184 days of age. The mean growth asymptotic mass (A) for *C. perspicillata* obtained through the logistic growth equation was 16.601 which fall within the mean (± *SD*) mass for adults. The growth rate constant (K) for mass of *C. perspicillata* was 0.042 with the *C. perspicillata* inflection point (I, the highest rate of growth) occurred around 19.495 days of age. *A. jamaicensis* had a significantly larger growth rate constant than that of *C. perspicillata* ($t = 21.06$, $p < 0.0001$).

Intra-specific variation was minimal for mass of both species throughout development signified by a high regression r^2 values of 0.991 for *A. jamaicensis* and 0.992 for *C. perspicillata* (Figure 18) acquired from best-fit polynomial.

Forearm

The mean ± *SD* forearm length for one day old *A. jamaicensis* was 29.34 mm ± 1.82 mm which corresponds to 52% of adult size while *C. perspicillata* forearm was 23.77 mm ± 1.19 mm, 53.5% of adult size. There was a significant difference in the percentage of adult forearm length of pups at birth between species ($t = 5.49$, $p < 0.0001$). The length of the forearm in both species increased in a linear fashion for the first four weeks (Figure 19) reaching a plateau with the rate of growth stabilizing. *A. jamaicensis* reached 90% of adult forearm length at 28 days of age whereas 100% of adult forearm length was reached at 43 days of age. *A. jamaicensis* began flying at 94% of adult forearm length and was able to fully maneuver like an adult at 77 days of age, corresponding to 99% of adult forearm length. *C. perspicillata* reached 90% of adult forearm length at 32 days of age while reaching 100% adult forearm length at 60 days.

C. perspicillata began flying with a forearm at 82% of adult size while being able to maneuver like an adult with a forearm of 99% of adult size.

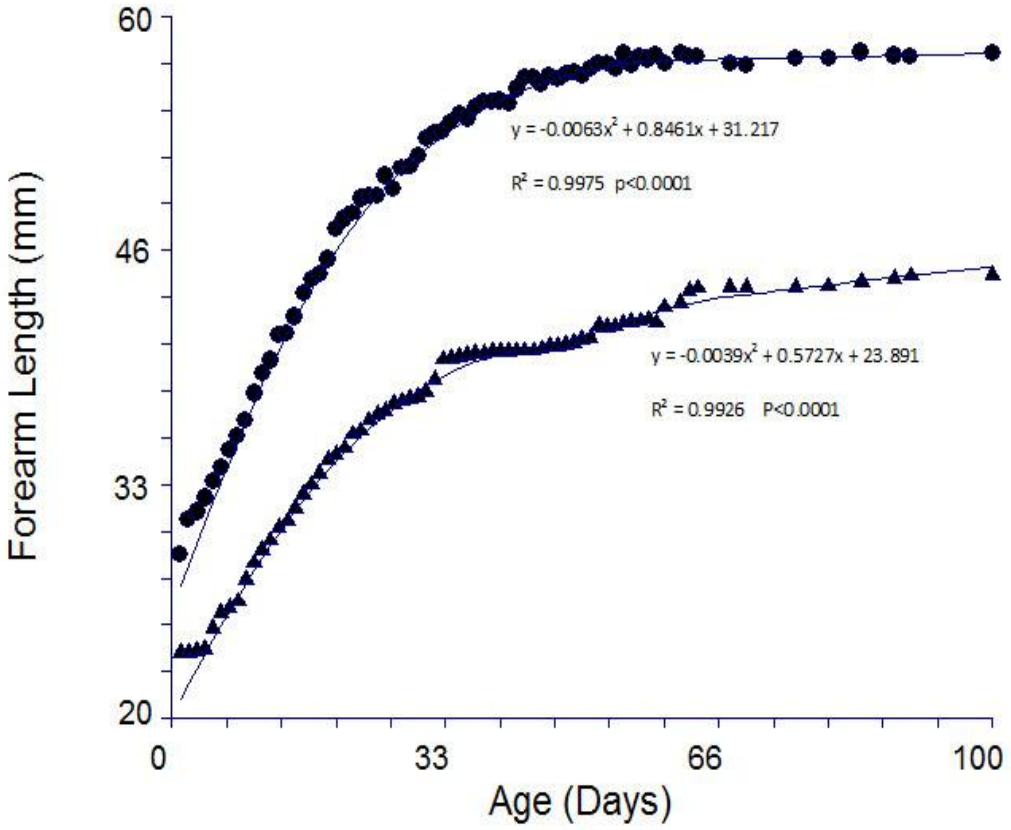

Figure 19. Daily mean forearm for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

Intra-specific growth of the forearm showed little variation among individuals throughout growth and development with high regression r^2 values of 0.9975 for *A. jamaicensis* and 0.9926 for *C. perspicillata*, acquired from best-fit polynomial regression lines (Figure 19). Logistic growth equations provided a mean growth asymptote of 58.20 mm for *A. jamaicensis* and 44.41 mm for *C. perspicillata* which falls within the actual

measured forearm lengths (mean ± *SD*) in adults of both species. The growth rate constant for *A. jamaicensis* was 0.076 whereas *C. perspicillata* had a forearm growth rate constant of 0.063 which was significant from *A. jamaicensis* that exhibited faster post-partum growth rate ($t = 20.76$, $p < 0.0001$) (Table 3). The point of growth inflection (I) for both species was near 1 day of age, indicating that growth rate was increasing at the overall fastest rate during the first 24 hours after birth.

The allometric relationship between forearm growth and the increase in mass was found to be different between species. Linear regression of log-transformed data comparing forearm and mass showed that both species had little variation with high regression r^2 values, with *A. jamaicensis* having an r^2 of 0.82 and *C. perspicillata* having an r^2 of 0.94 (Figure 20). The slopes of the regression lines using ANCOVA were found to be similar between species [$F(3, 138) = 0.19$, $p = 0.6622$], however, there was a significant difference in the Y-intercept [$F(3, 138) = 75.06$, $p < 0.0001$] indicating the regression lines cross the y-intercept at significantly different locations indicating a difference in the regression lines between species. In spite of the non-significant slopes allometric scaling indicated that the slope of the forearm and mass regression line of *C. perspicillata* was greater than 1, indicating positive allometry, meaning a greater increase in forearm for each increase in mass. The slope for *A. jamaicensis* showed negative allometric scaling with a slope of less than 1, indicating that mass was increasing faster than forearm growth.

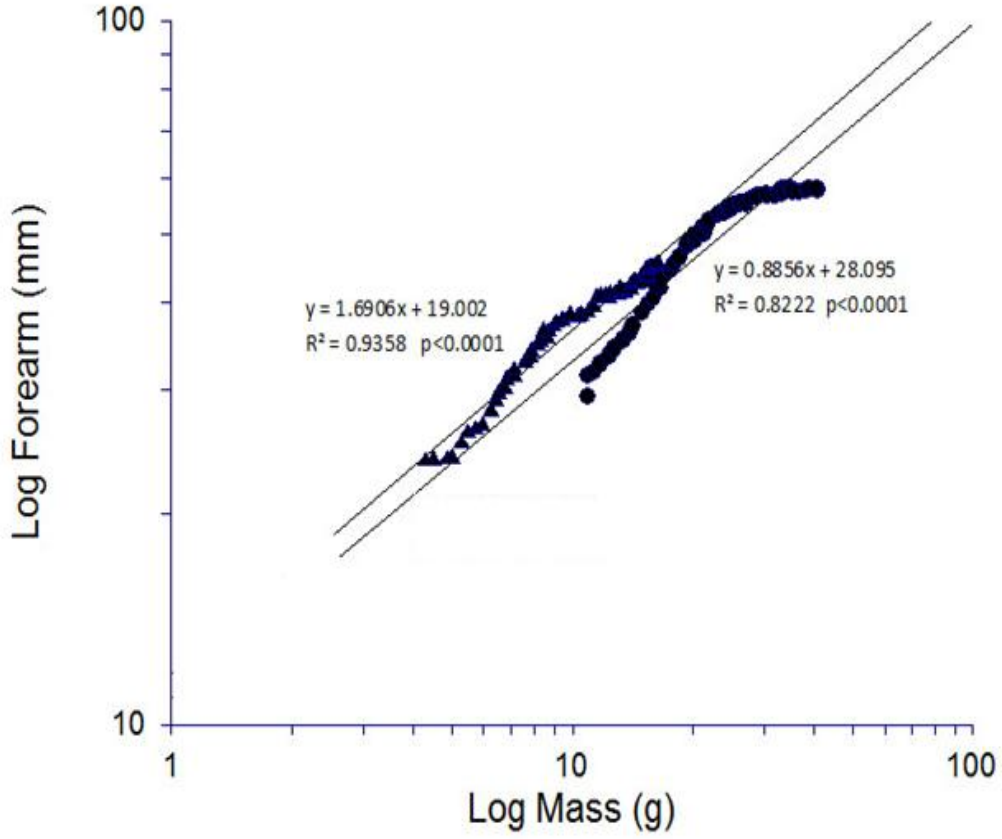

Figure 20. Relationship of mass (Log g) and forearm length (Log mm). *Artibeus jamaicensis* indicated by the circles has a slope less than one indicating negative allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are not significantly different [$F(3, 138) = 0.19$, $p = 0.6622$].

Wingspan

The mean ± *SD* wingspan at birth for *A. jamaicensis* was 206 mm ± 8.5 mm which corresponds to 51% of adult wingspan. *C. perspicillata* had a mean wingspan of 145 mm ± 8.44 mm, corresponding to 50% of the adult wingspan. There was not a significant difference between the wingspan of *A. jamaicensis* and *C. perspicillata* at 1 day post-partum (*A. jamaicensis* $n = 45$, *C. perspicillata* n = 25; $t = 1.06$, $p = 0.314$). *A.*

jamaicensis achieved 90% of adult wingspan at 35 days post-partum while reaching 100% of adult wingspan at 48 days post-partum. *C. perspicillata* achieved 90% of adult wingspan at 31 days post-partum while achieving 100% of adult wingspan at 45 days post-partum. *A. jamaicensis* achieved flight at 90% of adult wingspan while being able to maneuver like and adult at 100% of adult wingspan. *C. perspicillata* achieved flight at 82% of adult wingspan while being able to maneuver like an adult at 100% of adult wingspan.

Intra-specific variation was minimal during growth and development of wingspan, portrayed by high regression r^2 for *A. jamaicensis* of 0.9912 and an r^2 for *C. perspicillata* of 0.9794 obtained from best-fit polynomial regression (Figure 21). Growth asymptotes (A) for wingspan attained from logistic growth equations for *A. jamaicensis* was 442.25 mm and 345.1mm for *C. perspicillata*, which in both cases is higher than the mean ± 1 standard deviation for adult wingspan. Growth rate constants (K) were significantly different for both species ($t = 26.26$, $p < 0.0001$) with K for *A. jamaicensis* being 0.076 and 0.062 for *C. perspicillata*. The inflection point (I) showed that the rate of fasted growth of the wingspan occurred during day 4 post-partum for *A. jamaicensis* and day 6 post-partum for *C. perspicillata* (Table 3).

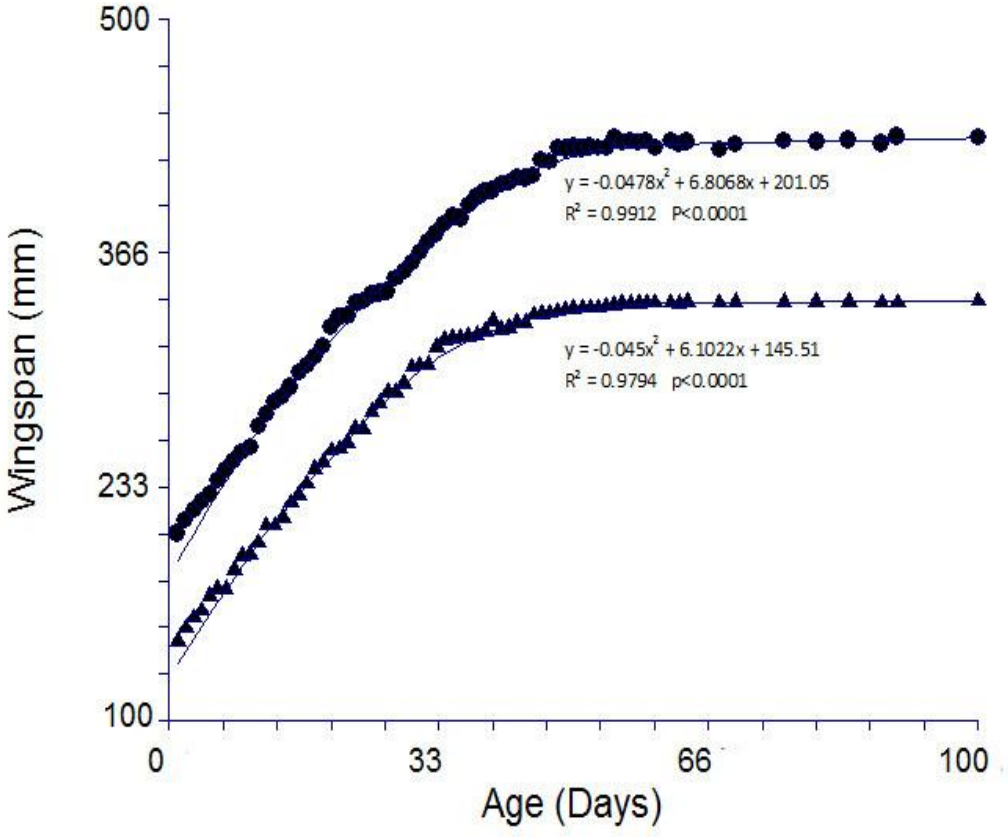

Figure 21. Daily mean wingspan for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

The allometric relationship of wingspan to mass was significantly different between the slopes of the two species [$F(3, 138) = 9.16$, $p = 0.0029$] obtained using an ANCOVA (Figure 22), with *C. perspicillata* having an overall steeper slope than *A. jamaicensis*. Linear regression of log-transformed data comparing wing area and mass showed that both species had little variation indicated by high regression r^2, with *A. jamaicensis* having an r^2 of 0.8971 and *C. perspicillata* having an r^2 of 0.9201 (Fig. 22). The slopes for both species were positive indicating that as the bats grew, wingspan

increased at a greater rate than did increase in mass resulting in, positive allometry (slopes ≥ 1) (Figure 22).

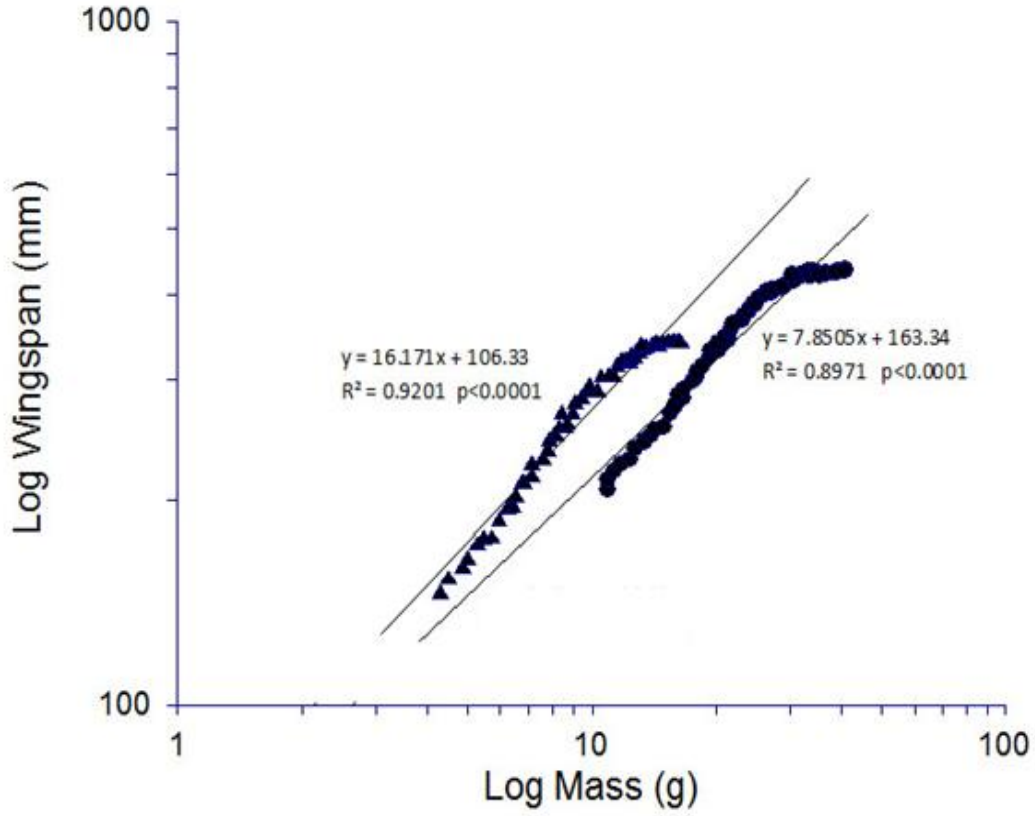

Figure 22. Relationship of mass (Log g) and wingspan (Log mm). Both *Artibeus jamaicensis* (circles) *Carollia perspicillata* (triangles) have slopes greater than one indicating positive allometry. ANCOVA indicate that the slopes of the two species are significantly different [$F(3, 138) = 15.88$, $p = 0.0001$] with *C. perspicillata* wingspan increasing more per one increase in mass.

Wing Area and Length

The mean ± *SD* area of the wing area (from the body distal) of one day old *A. jamaicensis* was 1700.23 ± 231.298 mm^2 which corresponds to 22% of adult wing area whereas the mean ± *SD* wing length for one day old *A. jamaicensis* was 65.8 ± 3.69 mm, corresponding to 46% of adult wing length. Mean area of the wing for *C. perspicillata*

was 998.23 ± 121.89 mm^2 which is 23.5% of adult measurements while wing length was 47.88 ± 4.13 mm corresponding to 48% of adult wing length. Wing area and wing length were found to be significantly higher in *C. perspicillata* than in AJ (t = 0.458, p = 0.0001, wing area; t = 8.02, p = 0.0001, wing length). Wing area in *A. jamaicensis* increased in a linear fashion for 35 days post-partum while *C. perspicillata* wing area increased linearly for the first 30 days (Figures 23 and 24) wing length increased linearly in *A. jamaicensis* for 40 days and 30 days post-partum for *C. perspicillata*.

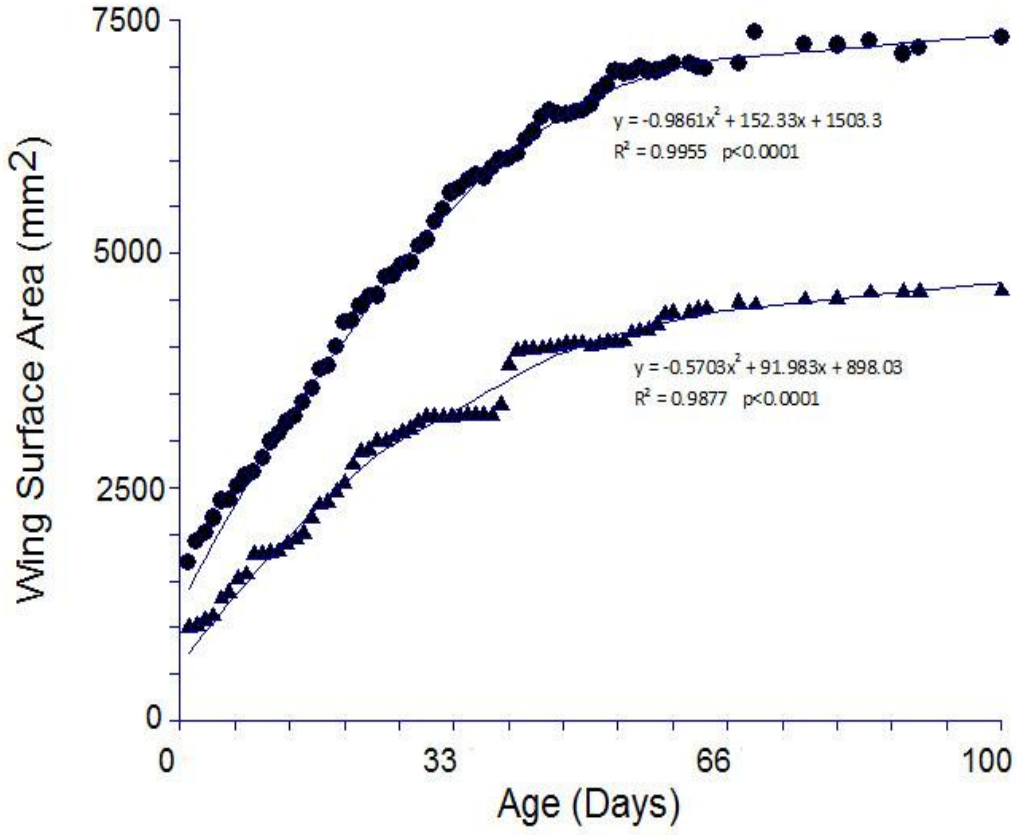

Figure 23. Daily mean wing surface area for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

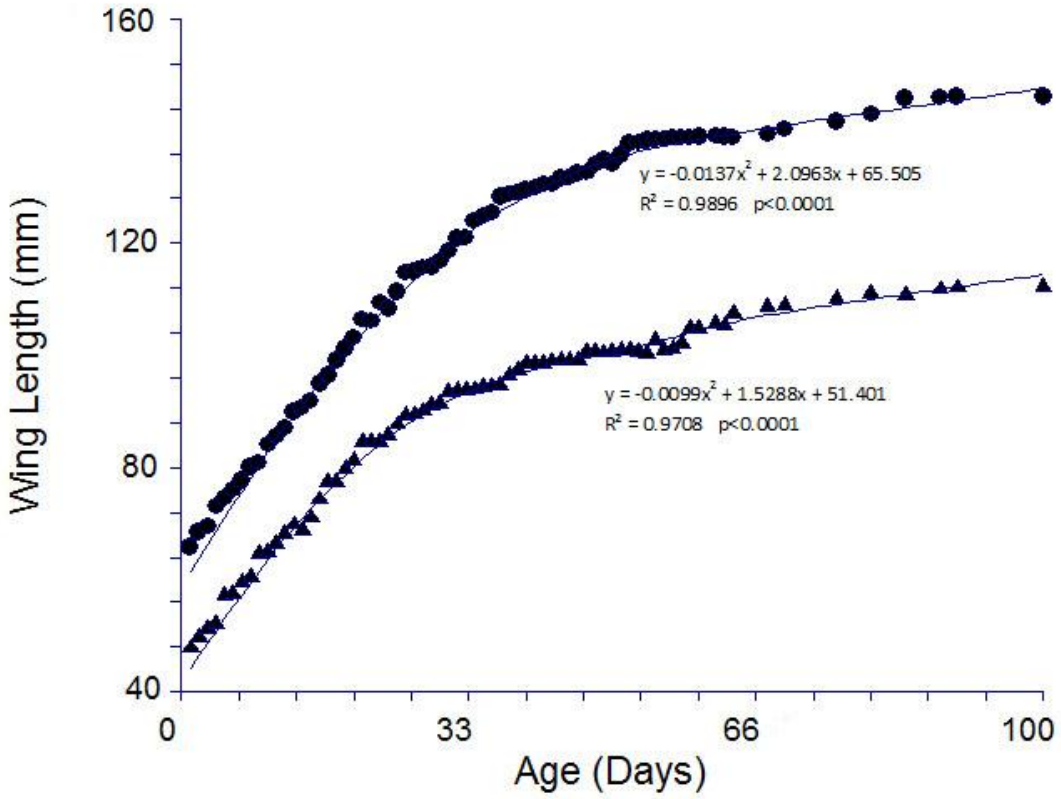

Figure 24. Daily mean wing length for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

A. jamaicensis reached 90% of adult wing area at 50 days post-partum and reached 100% adult wing area by 70 days post-partum while achieving 90% and 100% adult wing length at 42 days and 88 days post-partum. *A. jamaicensis* began sustained flight with a wing area and wing length of 77% and 86% of adult proportions whereas adult-like maneuverability was obtained when the juvenile wing area and wing length was at 99% and 98% of adult proportions. *C. perspicillata* attained 90% of adult wing area and wing length at 41 and 45 days post-partum while reaching 100% adult wing area and wing length at 70 and 80 days post-partum. *C. perspicillata* began flying with a wing

area and wing length of 67% and 78% of adult wing area and length while being able to maneuver like an adult at 100% and 96% of adult wing area and length.

Intra-specific variation was minimal pertaining to the growth of the wing area and length, represented by high correlation coefficients with an r^2 for *A. jamaicensis* of 0.9955 for wing area and 0.9896 for wing length while *C. perspicillata* had an r^2 of 0.9877 for wing area and 0.9708 for wing length, obtained from best-fit polynomial regression (Figs. 23 and 24). Growth asymptotes for wing area and length were obtained using logistic growth equations. Wing area for *A. jamaicensis* was 7334.175 mm^2 and 4562.78 mm^2 for *C. perspicillata*, which are both similar to the measured wing area in adults. Growth asymptotes for wing length were 145.514 mm for *A. jamaicensis* and 108.689 for *C. perspicillata*, which fall within the adult range of wing length. Growth rate constants for wing area were significantly different between species ($t = 6.91$, $p < 0.0001$) with a mean of 0.07 for *A. jamaicensis* and 0.067 for *C. perspicillata* and growth rate constants for wing length were 0.062 for *A. jamaicensis* and 0.058 for *C. perspicillata* which were significantly higher for *A. jamaicensis* ($t = 7.64$, $p < 0.0001$) representing a heterochronic relationship between the two species for both wing area and length. Mean point of inflection showed that wing area and length for *A. jamaicensis* were increasing at the fasted rate near 16 and 4.53 days post-partum while the wing area and length for *C. perspicillata* increased at the fasted rate near 17.5 and 3.76 days post-partum (Table 3).

The allometric relationship of wing area increase and mass increase was similar between species. Linear regression of log-transformed data comparing wing area and mass showed that intra-species variation for both species was minimal with correlation

coefficient for *A. jamaicensis* of r^2 of 0.9381 and *C. perspicillata* having an r^2 of 0.9687 (Figure 25). The slopes of the regression lines using ANCOVA were found to be similar between species [$F(3.\ 139) = 2.26$, $p = 0.1347$], however, the Y-intercept was found to be significantly different between species [$F(3.\ 139) = 351.48$, $p < 0.0001$] indicating allometric relationships with the regression lines crossing the Y-intercept at significantly different locations (Figure 25).

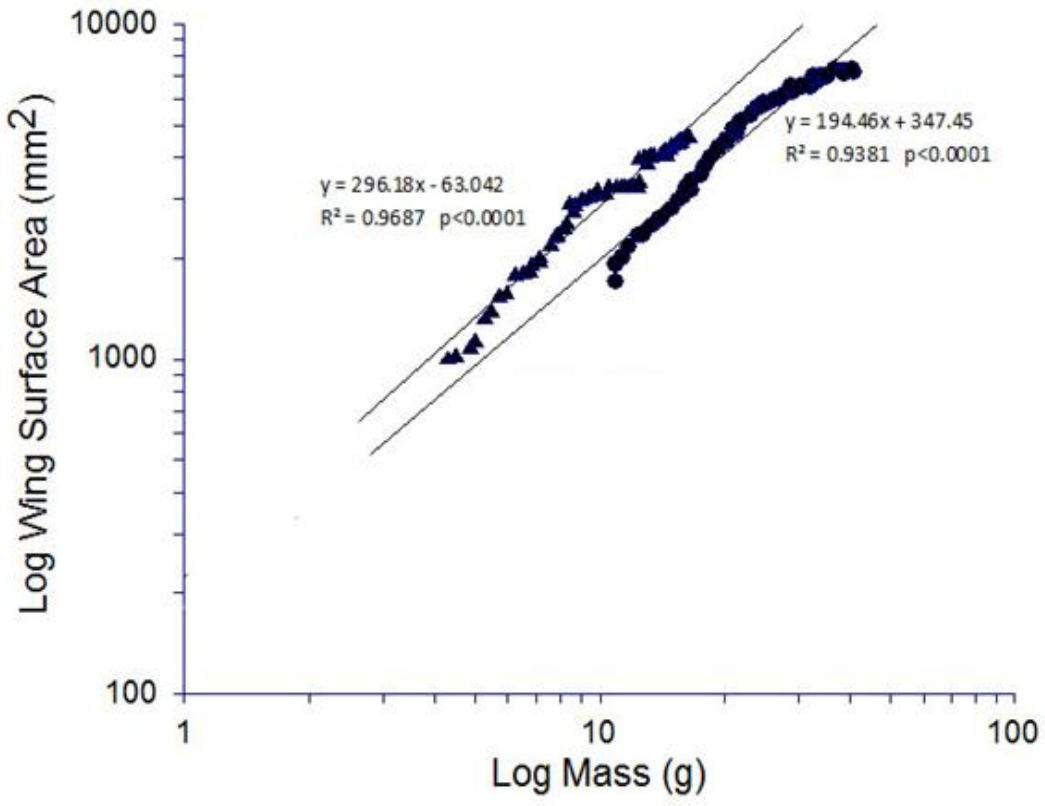

Figure 25. Relationship of mass (Log g) and wing area (Log mm^2). *Artibeus jamaicensis* indicated by the circles has a slope greater than one indicating positive allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are not significantly different [F(3, 138) = 2.26, $p = 0.1347$].

Slopes of the regression lines for wing length were significantly different between species [$F(3, 138) = 15.88$, $p = 0.0001$] (Figure 26) suggesting that the wing length of *C. perspicillata* increased more per increase in mass than *A. jamaicensis*. Slopes for both species indicated that as the bats grew, the wing area and length increased more per each increase in mass indicating positive allometry with slopes that are greater than 1 (Figures. 25 and 26).

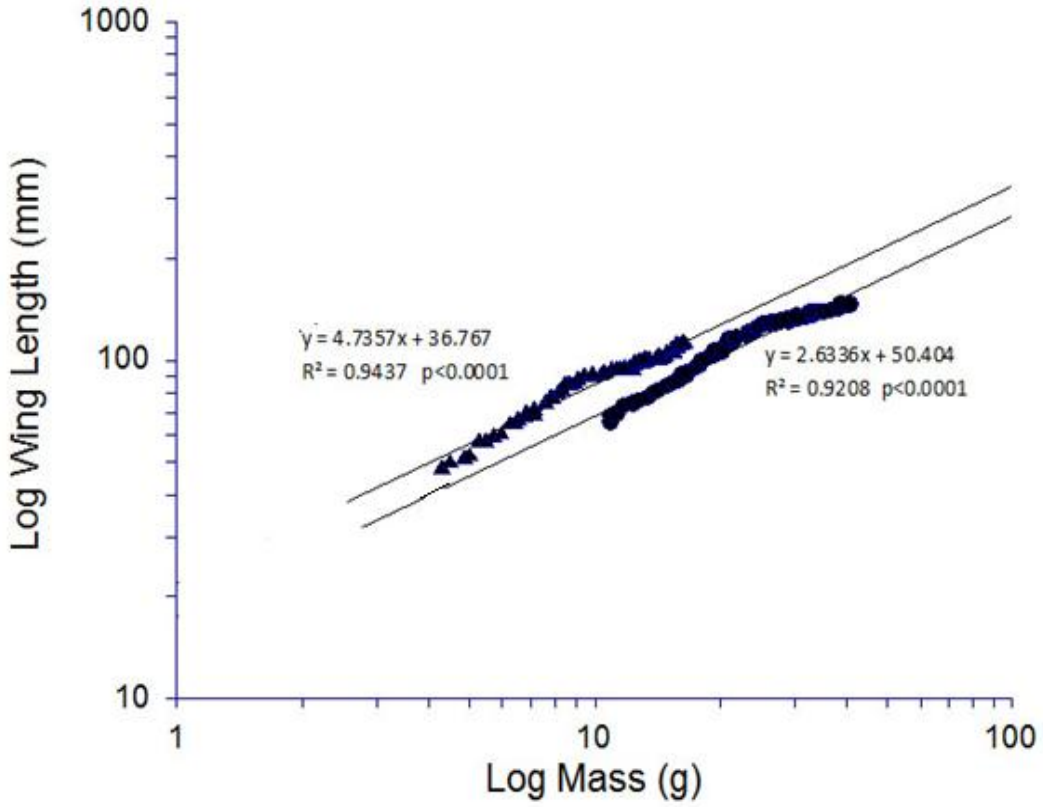

Figure 26. Relationship of mass (Log g) and wing length (Log mm^2). *Artibeus jamaicensis* indicated by the circles has a slope greater than one indicating positive allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are not significantly different [$F(3, 138) = 15.88$, $p = 0.0001$].

Patterns for wing loading (mass divided by the wing area) for both *A. jamaicensis* and *C. perspicillata* were similar with high values in pups when compared to adults. There was a rapid decrease in wing loading as the bats aged with adult levels reached at 4 days post-partum for *A. jamaicensis* and 10 days post-partum for *C. perspicillata* (Figure 27). During growth and development, individuals of both species surpass the wing loading values of the adult with *A. jamaicensis* (*n* = 45) reaching 129% of adult wing loading values at 33 days post-partum and *C. perspicillata* (*n* = 25) reaching adult wing loading values of 120% at 23 days post-partum. Wing loading proceeded to increase to adult values as juvenile weights increased over time to (Figure 27).

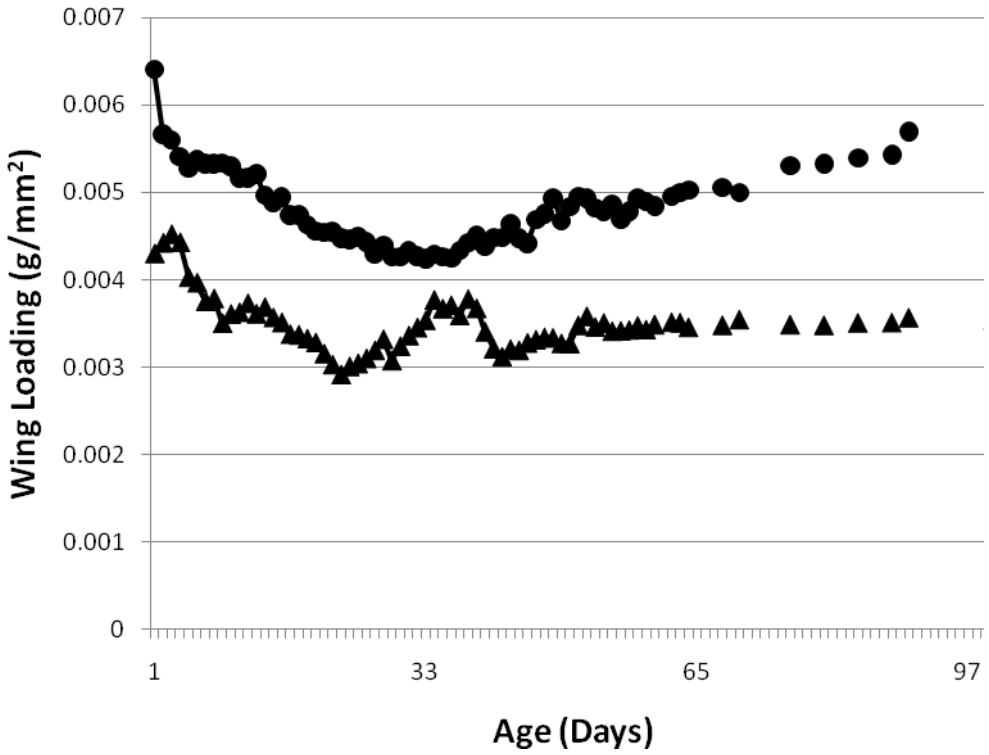

Figure 27. Daily mean wing loading values for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Wing loading represents the ratio of mass (g) of the bat to the wing area (mm^2).

The wing was further broken down into arm-wing (area extending distal from the body to the fifth-digit) and the hand-wing (area extending distal from the fifth-digit to the wing-tip). The mean ± *SD* arm-wing area for one day old *A. jamaicensis* (*n* = 45) was 1170.71 ± 144.02 mm^2 which corresponds to 28% of adult arm-wing area. *C. perspicillata* (*n* = 25) was 593.52 ± 80.87 mm^2. There was a significant difference between species in the percentage of adult arm-wing area of pups at birth with arm-wing area of *A. jamaicensis* at 28% and *C. perspicillata* at 30% ($t = 12.36$, $p = 0.0001$). Arm-wing area growth of *A. jamaicensis* increased in a linear fashion for the first 40 day while *C. perspicillata* arm-wing growth increased linearly for the first 28 days post-partum (Figure 28). *A. jamaicensis* reached 90% of adult arm-wing area at 48 days post-partum whereas 100% adult arm-wing area was reached at 68 days post-partum. *A. jamaicensis* achieved sustained flight with arm-wing area at 81% of adult arm-wing area with adult-like agility being achieved at 100% of adult arm-wing area. *C. perspicillata* reached 90% of adult arm-wing area 55 days post-partum while achieving 100% of adult arm-wing area at 70 days post-partum. *C. perspicillata* achieved flight at 67% of adult arm-wing area and was able to maneuver like an adult at 94% of that of adult arm-wing area.

The mean ± *SD* arm-wing length (length from the shoulder distal to the fifth metacarpal) for one day old *A. jamaicensis* (*n* = 45) was 30.27 ± 1.85 mm corresponding to 53% of adult arm-wing length. *C. perspicillata* (*n* = 25) arm-wing length was 22.64 ± 2.39 mm which was 54% of adult arm-wing length. The percentage of adult arm-wing length of *C. perspicillata* at birth was found to be significantly higher than that of *A. jamaicensis* ($t = 7.81$, $p = 0.0001$). The growth of the length of the arm-wing continued in a linear fashion for *A. jamaicensis* for the first 35 day post-partum and for the first 25

days post-partum for *C. perspicillata* (Figure 29) with both species leveling off thereafter. *A. jamaicensis* reached 90% of adult arm-wing length at 32 days post-partum corresponding to the first day of flight while reaching 100% at 80 days of age. *A. jamaicensis* were able to maneuver like an adult at 99% of adult arm-wing length. *C. perspicillata* achieved 90% of adult arm-wing length at 56 days of age while being able to maneuver like an adult at 98% of adult arm-wing length.

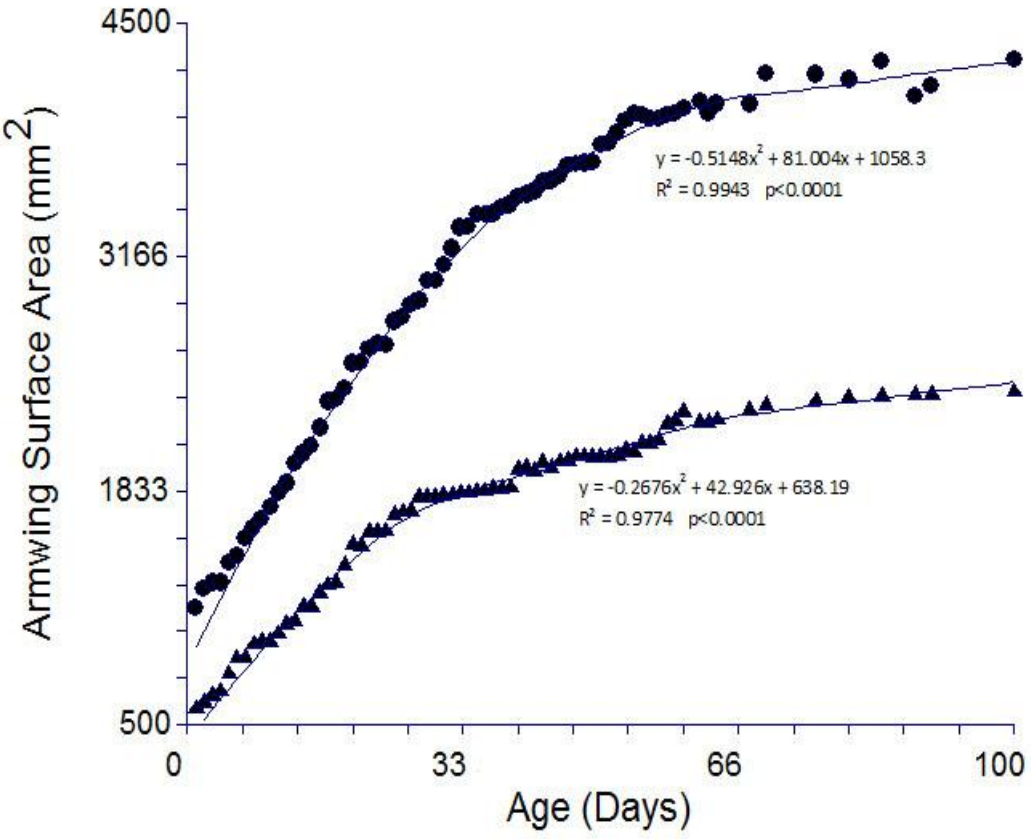

Figure 28. Daily mean arm-wing surface area for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

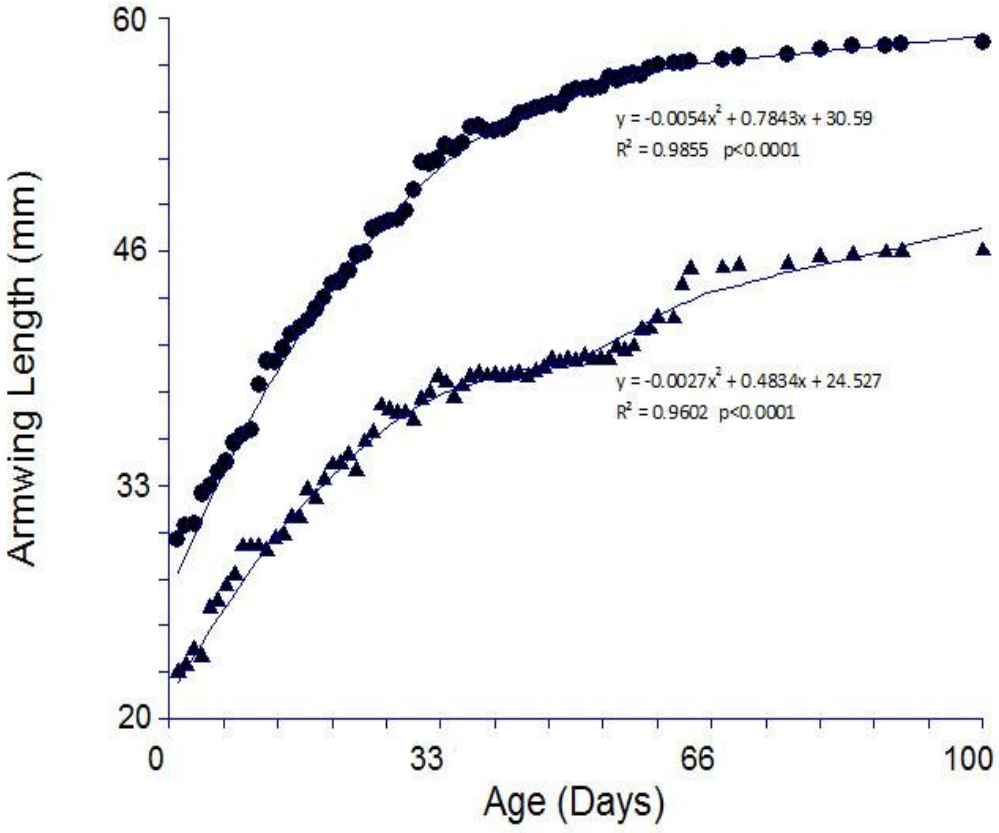

Figure 29. Daily mean arm-wing length for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

Minimal variation was found in the growth of the arm-wing in regards to area and length, represented by high regression r^2 values. *A. jamaicensis* had an r^2 of 0.9943 for area and 0.9855 for length while *C. perspicillata* had an r^2 of 0.9774 for area and 0.9602 for length obtained from best-fit polynomial regression (Figures 28 and 29). Growth asymptotes for arm-wing area and length were obtained using logistic growth equations. *A. jamaicensis* arm-wing area was 4215.511 mm^2 with length at 58.728 mm (Table 3). *C. perspicillata* growth asymptotes for arm-wing area and length were 2299.255 mm^2 and 46.105 mm, which are all similar to the measured arm-wing area and length in adults.

Growth rate constants were similar for arm-wing area with *A. jamaicensis* at 0.068 and *C. perspicillata* at 0.066 ($t = 1.34$, $p = 0.197$), however, arm-wing length was significantly different ($t = 34.15$, $p < 0.0001$) with a mean of 0.06 for *A. jamaicensis* and 0.045 for *C. perspicillata*. Mean point of inflection showed that arm-wing area and length for *A. jamaicensis* was increasing at the fasted rate near day 14.925 and day 0.443 post-partum whereas the arm-wing area and length for *C. perspicillata* increased at the fasted rate near day 13.464 and day 0.152 post-partum (Table 3).

The allometric relationship of arm-wing area and length and mass regression was similar for the slope of arm-wing area [$F(3, 138) = 0.01$, $p = 0.9185$] (Figure 30), however, the arm-wing length was found to be significantly different between species [$F(3, 138) = 13.29$, $p = 0.0004$] (Figure 31). The Y-intercept for arm-wing area was found to be significantly different, indicating the regression lines cross the y axis at a significantly different location [$F(3, 138) = 112.25$, $p < 0.0001$] (Figure 30) and that there was a significant allometric relationship. Linear regression of log-transformed data comparing arm-wing area and length and mass showed that both species had high regression r^2 values, indicating little variation with *A. jamaicensis* having an r^2 of 0.9391 and 0.8924 and *C. perspicillata* having an r^2 of 0.9564 and 0.9476 (Figures 30 and 31). The slopes for both species as mentioned were similar for area, both of which were less than one, indicating negative allometry with mass increasing at a greater rate than arm-wing area (Figures 30 and 31). There was a significant difference between the slopes of the species for arm-wing length, however, with *C. perspicillata* having a slope greater than one, indicating that as mass increased there was a greater increase in the arm-wing length which corresponds to positive allometry, however, *A. jamaicensis* had a slope of

less than one indicating negative allometry, with every increase in mass the increase in arm-wing length was less (Figure 31).

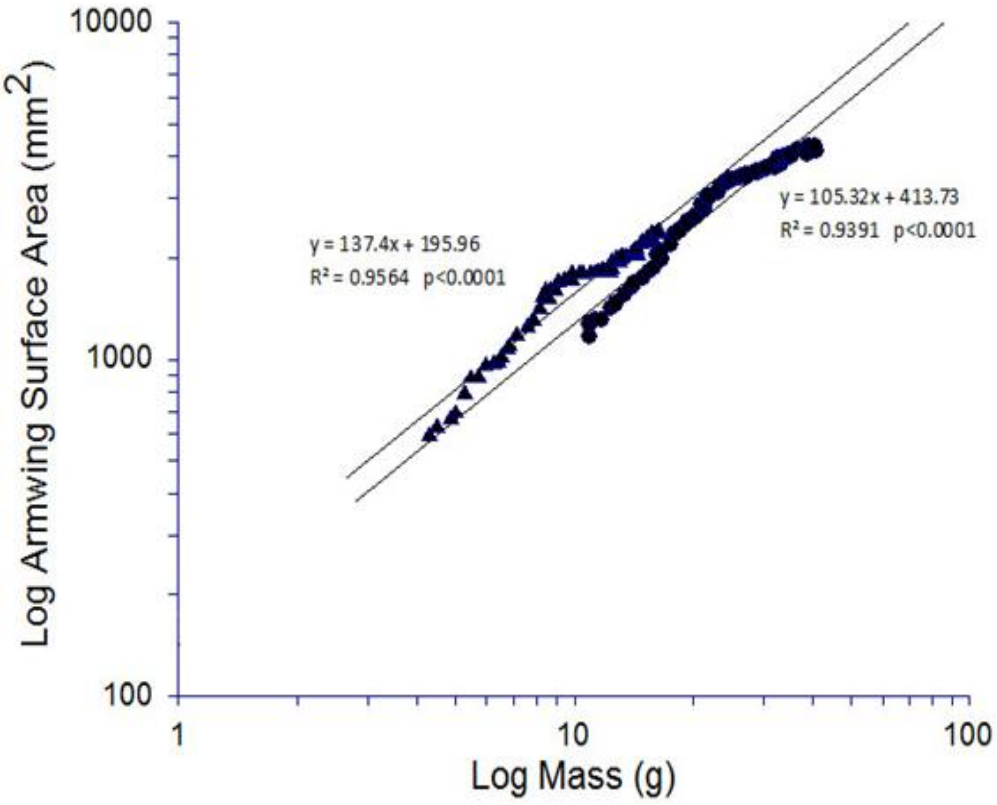

Figure 30. Relationship of mass (Log g) and arm-wing area (Log mm^2). *Artibeus jamaicensis* indicated by the circles has a slope less than one indicating negative allometry. *Carollia perspicillata* indicated by triangles has a slope less than one representing negative allometry. ANCOVA indicate that the slopes of the two species are similar [$F(3, 138) = 0.01$, $p = 0.9185$].

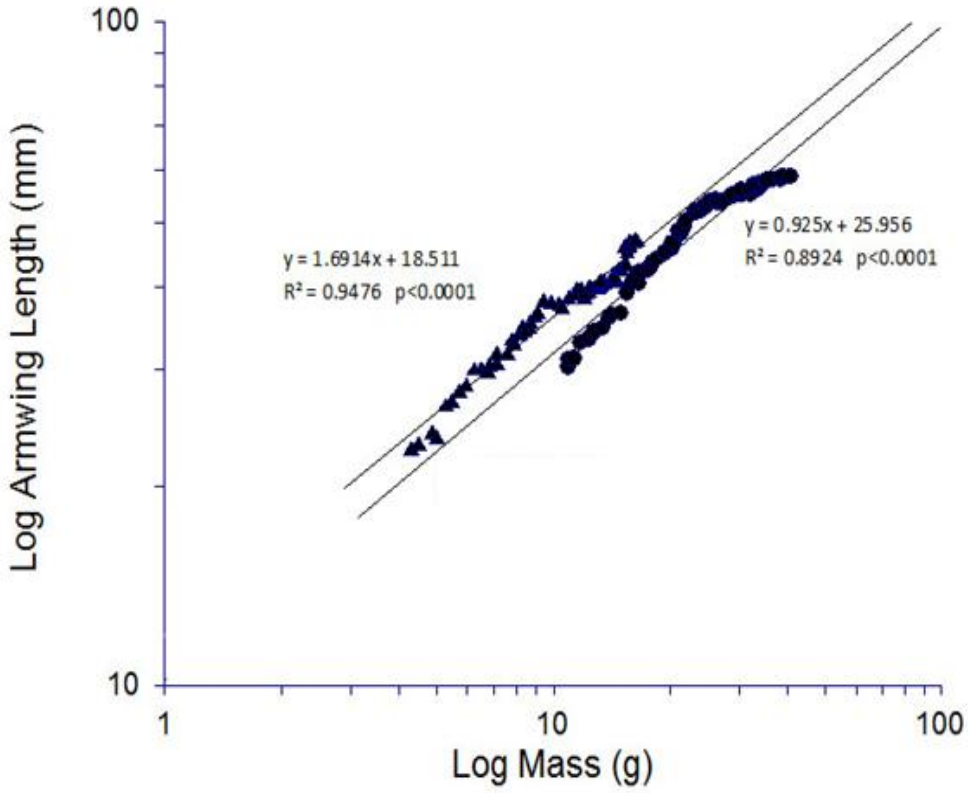

Figure 31. Relationship of mass (Log g) and arm-wing length (Log mm). *Artibeus jamaicensis* indicated by the circles has a slope less than one indicating negative allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are significantly different [$F(3, 138) = 13.29$, $p = 0.0004$].

The mean ± *SD* hand-wing area for one day old *A. jamaicensis* ($n = 45$) was 529.52 ± 101.36 mm^2 which corresponds to 22% of adult hand-wing area. *C. perspicillata* ($n = 25$) was 404.72 ± 79.08 mm^2 corresponding to 24% of adult size. There was a significant difference between species of the percentage of adult hand-wing of pups at birth with *A. jamaicensis* being born at 22% and *C. perspicillata* at 24% of adult size ($t = 7.00$, $p = 0.0001$). Hand-wing area of both *A. jamaicensis* and *C. perspicillata* increased in a linear fashion for the first 40 day (Figure 32). *A. jamaicensis* achieved 90% and 100% of adult

hand-wing area at 41 and 54 days post-partum respectively, achieving flight with the hand-wing area at 79% of adult hand-wing size with adult maneuverability being achieved at 100% of adult arm-wing area. *C. perspicillata* reached 90% and 100% of adult hand-wing area at 39 and 70 days post-partum. *C. perspicillata* achieved flight at 59% of adult hand-wing area and was able to maneuver like an adult at 99% of that of adult hand-wing area.

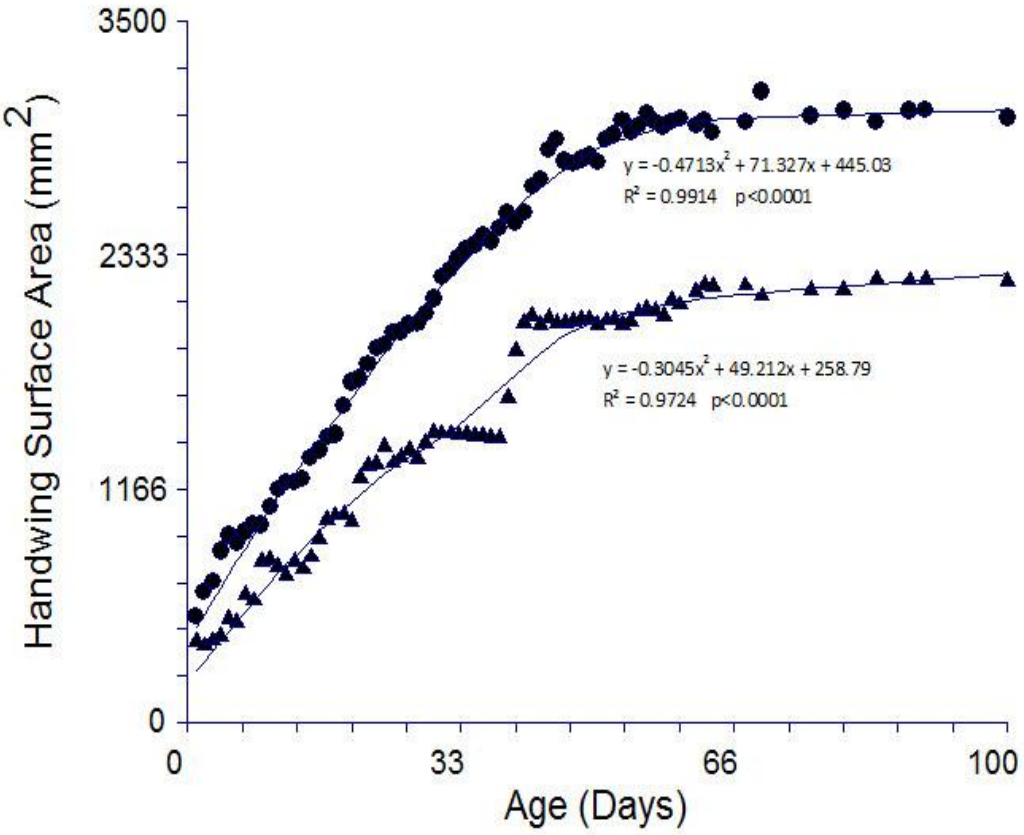

Figure 32. Daily mean hand-wing surface area for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

The mean ± *SD* hand-wing length for one day old *A. jamaicensis* (n = 45) was 35.53 ± 2.80 mm corresponding to 44% of adult hand-wing length. Hand-wing length of *C. perspicillata* (n = 25) was 25.24 ± 3.43 mm which was 45% of adult hand-wing length. The percentage of adult hand-wing length of *C. perspicillata* at birth was found to be significantly higher than that of *A. jamaicensis* (t = 3.99, p = 0.0003). The growth of the hand-wing continued in a linear fashion for *A. jamaicensis* for the first 40 day post-partum and for the first 25 days post-partum for *C. perspicillata* (Figure 33) with both species leveling off thereafter. *A. jamaicensis* reached 90% and 100% of adult hand-wing length at 46 and 88 days post-partum. *A. jamaicensis* were able to maneuver like an adult at 96% of adult hand-wing size. *C. perspicillata* achieved 90% and 100% of adult hand-wing at 38 and 78 days of age while being able to maneuver like an adult at 95% of adult hand-wing length.

Intra-specific variation corresponding to the growth of the hand-wing in regards to area and length was minimal, represented by high regression r^2. *A. jamaicensis* had an r^2 of 0.9914 for area and 0.9875 for length while *C. perspicillata* had an r^2 of 0.9724 for area and 0.9659 for length both obtained from best-fit polynomial regression (Figures 32 and 33). Growth asymptotes for hand-wing area and length were obtained using logistic growth equations, *A. jamaicensis* hand-wing area was 3118.181 mm^2 with length at 86.776 mm (Table 3). *C. perspicillata* growth asymptotes for hand-wing area and length were 2246.5 mm^2 and 63.316 mm, which are all similar to the measured hand-wing area and length in adults. Growth rate constants were significantly different for hand-wing area and length with *A. jamaicensis* at 0.075 and *C. perspicillata* at 0.07 (t = 11.308, p < 0.0001) for area and *A. jamaicensis* at 0.075 and CP 0.071 (t = 34.15, p < 0.0001). Mean

point of inflection showed that hand-wing area and length of *A. jamaicensis* was increasing at the fasted rate near 18.784 day and 7.458 days post-partum while the hand-wing area and length for *C. perspicillata* increased at the fasted rate near 21.353 and 6.333 days post-partum (Table 3).

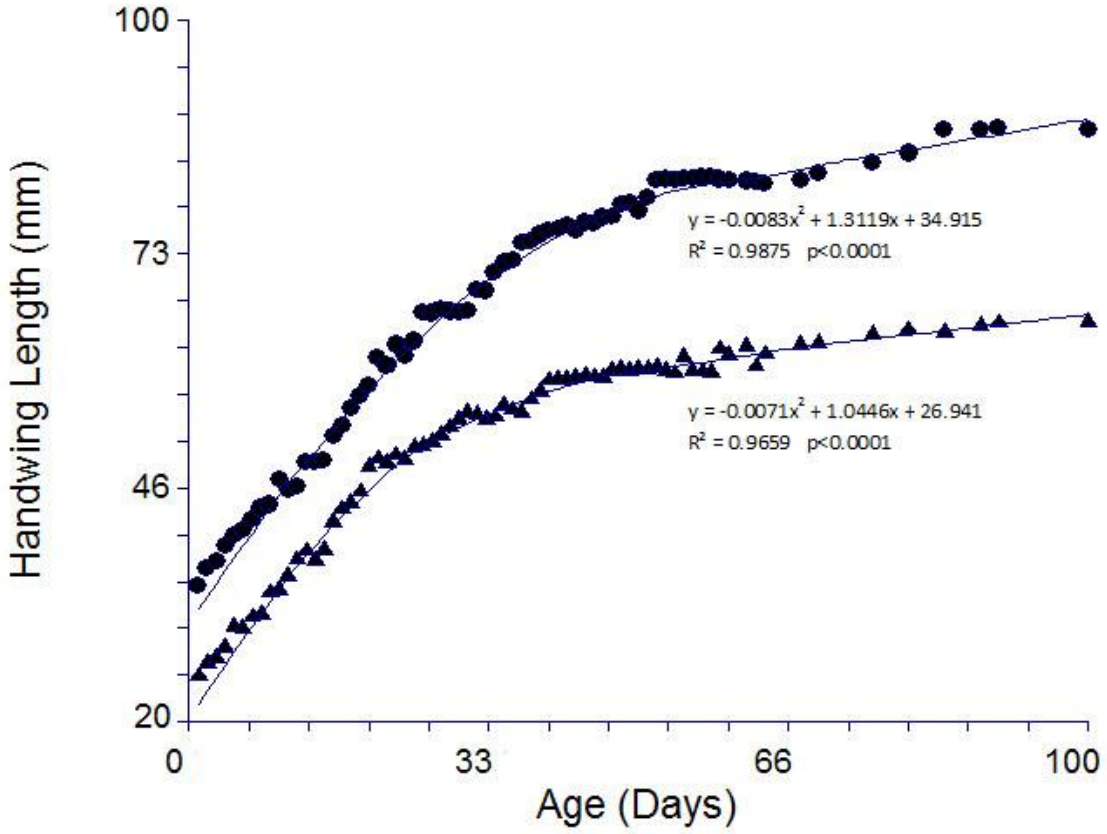

Figure 33. Daily mean hand-wing length for first 100 days post-partum. *Artibeus jamaicensis* are represented with circles and *Carollia perspicillata* with triangles. Lines represent best-fit polynomial regression.

The allometric relationship of hand-wing area and length increase and mass increase was significantly different for the slope of hand-wing area [$F(3, 138) = 25.42$, $p < 0.0001$] (Figure 34) and the slope of hand-wing length [$F(3, 138) = 16.63$, $p < 0.0001$] (Figure 35). Linear regression of log-transformed data comparing hand-wing area and length and mass showed that both species had little variation with high correlation coefficients, with *A. jamaicensis* having an r^2 of 0.9312 and 0.931 and *C. perspicillata* having an r^2 of 0.9555 and 0.9259 (Figures 34 and 35). The slopes for hand-wing area and length for both species were greater than one, indicating positive allometry with hand-wing area and length increasing at a greater rate than mass (Figures 34 and 35), with *C. perspicillata* hand-wing area and length increasing more per increase in mass than *A. jamaicensis*.

Tip shape index for *A. jamaicensis* and *C. perspicillata* were calculated for each flight stage as well as in adults. Wing shape is determined by the tip shape index which uses arm-wing and hand-wing area and length. The larger the tip shape index number the rounder the tip of the wing, which is indirectly used to determine the flight ability of the bat. The overall mean tip shape indices for *A. jamaicensis* and *C. perspicillata* were 0.96 and 1.56 respectively, indicating that *C. perspicillata* had rounder wing tips than *A. jamaicensis*, resulting in the ability to fly slower and be more maneuverable. Looking at the tip shape index at individual flight development stages for both *A. jamaicensis* and *C. perspicillata* we find that *C. perspicillata* is consistently higher than *A. jamaicensis*. In the flop stage, the tip shape index for *A. jamaicensis* was 0.68 and 1.35 for *C. perspicillata*. The tip shape index in the flutter stage for *A. jamaicensis* was 1.01 and 1.45 for *C. perspicillata*. The flap stage tip shape index for *A. jamaicensis* was 1.04 and

1.22 for *C. perspicillata*. The flight stage was broken down into the time from first flight through when the juvenile was able to maneuver like an adult then through adult age. The tip shape indices on the day of first flight for *A. jamaicensis* was 1.11 and 1.68 for *C. perspicillata*. The second flight stage tip shape index for *A. jamaicensis* was 0.95 and 2.09 for *C. perspicillata* resulting in an overall rounder wing-tip for *C. perspicillata* in the adult form than *A. jamaicensis*.

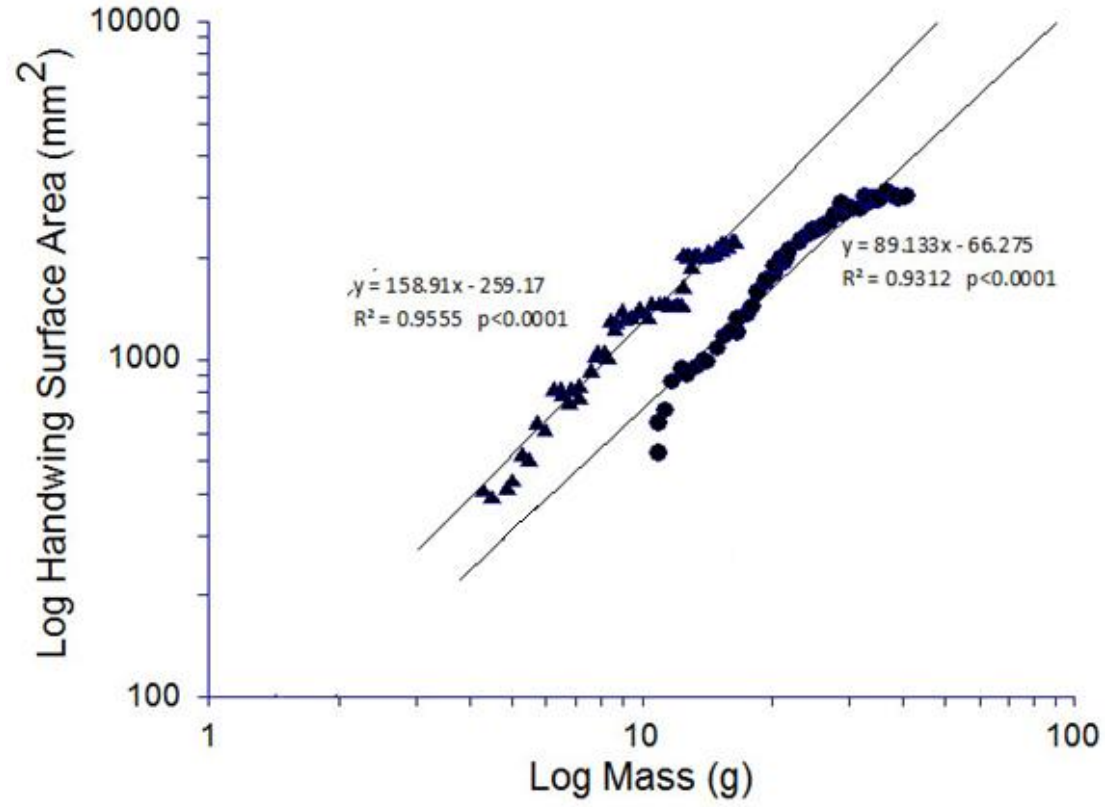

Figure 34. Relationship of mass (Log g) and hand-wing area (Log mm^2). *Artibeus jamaicensis* indicated by the circles has a slope greater than one indicating positive allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are significantly different [$F(3, 138) = 25.42$, $p < 0.0001$].

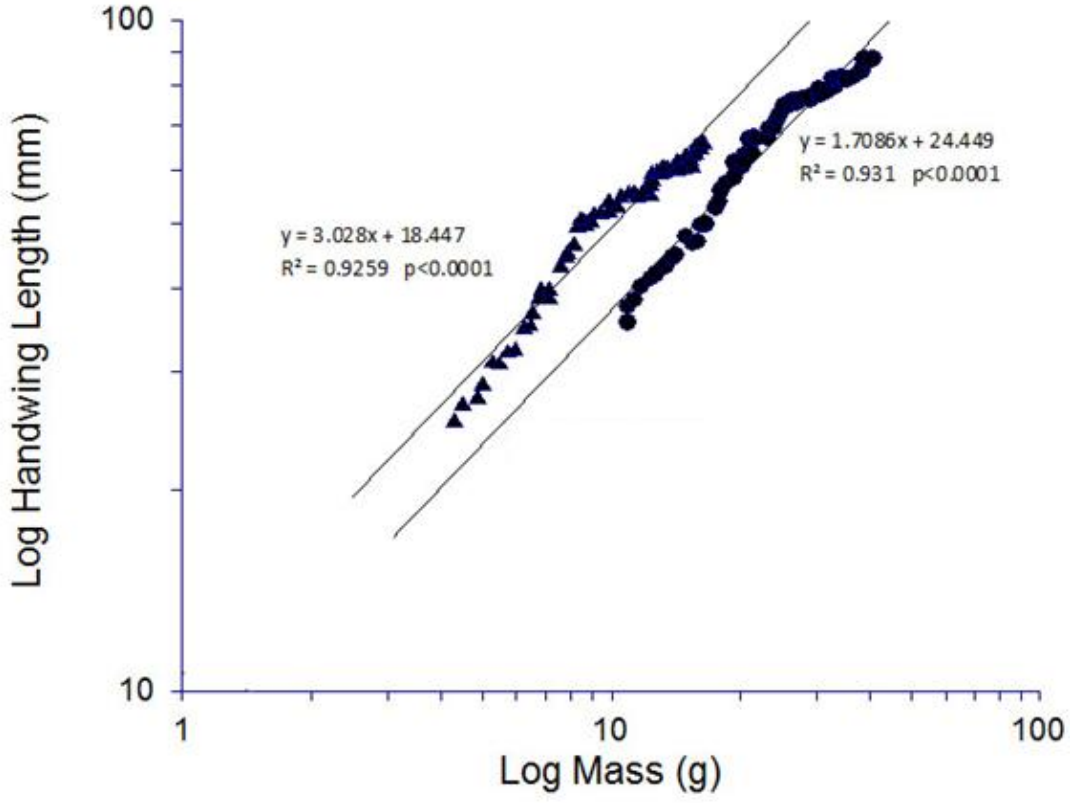

Figure 35. Relationship of mass (Log g) and hand-wing length (Log mm). *Artibeus jamaicensis* indicated by the circles has a slope greater than one indicating positive allometry. *Carollia perspicillata* indicated by triangles has a slope greater than one representing positive allometry. ANCOVA indicate that the slopes of the two species are significantly different [$F(3, 138) = 16.63$, $p < 0.0001$].

Digit Ossification

Length of the total epiphyseal gap of the metacarpal-phalangeal joint of the fourth digit increased in both *A. jamaicensis* (n = 20) and *C. perspicillata* (*n* = 20) from birth, reaching a maximal length in the flutter stage of flight development then decreased until the gap was closed at the adult bat size in both species (Figure 36). The mean total gap (from the distal end of the metacarpal to the proximal end of the phalange) during each flight stage was found to be significantly different in each flight stage using Student's

t-tests of measurements that were adjusted for body size. The mean total gap in the flop stage was 2.708 ± 0.15 mm in *A. jamaicensis* and 2.09 ± 0.14 mm in *C. perspicillata* with the total gap being significantly larger in *C. perspicillata* than *A. jamaicensis* ($t = 16.03$, $p < 0.0001$). The mean total gap in the flutter stage increased to 3.75 ± 0.08 mm in *A. jamaicensis* and 3.216 ± 0.2 mm in *C. perspicillata* with *C. perspicillata* having a significantly larger total gap ($t = 17.09$, $p < 0.0001$). The mean total gap decreased as the juvenile passed into the flap stage with total gap for *A. jamaicensis* being 3.228 ± 0.3 mm and 2.762 ± 0.1 mm in *C. perspicillata* with *C. perspicillata* being significantly larger than *A. jamaicensis* ($t = 16.104$, $p < 0.0001$). The mean total gap in the flight stage for *A. jamaicensis* was 2.768 ± 0.13 mm and 1.41 ± 0.15 mm in *C. perspicillata* with *A. jamaicensis* being significantly larger than *C. perspicillata* ($t = 4.31$, $p = 0.002$).

Secondary ossification centers began to form in the epiphyses of the proximal phalanges and the distal metacarpals of the fourth digit during the flap stage. Prior to the flap stage the entire joint was completely cartilage, lacking secondary ossification centers. At the time when the centers of ossification began to form, the distal and proximal epiphyseal gaps became observable.

The proximal epiphyseal gap of the phalange first appeared in the flap stage and remained open through the initial flight stage, becoming fused prior to adult size. The proximal epiphyseal gap in the flap stage for *A. jamaicensis* was 1.11 ± 0.23 mm and 1.41 ± 0.09 mm for *C. perspicillata* with *C. perspicillata* having a significantly larger proximal gap ($t = 14.719$, $p < 0.0001$) (Figure 37). The mean proximal gap in the flight stage was 0.79 ± 0.07 mm for *A. jamaicensis* and 1.082 ± 0.08 mm for *C. perspicillata* with *C. perspicillata* having a significantly larger proximal gap in the flight stage than *A.*

jamaicensis (t = 15.882, p < 0.0001) (Figure 37). The proximal gap had closed and was completely ossified in the adults of both species. The proximal gap decreased in size significantly from the flap stage to the flight stage for both species (*A. jamaicensis*, t = 3.23, p = 0.009; *C. perspicillata*, t = 6.88, p < 0.0001) (Figure 37) indicating that the bone was continuing to ossify.

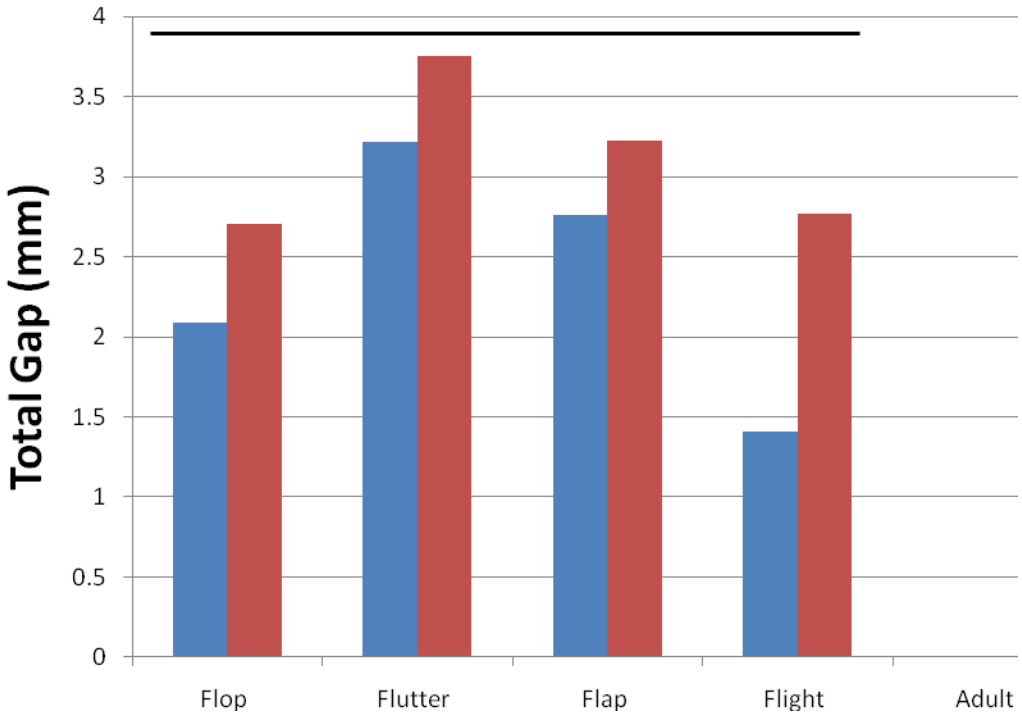

Figure 36. Mean post-partum changes in the epiphyseal total gap length. Measurements from the fourth metacarpal/phalangeal joint obtained at each of the four flight stages and adults. *Artibeus jamaicensis* are represented by blue and *Carollia perspicillata* by red. Student's t-test were performed on data that was adjusted for body size (* = p < 0.01).

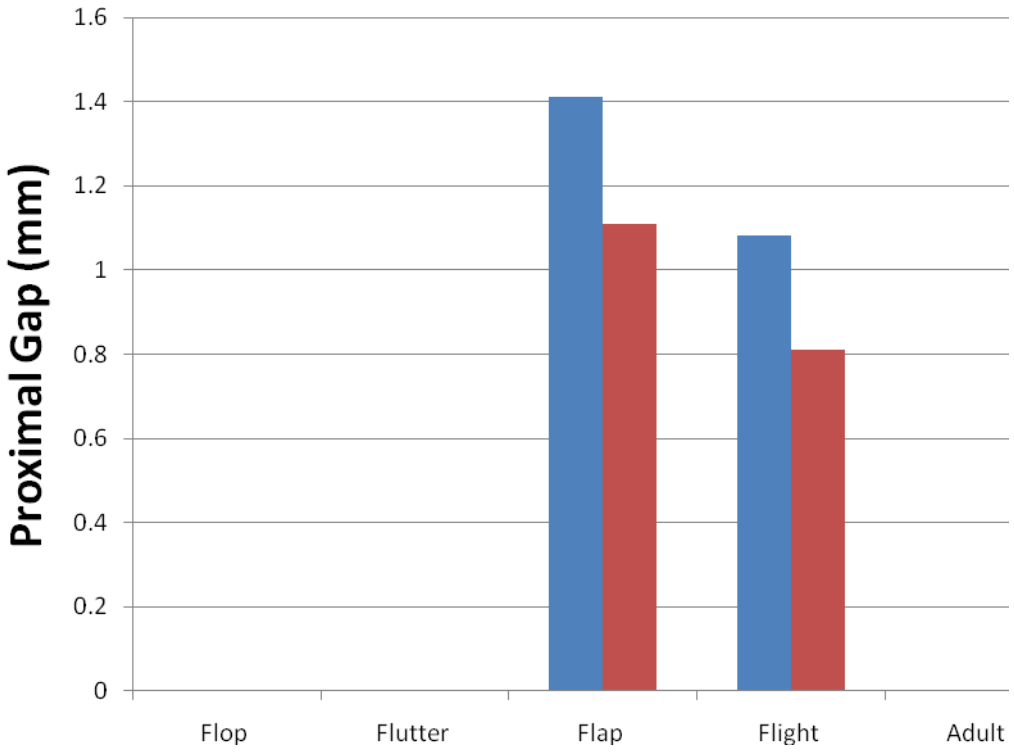

Figure 37. Mean post-partum changes in the proximal gap length. Measurements from the fourth metacarpal/phalangeal joint obtained at each of the four flight stages and adults. *Artibeus jamaicensis* are represented by blue and *Carollia perspicillata* by red. Student's *t*-test were performed on data that was adjusted for body size (* = $p < 0.001$).

The mean distal epiphyseal gap of the metacarpal was first observed in the flap stage and remained open through the flight stage, however, was closed by adult size. The distal epiphyseal gap in the flap stage for *A. jamaicensis* was 0.366 ± 0.13 mm and 0.726 ± 0.06 mm for *C. perspicillata* with *C. perspicillata* having a significantly larger distal gap than *A. jamaicensis* ($t = 14.993$, $p < 0.0001$) (Figure 38). The mean distal gap in the flight stage was 0.276 ± 0.08 mm for *A. jamaicensis* and 0.2 ± 0.09 mm for *C. perspicillata* with both species having similar lengths of distal gaps in the flight stage ($t = 0.717$, $p = 0.489$) (Figure 38). The distal gap had closed and was completely ossified in the adults of both species. The distal gap decreased in size from the flap stage to the flight stage,

however, the decrease was not a significant decrease in *A. jamaicensis* (*A. jamaicensis*, *t* = 1.48, p = 0.168; *C. perspicillata*, t =11.82, p < 0.0001) (Figure 35) indicating that the bone was continuing to ossify, however, at a greater rate in *C. perspicillata*.

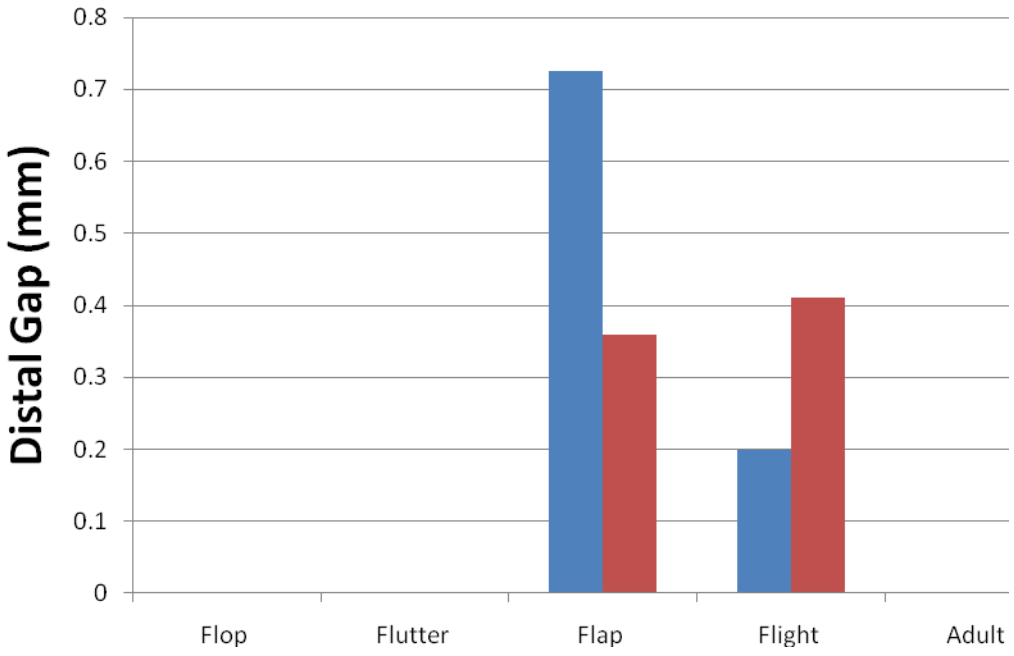

Figure 38. Mean post-partum changes in the distal gap length. Measurements from the fourth metacarpal/phalangeal joint obtained at each of the four flight stages and adults. *Artibeus jamaicensis* are represented by blue and *Carollia perspicillata* by red.Student's *t*-test were performed on data that was adjusted for body size (* = $p < 0.0001$).

Muscle Development

Fiber analysis was performed on muscles that were taken from the pectoralis major and acromeodeltoideus on the first day that a juvenile bat was determined to be within a flight development stage (i.e., flop, flutter, flap, flight). *A. jamaicensis* fast-twitch fiber cross-sectional area for pectoralis major for flop, flutter, flap and flight stages were 16%, 22%, 42%, and 100% of adult size and 38%, 36%, 54%, and 70% of adult

size in the acromeodeltoideus. *C. perspicillata* fast-twitch cross-section area for pectoralis major was 25%, 51%, 57%, and 69% of adult size and 29%, 52%, 88%, and 91% adult size of acromeodeltoideus areas.

Slow-twitch fiber cross-sectional area compared with adult fiber area for flop, flutter, flap and flight stages were 19%, 44%, 68%, and 99% in pectoralis major and 40%, 57%, 62%, and 100% in acromeodeltoideus of *A. jamaicensis*. *C. perspicillata* cross-sectional area for pectoralis major was 32%, 43%, 68%, and 99%, and 27%, 46%, 86%, and 98% for acromeodeltoideus.

Comparisons to determine if there were fiber size differences between flight stages of *A. jamaicensis* pectoralis major were made using Student's t-test on data adjusted for body size using wing surface area as the standard (Figure 39). The flop and flutter stage were significantly different in fast- and slow-twitch fiber size (fast, $t = 2.82$, $p = 0.006$; slow, $t = 6.93$, $p < 0.0001$). The flutter and flap stages were found to be similar in size for fast-twitch fibers ($t = 0.893$, $p = 0.374$), however, slow-twitch fiber types were significantly different in size between flutter and flap ($t = 3.23$, $p = 0.002$), the flap and flight stages were also significantly different in size for fast-twitch fiber types ($t = 13.323$, $p < 0.0001$), however, the slow-twitch fiber size was similar ($t = 0.286$, $p = 0.776$). The flight and adult stages were significantly different in size for both fast- and slow-twitch fibers (fast, $t = 7.575$, $p < 0.0001$; slow, $t = 6.478$, $p < 0.0001$).

Comparisons to determine if there were fiber size differences between flight stages of *A. jamaicensis* acromeodeltoideus were made using Student's t-test on data adjusted for body size using wing surface area as the standard (Figure 40). The flop and flutter stage were significantly different in fiber size for fast-twitch fibers ($t = 5.759$, $p <$

0.0001), however, they were similar in size for slow-twitch fibers (t = 1.476, p = 0.152), the flutter and flap stages were significantly different in size for both fast- and slow-twitch fiber types (fast, t = 4.559, p < 0.0001; slow, t = 2.352, p = 0.028), the flap and flight stage were similar in size for fast-twitch (t = 1.533, p = 0.128), however, they were significantly different in size for slow-twitch fibers (t = 2.509, p =0.0233) and the flight and adult stages were similar in size for fast-twitch fibers (t = 1.143, p = 0.256) and significantly different in size for slow-twitch fibers (t = 11.948, p < 0.0001).

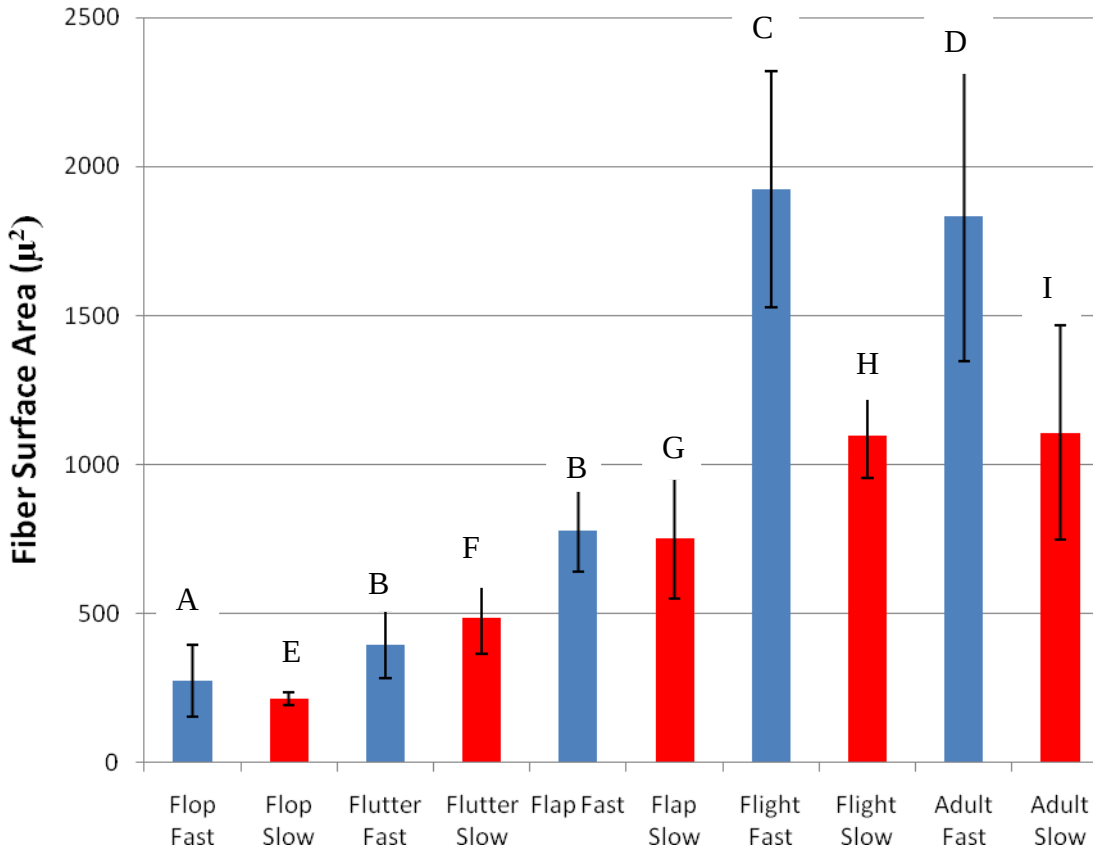

Figure 39. Cross-sectional fiber diameter of the pectoralis major from *A. jamaicensis*. Represented are fast- and slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Letters represent significant differences (p <0.05) in fiber surface area, comparing flight stages for fast- or slow-twitch. Bars with the same letter are not significantly different. Error bars represent one standard deviation from the mean.

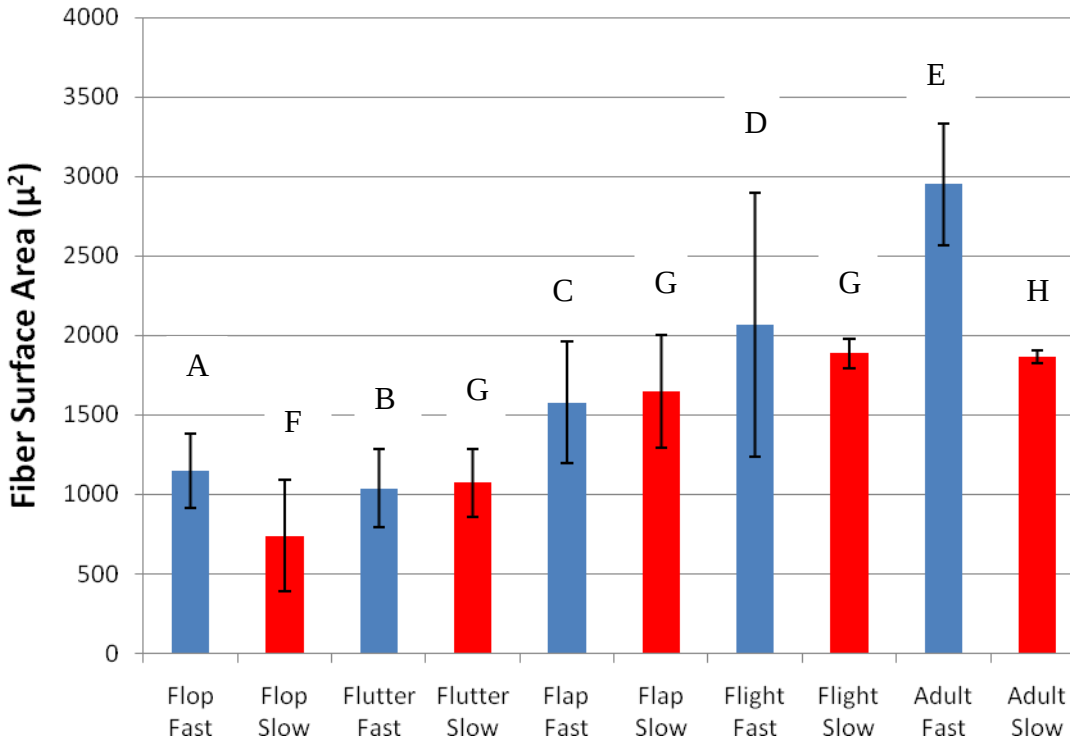

Figure 40. Cross-sectional fiber diameter of the acromeodeltoideus from *A. jamaicensis*. Represented are fast- and slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Letters represent significant differences ($p < 0.05$) in fiber surface area, comparing flight stages for fast- or slow-twitch. Bars with the same letter are not significantly different. Error bars represent one standard deviation from the mean.

Comparisons to determine if there were fiber size differences between flight stages of *C. perspicillata* pectoralis major were made using Student's t-test on data adjusted for body size using wing surface area as the standard (Figure 41). The flop and flutter stage were significantly different in size ($t = 13.488$, $p < 0.0001$) in fast-twitch fibers and similar in size in slow-twitch fibers ($t = 1.905$, $p = 0.089$), the flutter and flap stages were significantly different in size for both fast ($t = 21.364$, $p < 0.001$) and slow-twitch fiber types ($t = 2.642$, $p = 0.019$). The flap and flight were significantly different in size for fast-twitch fiber types ($t = 7.589$, $p < 0.0001$) and similar in size for

slow-twitch fibers (t = 1.016, p = 0.324). The flight and adult stages were significantly different in size for fast-twitch fibers (t = 2.067, p = 0.041) and for slow-twitch fibers (t = 3.929, p = 0.0005).

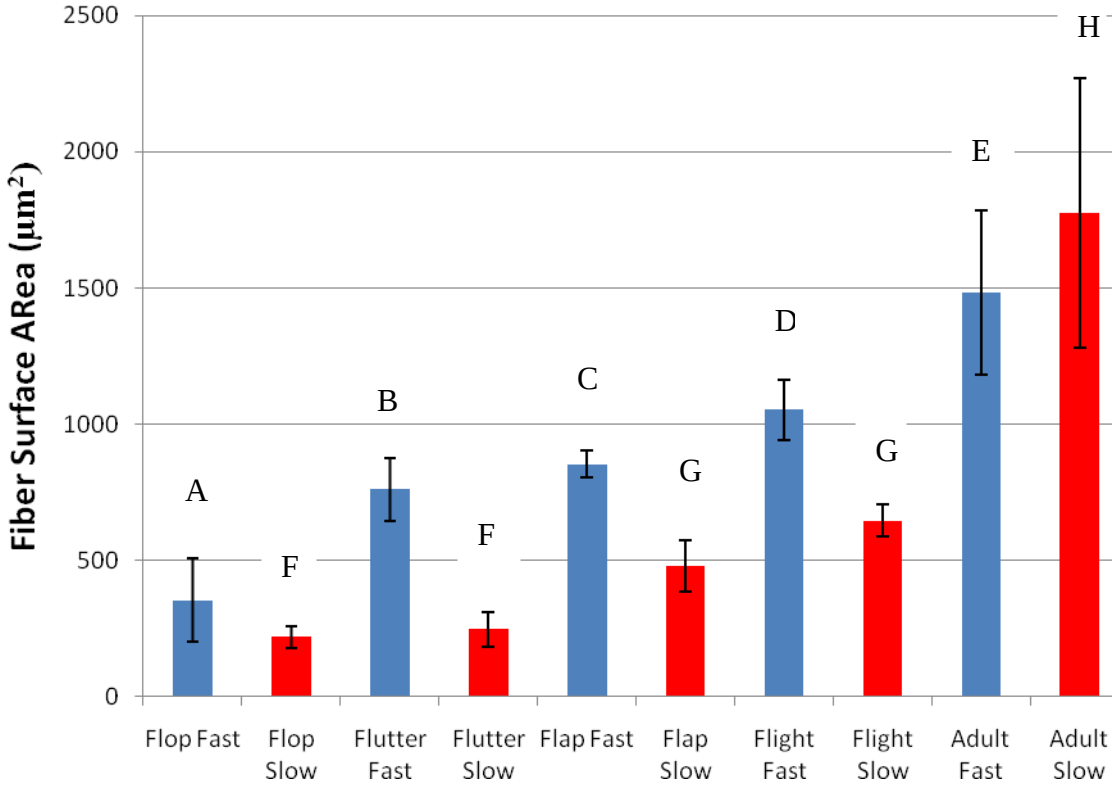

Figure 41. Cross-sectional fiber diameter of the pectoralis major from *C. perspicillata*. Represented by fast- and slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Letters represent significant differences ($p < 0.05$) in fiber surface area, comparing flight stages for fast- or slow-twitch. Bars with the same letter are not significantly different. Error bars represent one standard deviation from the mean.

Comparisons to determine if there were fiber size differences between flight stages of *A. jamaicensis* acromeodeltoideus were made using Student's *t*-test on data adjusted for body size using wing surface area as the standard (Figure 42). The flop and

flutter stages were significantly different in size for both fast- and slow-twitch fibers (fast, $t = 12.544$, $p < 0.0001$; slow, $t = 3.743$, $p = 0.001$). The flutter and flap stages were significantly different for fast-twitch fibers ($t = 7.253$, $p < 0.0001$) and similar in size for slow-twitch fibers ($t = 0.594$, $p = 0.559$). The flap and flight stages were significantly different in size for fast-twitch fibers ($t = 7.253$, $p < 0.0001$) and similar in size for slow-twitch fibers ($t = 1.522$, $p = 0.149$). The flight and adult stages were significantly different in size for both fast- and slow-twitch fibers (fast, $t = 7.706$, $p < 0.0001$; slow, $t = 6.596$, $p < 0.0001$).

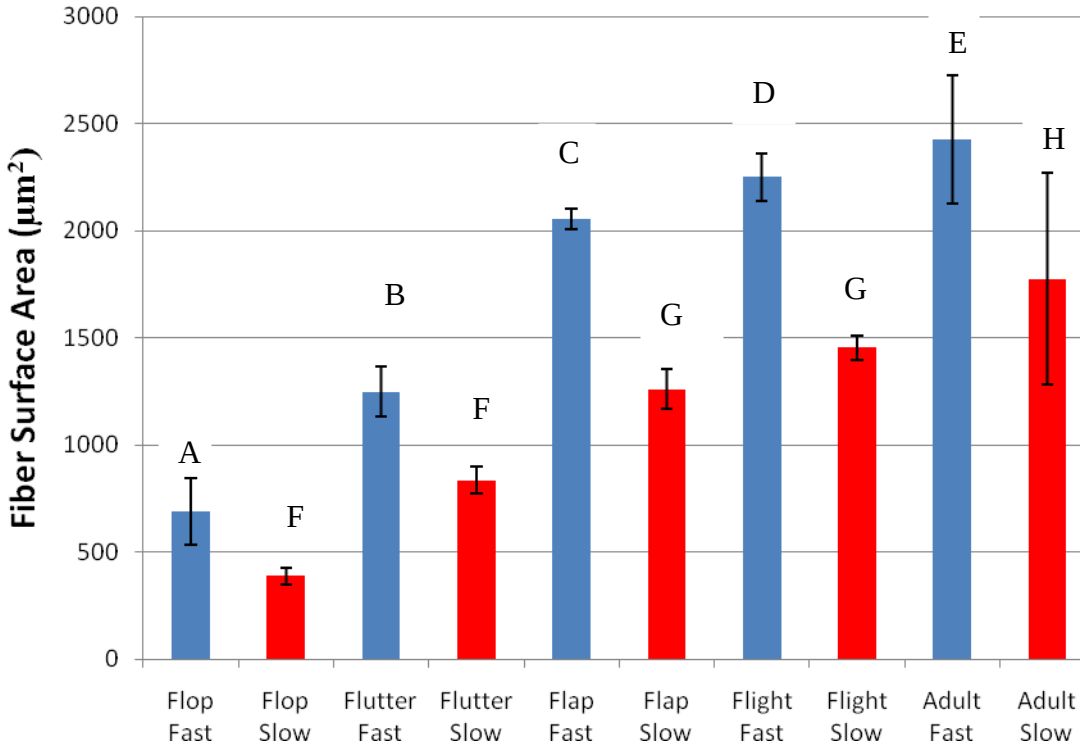

Figure 42. Cross-sectional fiber diameter of the acromeodeltoideus from *C. perspicillata*. Representing fast- and slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Letters represent significant differences ($p < 0.05$) in fiber surface area, comparing flight stages for fast- or slow-twitch. Bars with the same letter are not significantly different. Error bars represent one standard deviation from the mean.

When comparing the surface area of fast- and slow-twitch fibers adjusted for size of the pectoralis major (Figures 43 and 44) between species I found that when comparing individual flight stages of fast-twitch fibers there was a significant difference between the flop (t = 9.268, P < 0.0001), flutter (t = 31.6, p < 0.0001), flap (t = 33.622, p < 0.0001), adult (t = 6.615, p < 0.0001), however, the flight stage was similar in surface are between the species (t = 0.352, p = 0.725). Slow-twitch fibers comparisons showed that there were significant differences between flop (t = 10.513, p < 0.0001) flutter (t = 2.223, p = 0.034), flap (t = 2.43, p = 0.018), flight (t = 5.727, p < 0.0001), adult (t =13.986, p < 0.0001).

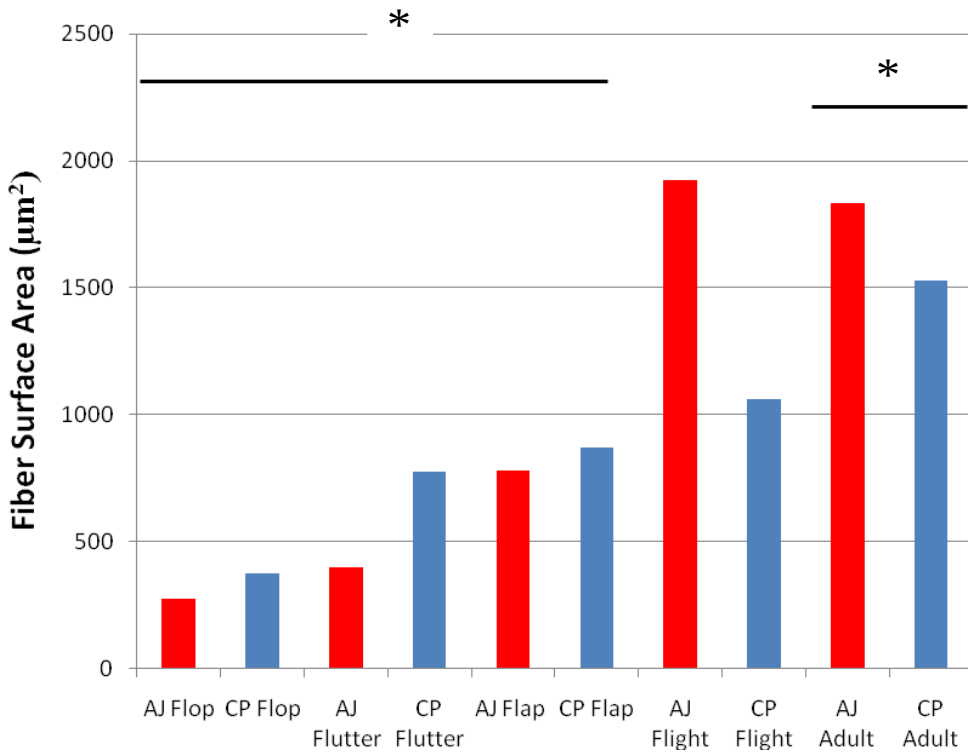

Figure 43. Cross-sectional fiber diameter of fast-twitch fibers from the pectoralis major. *Artibeus jamaicensis* (red) and *Carollia perspicillata* (blue) pectoralis major muscle representing fast-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement Lines represent significant differences (with * p < 0.05) in fiber surface area, comparing flight stages for fast-twitch fibers.

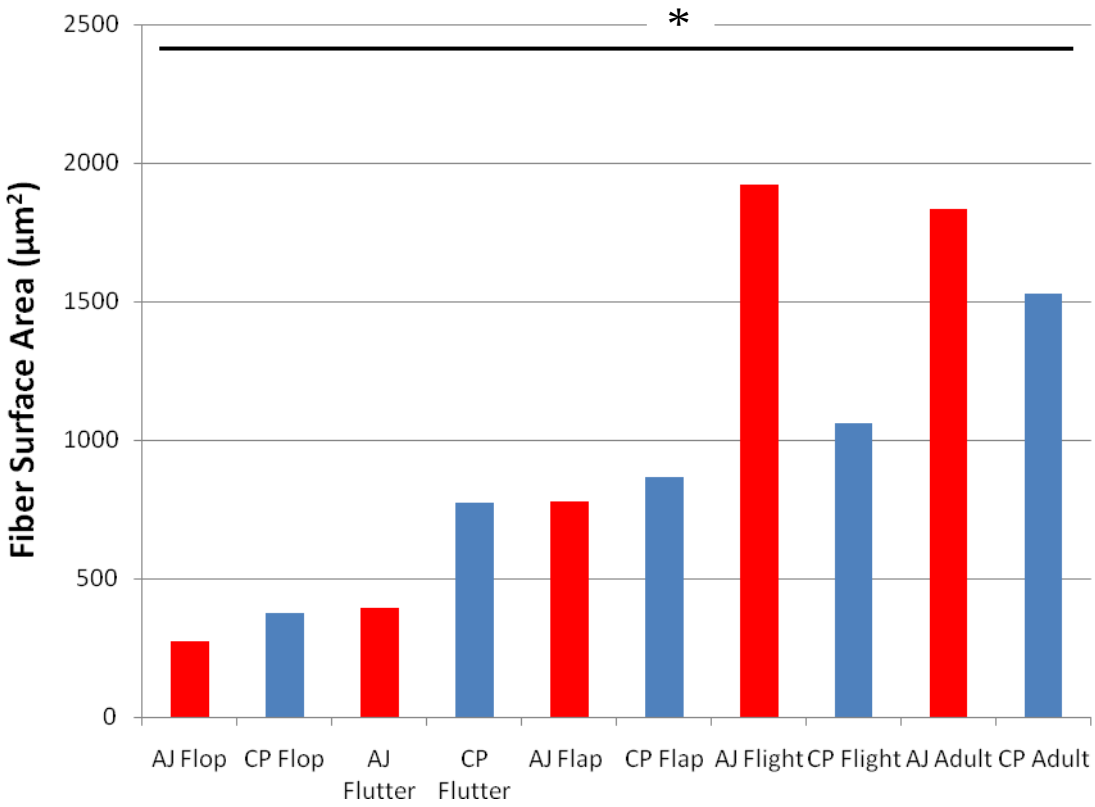

Figure 44. Cross-sectional fiber diameter of slow-twitch fibers from the pectoralis major. *Artibeus jamaicensis* (red) and *Carollia perspicillata* (blue) pectoralis major muscle representing slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement Lines represent significant differences (with * $p < 0.05$) in fiber surface area, comparing flight stages for fast-twitch fibers.

When comparing individual flight stages of fast-twitch fibers of the acromeodeltoideus (Figures. 45 and 46) there was a significant difference between the flutter ($t = 16.625$, $p < 0.0001$), flap ($t = 11.354$, $p < 0.0001$), flight ($t = 11.145$, $p < 0.0001$) adult ($t = 9.521$, $p < 0.0001$), however, the flop ($t = 1.863$, $p = 0.065$) stage was similar in surface are between the species. Slow-twitch fibers comparisons showed that there were significant differences between flop ($F = 4.09$, $p = 0.0462$), flutter ($t = 3.788$,

$p = 0.0009$), and flap ($t = 4.089$, $p = 0.0006$), flight ($t = 6.457$, $p < 0.0001$), however, flop ($t = 0.588$, $p = 0.562$), and adult ($t = 0.66$, $p = 0.4205$) were similar.

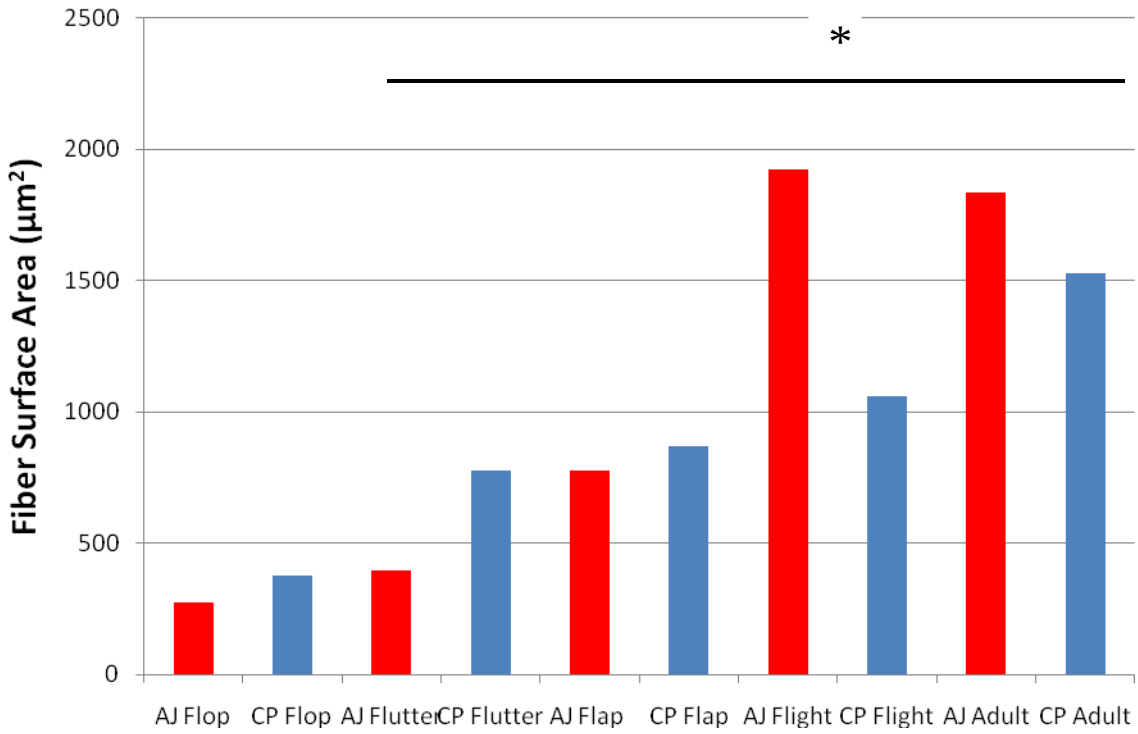

Figure 45. Cross-sectional fiber diameter of fast-twitch fibers from the acromeodeltoideus. *Artibeus jamaicensis* (red) and *Carollia perspicillata* (blue) acromeodeltoideus muscle representing fast-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Lines represent significant differences (with * $p < 0.05$) in fiber surface area, comparing flight stages for fast-twitch fibers.

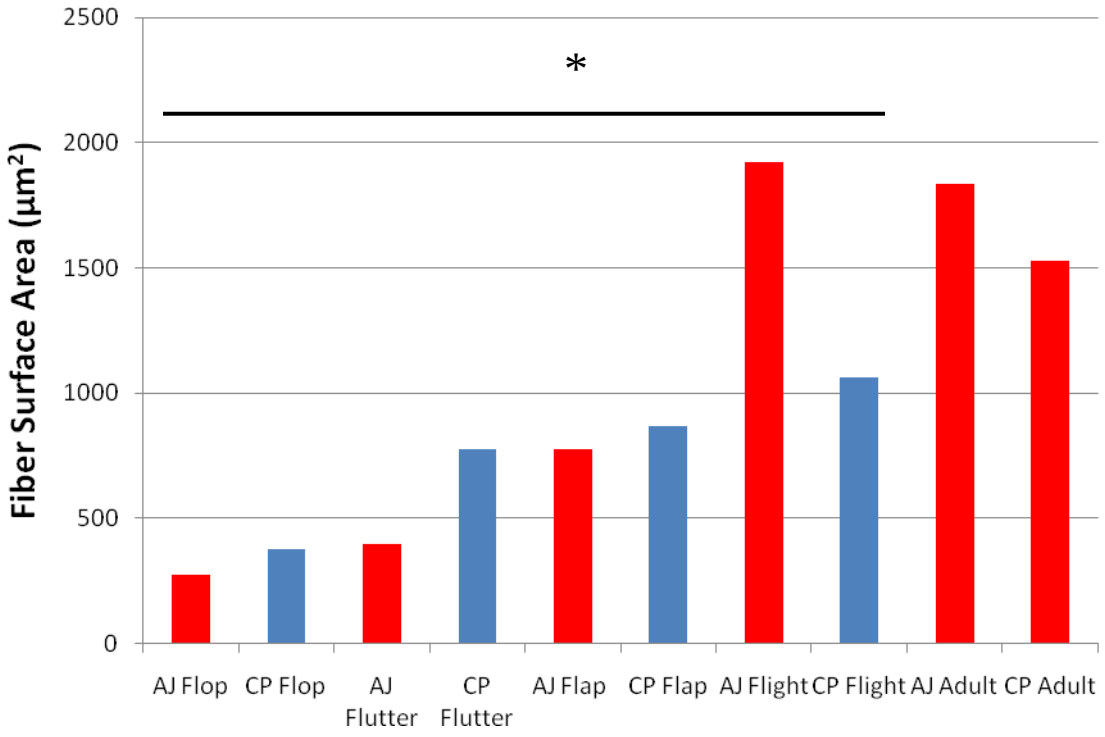

Figure 46. Cross-sectional fiber diameter of slow-twitch fibers from the acromeodeltoideus. *Artibeus jamaicensis* (red) and *Carollia perspicillata* (blue) acromeodeltoideus muscle representing slow-twitch fibers for the four flight developmental stages as well as adults. Significance was based on data adjusted for size (fiber area/wing surface area) using wing surface area as the size measurement. Lines represent significant differences (with * $p < 0.05$) in fiber surface area, comparing flight stages for fast-twitch fibers.

The percentage of fast- and slow-twitch fibers of *A. jamaicensis* pectoralis major were compared to determine if there were significant differences between flight stages for each muscle type. I found that fast- and slow-twitch fibers of the pectoralis major of *A. jamaicensis* were significantly different throughout flight stages using one-way ANOVA [Fast-twitch, $F(4, 145) = 112.073$, $p < 0.0001$; Slow-twitch, $F(4, 145) = 70.103$, $p < 0.0001$] (Figure 47) with fast-twitch fibers decreasing in total percentage until the flight

stage. Slow-twitch fibers increased in percentage through flight stage and adults (Figures 61-65 in Appendix D for immunohistochemistry muscle examples).

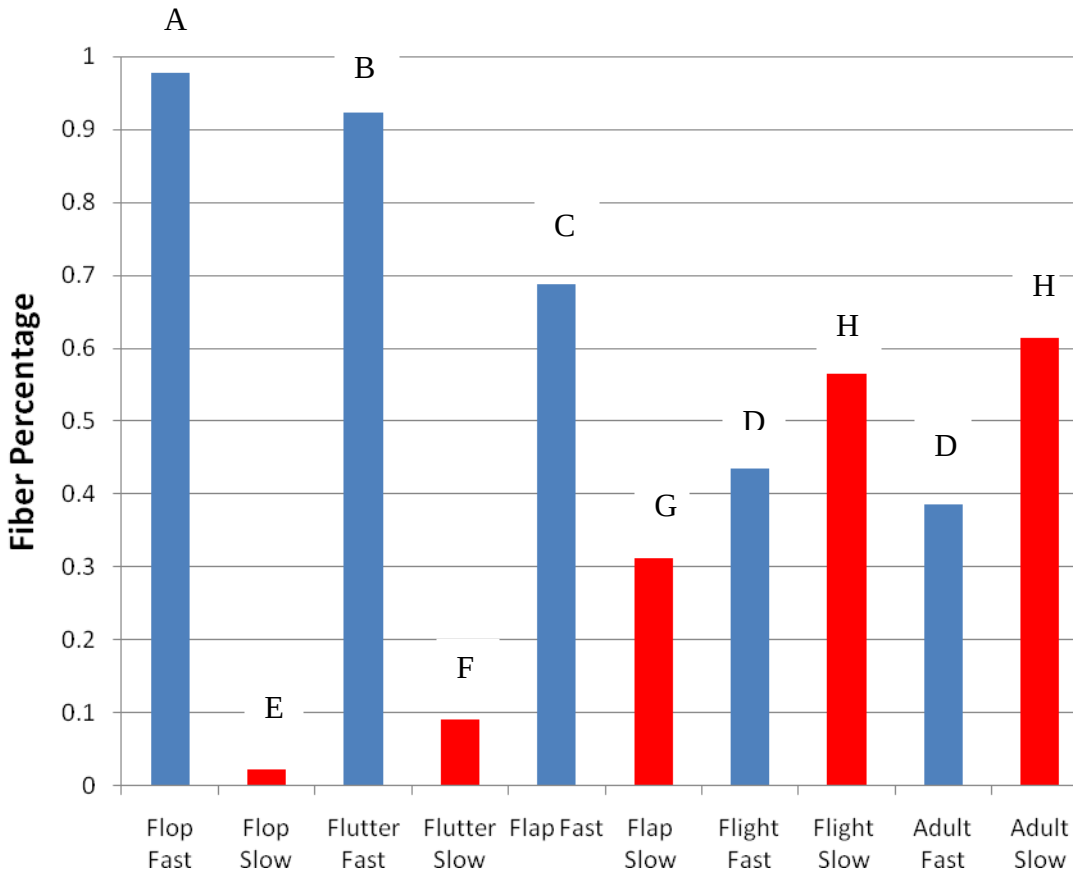

Figure 47. Percentage of fibers in *A. jamaicensis* pectoralis major. Representing fast- (blue) and slow-twitch (red) fibers for the four flight developmental stages as well as adults. Letters represent significant differences ($p < 0.05$) in fiber percentage, comparing flight stages for fast- or slow-twitch. Bars with the same letter are not significantly different.

Differences between each flight stage were compared using Tukey's test with one-way ANOVA. *A. jamaicensis* fast-twitch fiber percentage were significantly different between flop (98%) and flutter (91%) ($F = 5.48$, $p = 0.042$), flutter (91%) and flap (69%) ($F = 15.844$, $p < 0.0001$), flap (69%) and flight (44%) ($F = 23.37$, $p < 0.0001$), however, the percentage of fast-twitch fibers were similar between flight (44%)

and adult (39%) ($F = 2.72$, $p = 0.07$). Slow-twitch fiber percentage in *A. jamaicensis* pectoralis major were significantly different between flop (2%) and flutter (9%) ($F = 8.15$, $p < 0.0001$), flutter (9%) and flap (31%) ($F = 6.06$, $p < 0.0001$), flap (31%) and flight (56%) ($F = 14.05$, $p < 0.0001$), however, the percentage of slow-twitch fibers were similar between flight (56%) and adults (61%) ($F = 1.78$, $p = 0.076$).

The percentage of fast- and slow-twitch fibers of *A. jamaicensis* acromeodeltoideus were compared to determine if there were significant differences between flight stages for each muscle type. I found that fast- and slow-twitch fibers of the acromeodeltoideus of *A. jamaicensis* were similar throughout flight stages using one-way ANOVA [Fast-twitch, $F(4, 145) = 0.454$ $p = 0.756$; Slow-twitch, $F(4, 145) = 0.253$, $p = 0.554$] (Figure 48) with both fast- and slow-twitch remaining similar across all stages with fast-twitch fibers resulting in near 100% of all fibers in the acromeodeltoideus muscle (see Figures 66-70 in Appendix D for immunohistochemistry examples).

The percentage of fast- and slow-twitch fibers of *C. perspicillata* pectoralis major [$F(4. 145) = 0.45$, $p = 0.674$] and acromeodeltoideus [$F(4. 145) = 0.226$, $p = 0.342$] remained similar throughout development (Figures 49 and 50). In both *C. perspicillata* pectoralis major and acromeodeltoideus the fast-twitch fibers accounted for greater than 90% of all fibers (see Figures 71-75 in Appendix D for pectoralis major and Figures 76-80 for acromeodeltoideus immunohistochemistry muscle examples).

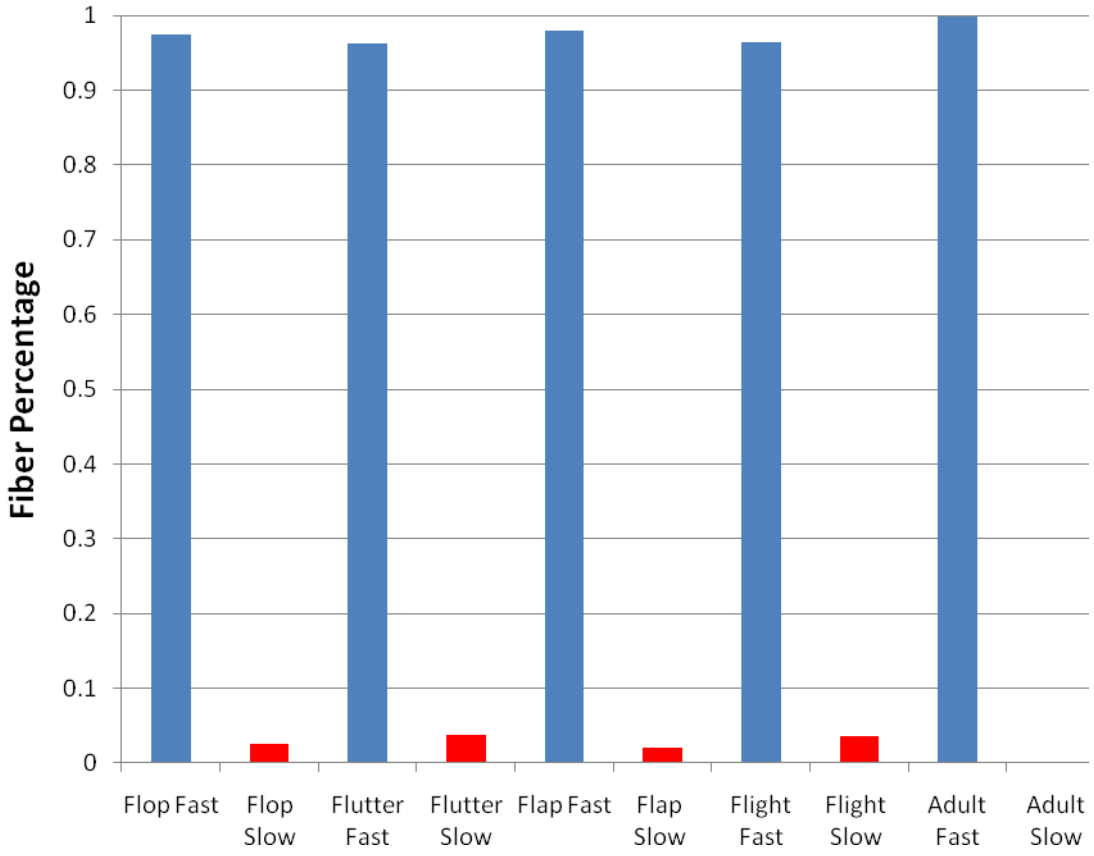

Figure 48. Percentage of fibers in *A. jamaicensis* acromeodeltoideus. Representing fast (blue)- and slow-twitch (red) fibers for the four flight developmental stages as well as adults. There was not a significant difference between flight stages for both fast- and slow-twitch fibers.

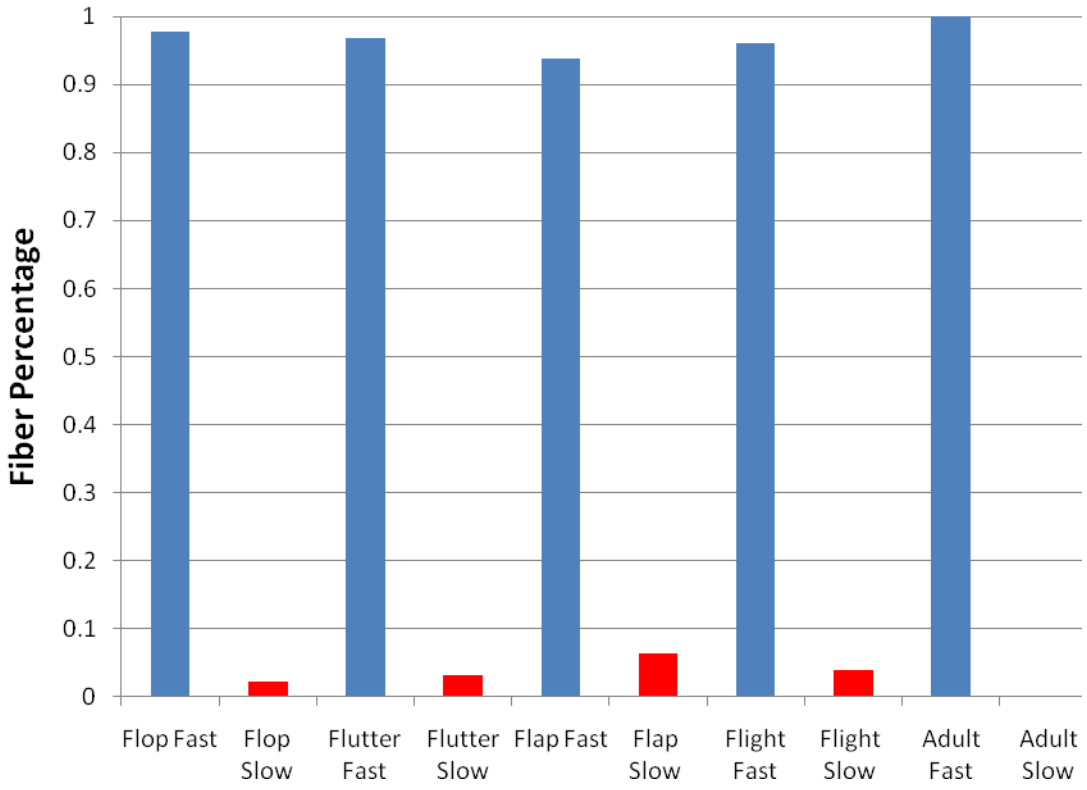

Figure 49. Percentage of fibers in *C. perspicillata* pectoralis major. Representing fast (blue)- and slow-twitch (red) fibers for the four flight developmental stages as well as adults. There was not a significant difference between flight stages for both fast- and slow-twitch fibers.

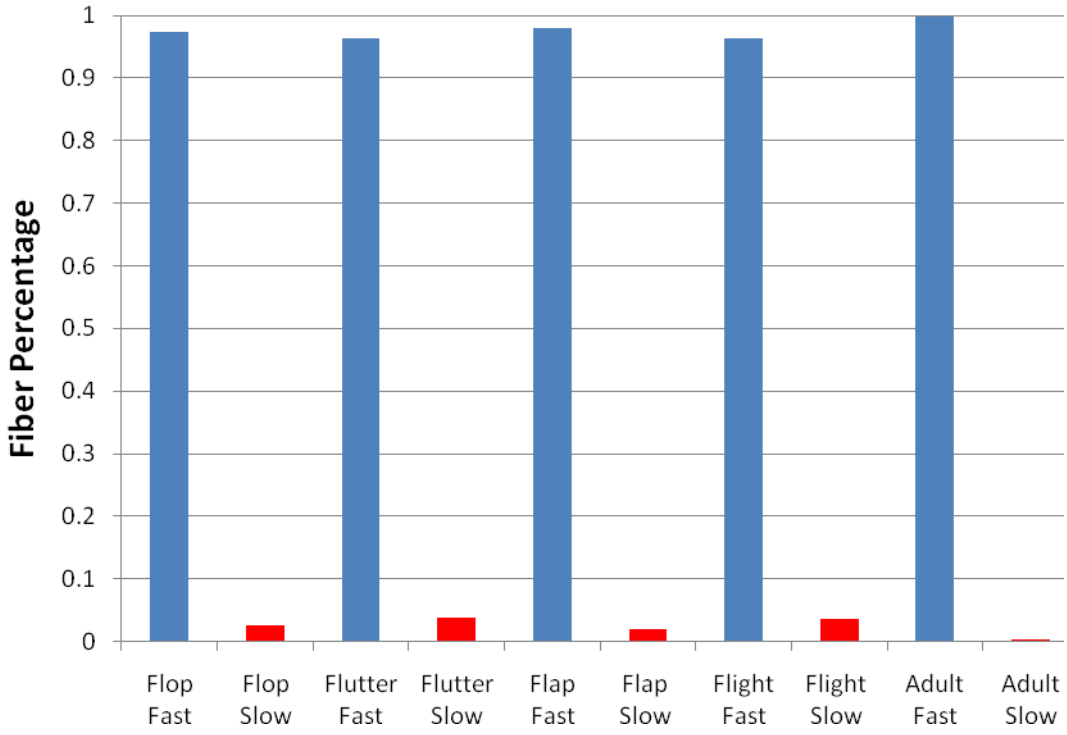

Figure 50. Percentage of fibers in *C. perspicillata* acromeodeltoideus. Representing fast (blue)- and slow-twitch (red) fibers for the four flight developmental stages as well as adults. There was not a significant difference between flight stages for both fast- and slow-twitch fibers.

Multivariate Analysis

To integrate all aspects of growth and development principle component analysis (PCA) was run on morphological traits including: forearm length, mass, wing surface area, wingspan, arm-wing area and length, hand-wing area and length, and wing length. Ossification and muscle properties including: full epiphyseal gap, percentage of fast- and slow-twitch fiber of the pectoralis major, percentage of fast- and slow-twitch fibers of the acromeodeltoideus, surface area of the fast- and slow-twitch fibers of the pectoralis major and acromeodeltoideus. PCA was performed on each flight development stage to determine interactions among variables from all parts of the study. Eigenvalues for the

factors in the flop stage indicated that Factor 1 (58.27%), Factor 2 (7.75%), Factor 3 (6.97%), and Factor 4 (5.37%) were responsible for 78.36% of the sample variation. Factors 5-19 were not used in further analysis because the scree-plots (Cattell, 1966) indicated them to be insignificant in relation to the overall variation. The eigenvectors are shown in Table 4. Eigenvectors for forearm, mass, wing surface area, wingspan, arm-wing area and length, hand-wing area and length, wing length, full epiphyseal gap, and the area of fast-twitch fibers in the acromeodeltoideus were similar for Factor 1. Factor 2 eigenvectors were percentage of slow-twitch fibers in the pectoralis major, and the area of the fast- and slow-twitch fibers in the pectoralis major. Factor 3 was made up of the percentage of fast-twitch fibers of the pectoralis major and acromeodeltoideus, the area of slow-twitch fibers in the acromeodeltoideus and flight development. Factor 4 consisted of percentage of slow-twitch fibers in the acromeodeltoideus (Table 4). *C. perspicillata* and *A. jamaicensis* were shown to be distinct from each other when comparing Factor 1 with all other factors on the basis of factor 1 one being strongly influenced by size. Factors 2 and 3 were distinctly different between species, however, factors 3 and 4 were similar between species (Figures 51-53).

Eigenvalues for the factors in the flutter stage indicated that Factor 1 (63.89%), Factor 2 (8.63%), and Factor 3 (4.91%) were responsible for 77.43% of the sample variation. Factors 4-19 were not used in further analysis because the scree-plots (Cattell, 1966) indicated them to be insignificant in relation to the overall variation. The eigenvectors are shown in Table 5. Factor 1 eigenvectors included forearm, mass, wing surface area, wingspan, arm-wing area and length, hand-wing area and length, wing

Table 4

Eigenvectors for Factors 1, 2, 3, and 4 for the Flop Stage of Flight Development

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Forearm Length	-0.284616			
Mass	-0.286818			
Surface Area	-0.289078			
Wingspan	-0.290849			
Arm-wing Area	-0.286816			
Hand-wing Area	-0.265757			
Arm-wing Length	-0.279007			
Hand-wing Length	-0.271045			
Wing Length	-0.290245			
Epiphyseal Gap	-0.263135			
Percentage of Pectoralis Fast Fibers			0.443649	
Percentage of Pectoralis Slow Fibers		-0.526335		
Percentage of Acromeodeltoideus Fast Fibers			-0.328026	
Percentage of Acromeodeltoideus Slow Fibers				-0.699354
Area of Fast Fibers in Pectoralis		-0.424309		
Area of Slow Fibers in Pectoralis		0.399123		
Area of Fast Fibers in Acromeodeltoideus	-0.239924			
Area of Slow Fibers in Acromeodeltoideus			0.450805	
Flight Development			-0.566477	

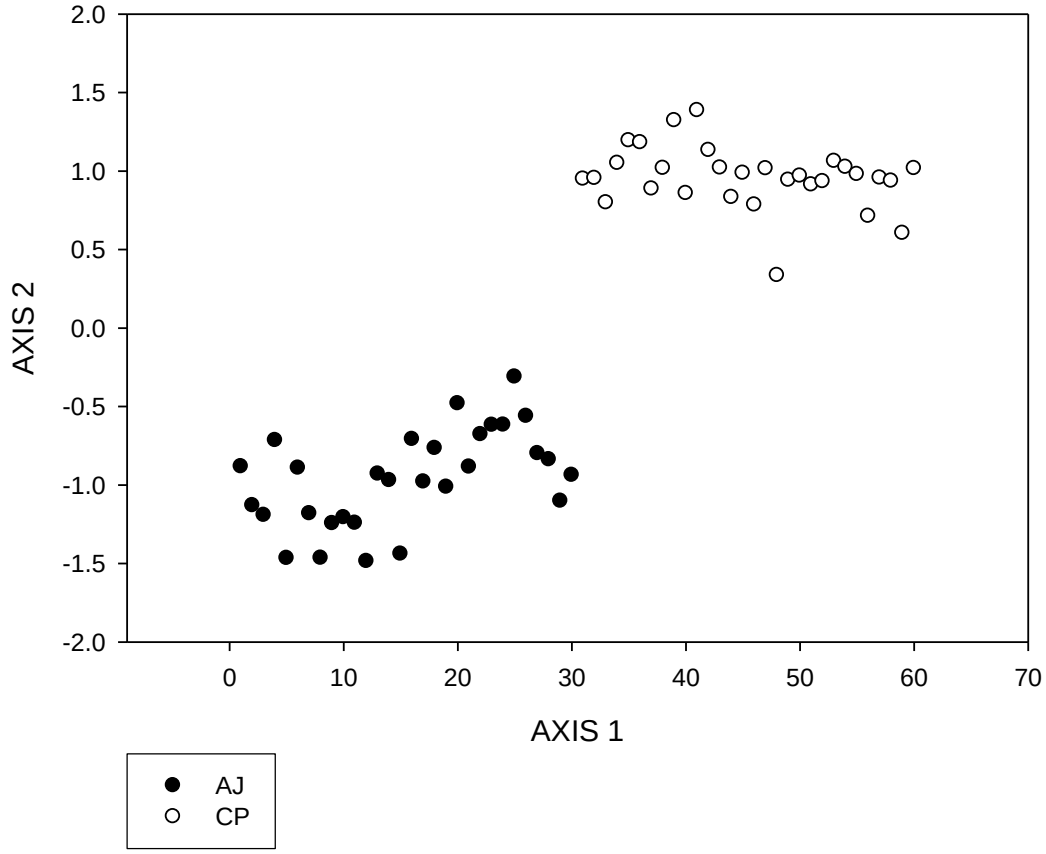

Figure 51. Factor 1 and 2 scores for the flop stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 1 and Factor 2 showing distinct clustering for each species.

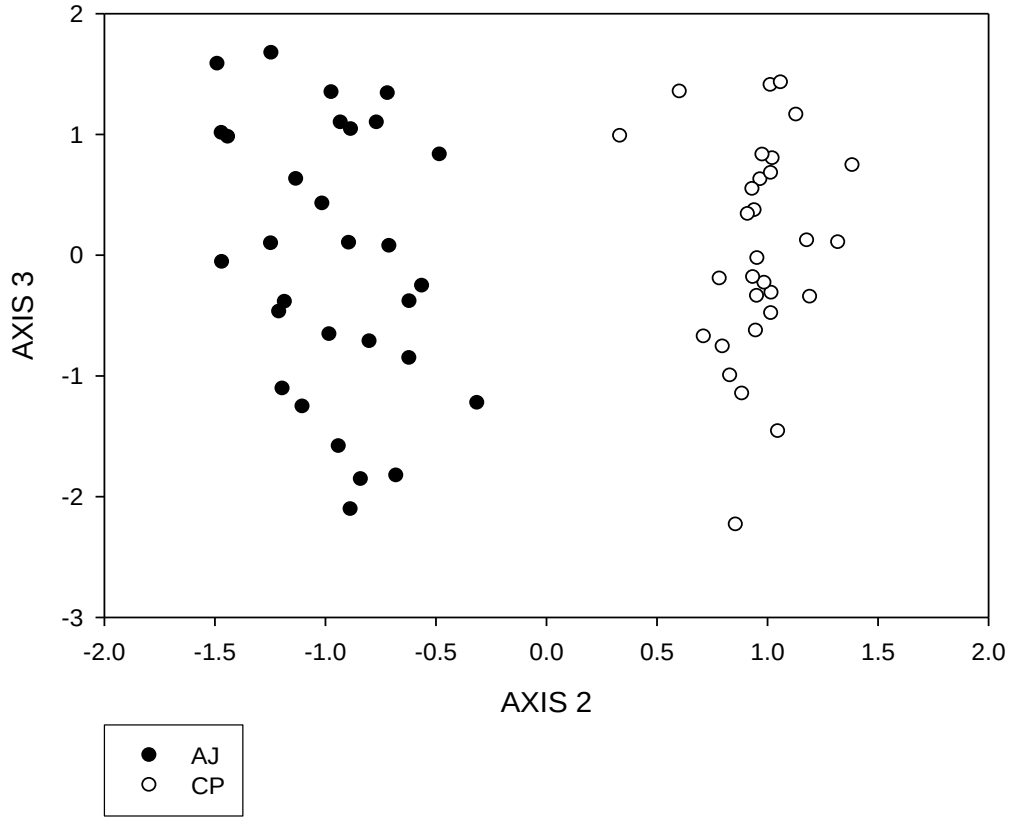

Figure 52. Factors 2 and 3 scores for the flop stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 2 and Factor 3 show distinct clustering for each species.

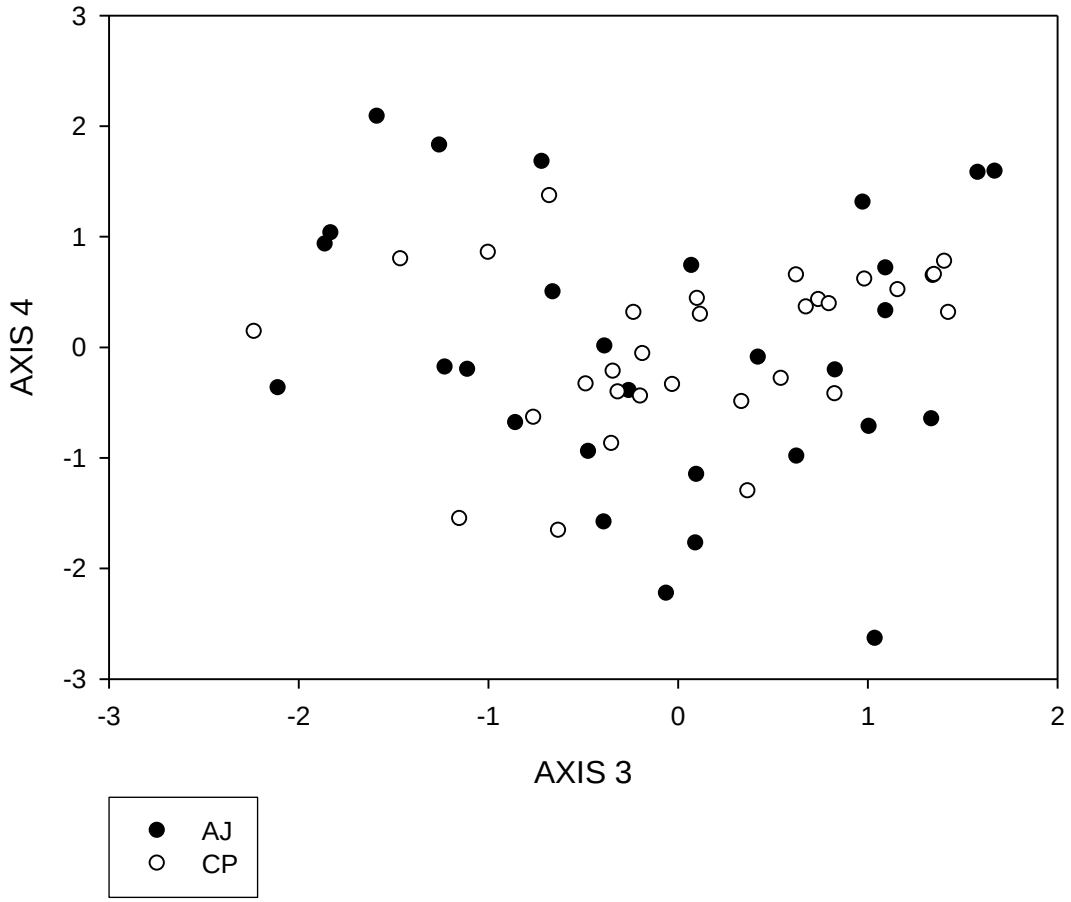

Figure 53. Factor 3 and 4 scores for the flop stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 3 and Factor 4, showing a lack of clustering for each species.

Table 5

Eigenvectors for Factors 1, 2, and 3 for the Flutter Stage of Flight Development

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Forearm Length	-0.272839			
Mass	-0.279833			
Surface Area	-0.276498			
Wingspan	-0.278897			
Arm-wing Area	-0.275738			
Hand-wing Area	-0.264699			
Arm-wing Length	-0.267589			
Hand-wing Length	-0.271297			
Wing Length	-0.280379			
Epiphyseal Gap	-0.233284			
Percentage of Pectoralis Fast Fibers			-0.325896	
Percentage of Pectoralis Slow Fibers			0.220274	
Percentage of Acromeodeltoideus Fast Fibers	-0.259418			
Percentage of Acromeodeltoideus Slow Fibers		-0.589884		
Area of Slow Fibers in Pectoralis		-0.636656	0.370771	
Area of Fast Fibers in Acromeodeltoideus				
Flight Development			-0.776381	

length, full epiphyseal gap, and percentage of fast-twitch fibers in acromeodeltoideus. Factor 2 eigenvectors were percentage of slow-twitch fibers in the acromeodeltoideus, and the area of the fast-twitch fibers in the acromeodeltoideus. Factor 3 eigenvectors included the percentage of fast- and slow-twitch fibers of the pectoralis major and the area of slow-twitch fibers of the pectoralis major as well as the day of first flutter (Table 5). *C. perspicillata* and *A. jamaicensis* were shown to be distinct from each other when comparing Factor 1 with Factor 2, and Factor 3 based on size, however, there was not a distinct difference between species when comparing Factors 2 and 3 (Figures 54-55).

Eigenvalues for the factors in the flap stage indicated that Factor 1 (51.97%), Factor 2 (9.62%), Factor 3 (6.95%), and Factor 4 (5.89%) were responsible for 74.43% of the sample variation. Factors 5-19 were not used in further analysis because the scree-plots (Cattell, 1966) indicated them to be insignificant in relation to the overall variation. The eigenvectors are shown in Table 6.

Eigenvectors for Factor 1 included forearm, mass, wingspan, arm-wing area and length, hand-wing area and length, wing length, and day of first achieving the flap flight stage. Factor 2 eigenvectors were percentage of fast- and slow-twitch fibers in the pectoralis major and area of slow-twitch fibers of the pectoralis major. Factor 3 was made up of the percentage of fast-twitch fibers of the acromeodeltoideus and area of fast-twitch fibers of the acromeodeltoideus. Factor 4 was made up of the area of slow-twitch fibers of the acromeodeltoideus (Table 6). *C. perspicillata* and *A. jamaicensis* were shown to be distinct from each other when comparing Factor 1 with all other factors based on Factor 1 being a size component (Figures 56-57).

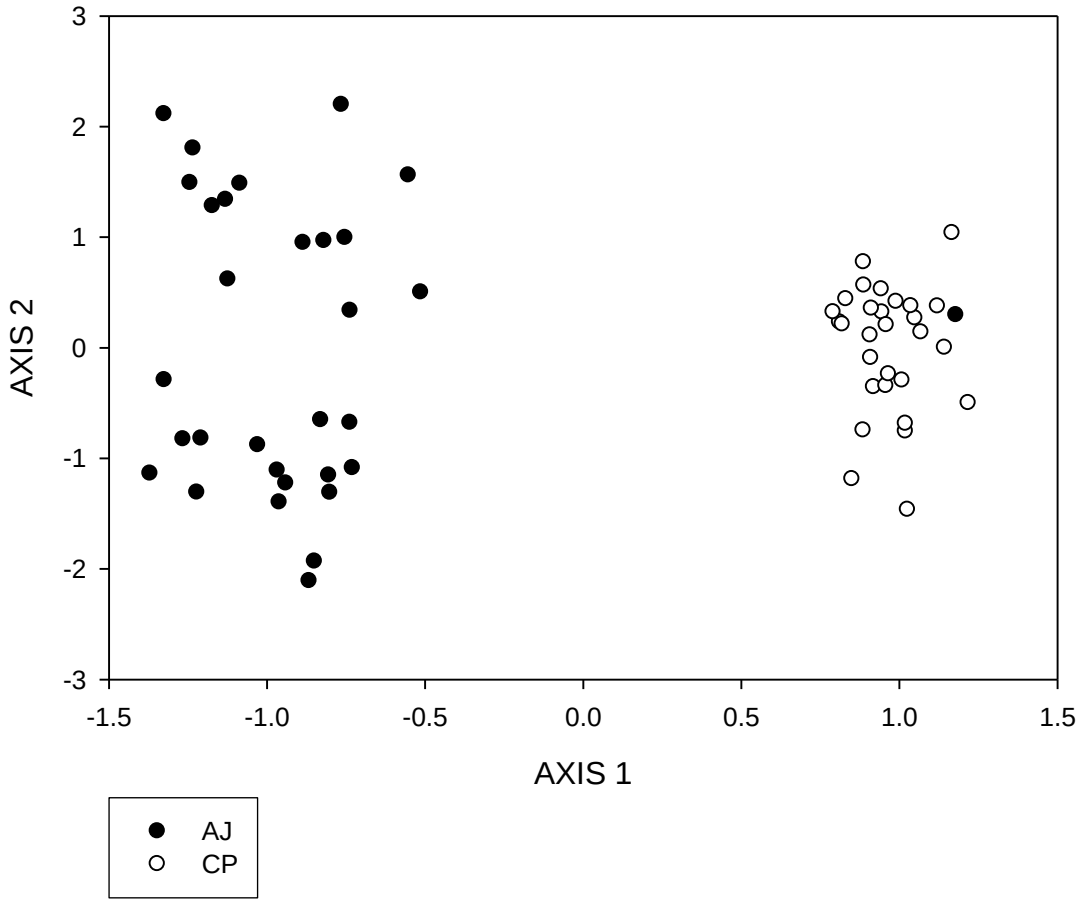

Figure 54. Factors 1 and 2 scores for the flutter stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 1 and Factor 2 show distinct clustering for each species.

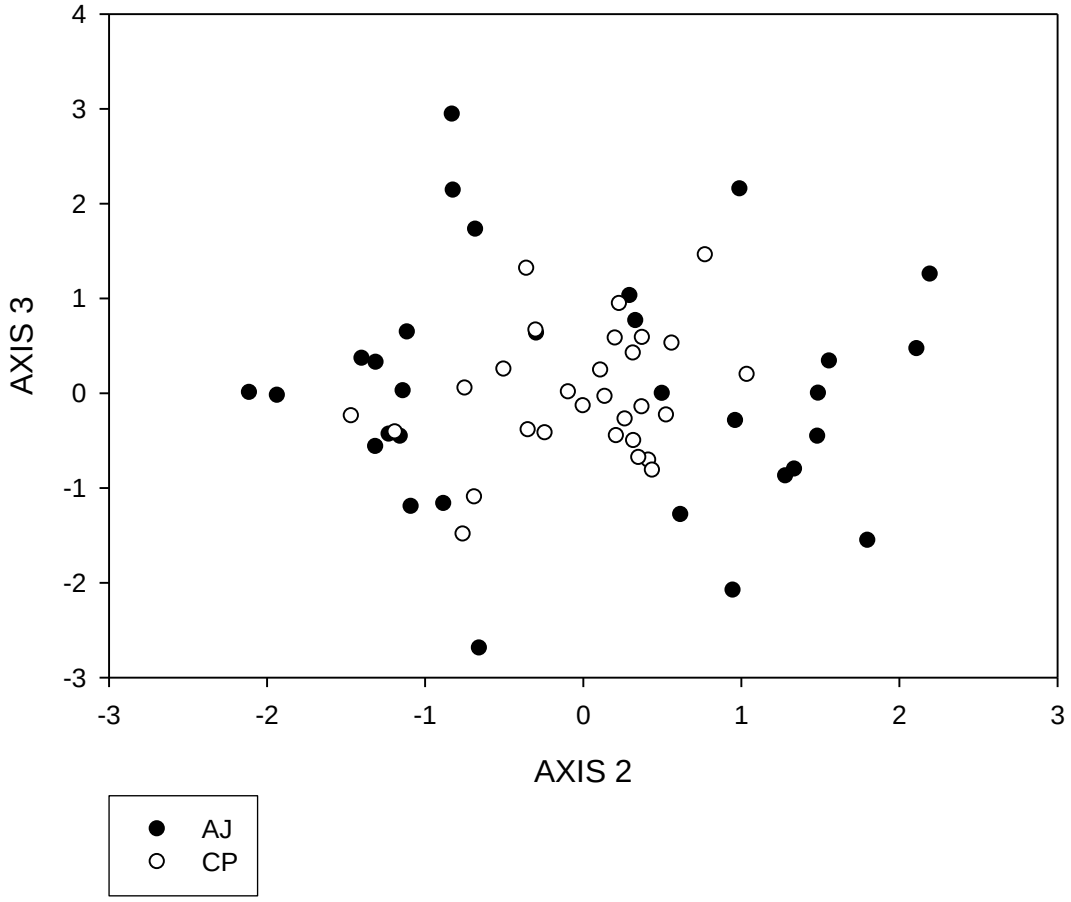

Figure 55. Factors 2 and 3 scores for the flutter stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 2 and Factor 3, showing the lack of distinct clustering for each species.

Table 6

Eigenvectors for Factors 1, 2, and 3 for the Flap Stage of Flight Development

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Forearm Length	-0.308883			
Mass	-0.311393			
Wingspan	-0.306024			
Arm-wing Area	-0.309251			
Hand-wing Area	-0.286677			
Arm-wing Length	-0.306323			
Hand-wing Length	-0.300159			
Wing Length	-0.310277			
Percentage of Pectoralis Fast Fibers		0.535309		
Percentage of Pectoralis Slow Fibers		-0.305829		
Percentage of Acromeodeltoideus Fast Fibers			0.716142	
Area of Slow Fibers in Pectoralis		-0.461425		
Area of Fast Fibers in Acromeodeltoideus			-0.447579	
Area of Slow Fibers in Acromeodeltoideus				0.801192
Flight Development	-0.238430			

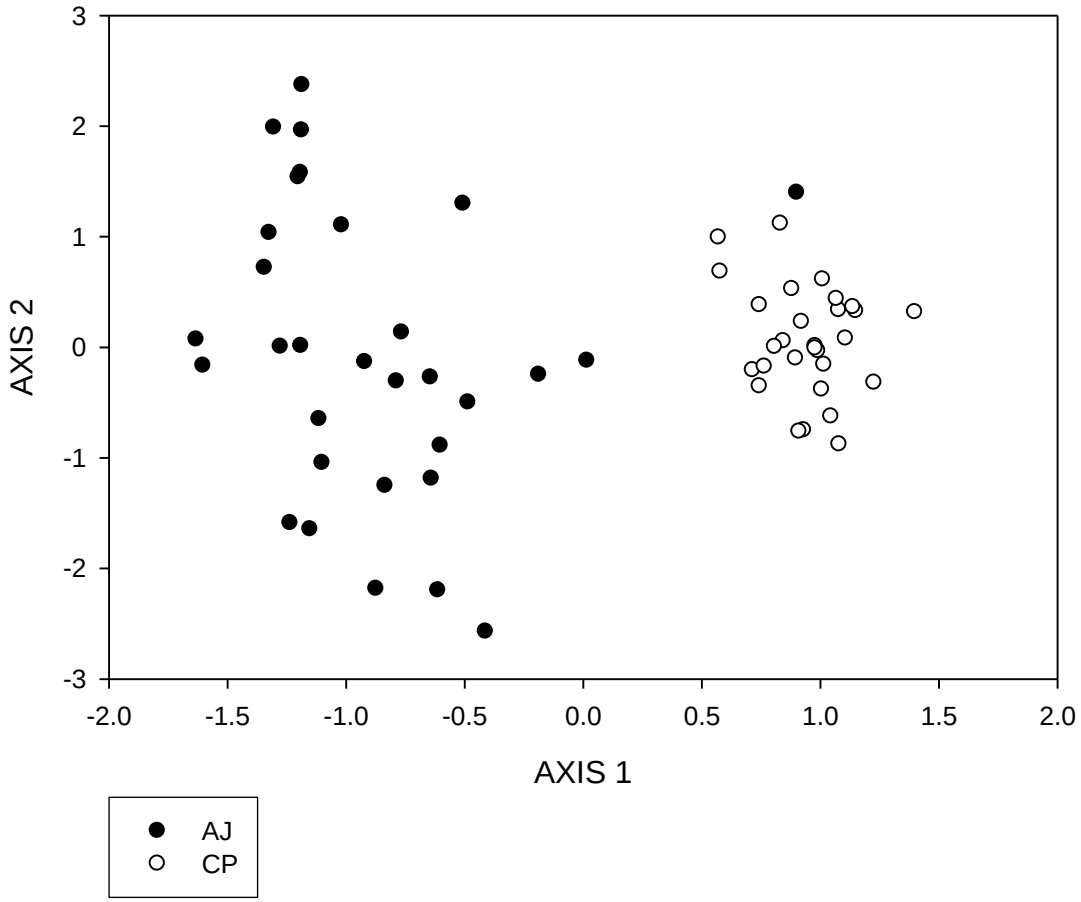

Figure 56. Factors 1 and 2 scores for the flap stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 1 and Factor 2 show distinct clustering for each species.

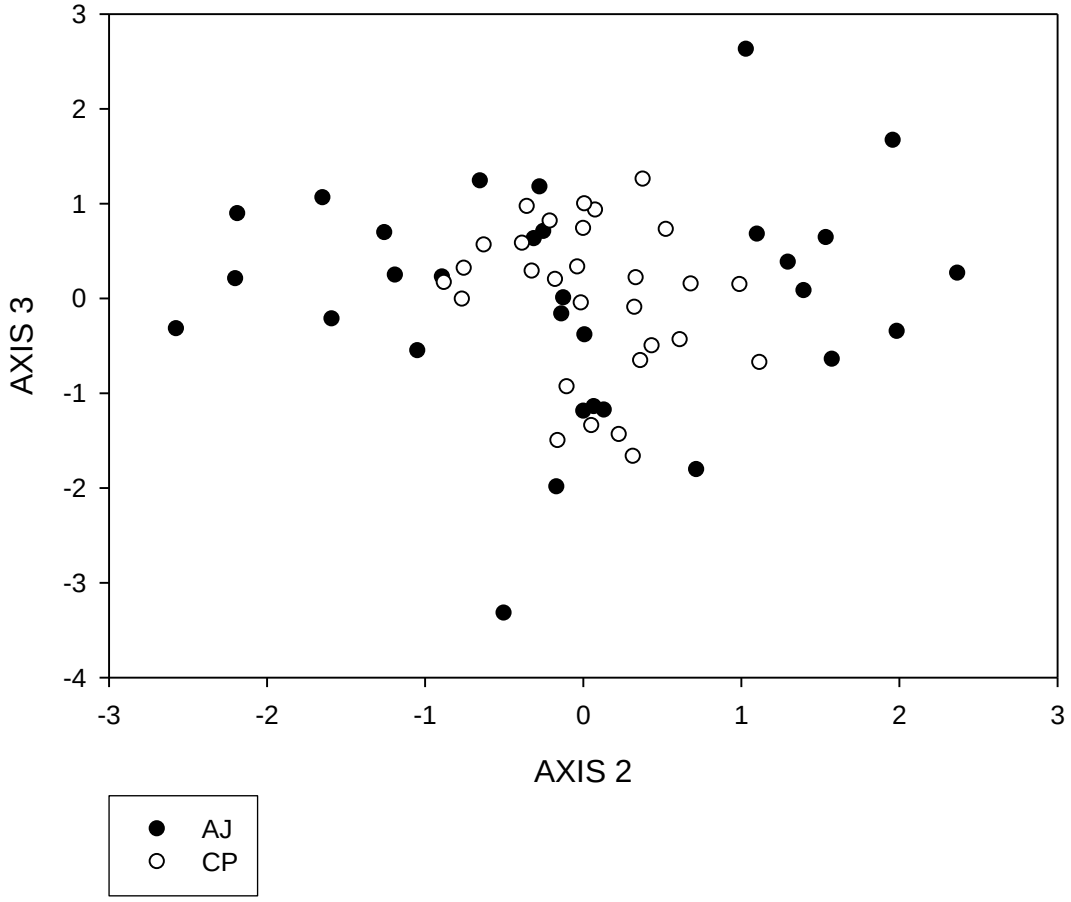

Figure 57. Factors 2 and 3 scores for the flap stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 2 and Factor 3, showing an example of the lack of distinct clustering for each species..

Eigenvalues for the factors in the flight stage indicated that Factor 1 (66.15%), Factor 2 (7.56%), and Factor 3 (6.20%) were responsible for 79.91% of the sample variation. Factors 4-19 were not used in further analysis because the scree-plots (Cattell, 1966) indicated them to be insignificant in relation to the overall variation. The eigenvectors are shown in Table 7. Eigenvectors for Factor 1 included forearm, mass, wing surface area, arm-wing area and length, hand-wing area and length, wing length, full epiphyseal gap, and fiber area of fast- and slow-twitch in the pectoralis major. Factor 2 eigenvectors were percentage of slow-twitch fibers in the acromeodeltoideus. Factor 3 was made up the percentage of fast- and slow-twitch fibers of the pectoralis major and the fiber area for fast-twitch fibers in acromeodeltoideus (Table 7). *C. perspicillata* and *A. jamaicensis* were shown to be distinct from each other when comparing Factor 1 with all other factors as factor one pertained to size. Factor 2 and Factor 3 were similar between species (Figures 58-59).

Table 7

Eigenvectors for Factors 1, 2, and 3 for the Flight Stage of Flight Development

Variables	Factor 1	Factor 2	Factor 3
Forearm Length	-0.282070		
Mass	-0.280343		
Surface Area	-0.282583		
Arm-wing Area	-0.284244		
Hand-wing Area	-0.267083		
Arm-wing Length	-0.282164		
Hand-wing Length	-0.277239		
Wing Length	-0.285005		
Epiphyseal Gap	-0.278794		
Percentage of Pectoralis Fast Fibers			-0.356282
Percentage of Pectoralis Slow Fibers			0.278124
Percentage of Acromeodeltoideus Slow Fibers		0.657771	
Area of Fast Fibers in Pectoralis	-0.236119		
Area of Slow Fibers in Pectoralis	-0.243911		
Area of Fast Fibers in Acromeodeltoideus			-0.609062

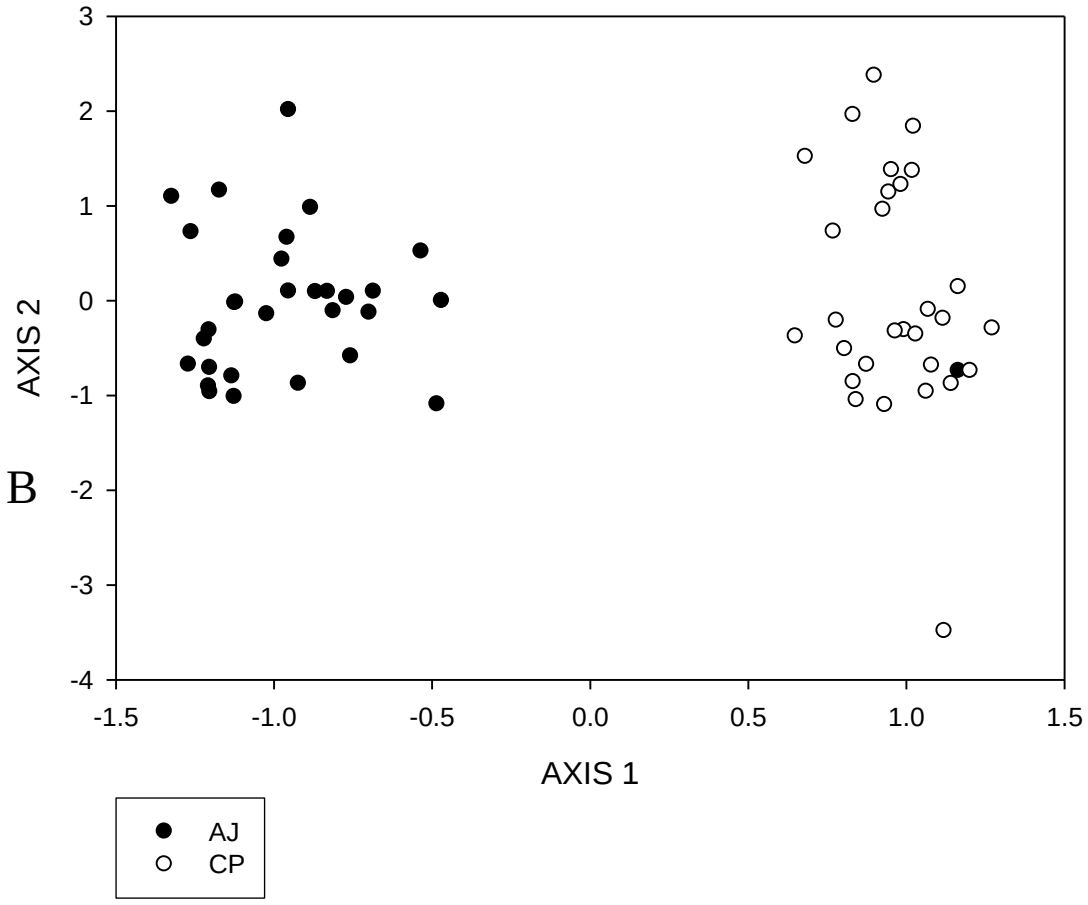

Figure 58. Factors 1 and 2 scores for the flight stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 1 and Factor 2 show distinct clustering for each species.

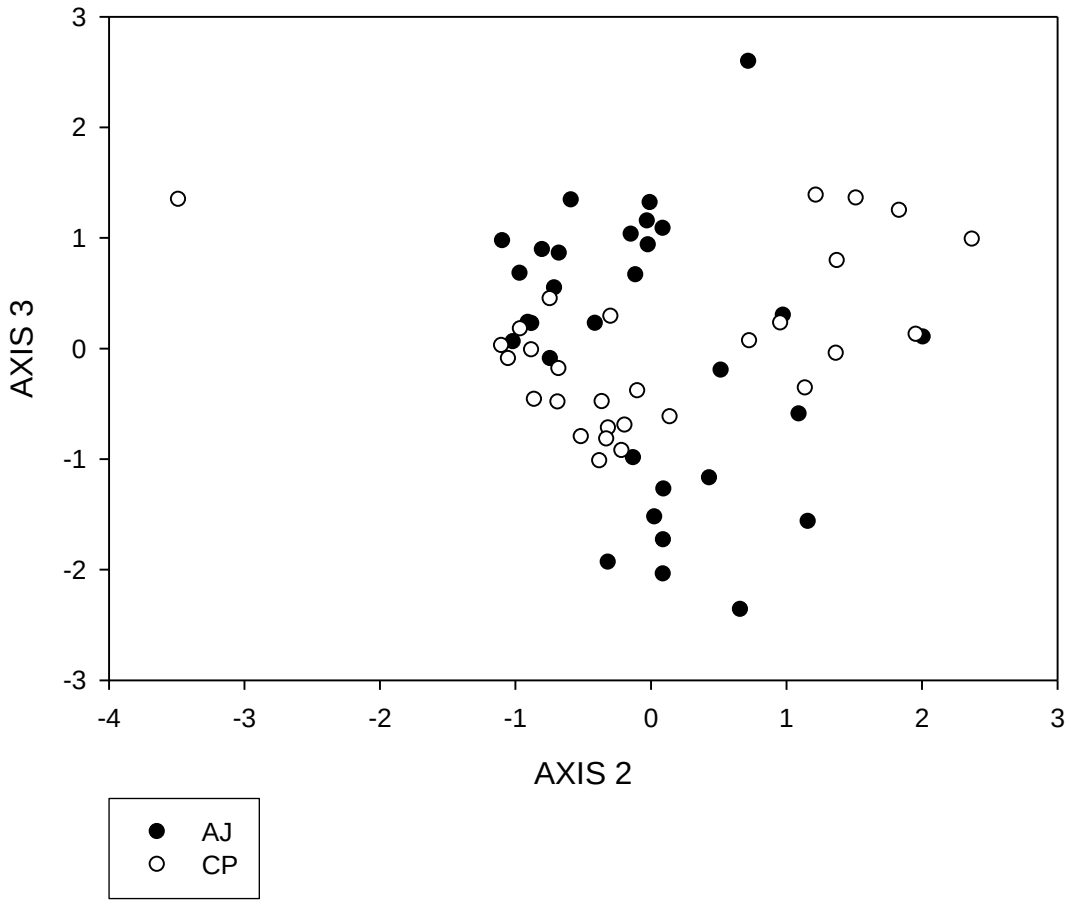

Figure 59. Factors 2 and 3 scores for the flight stage of flight development. Comparison of PCA factor scores for *Artibeus jamaicensis* (AJ) and *Carollia perspicillata* (CP) in the flop stage of flight development. Comparing Factor 2 and Factor 3, showing the lack of distinct clustering for each species due to the lack of the size factor.

CHAPTER V

DISCUSSION

Results from my study indicate that there are significant developmental differences, both in flight behavior and morphometrics, between *Artibeus jamaicensis* and *Carollia perspicillata.* Phenotypic diversity is thought to be a result of extensive variation in the genetic make-up of closely related taxa (Raff, 1996). In my research, developmental differences were used as a proxy for phylogenetic differences to provide information on Chiropteran evolution, resulting in evolutionary divergence from a common ancestor. Powered flight in bats allowed for an extensive adaptive radiation that led to one of the most diverse orders within mammals with different growth, ecological and life history trends. *C. perspicillata,* being the more ancestral (Baker et al., 2003) of the two species is born in a more precocial state. It has been found that as an order, bats exhibit both precocial and altricial characteristics (Kurta & Kunz, 1987). Bats are born at a relatively high birth weight, which are generally larger than most mammals of comparable size, representing the precocial end of the spectrum. However, there are different ends of the spectrum within the order Chiroptera. Within megachiroptera, juvenile *Pteropus giganteus* are born more altricial being able to fly at 9-12 weeks and weaning occurs between 15-20 weeks of age (Neuweiler, 1962). Some of the most precocial bats are within the microchiropteran Emballonurids are born large and are able to fly at two weeks and are weaned at 6-8 weeks of age (Bradbury & Emmons, 1974) Giving birth to juveniles that are large can be advantageous. Some of the advantages

include reduced heat loss due to surface to volume ratio, reduced mortality, and shorter postnatal developmental period. In-comparison, bat wing development and locomotor function are more altricial, with most bats not being able to fly until they have achieved 90% of adult wing dimensions and 70% of adult mass (Kunz, 1987). *A. jamaicensis* and *C. perspicillata* with large wings and low wing loading are capable of having a neonate that is born in a larger more precocial state (Norberg, 1981; Norberg & Rayner, 1987). *A. jamaicensis* and *C. perspicillata* along with the majority of the species of the family Phyllostomidae, are considered to be precocial when compared to other bats, however, as mentioned *C. perspicillata* is born in a more precocial state with all morphological traits except wingspan being more developed at birth when compared to adults. In addition to morphological traits it was found that *C. perspicillata* are born with more fur allowing for better thermoregulation. One aspect where bats are considered altricial is the fact that they cannot forage effectively until they are able to fly and maneuver like an adult (Buchler, 1980).

When taking into account growth, ecology and natural history traits one must take into account the developmental sequence of events. These events define the ontogeny of the individual and are of significant importance when viewed in an evolutionary context. Changes in any of the sequences have been shown to be a mechanism of vertebrate evolution (Gould, 1977; McKinney & McNamara, 1991; Smith, 2003).

Ontogenetic Implications of Bat Ecology and Co-existence

Developmental stages may be regarded as a series of behavior events through which an organism passes through (Binneda-Edmonds, 2002). These events should be well defined and distinct changes that are distinguishable in the behavior or morphology

of the organism with a finite time period. Powers et al. (1991) divided flight development into such developmental events of: flop, flutter, flap and flight.

Flight Development

Flight capabilities in *Artibeus jamaicensis* and *Carollia perspicillata* develop rapidly. Attainment of flight which allows for the juvenile to be independent of maternal care is an important aspect of growth and development. The ontogeny of flight in both *A. jamaicensis* and *C. perspicillata* follow the pattern found by Powers et al. (1991) of flop, flutter, flap, and flight with minor changes. In this study, I was able to determine that both species in the flop stage showed some wing movement though minor. This is in contrast to what past research has found in *Myotis lucifugus* (Powers et al., 1991) and *Hipposideros Pomona* (Lin et al., 2011), with wing movement not occurring until day 10 post-partum.

There was not a significant difference between species in the time in which they began to flop and flutter. Both species remained in the flop stage for a very short period, increasing the amount of wing movement as they fell, moving into the flutter stage within 2 days post-partum. This is an indication that the neuromuscular development of the muscles used for wing movement are beginning to develop and function rapidly (Kunz & Stern, 1995). Interestingly many juveniles of both species skipped the flop stage and were found to flutter on day one post-partum. *C. perspicillata* advanced into and remained in the flutter and flap stages for a significantly shorter period of time than *A. jamaicensis*. *C. perspicillata* began flying in straight lines significantly earlier than *A. jamaicensis*. *C. perspicillata* began to show straight flight at 23 days of age while *A. jamaicensis* did not start flying in straight lines until 32 days of age. As mentioned

previously it has been shown that most microchiropteran bats cannot fly until they have reached 90% if adult wing dimensions and 70% of adult mass (Hamilton & Barclay, 1994). *A. jamaicensis* achieved straight flight with a mass of 59%, forearm of 94%, wingspan of 90%, wing area of 77%, and wing length of 86%. The mass and wing dimensions (excluding the forearm and wingspan) of *A. jamaicensis* on the day of first flight were well below that of what has previously been found. *C. perspicillata* obtained straight flight with a mass of 56%, forearm of 82%, wingspan of 82%, wing area of 67%, and wing length of 78% with all percentages below the 70% for mass and 90% mark for wing dimensions.

The majority of the dimensions of the wing and mass were below the findings of Hamilton and Barclay (1994) which indicates that the juveniles were not at adult flight stages and ability, however, I found that all aspects of wing dimensions are well above 90% and mass is above 70% when the juveniles are capable of adult-like flight agility. In conclusion my results support my hypothesis and predictions that the more precocial *C. perspicillata* would become volant prior to *A. jamaicensis.*

Fight Ability and Maneuverability

The constraints imposed by developmental stages and morphology of bat species directly impact their flight ability and foraging ecology (Norberg, 1994). The size and shape of the wings in conjunction with the mass of the bat can be a guide as to where a bat species will forage and the speed as to which it may fly (Norberg, 1994). This relationship implies that bats that are able to fly slower will be more maneuverable, capturing aerial insects or foraging in dense clutter.

In this study, body size was found to be an important aspect of flight ability. As Stockwell (2001) found and stated, the mechanical and aerodynamic effect of body size should not be factored out of maneuverability analysis. Mass can have an impact on the turning ability of the bat and the force placed upon the wing membrane as the bat turns. Bats have the ability to increase maneuverability by flying more slowly. This is characteristic of bats that forage in dense understory (Norberg, 1981, 1987, 1990; Norberg & Rayner, 1987). This is not an option for many large bats, as there is a need for the bat to fly at a sufficient speed to create a sufficient amount of lift (Norberg & Rayner, 1987). With this in mind larger bats are not able to create the amount of lift that is necessary for higher maneuverable slow flight. In addition, wing shape is highly correlated to flight ability. The wider the wing tip the more capable the bat is of cambering (bending the wing in a concave shape) their wing (Norberg, 1972; Vaughan, 1970) allowing for slower flight.

At the time of first flight bats are lacking in maneuverability skills with flight attempts resulting in a downward path that has been considered a type of practice behavior (Hughes et al., 1995). These practice flights have been observed in many bat species with many occurring within the maternity roost site, species include: *Myotis velifer* (Kunz, 1974), *Myotis lucifugus* (O'Farrell & Studier, 1973), *Rhinolophus cornutus* (Yokoyama & Uchida, 1979a) and *Rhinolophus ferrumequinum* (Hughes et al., 1989).

I found that both *A. jamaicensis* and *C. perspicillata* were similar to what Hughes et al. (1995) found in that at the point of first flight both species had limited flight ability and were unable to fly in a maneuverable manner. It is also thought that limb and body size and physiological functions such as: cellular differentiation, muscle strength, and

sensory-motor coordination all constrain flight performance (Ricklefs, 1979a). In the current test of maneuverability the dowels were placed at distances that were specific to the wingspan of each individual bat with spaces of 100%, 75%, and 50% of wingspan. I found that the larger *A. jamaicensis,* as one would expect, was overall less maneuverable than *C. perspicillata*. Timing of adult-like maneuverability was also significantly different between species with *A. jamaicensis* being able to fly like an adult at 45 days post first-flight while *C. perspicillata* 40 days post first-flight. This timing turned out to be highly different than the findings of Bucher (1980) who found that *Myotis lucifugus* in roughly 7-10 days progressed from first flight attempts to adult-like flight behaviors.

I found the as the dowel spacing decreased, representing a more cluttered environment, both species decreased in maneuverability ability. *A. jamaicensis* was capable of flying with adult-like agility at full wingspan spacing at 35 days post first-flight while decreasing in ability as the spacing decreased, 75% spacing was adult-like at 45 days post first-flight and 50% spacing was adult-like at 65 days post first-flight. Similar findings were found for *C. perspicillata* with adult-like maneuverability occurring at full, 75% and 50% dowel spacing at 25, 35 and 50 days post first-flight.

These results support my hypothesis and predictions that *C. perspicillata* has wing dimensions that support more maneuverable flight throughout development. The results also support the foraging habits of bat species that vary in mass and wing structure as to where they forage for fruit. *A. jamaicensis* is found in the upper canopy of the rain forest (Ortega & Castro-Arellano, 2001) while *C. perspicillata* are found in the understory to mid-canopy foraging for fruit (Cloutier & Thomas, 1992).

Differences in body size are a means by which species can avoid overlap of resources (Schoener, 1974) allowing for species coexistence. Size, thus, can impose significant constraints on the ways in which organisms interact with their surrounding environment which can influence the strength and type of interactions with other species (Schoener, 1974). How organisms are utilized resources and obstacles such as predation are generally related to body size. With this in mind many species will undergo ontogenetic shifts in food and habitat use throughout their developmental period (Werner & Gilliam, 1984) creating a complex interaction in the natural communities.

Populations compete with different types of competitors and predators and encounter different types of obstacles based on the stage of life they are presently in (Werner & Gilliam, 1984). The species' size and developmental stage and subsequent interactions can shape their life histories and the overall dynamics of the communities, creating ontogenetic niches (Werner & Gilliam, 1984). Ontogenetic niches as defined by Werner and Gilliam (1984) refers o the resource use patterns of an organism that develop as it goes from birth to adulthood. Ontogenetic resource shifts can deeply complicate species interactions and community dynamics. In many cases the dynamics are not specifically affected by the adults but the juveniles as they progress through the different niches as they become more adult-like in their abilities (Adams, 1996, 1997; Frazer & Ehrhart, 1985). This is an obvious when taking into account the life history of organism that have different stages such as a larval form and then undergo metamorphosis. Stage specific interactions can incur profound outcome on the interactions of the species within a community (Werner & Gilliam, 1984).

Many species can use similar resources when they are small but change their niche as they become larger and more mature. Based on observations in bats there is a selective advantage for fast growth (Boyd & Myhill, 1987; De Fanis & Jones, 1995; Jin et al., 2010; Liu et al., 2009; Reiter, 2004; Stern & Kunz, 1998). An increase in development decreases the time the juvenile spends in the smaller, more vulnerable size decreasing the overall risk of mortality. Within bat communities there is little known about the juveniles and their ontogenetic niches. Buchler (1980) found that juvenile *Myotis lucifugus* found that younger bats left the roost at different times than adults and they were also found to avoid cluttered locations. Research on diet of bats at different developmental stages has shown that the diet of juvenile bats was significantly different from that of an adult (Adams, 1997). Adams (1996, 1997) found specific trends in *Myotis lucifugus* when comparing foraging habits. He found that adults foraging in less cluttered habitats were significantly higher before juveniles became volant. Once juveniles became volant adults shifted their foraging to more cluttered habitats and the juveniles were captured more often in the low clutter areas.

I found that there is a significant difference in flight ability for both *A. jamaicensis* and *C. perspicillata* that could have an effect on both the population and community level. There is a time period where the juveniles soon after volancy cannot maneuver at the ability of an adult, limiting them to less cluttered foraging locations. This theoretically segregates the juveniles into habitats that are different from the adults for a time period until they are capable of adult-like flight agility. Within this time period, juveniles are at their greatest risk of mortality as they are passing from less maneuverable to more maneuverable. As a juvenile gets closer to the flight ability of an

adult they reduce the risk of mortality due to a higher ability to fly and forage effectively. Adams (1996, 1997) referred to this as an adaptive ontogenetic landscape moving from moving from peak to peak through maladaptive valleys with those that survive being the ones that successfully maneuver and forage at a specific size and age.

A maneuverability difference was also found between the two species. This is of significant importance, allowing for *A. jamaicensis* and *C. perspicillata* to use different habitat types, coexisting within the same community. If the two species overlapped in the food types and foraging location, there would be competition which could essentially eliminate one of the species from the community.

Essentially, we are seeing age and size specific resource partitioning on a species and community level. Juveniles are capable of only using specific resources based on their flight ability while adults like Adams (1996, 1997) found in *Myotis lucifugus* are moving to different foraging locations as to not overlap with the just volant juveniles. The difference in size, wing shape and flight ability between *A. jamaicensis* and *C. perspicillata* allows for resources to be partitioned with different locations in the height within the forest. With this in mind, juveniles have an influence on the ecology of a population and community

The Evolution and Development of Wing Form and Body Size

Phenotypic variations are thought to reflect diversity on the gene level in closely related taxa (Raff, 1996). Bat diversity, specifically in size and wing shape, are the result of genetic variation that has evolutionarily diverged from a common vertebrate limb. Key divergent mechanisms that have led to the diversity of wing phenotypes are primarily the change in developmental rates, termed heterochrony (Gould, 1977). In

addition to heterochrony morphological traits grow at an accelerated rate when compared to body size resulting in differential shape changes; this can be described by ontogenetic allometry. Closely related mammals have been found to differ in their ontogenetic pathways of both shape and growth rate (O'Higgins & Jones, 1998). The understanding of heterochronic shifts and allometric comparisons during development of wing morphology, body size and muscle size in closely related organisms is key to understanding the evolution of the diversity of flight and form in the order Chiroptera.

Bat Development

In this study the development of two closely related New World fruit bats was compared. *Artibeus jamaicensis* and *Carollia perspicillata* are within the same family, however, separate phylogenetically at the subfamily level, with *C. perspicillata* being the more ancestral species (Baker et al., 2003). They differ in body size and have overall different wing structure, which has led to their differing foraging habitats of either in dense vegetation in the jungle understory as with *Carollia perspicillata* or within or near the canopy as with *Artibeus jamaicensis* (Cloutier & Thomas, 1992; Fleming, 1988; Ortega and Castro-Arellano, 2001). Both species have large, wide wings which enables them to fly slower and carry large loads to a roost site (Cloutier & Thomas, 1992; Fleming, 1988; Ortega & Castro-Arellano, 2001). It is important in the understanding of flight and development to examine aspects of ontogeny such as, wing morphology, body size, muscle, and bone development and how they pertain to flight development and ability.

With this in mind, I found that there are significant developmental differences between the two species that help explain their divergence from a common ancestor.

Many of these differences are based on heterochronic growth rate changes and differences in allometric scaling.

As mentioned previously, *C. perspicillata* were born with morphological traits that were closer to adult dimensions than *A. jamaicensis* with wingspan being the only exception. With this in mind, I found that for *A. jamaicensis* to obtain a much larger size both in body and wing dimensions, they grew at an accelerated rate when compared to *C. perspicillata* in the majority of measured body parts, including mass, forearm, wingspan, wing area, wing length, arm-wing length, hand-wing area and length. Arm-wing area turned out to be the only trait that did not have a significantly higher growth rate constant for *A. jamaicensis* when compared to *C. perspicillata*. In this study. growth was measured using the logistic growth equations that take into account the non-linear growth curves that were found in all traits measured (Ricker, 1979; Zullinger et al., 1984). Importantly, growth rate constants taken form nonlinear equations are independent of body size and period of growth allowing for comparisons to be made between species (Kunz & Robson, 1995).

The pattern of postnatal growth in both *A. jamaicensis* and *C. perspicillata* is similar to that of many bat species (Kunz & Stern, 1995). The length and area of the wing and body mass increase in a linear formation then eventually plateau as the juvenile reached an asymptotic value which is within the range of adult measurements. By the time the juveniles were flying like an adult, wing dimensions were similar to that of an adult, however, the mass of the juveniles were still proportionally lower than that of the adults (Hamilton & Barclay, 1994; Kunz & Stern, 1995).

These findings are consistent with my hypothesis and predictions that there would be significant differences between the growth rates of *A. jamaicensis* and *C. perspicillata* allowing for the overall size difference that is seen between the two.The patterns of higher growth rate constants are consistent with rate changes that are described by heterochrony. With *C. perspicillata* being the ancestral species, *A. jamaicensis* showed peramorphic heterochrony with its growth rates being at a more accelerated rate when compared to *C. perspicillata*. Peramorphic heterochrony, specifically acceleration, has been found to be the main developmental factor in sexually dimorphic species with either the male or the female growing at a faster rate (Jarman, 1983). This was nicely shown by O'Higgins and Dryden (1993) with male apes growing at a faster rate than females, accounting for the males overall larger size. Accelerated growth has also been found to be localized to specific body parts. Hafner and Hafner (1988) found that the tail vertebrae of kangaroo rats grow at an accelerated rate when compared with other closely related rodents.

Growth rate constants have not been compared between closely related bat species in the same study to this date. Growth rates of many species of bats have been examined; however, they were not directly compared to other species (Boyd & Myhill, 1987; De Fanis & Jones, 1995; Jin et al., 2010; Kunz & Anthony, 1982; Liu et al., 2009; Reiter, 2004).

My research on wing dimensions showed that the wing loading (mass divided by the wing area) followed patterns found in previous research on bat development (Norberg, 1990, 1994). Wing loading decreased rapidly in both species with *A. jamaicensis* achieving adult-like wing loading at 4 days post-partum while *C.*

perspicillata achieved adult-like wing loading at 10 days post-partum which is roughly 29 days prior to first flight for *A. jamaicensis* and 13 days prior to first flight for *C. perspicillata*. This is congruent with the accelerated growth seen in *A. jamaicensis* in both mass and wing area. *A. jamaicensis* and *C. perspicillata* had lower body mass at the onset of flight and, thus, a lower wing loading than that found in adults. Wing loading for both species remained lower than the adult mean until *A. jamaicensis* reached 68 days post-partum and *C. perspicillata* reached 48 days post-partum which minimizes the power needed during flight development.

My data showed that wing loading and flight capability do not simply move toward adult values as they develop. Wing loading at birth exceeded that of values in adults to values that were significantly lower than that of adults. After a period, the juvenile wing loading values gradually increased to be similar to that of the adult wing loading values. Declines in wing loading below that of adult values during development have been found in *Nycticeus humeralis (*Jones, 1967), *Antrozous pallidus* (Davis, 1969), *Rhinolophus ferruminequinum*, *Pipistrellus pipistrellus* (Hughes et al., 1989, 1995), *M. lucifugus* (Powers et al., 1991), *Plecotus auritus* (De Fanis & Jones, 1995), and *Phyllostomus hastatus* (Stern, Kunz, & Bhatt,1997).). Results for *A. jamaicensis* and *C. perspicillata* are similar to that of the wing loading results of *P. hastatus,* with wing loading values that were below that of adult levels at 7 weeks increasing to levels of adult wing loading at 14 weeks post-partum (Stern et al., 1997).

When looking at allometric comparisons both species showed a more rapid increase of wing area than mass with *C. perspicillata* with the steeper slope indicating a larger increase in wing area per increase in mass allowing for an overall lower wing

loading. Interestingly both species achieved similar adult-like wing loading near the time they became comparable in maneuverability with adults. Wing loading, however, was found to be much higher in *A. jamaicensis* than in *C. perspicillata* which follows what Norberg and Rayner (1987) found with smaller more maneuverable bats having lower wing loading, allowing for more agile flight ability. Powers et al. (1991) found that *Myotis lucifugus* reached adult-like wing loading at 15 days post-partum. Having low wing loading at the onset of flight is highly important. Flight performance and the cost of transport are highly correlated with wing loading (Norberg, 1990; Norberg & Rayner, 1987). This allows for increased maneuverability and decreases the cost of flight while the juveniles are learning how to fly and forage (Aldridge, 1987; Hughes et al., 1995).

The hand-wing (area from the fifth digit to the wing tip) in both species appeared to be underdeveloped at birth which is comparable to what has been found in other species (Hughes et al., 1989; Powers et al., 1991; Taft & Handley, 1991). The hand-wing was found to increase in size more rapidly than the arm-wing during post-partum development in both species as seen by higher growth rate constants from logistic growth equations. *A. jamaicensis* had a hand-wing growth rate constant of 0.075 compared to the arm-wing with a growth rate constant of 0.062 for area and a hand-wing growth constant of 0.075 for length and an arm-wing growth constant of 0.06. *C. perspicillata* was similar with a growth rate constant for the hand-wing and arm-wing area of 0.07 and 0.066. Growth rate constants for *C. perspicillata* hand-wing and arm-wing length were 0.071 and 0.045. *A. jamaicensis* and *C. perspicillata* had similar growth rates for arm-wing area, however, there was a significantly faster rate of growth in the arm-wing length, hand-wing area and hand-wing length of *A. jamaicensis* than *C. perspicillata*.

Subsequently, the hand-wing in both species is longer than arm-wing which directly impacts the tip shape index. This is a measure of the wing-tip shape.

A high tip index indicates a rounded wing tip and a low index indicates pointed wing tips. Bats with more elongated, round wing tips have the ability to fly slow and even hover with the distal end of the wing generating the majority of the force (Findley et.al., 1972; Norberg, 1976; Norberg & Rayner, 1987; Rayner, 1986). As an adult *C. perspicillata* (1.95) has a significantly higher tip shape index than *A. jamaicensis* (0.94). This provides information that *C. perspicillata* has a wing tip that is rounder than *A. jamaicensis* providing greater lift when combined with lower wing loading provides for an increase in the bats maneuverability and capability of flying at a slower speed. My maneuverability tests backed up the morphological results with *C. perspicillata* being significantly more maneuverable than *A. jamaicensis*. This has also been shown with the location of their foraging and roosting sites (Cloutier & Thomas, 1992; Ortega & Castro-Arellano, 2001).

Allometric scaling determines the relative shape change in a trait when compared to size which in most cases is the organism's mass (Raff, 1996). Allometry is described as being either positive, the trait increases faster than mass, negative, the trait increases slower than mass, or isometry, the trait and mass increase in a similar fashion (McKinney & McNamara, 1991).

I found that for both *A. jamaicensis* and *C. perspicillata* followed the positive allometry developmental pattern. *C. perspicillata* had positive allometry in all morphometric traits including forearm, wing surface area, wingspan, arm-wing area, hand-wing area, wing length, arm-wing length, and hand-wing length. *A. jamaicensis*

had positive allometry in morphometric traits in the majority of traits including: wing surface area, wingspan, arm-wing area, hand-wing area, wing length, and hand-wing length. *A. jamaicensis* unlike *C. perspicillata* had negative allometry in two traits, forearm and arm-wing length, however, the arm-wing length had a slope of 0.925 which is near isometry.

In both species, it was obvious that wing dimensions outpaced the increase in mass. Wing dimensions reached adult measurements in most cases long before the juvenile reached the mass of an adult. These finding were similarly found in *P. subflavus*, at the time of first flight wing dimensions were near adult proportions, however, their mass was proportionally lower (Hamilton & Barclay, 1994; Kunz & Stern, 1995).

Positive allometry during development has been thought of as potentially trading off energy investment (Ricklefs, 1979a) with the growth of wing structures receiving more energy than that of mass. As juveniles grow development of the wing has been seen to grow at a faster pace than mass. This has been seen in many species of bats such as *Pipistrellus pipistrellus* (Hughes et al., 1995), *Pipistrellus minus* (Isaac & Marimuthu, 1997), *Pipistrellus subflavus* (Hoying & Kunz, 1998), and *Myotis blythii* (Sharifi, 2004). *C. perspicillata* had the larger slopes meaning that the wing dimension increased more per increase in mass than did *A. jamaicensis* which may give an advantage to locomotor performance, specifically an earlier volancy. A difference in wing ontogeny and body mass seems to be adaptive allowing for easier flight development when juveniles are first learning how to fly (Stern et al., 1997). Allometric scaling is also supported by the state the species is in at birth. Animals that are born in a more precocial state theoretically

could use more of its resources in becoming independent. In the case of bats, that would be the development of the flight apparatus allowing for earlier volancy. *C. perspicillata* was found to be born in a more precocial state and therefore can use more of its resources to wing growth. I found this to be true with *C. perspicillata* having steeper slopes in all of the traits analyzed.

Digit Ossification

Ossification of the fourth digit metacarpal-phalangeal joint has been found to be the last joint to ossify and has been used as an accurate measurement of skeletal growth and when the juvenile has completed skeletal growth (Kunz, 2009). Epiphyseal gaps in *A. jamaicensis* and *C. perspicillata* follow the trends that have been found in many other bat species (Hood et al., 2002; Hoying & Kunz, 1998; Rajan & Marimuthu, 1999; Reiter, 2004; Stern & Kunz, 1998). In my research, I measured epiphyseal gap lengths on the first day of each flight stage. I found that the total gap increased to a maximum length in the flutter stage and steadily decreased in size to complete closure in the adults. This finding was similar for both species with the flutter stage having the largest total epiphyseal gap. Overall *A. jamaicensis* had a larger epiphyseal gap than did *C. perspicillata* in all flight stages.

At the first day of the flutter stage, the both species lacked distinct distal and proximal gaps, however, by the start of the flap stage, both species had distinct distal and proximal gaps present. The proximal gap was significantly larger in *A. jamaicensis* that in *C. perspicillata* in the flap and flight stages. By the time both species were of adult age, proximal gap had completely ossified and was not present. The distal gap was present only in the flap and flight stages as was the proximal gap. There were significant

differences between *A. jamaicensis* and *C. perspicillata*, however, *C. perspicillata* had a larger distal gap in the flap stage and *A. jamaicensis* had a significantly larger distal gap in the flight stage. The distal gap in both species was completely ossified by the time the juveniles were considered adult age. The distal gap in both species was much smaller than the proximal gap.

The epiphyseal gaps in both species were open as the juveniles were learning to fly as well as at the time of flight indicating a significant amount of growth and wing size modification still occurring after flight was achieved. The total epiphyseal gap was completely ossified at the adult stage indicating that the wing had stopped growing and had reached adult dimensions (Kunz et al., 2009).

Muscle Development

Muscles of locomotion in mammals are composed of up to three different fiber types, belonging to motor units that have distinct functional properties resulting in varying performance capabilities. There are many classification paradigms that are based on the properties of myosin heavy chains which can be broken down into type I, type IIa, and type IIb motor units (Brooke & Kaiser, 1970; Guth & Samaha, 1969, 1970). In this study, I chose to use immunohistochemistry which uses antibodies that attach to either fast or slow myosin isoforms on muscle fibers of the pectoralis major and the acromeodeltoideus. This method is useful in identifying muscle type, however, it does not allow for identification of metabolic activity such as oxidative or glycolytic. Flight muscles are extremely important to bats for both producing the appropriate power for flight as well as creating the force for maneuverability. The pectoralis muscles are used for sustained forward motion, specifically performing the up and downstroke motion of

flight (Hermanson & Altenbach, 1981, 1985; Vaughan, 1970). The acromeodeltoideus muscles function as one of the power centers for maneuverability during flight (Powers et al., 1991). Flight muscles in the adult bat have been found to be completely fast-twitch (Armstrong, 1977; Foehring & Hermanson, 1984; George & Jyoti, 1955; Hermanson & Foehring, 1988; Hermanson et al., 1991; Strickler, 1980). The ontogeny of the flight muscles have been studied predominantly in the insectivorous bat *Myotis lucifugus* (Kunz and Anthony, 1982; Powers et al., 1991; Schutt et al., 1994) with the focus being on the pectoralis muscle. Powers et al. (1991) performed a detailed study on *Myotis lucifugus* using histology to identify myosin heavy chain types and metabolic pathways used. She studied the pectoralis major and the acromiodeltoidus and found that by the time of weaning both muscle groups were homogeneous with the predominant muscle being fast-twitch. Muscle fibers have been found to increase in size as the animal ages, indicating increased power through use overall development due to aging (Powers et al., 1991; Schutt et al., 1994). These findings were similar to what they found in the adults.

Hermanson and Foehring (1988) found two fiber types in the pectoralis muscle of adult *Artibeus jamaicensis* using histological methods that identified the type of myosin ATPase present in the fibers. They classified both fibers as fast-twitch with one being type one and the other being type two.

With these findings in mind, I performed immunohistochemistry on flight muscles for the first time in bats. Immunohistochemistry can be more specific than that of the histology that has been used in the past. Surprisingly, I found that muscle development of *C. perspicillata* followed the developmental trends of what has been found with the histological analysis that has been performed to date (Armstrong, 1977;

Foehring & Hermanson, 1984; George & Jyoti, 1955; Hermanson & Foehring, 1988; Hermanson et al., 1991; Powers et al., 1991; Strickler, 1980). I found that, in both the pectoralis major and the acromeodeltoideus, the major muscle fiber type was fast-twitch. Fast- and slow-twitch fibers were present; however, the fast-twitch fibers represented greater than 90% of all fibers analyzed.

Fast-twitch muscle fibers in *C. perspicillata* were found to increase in diameter as the juveniles transitioned through flight stages. In the pectoralis major, the fast-twitch fibers were significantly larger in diameter as the juvenile aged. There was a period when the fast-twitch fibers in acromeodeltoideus during the flap and flight stages were similar in size. The fiber diameter of the slow-twitch fibers increased in size throughout growth also with a few stages that were similar in size. In the pectoralis major, the diameter was similar in the flight and adult stages insinuates that the fibers were at the adult stage by the time they began flying. Similarly, the diameter of the slow-twitch fibers in the flap and flight stages were similar, however, they continued to increase through the adult stage representing increases growth throughout development.

The muscle immunohistochemistry of *A. jamaicensis* proved to be more interesting than expected. The fiber type of the pectoralis major started off in a similar fashion as has been found in all other bats. The majority of the fibers were fast-twitch, greater than 90% in the flop and flutter flight stage. However, as the bats progressed through flight stages, the amount of slow-twitch and fast-twitch fibers of the pectoralis major changed. There were significantly more fast-twitch fibers in the flap stage, however, 30% of the total fibers were slow-twitch. By the flight stage, there had been a switch in the fiber types with slow-twitch fibers now being the more predominant fiber

type. I found that there were significantly more slow-twitch fibers in the pectoralis major in the flight and adult stages. This is contrary to any histology results of the pectoralis major to date. The adult *A. jamaicensis* had roughly 60% of the fibers in the pectoralis major being slow-twitch.

The fibers of the acromeodeltoideus in *A. jamaicensis* were similar to that of *C. perspicillata* and all other bats surveyed to this point. They major fiber type was fast-twitch with all flight stages having greater than 90% fast-twitch fibers. Fiber diameter increased in size after the flutter stage with the flap, flight, and adult stages being significantly different, indicating that the acromeodeltoideus like in *C. perspicillata* was still increasing in size at the time of flight and throughout the period where the juveniles were mastering flight ability. The Pectoralis major followed a similar pattern as the acromeodeltoideus with the flop and flutter stages being similar in fiber diameter. Fiber size continued to increase there after; however, the diameter was similar between the flight and adult stages indicating that the fast-twitch fibers were adult-like at the time of first flight.

The slow-twitch increased in size through stages until the flight stage. The flight and adult fiber diameter for slow-twitch fibers were similar in both the pectoralis major and the acromeodeltoideus indicating as with fast-twitch fibers that the slow-twitch fibers were adult-like at the start of the flight stage.

As one would expect, do to the size difference, there was a significant difference of fiber size in both the pectoralis major in all flight stages between *A. jamaicensis* and *C. perspicillata* for both the fast- and slow-twitch fibers. Fast-twitch fibers of the acromeodeltoideus were significantly larger in *A. jamaicensis* in all developmental stages

except for the flight stage. The slow-twitch fibers of the acromeodeltoideus were larger in *A. jamaicensis* in the flop, flutter, and flap stages; however, they were similar in size to *C. perspicillata* in the flight and adult stages.

As mentioned, the fiber type and diameter of *A. jamaicensis* acromeodeltoideus and *C. perspicillata* pectoralis major and acromeodeltoideus follow the trends that have been previously found in bat studies. This includes that majority of the fibers being fast-twitch and an overall increase in muscle fiber diameter as the juvenile ages. In this study, I found that the pectoralis major of *A. jamaicensis* does not follow the common trend in bats with a fiber type switch occurring during development.

The pectoralis of *A. jamaicensis* transitions form 98% fast-twitch in the flop stage of flight development to 39% fast-twitch fibers in adults. This begs the question as to why this fiber transition occurred. It has been shown that myofibrillar protein structure, metabolic enzymes, contractile properties are not fixed being dynamic in structure and function and have the ability to respond to altered demands on function which in turn can change the phenotypic profile of the fiber (Pette & Staron, 2001). The phenotype of the fibers are affected by many different parameters such as aging, mechanical loading/unloading, hormones, exercise training, and by innervations/neuromuscular activity (Pette & Staron, 2000).

One of the major differences in *A. jamaicensis* and *C. perspicillata* is their body size and the resulting behavioral adaptations that have occurred due to this difference. Studies on a wide variety of mammals have shown that body mass increases the expression of slow-twitch isoforms with a decrease in the number of fast-twitch isoforms (Aigner et al., 1993; Hamalainen & Pette, 1995). Innervations and neuromuscular

activity has been thought of as one of ways that may induce fiber type transitions. This has been shown through denervation studies, showing that fast-twitch fibers become slow-twitch when reinnervated by a slow nerve (Buller, Eccles, & Eccles, 1960). Exercise training has also been found to induce the transformation of fast- to slow-twitch changes (Andersen & Henriksson, 1977). These changes correspond to the altered use of the muscle, such as increasing slow-twitch fibers with an increase in endurance training. Mechanical loading seems to be the element that most closely fits the current situation. Ianuazzo, Gollnick, and Armstrong. (1976) and Ianuazzo et al. (1989) found that changes occurred in muscle fibers that were overloaded with the change being an increase in slow-twitch fibers. This has been found in a rodent model comparing rat and mouse soleus muscle during postnatal development. Wigston and English (1992) found that the soleus muscle of male Fisher 344 rats had a shift from 54% to 94% slow-twitch fibers between weeks 1 and 52. They did not find this to be the case in C57BL/6J mice. The soleus of the mice remained similar with the majority of the fibers being fast-twitch. They accounted for the fiber switch, which must result of the conversion of entire motor units, being higher attributed to body weight and secondarily the amount of muscle usage. Lastly, muscle fiber switching has been shown to be common as an organism ages (Larsson & Ansved, 1995) with fast-twitch decreasing in an age-dependent manner.

The transition of the pectoralis major muscle fibers of *A. jamaicensis* demonstrates that fully developed and differentiated muscle cells are able to change gene expression under differing conditions. With *C. perspicillata* being the more ancestral species with a much smaller body one would hypothesis that the pectoralis major of *A. jamaicensis* transitioning from mainly fast-twitch to majority slow-twitch is correlated to

the increase in body size. With the increase in body size there is an increase in the overall load on the muscles which in turn increases the demand on the muscles. The pectoralis major is the muscle that functions with the downstroke which is the power for forward flight. *A. jamaicensis* is known to carry fruit from a tree to a roost site that is some distance away (Ortega & Castro-Arellano, 2001). This habit may put increased stress on the muscles increasing the need for more endurance which is possible by having an increase in slow-twitch fibers.

Evolutionary Implications

Bat evolution is widely unknown and somewhat controversial, especially in regards to the origin and development of flight. Based on the lack of evolutionary history, phylogenetic studies have tried to link bats together based on phenotypes and genotypes (Baker et al., 2003; Jones & Teeling, 2006; Wetterer et al., 2000). The evolution of the bat wing and the ability to use them for flight has given bats the opportunity to exploit new habitats and ecosystems. Due to the lack of fossil evidence, research has advanced into the molecular mechanisms, regarding the formation and elongation of the bat wing. New insights into regulatory proteins have shown possibilities for the development of the wing and elongation of the fingers (Chen et al, 2005; Cretekos et al, 2008; Sears et al, 2006; Sears, 2008; Weatherbee et al, 2006; Weatherbee, 2008).

Based on the lack of knowledge of ancestral forms and what developmental events occurred during the divergence of bats from a proto-bat to the large number of species we see today, one can use ontogeny as a proxy for phylogeny. In this study, I found that peramorphic heterochronic rate changes occurred between *A. jamaicensis* and

C. perspicillata ultimately changing the size of both the body and wing of *A. jamaicensis* when compared with the more ancestral *C. perspicillata*. *A. jamaicensis* grew at an accelerated rate in both mass and wing morphology. I also found allometric differences with the more precocial *C. perspicillata* having increased growth in the wing in comparison to overall mass increase. This provided a mechanism for *C. perspicillata* to achieve flight at an earlier stage than *A. jamaicensis* due to the wing dimensions becoming adult-like more quickly. Additionally, the evolution of muscle differences in due to an increase in mass is evident in the pectoralis major. *A. jamaicensis* is twice as large as *C. perspicillata* and, therefore, had an increase in slow-twitch fibers that increase the endurance and ability to carry more weight. These aspects of development provide mechanisms for divergence that is not seen in the analysis of adult bats.

These findings add strength to the thought that ontogeny can indeed be used as a proxy when looking at evolutionary divergence of closely related organism. Adams (2000) summarized this as follows:

> Integrating an ontogenetic perspective into investigations of complex systems provides a more insightful and balanced interpretation because 1) it is selection on developmental variation that produces phenotypic variation among adults in populations, 2)commonality in developmental patterns may indicate common ancestry (and lack thereof may be indicative of convergence), and 3) preadult individuals directly influence the dynamics of populations and communities through time. (p. 2)

My research has provided evidence that ontogeny is an important route to look at when trying to decipher the evolution of species. This information can be combined with phylogenetic information, providing possible mechanisms as to what factors could have influenced the divergence of closely related species from a common ancestor.

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APPENDIX A

CLADOGRAM OF THE FAMILY PHYLLOSTOMIDAE

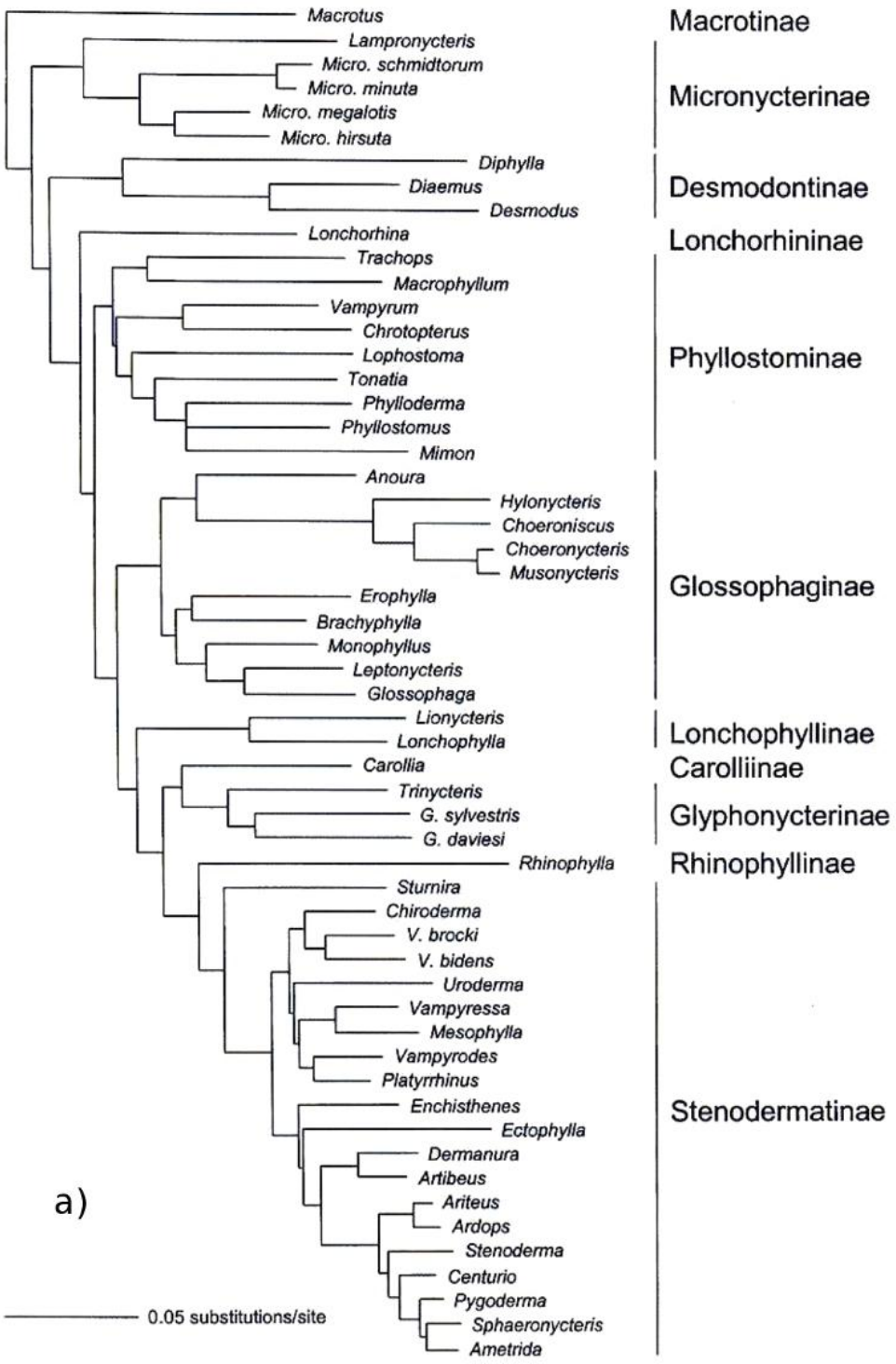

Figure 60. Phyllostomidae cladogram. The cladogram was acquired using Bayesian analysis of *RAG2* data. Indicates that the genus Carollia is more ancestral than the genus Artibeus. (From Baker et al., 2003).

APPENDIX B

MANEUVERABILITY DATA SHEET

Table 8

Maneuverability Course Data Sheet

Maneuverability											
Date	Species	Age	1x ws (1)	1x ws (2)	1x ws (3)	0.75x ws (1)	0.75x ws (2)	0.75x ws (3)	0.5x ws (1)	0.5x ws (2)	0.5x ws (3)

Tests measured at full (1x) wingspan (ws), 75% (0.75x) wingspan (ws) and half (0.5x) wingspan (ws). Bats were flown through each spacing three times.

APPENDIX C

MOPRHOMETRIC DATA SHEET

Table 9

Morphometric Data Sheet

Date	Species	Age	Band #	Forearm Length (mm)	Weight (g)	Wing Surface Area	Wingspan	Trial #	Distance (cm)

APPENDIX D

PHOTOMICRAPHS OF IMMUNOHISTOCHEMISTRY OF THE PECTORALIS MAJOR AND ACROMEODELTOIDEUS STAINED FOR FAST- AND SLOW-TWITCH FIBERS

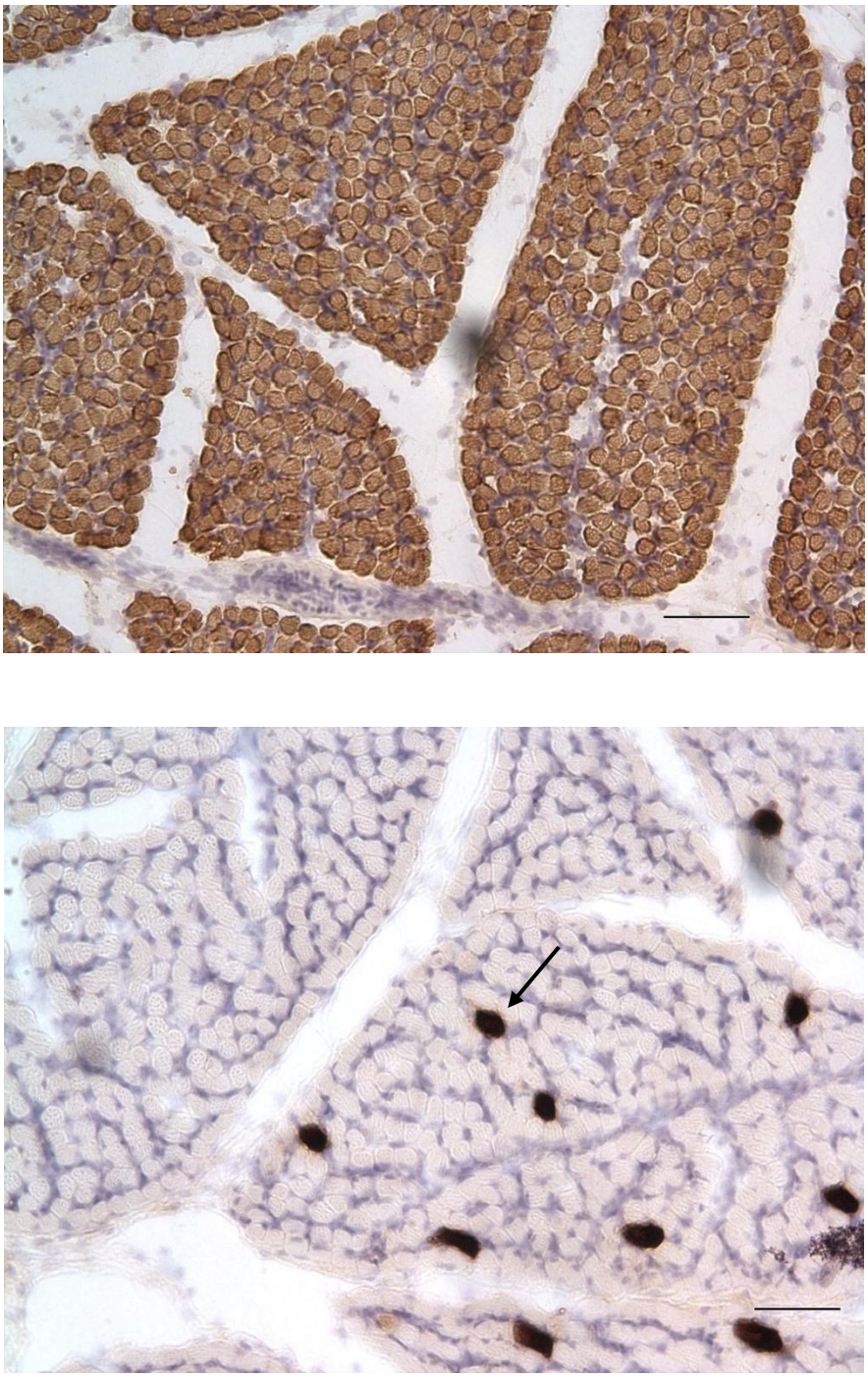

Figure 61. Cross sections of *A. jamaicensis* pectoralis major from the flop stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

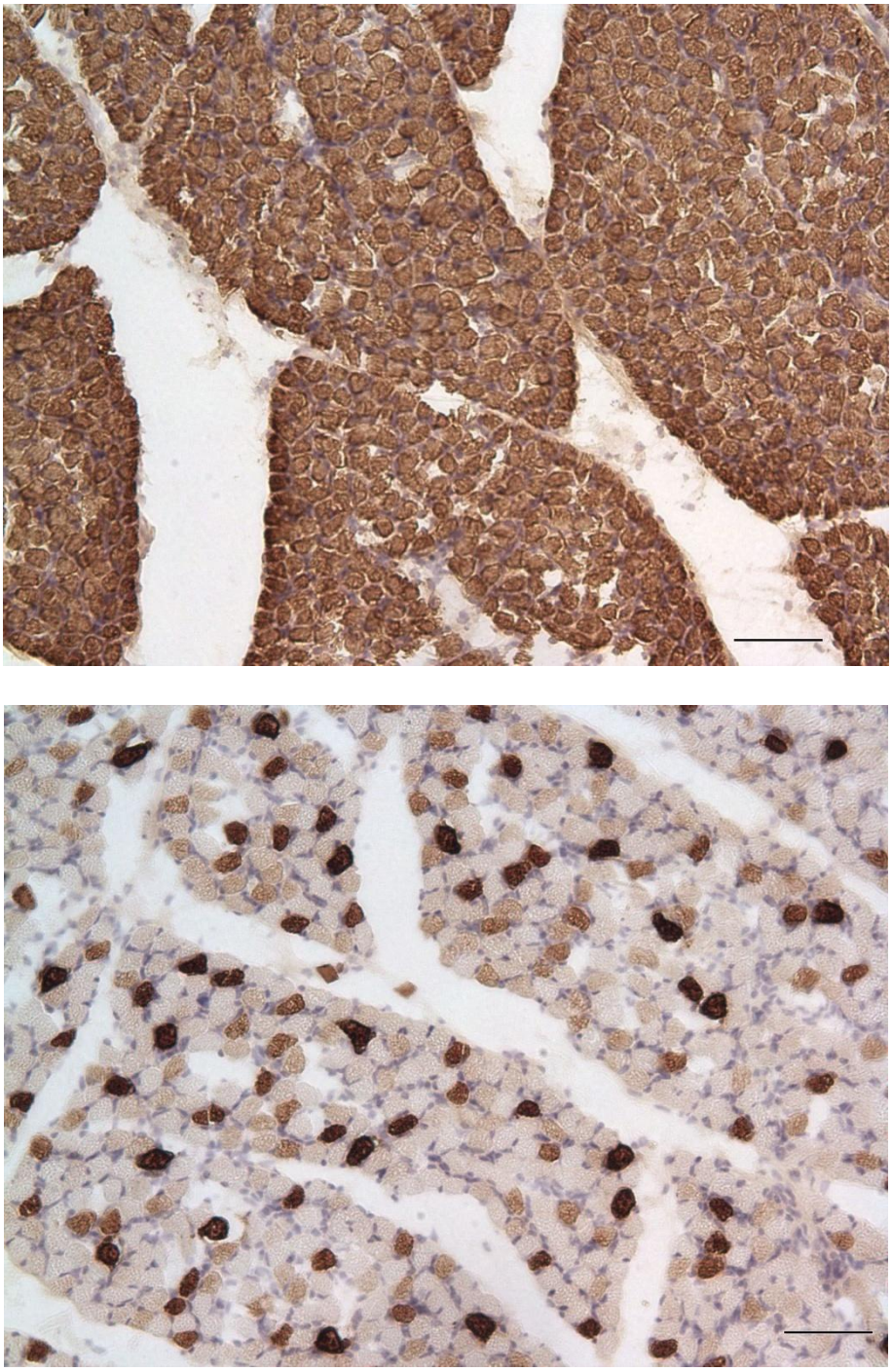

Figure 62. Cross sections of *A. jamaicensis* pectoralis major from the flutter stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

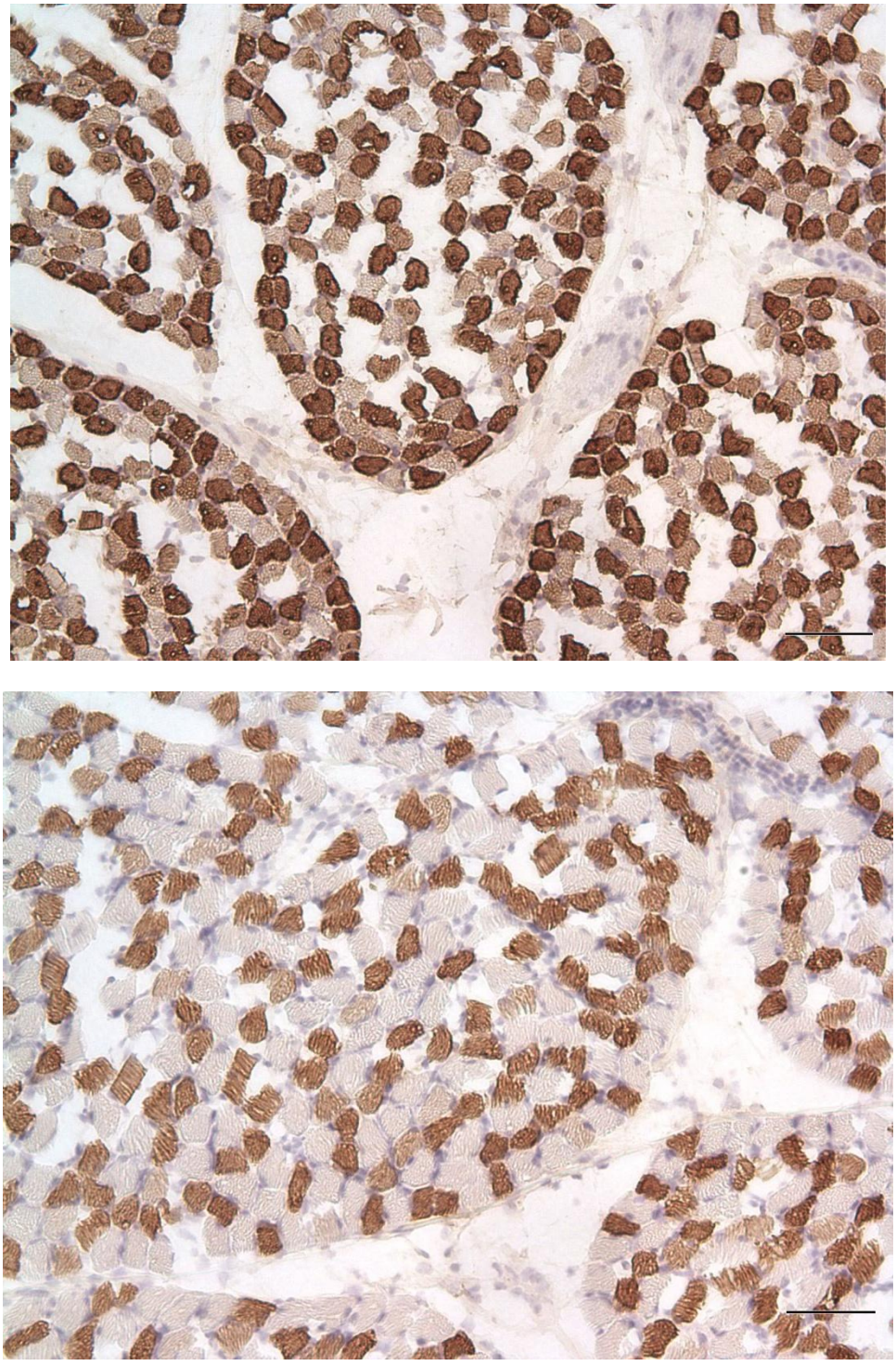

Figure 63. Cross sections of *A. jamaicensis* pectoralis major from the flap stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

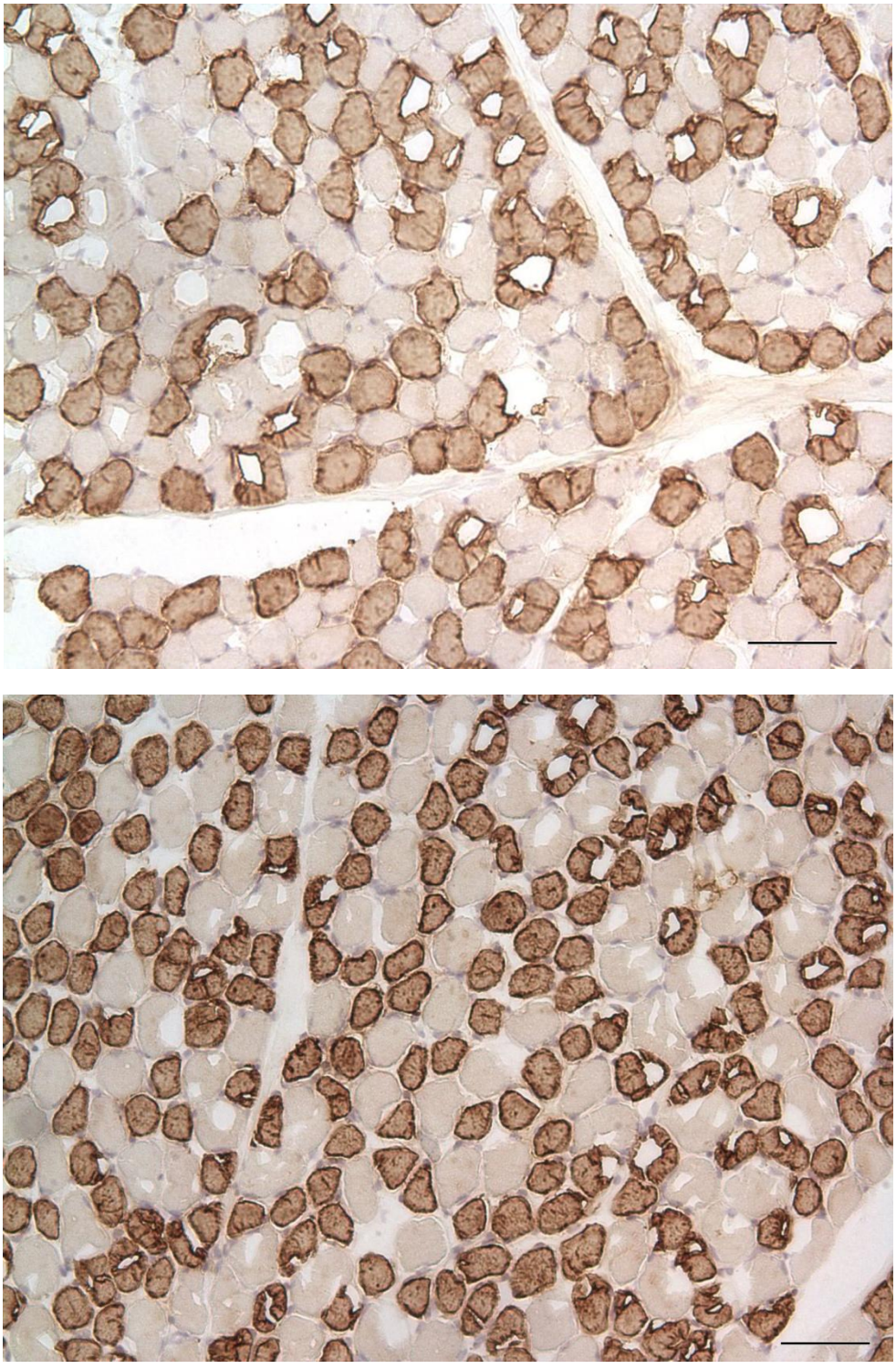

Figure 64. Cross sections of *A. jamaicensis* pectoralis major from the flight stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

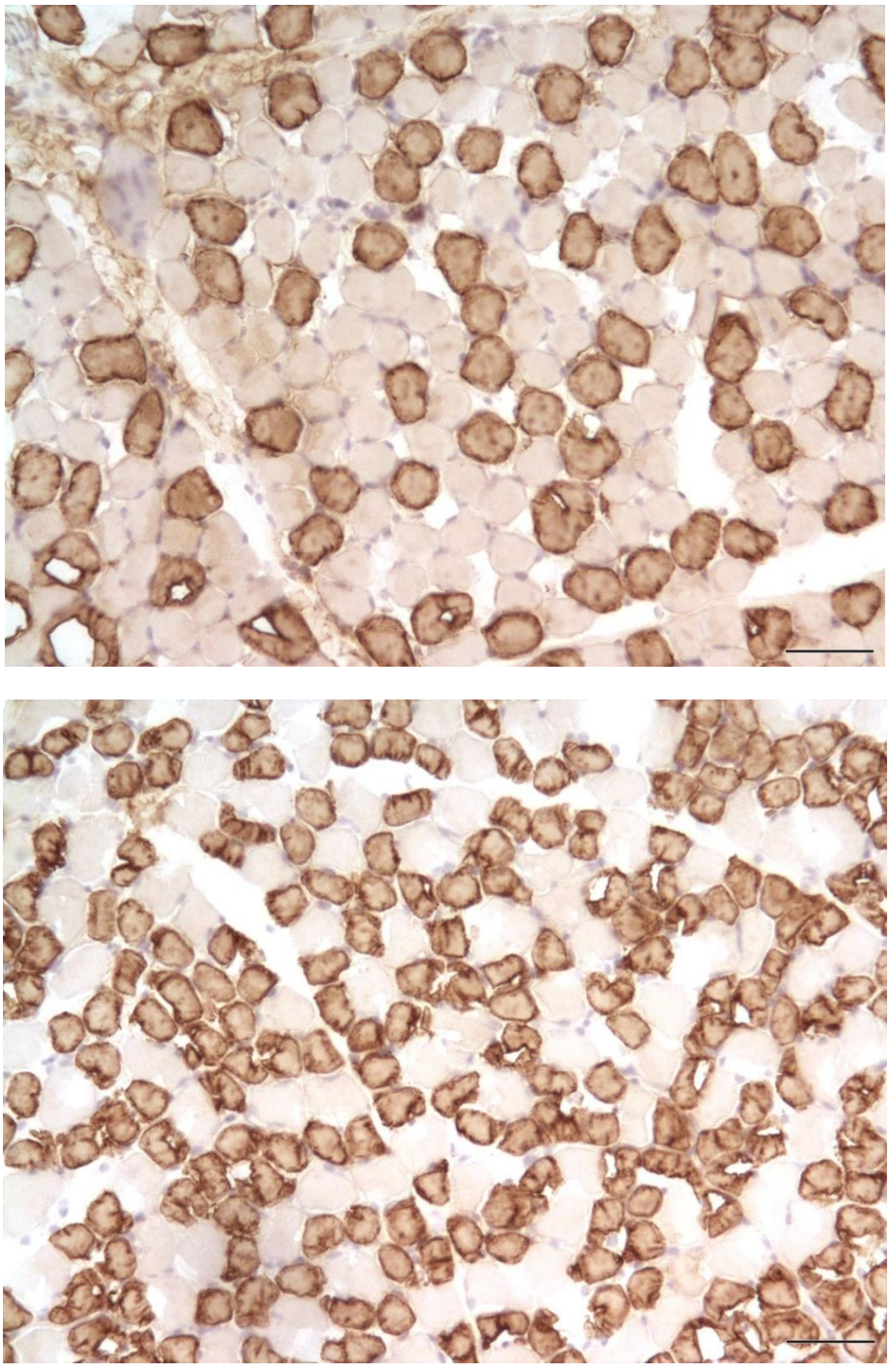

Figure 65. Cross sections of *A. jamaicensis* pectoralis major from the adult stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

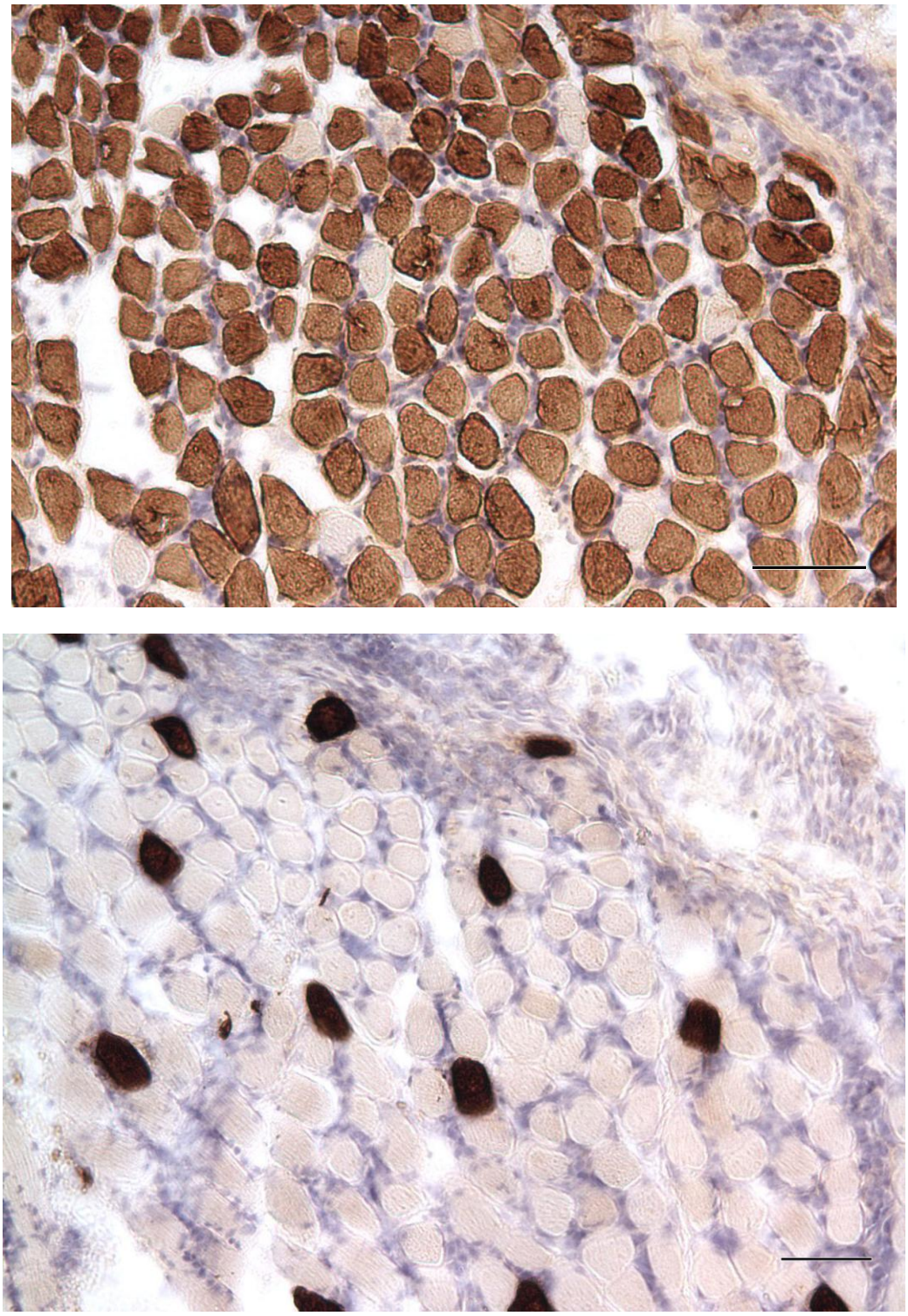

Figure 66. Cross sections of *A. jamaicensis* acromeodeltoideus from the flop stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

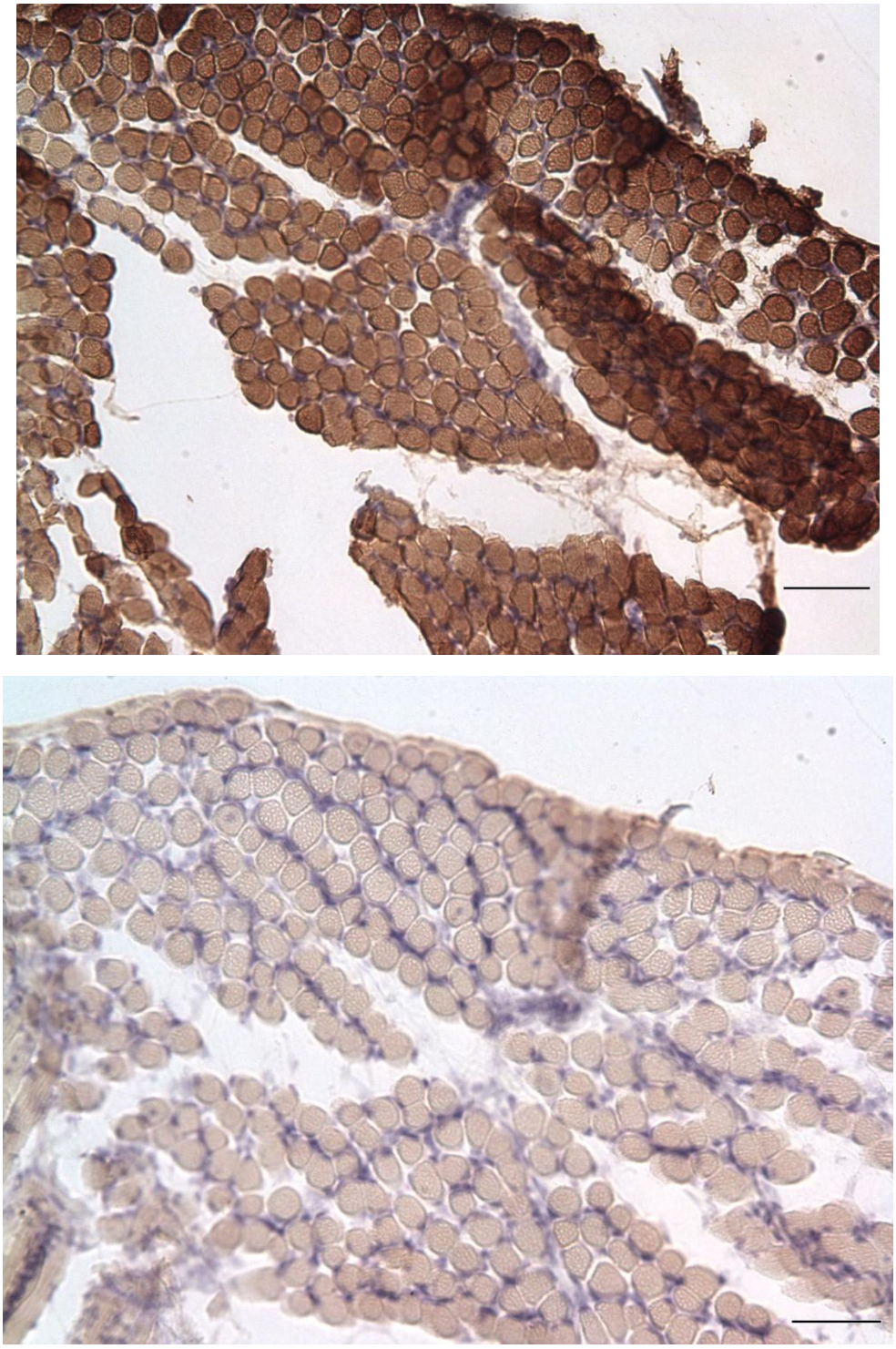

Figure 67. Cross sections of *A. jamaicensis* acromeodeltoideus from the flutter stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

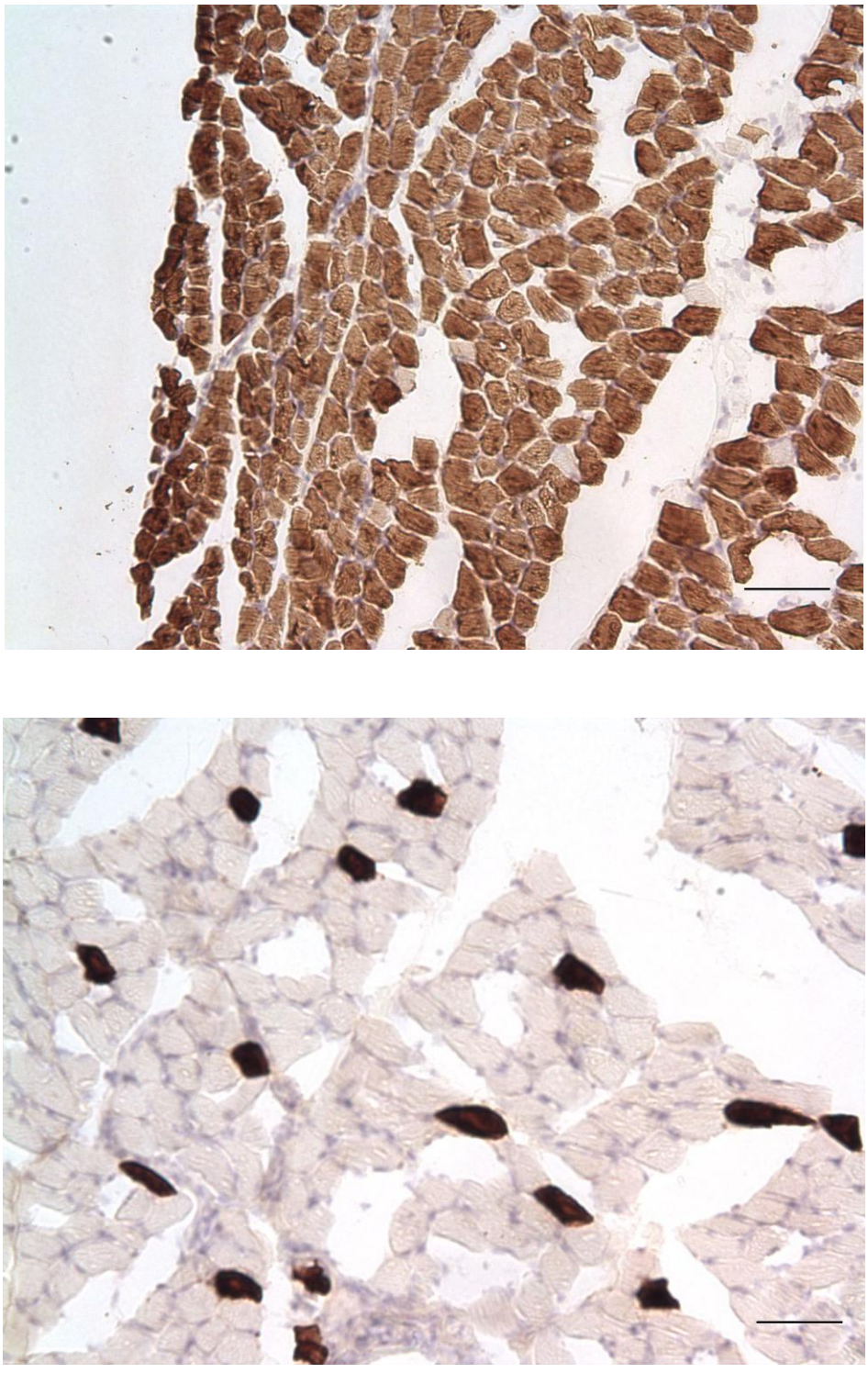

Figure 68. Cross sections of *A. jamaicensis* acromeodeltoideus from the flap stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

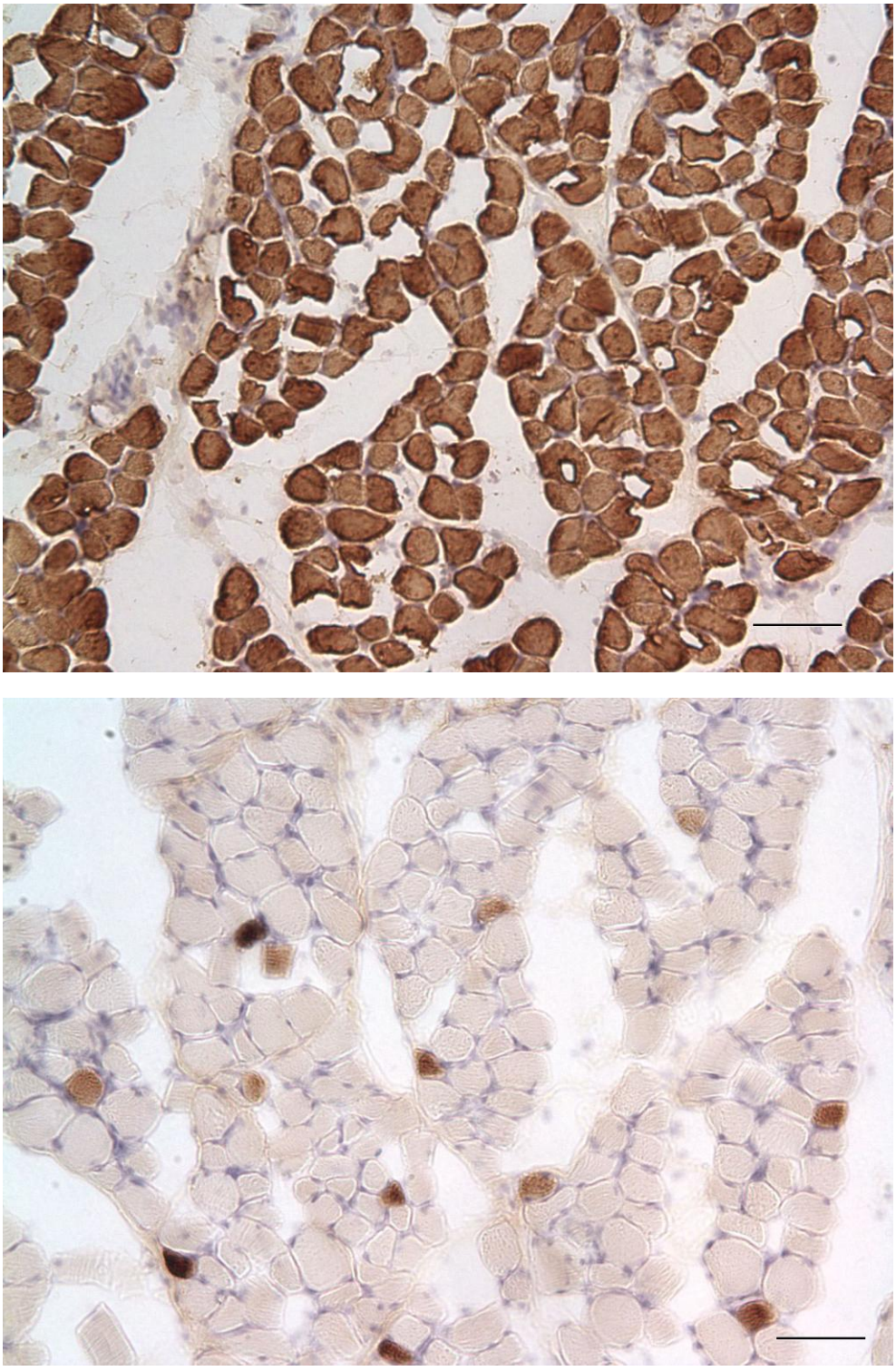

Figure 69. Cross sections of *A. jamaicensis* acromeodeltoideus from the flight stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

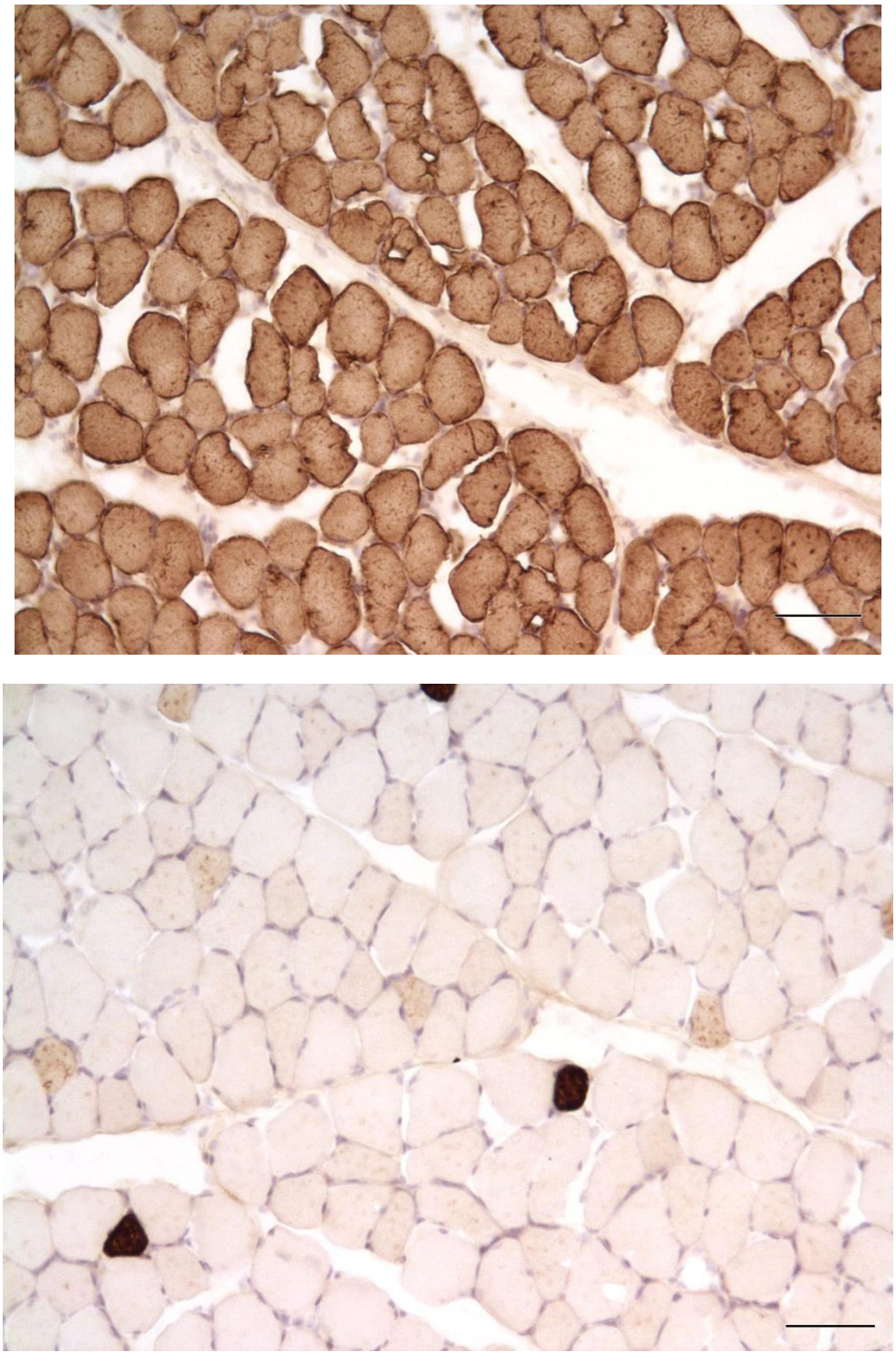

Figure 70. Cross sections of *A. jamaicensis* acromeodeltoideus from the adult stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

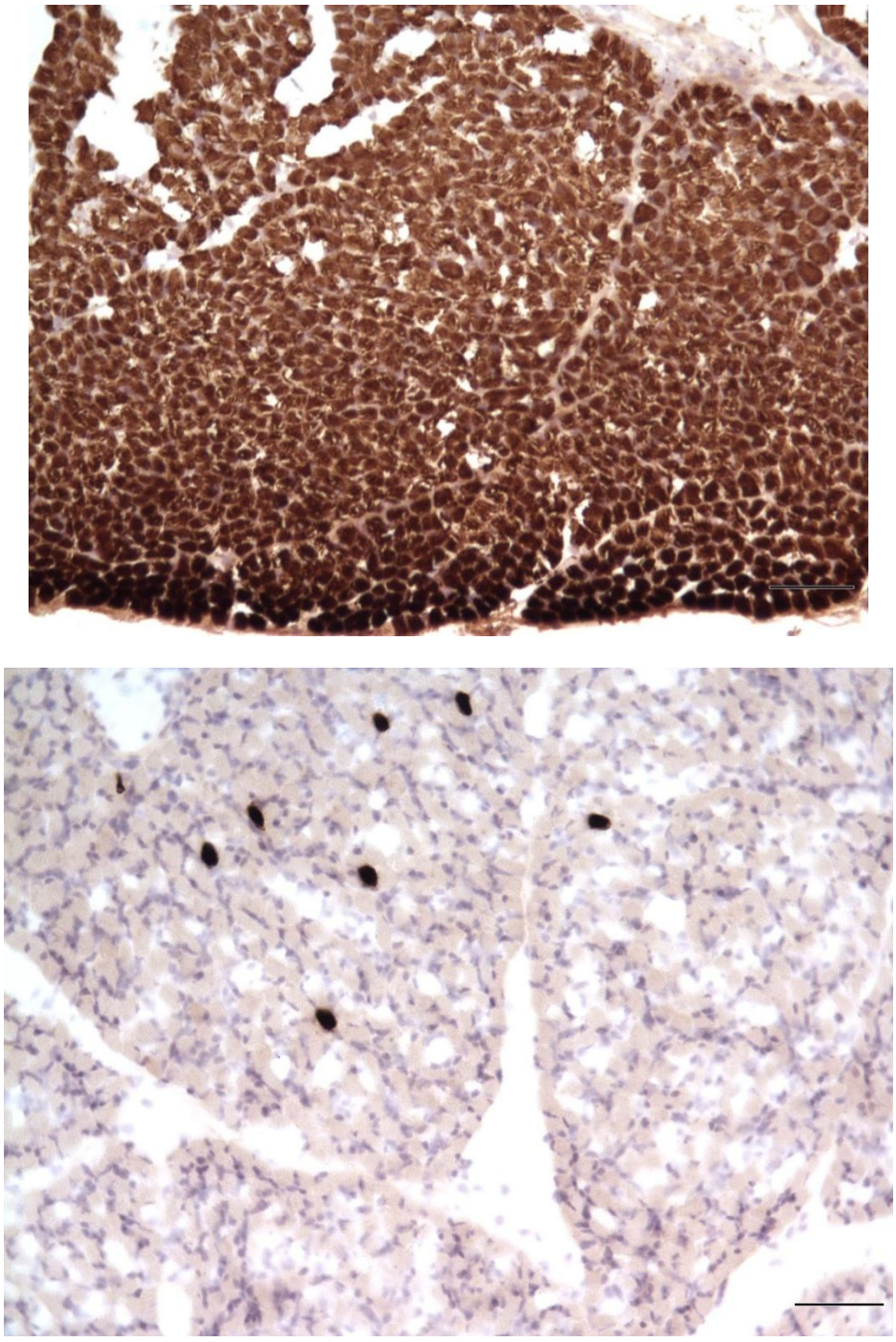

Figure 71. Cross sections of *C. perspicillata* pectoralis major from the flop stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

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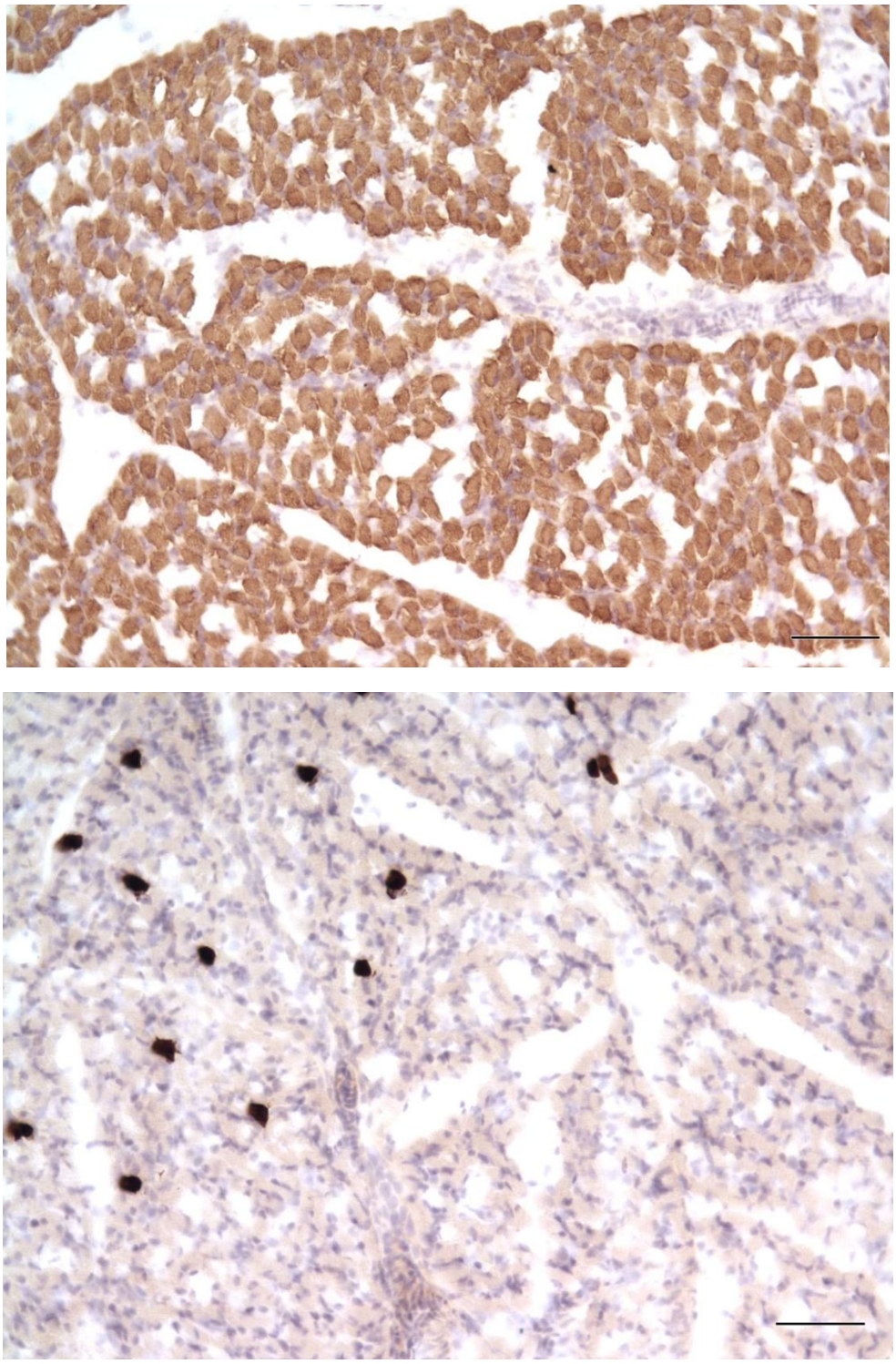

Figure 72. Cross sections of *C. perspicillata* pectoralis major from the flutter stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

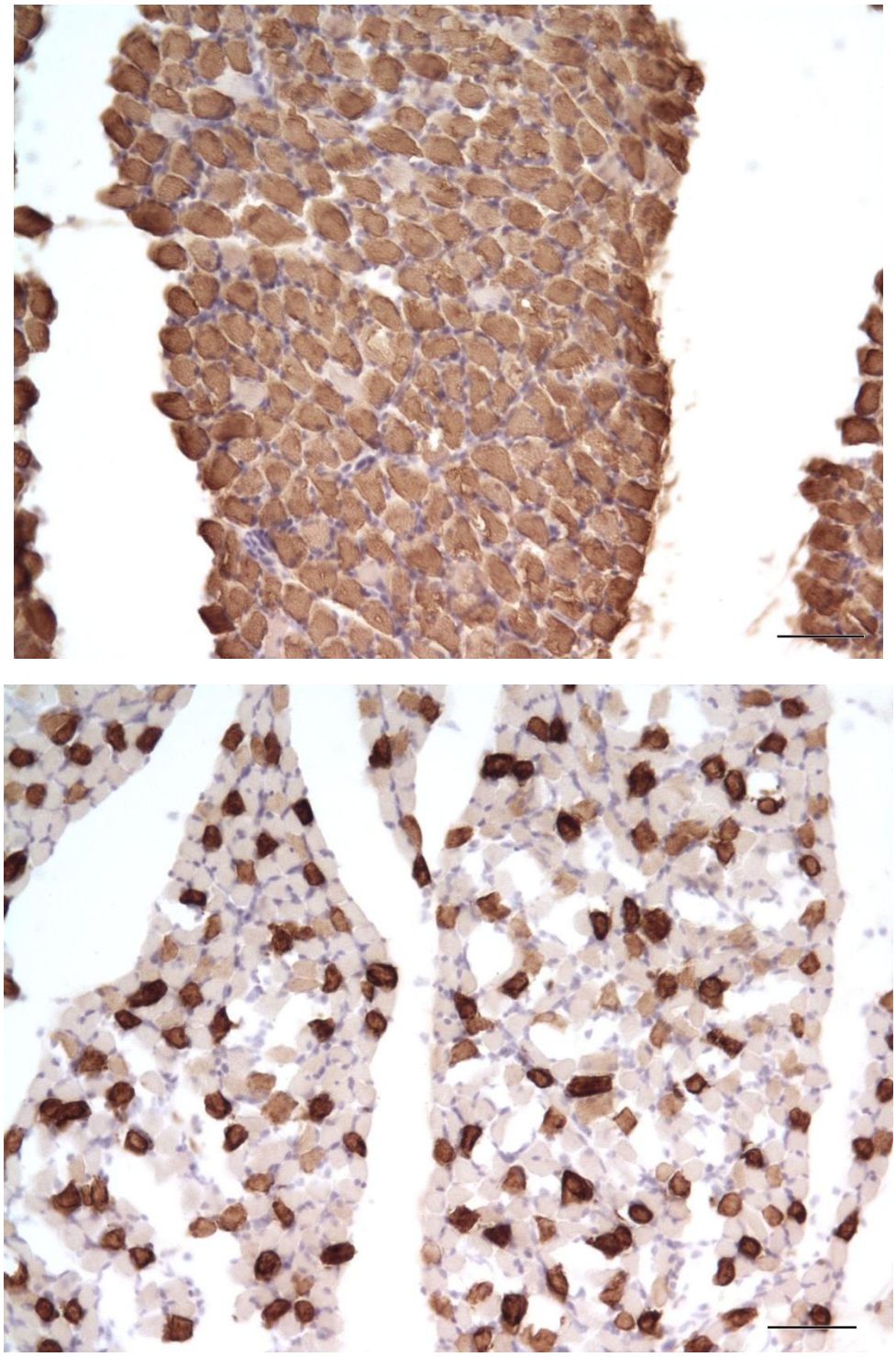

Figure 73. Cross sections of *C. perspicillata* pectoralis major from the flap stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

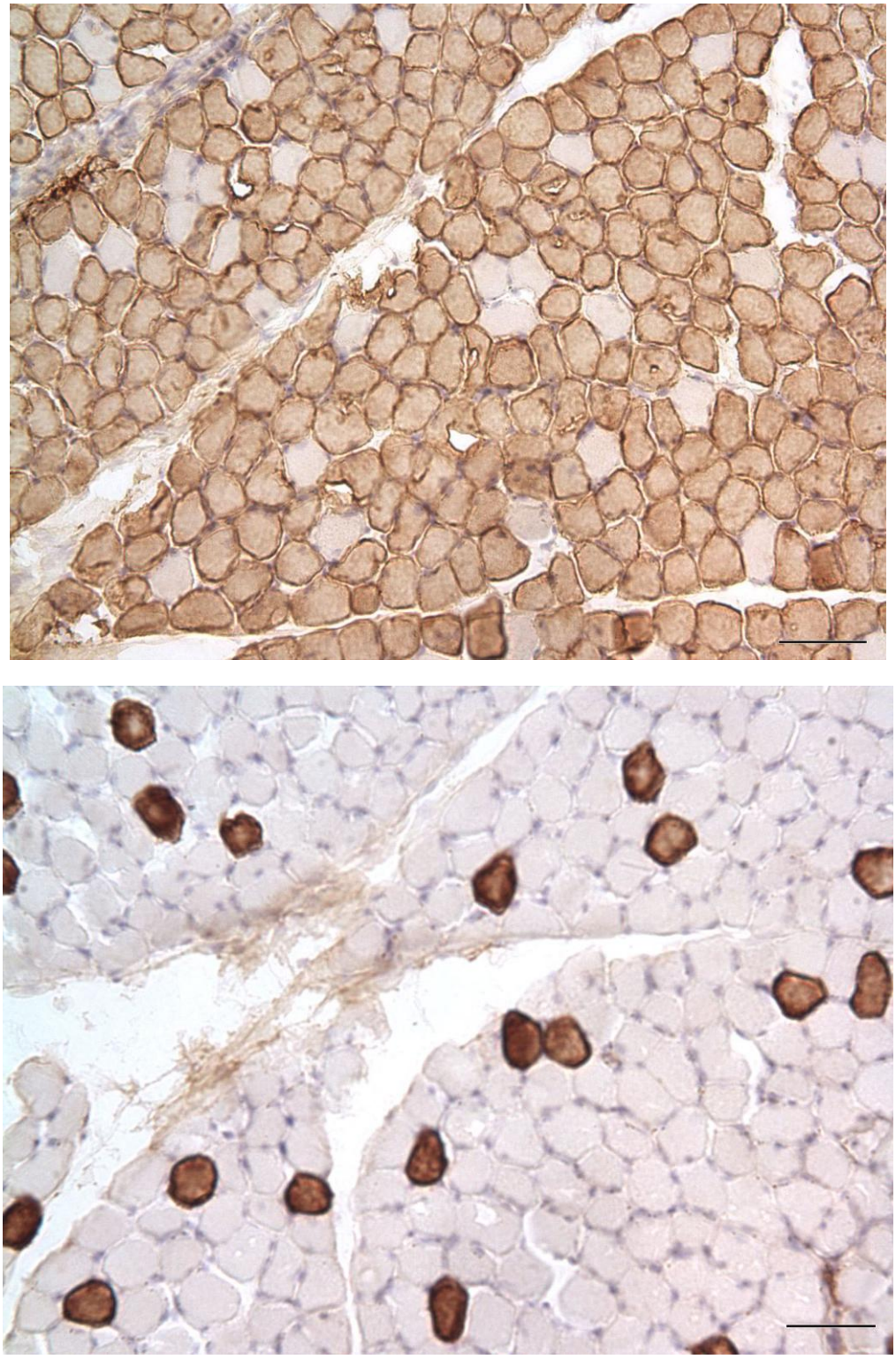

Figure 74. Cross sections of *C. perspicillata* pectoralis major from the flight stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

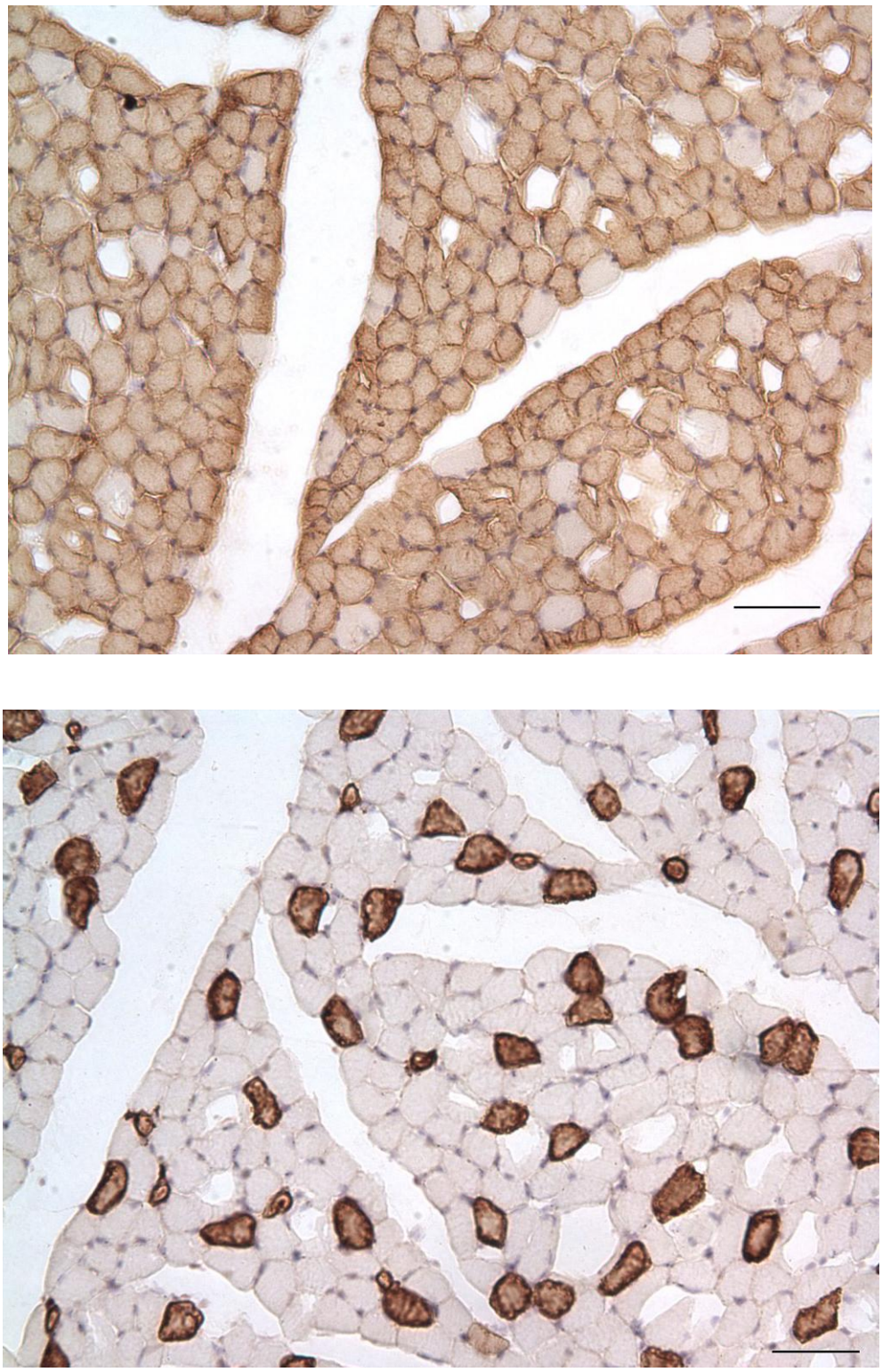

Figure 75. Cross sections of *C. perspicillata* pectoralis major from the adult stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

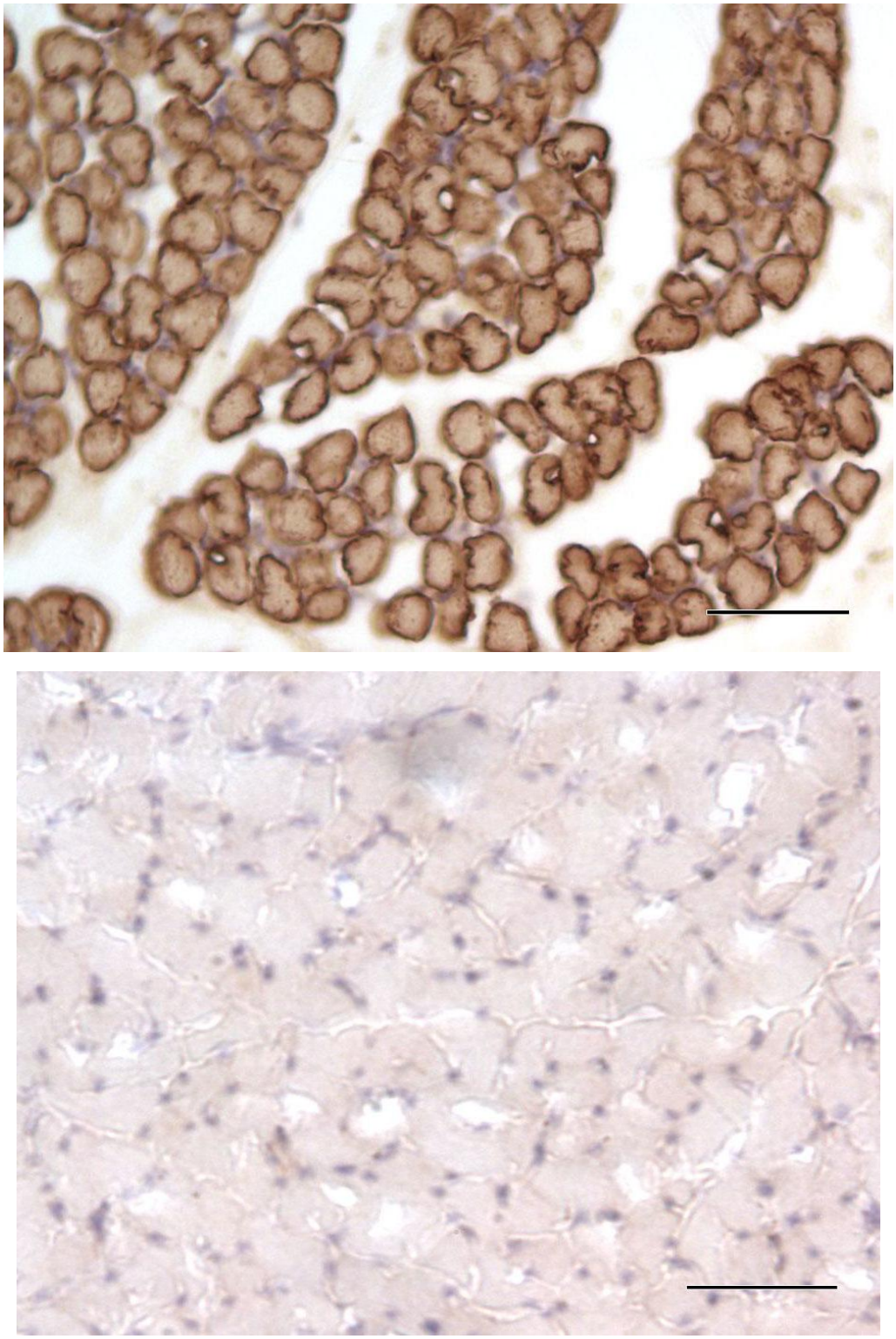

Figure 76. Cross sections of *C. perspicillata* acromeodeltoideus from the flop stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

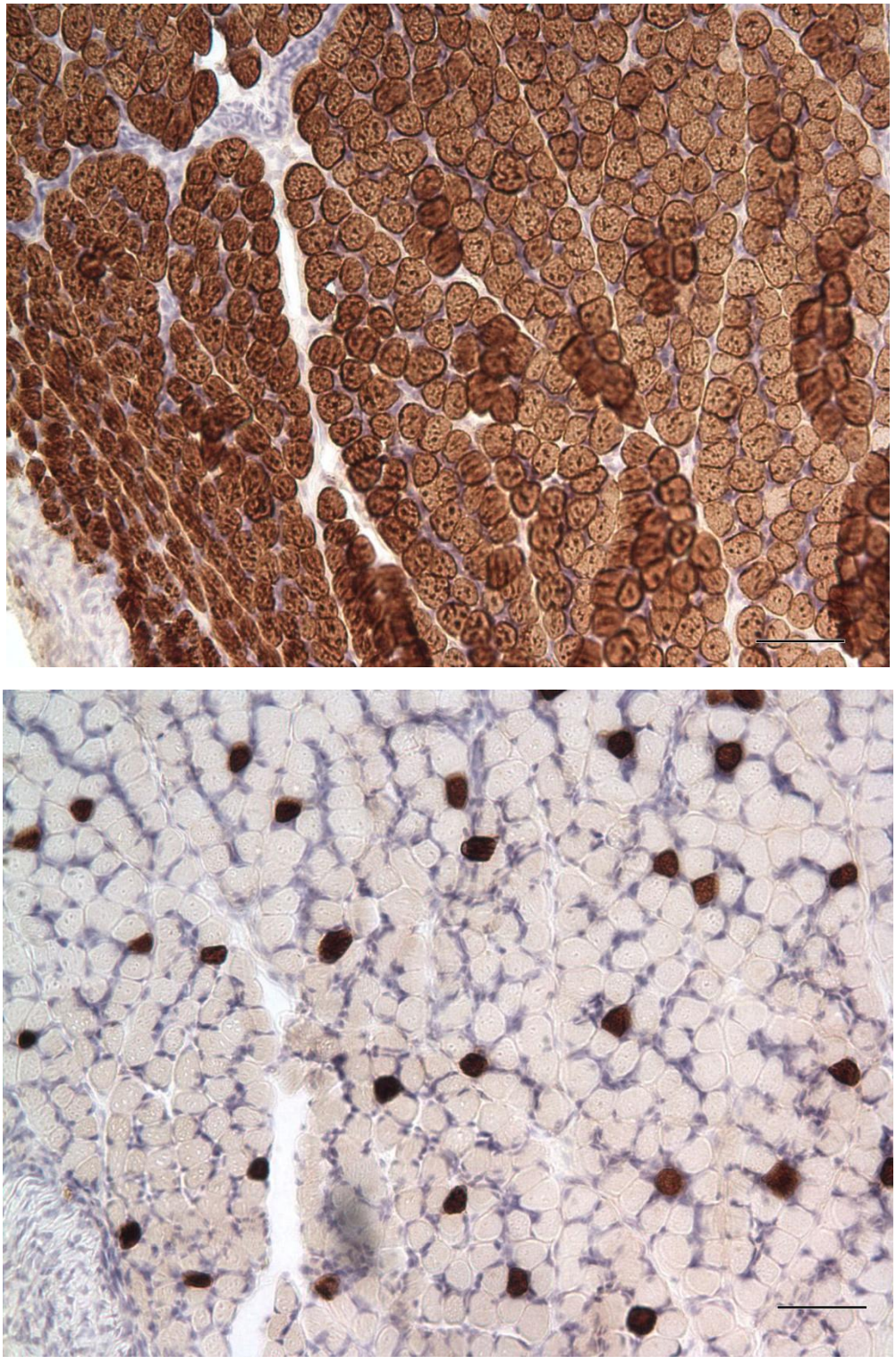

Figure 77. Cross sections of *C. perspicillata* acromeodeltoideus from the flutter stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

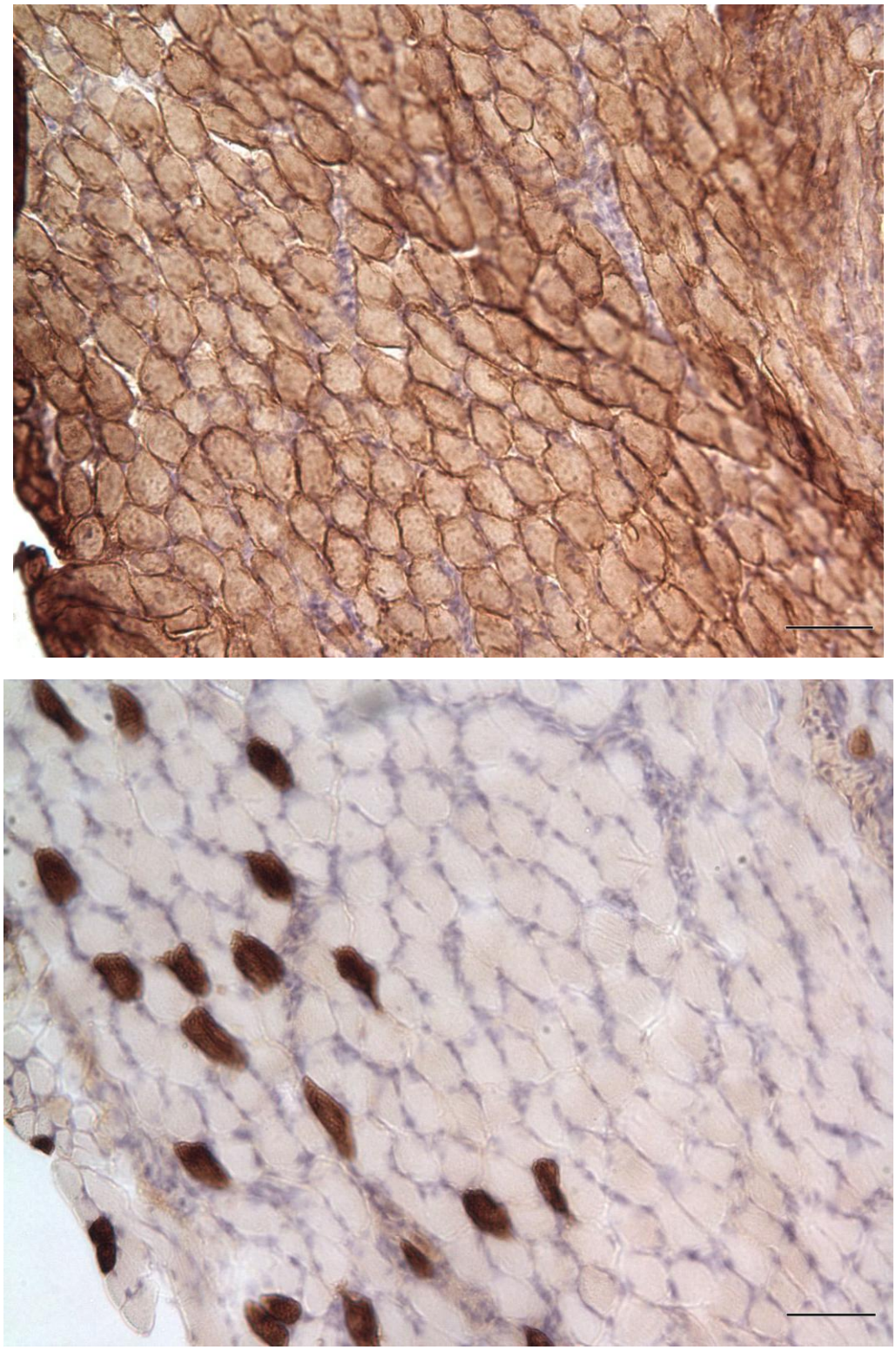

Figure 78. Cross sections of *C. perspicillata* acromeodeltoideus from the flap stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

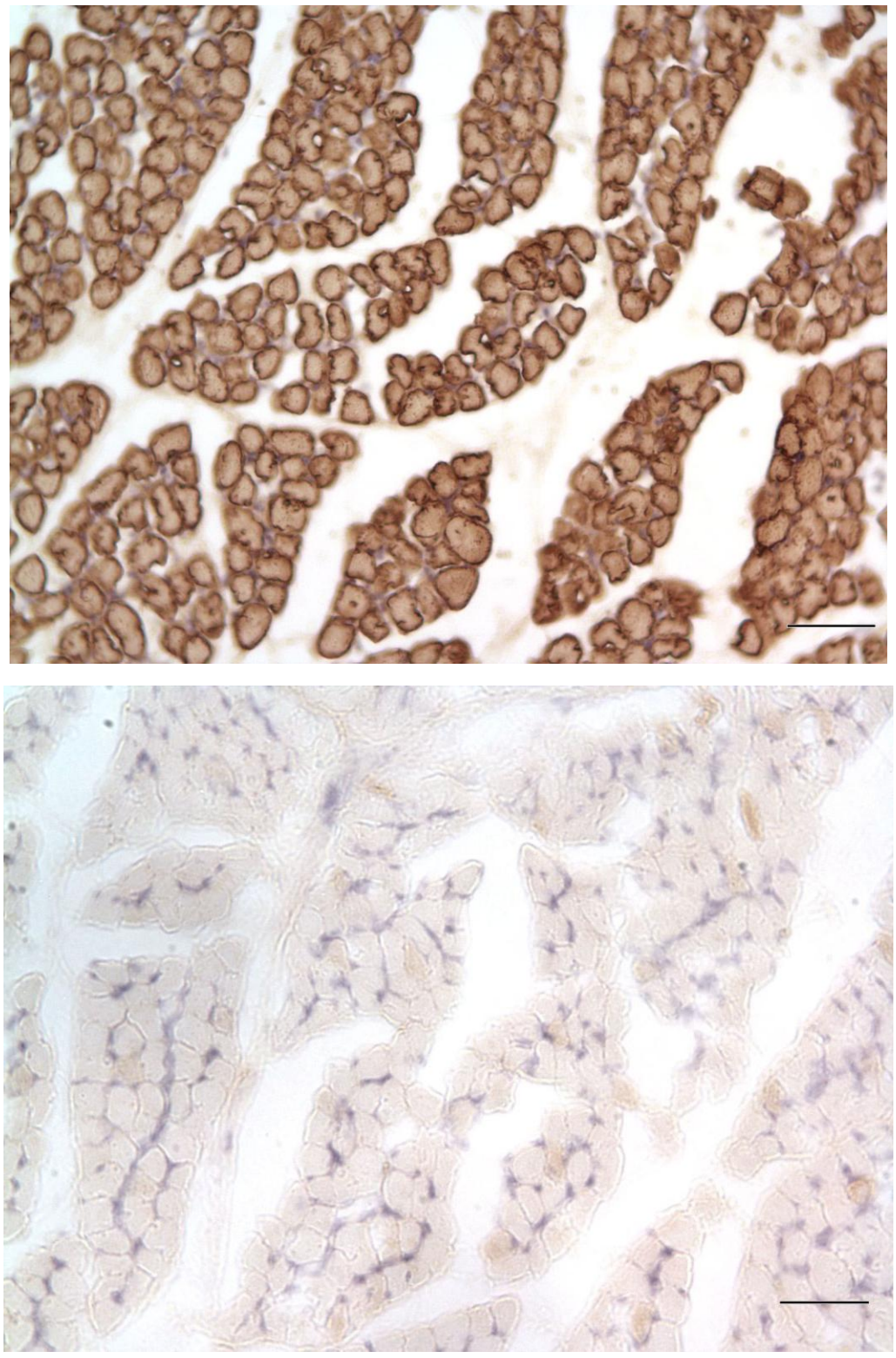

Figure 79. Cross sections of *C. perspicillata* acromeodeltoideus from the flight stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.

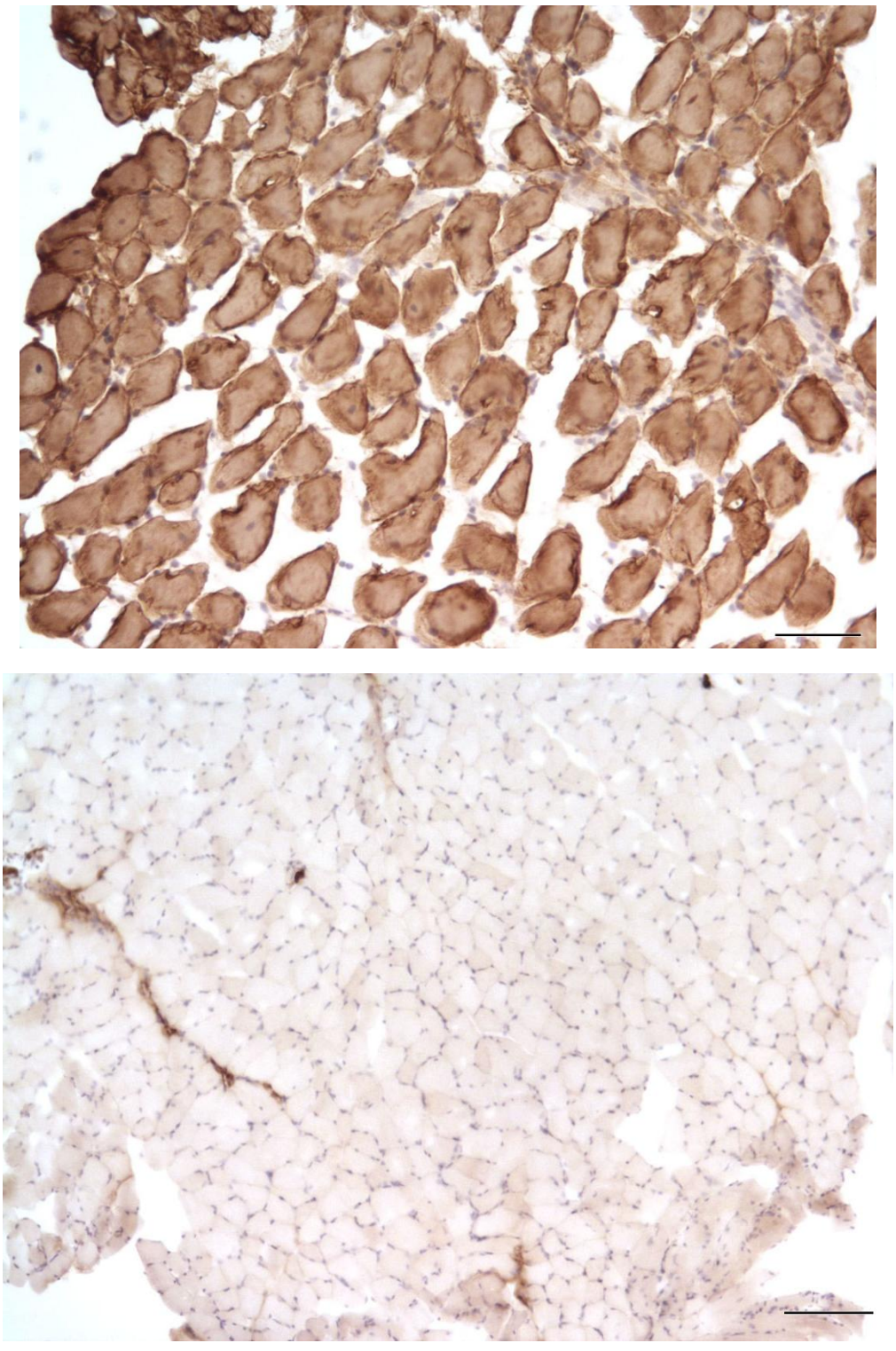

Figure 80. Cross sections of *C. perspicillata* acromeodeltoideus from the adult stage. The top picture is stained for fast-twitch fibers while the lower picture is stained for slow-twitch fibers. Magnified 200 times. Scale bar = 100 µm.