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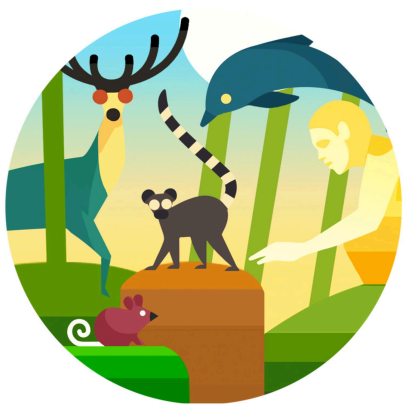

Morphometry as a tool to monitor animal growth: research on the effect of different diets administered to Costa Rican newborn and juvenile wild opossums.

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Summary

Animal morphometry plays a significant role in various aspects of wildlife research and care, including estimation of age, hand-rearing, and health management. However, over the past several decades, the research on animal morphometry has received limited attention, with most studies focusing on adult animals or museum specimens. Investigations into neonatal morphometry and the developmental patterns of newborn animals are scarce in recent literature, and protocols for assessing growth have only been developed for a limited number of species.

In the present research project, morphometry is applied alongside hand-rearing to investigate the growth patterns of six rescued opossums (five *Didelphis Marsupialis*, or Common Opossums, and one *Caluromys Derbianus*, or Derby's Woolly Opossum) at the Alturas Wildlife Sanctuary in Costa Rica over a two-month period. Their growth trajectories were examined through morphometric analysis of specific body parts. By analyzing measurements of selected body parts, this study aims to contribute to the development of a database on the normal growth patterns of rescued animals.

The data collected proved beneficial to the staff at Alturas Wildlife Sanctuary for monitoring overall growth and assisting in decision-making related to the care of rescued animals. The handling and manipulation of the animals were conducted within the framework of routine, standard procedures, ensuring that all interactions were both non-invasive and non-stressful for the individuals. These methods were carefully designed to align with natural handling practices, minimizing any potential disturbance and maintaining the animals' welfare throughout the process. Further research conducted in wildlife rescue centers could facilitate the development of detailed growth charts for various species, providing valuable support to rescuers in monitoring and successfully rearing these animals.

Introduction

Potential Applications of Morphometry in Animal Research

The study of external morphometry in animals serves not only to expand scientific understanding of animal morphology but also provides insights into a variety of other critical aspects. For instance, morphometric traits have proven reliable for sex determination in species such as the Blue-footed Booby (*Sula nebouxii*), where specific body measurements are used to differentiate males from females (Zavalaga & Paredes, 1997). This approach becomes especially valuable in species for which the evaluation of external genitalia is either not feasible or highly challenging, due to either age-related development or inherent characteristics of the species. In such cases, morphometric analysis offers an effective, non-invasive alternative for determining sex and contributes to minimizing handling stress for the individuals involved. Furthermore, relating body metrics like weight and total length to ecological factors, mortality rates, and species conservation is an area of ongoing interest (Kruuk et al., 1987; Kruuk & Conroy, 1991; Kruuk, 1995).

Morphometric data also offer valuable insights into age classification, growth dynamics, and development across various species. Depending on the specific goals of a study, there are two primary approaches to obtain body measurements—using specimens preserved in museums or working with live animals—each presenting its own advantages and limitations (Richard-Hansen et al., 1999). Recent morphometric analyses of skeletal growth and development in 63 wild-collected Ugandan Cercopithecus aethiops have demonstrated the utility of such studies in understanding growth patterns, life stages, and developmental milestones, with potential applications to both extant and extinct catarrhines (Bolter & Zihlman, 2003).

However, reliance on museum specimens presents certain challenges, particularly when weights or complete measurements are unavailable, which can limit the scope of research on large mammals (Richard-Hansen et al., 1999). In these cases, studies on live animals, whether conducted in the field or involving captive or rescued individuals, are often more appropriate. For example, age estimation studies in wild harbor seals (*Phoca vitulina*) have yielded important findings, particularly in research involving rescued or captive animals (Garshelis, 1984).

A tool for Welfare Assessment in Captive Animals

Captivity allows for the repeated, longitudinal measurement of individual animals—something rarely feasible in fieldwork. Such data are particularly valuable for tracking growth, assessing health, and evaluating the impact of management practices (Kleiman et al., 2010). In zoos, routine morphometric assessments are conducted to monitor animal health. For instance, at London Zoo, an annual weigh-in of all animals is used to ensure optimal care and welfare. Collected data are shared through the Zoological Information Management System (ZIMS), facilitating international collaboration and research (Field, 2014).

The unexplored Field of Neonatal Morphometry

Despite the established importance of morphometric data in wild and captive animal research, there is still a notable gap in the availability of such data, particularly for newborns and juveniles. This gap is often justified by the challenges of analyzing data across different age classes, leading to the exclusion of non-adult individuals in many studies (Richard-Hansen et al., 1999). Human medicine, in contrast, has long benefited from morphometric studies that underpin growth charts and references for infant development (Cole, 2012). The value of these studies is evident in their ability to identify abnormal growth patterns due to metabolic disorders or malnutrition early on (Nichols, 2016). While similar findings have been reported in studies on animals, such as growth disturbances in Cebus monkeys (Fleagle & Samonds, 1975), our understanding of neonatal growth in animals remains far inferior compared to the knowledge on neonatal growth in humans.

Possible impact of diet on growth charts

Dietary intake is a crucial determinant of growth and development in animals, influencing key morphometric parameters such as body weight, length, and girth. Variations in dietary composition, particularly the type and quality of protein, can significantly impact growth trajectories, as demonstrated in studies on both wildlife and managed populations. While nutritional requirements are often studied in controlled environments, data on how dietary differences shape growth in neonatal and juvenile animals, particularly in a rescue or rehabilitation setting, remain limited. This gap is especially relevant for species like opossums, where proper dietary planning is critical to support health and development during hand-rearing. By examining the role of dietary variations, this study aims to provide insights into optimizing care and developing species-specific growth references, contributing to the broader application of morphometry in wildlife rehabilitation and conservation.

Limitations of Morphometry

Monitoring animal growth to detect potential morphological abnormalities is crucial in both breeding and rehabilitation programs. Tracking body weight and measurements can provide critical insights into an individual's health and development, especially in hand-rearing programs. However, morphometric data often show significant variability between individuals and populations of the same species due to factors such as environmental conditions, diet, and social structure. These variations have been observed in both adult and juvenile animals, underscoring the limitations of applying laboratory-derived data to wild populations (Gay & Best, 1996; James, 1970; Fuentes & Jaksic, 1979; Delany & Monro, 1985).

Furthermore, inconsistencies in methods across different studies and regions have been noted, complicating the comparison of findings due to the lack of standardized measurement protocols (Jewell & Fullagar, 1966). In some cases, methods of measurement are inadequately recorded or defined, further limiting the reproducibility and comparability of morphometric studies. While attempts have been made to develop standardized growth measurement protocols for certain species (Marker & Dickman, 2003), a universally accepted and applied system remains elusive.

Aim of the Present Study

The primary objectives of this research were: 1) to gather data on the growth of young, rescued opossums, with the aim to enhance understanding of their typical growth patterns and to contribute to the development of a comprehensive growth database; 2) to evaluate the impact of dietary protein type on growth; and 3) to aid in the standardization of methods for morphometric data collection.

Such a resource could assist wildlife rescuers in monitoring the health and development of animals and predicting potential pathologies.

Common Opossums (*Didelphis marsupialis*)

The common opossum (CO) is one of the most widespread and recognizable marsupials found in Costa Rica and throughout much of Central and South America (Figure 1). Known for its adaptability to various environments, this species is found in forests, agricultural lands, and even urban areas, demonstrating remarkable ecological flexibility (Eisenberg, 1989). As an opportunistic omnivore, *D. marsupialis* plays an important role in both its natural habitat and human-impacted environments, where it contributes to seed dispersal and pest control (Atramentowicz, 1988).

Didelphis marsupialis is found across a wide range of ecosystems in Costa Rica, from lowland rainforests to secondary growth forests and anthropogenically impacted areas. These opossums are especially common in tropical and subtropical environments, where they thrive in moist and humid conditions. They are nocturnal and solitary animals, often taking shelter in tree hollows, burrows, or even human-made structures during the day (Julien-Laferriere & Atramentowicz, 1990). This adaptability allows *D. marsupialis* to maintain its presence even in areas heavily impacted by deforestation and urbanization (Emmons & Feer, 1997).

Figure 1: A juvenile common opossum (Didelphis marsupialis) at Cetas-IBAMA, a wildlife rehab center in Manaus, Brazil. Photo by Joel Sartore, National Geographic Photographer and Speaker.

As a generalist feeder, the common opossum's diet consists of fruits, insects, small vertebrates, and carrion (Atramentowicz, 1988). In Costa Rica, *D. marsupialis* often plays an important role in controlling populations of agricultural pests, such as insects and small rodents. In addition to its pest control capabilities, *D. marsupialis* aids in seed dispersal for various plant species, contributing to forest regeneration in fragmented landscapes (Charles-Dominique et al., 1981). While *Didelphis marsupialis* is currently not considered to be under threat, its wide distribution and adaptability make it an essential species for maintaining ecosystem balance. The species' ability to thrive in disturbed habitats provides valuable insight into the dynamics of biodiversity conservation in rapidly changing environments, such as those in Costa Rica.

The species is known for its unique reproductive strategy. Female opossums give birth to a large number of underdeveloped young, which continue their development in the mother's pouch for several weeks (Petrides, 1949). After weaning, the young often cling to the mother's back for further protection and facilitated mobility until they are fully independent. The weaning process is typically completed around 90 days of age, a pattern observed across various populations of *D. marsupialis* (Julien-Laferriere & Atramentowicz, 1990).

Due to its adaptability, *Didelphis marsupialis* frequently interacts with human populations in Costa Rica. While beneficial for controlling pests, these opossums can also be considered a nuisance when they raid trash bins or enter homes in search of food. However, they generally pose little threat to humans and often rely on tonic immobility which is better known to humans as their notorious "playing dead" defense mechanism when confronted by predators (Hunsaker, 1977). Despite this proximity to humans, zoonotic transmission risks from *D. marsupialis* remain relatively low. Although they can carry certain pathogens such as *Trypanosoma cruzi* (responsible for Chagas disease) and *Leptospira* (which can cause leptospirosis), opossums are not primary reservoirs for these diseases in human populations. Their solitary behavior, nocturnal foraging, and low interaction frequency with humans further reduce the likelihood of direct disease transmission, minimizing zoonosis concerns in most human-opossum encounters (Gardner & Hildreth, 1992; Hunsaker, 1977).

Additionally, *D. marsupialis* is resilient to a variety of environmental stressors, including habitat fragmentation. Studies show that the species can tolerate disturbed habitats better than many other mammals, allowing it to thrive in fragmented tropical forests (Ceballos & Miranda, 1986). This makes the common opossum a crucial species in the research and understanding of effects of habitat changes and urbanization on wildlife in Costa Rica.

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In clinical settings, common opossums often present with a variety of health challenges, especially when rescued from the wild. These include malnutrition, dehydration, parasitic infections, and trauma from encounters with vehicles or predators (Wright et al., 1992).A thorough clinical assessment is essential, focusing on hydration status, overall body condition, traumatic lesions and parasite load. Opossums are highly susceptible to stress, so minimizing handling and providing a calm environment is critical to reduce morbidity (Kreeger & Arnemo, 2012). Gastrointestinal parasites like *Toxocara* and *Capillaria* are frequently observed. Therefore, regular fecal exams and appropriate antiparasitic treatments are necessary (Gardner & Hildreth, 1992). Nutritional support is crucial, especially for young or malnourished individuals, and should include a balanced diet with adequate type and amount of protein, fats, and calcium to prevent metabolic bone disease, which is a common concern in hand-rearing efforts (Fowler, 2008).

Derby's Woolly Opossum (*Caluromys derbianus*)

Derby's woolly opossum (DWO) is a unique marsupial native to Central and South America, particularly prevalent in Costa Rica (Figure 2). This small nocturnal mammal is characterized by its soft, dense fur and prehensile tail, which aids in its arboreal lifestyle. The species primarily inhabits tropical rainforests, where it plays a crucial role in the ecosystem as a frugivore and pollinator. Its diet primarily consists of fruits, nectar, and insects, and it is known to feed on small vertebrates when the opportunity arises (Eisenberg, 1989; Emmons & Feer, 1997). The ecological significance of *C. Derbianus* extends beyond its feeding habits, as its foraging behavior contributes to seed dispersal and the pollination of various plant species, thereby maintaining the health and diversity of forest ecosystems.

In Costa Rica, Derby's woolly opossum is typically found in both lowland rainforests and montane cloud forests, where it is well-adapted to life in the canopy. The opossum is a solitary animal, which reduces competition for resources with other frugivores and insectivores. Its arboreal nature is complemented by its adaptations for climbing which allow it to navigate the branches and trunks of trees (Ceballos & Miranda, 1986). However, habitat loss due to deforestation and agricultural expansion pose significant threats to the population, making conservation efforts vital. Despite not being classified as endangered, the decline in forest cover in Costa Rica could lead to increased vulnerability of this species in the future (Ceballos & Miranda, 1986; Eisenberg, 1989).

Figure 2: A Derby's woolly opossum (Caluromys Derbianus) at the Membeca Lagos Farm, near Rio de Janeiro, Brazil. Photo by Joel Sartore, National Geographic Photographer and Speaker.

Morphologically, Derby's woolly opossum exhibits several adaptations that enable it to thrive in its arboreal environment. Adults typically weigh between 200 and 300 grams and measure around 25 to 35 centimeters in length, excluding the tail (Charles-Dominique et al., 1981; Emmons & Feer, 1997). Their large eyes are adapted for nocturnal vision, while their fur provides insulation and camouflage in the forest canopy.

C. Derbianus is known for its agility and dexterity. It employs a range of vocalizations to communicate with conspecifics, particularly during mating season. The reproductive cycle of Derby's woolly opossum is relatively slow compared to the common opossum, with females typically giving birth to one or two young after a gestation period of about two weeks. The young are born altricial and remain attached to the mother's teats for several weeks, highlighting the maternal investment in offspring development (Eisenberg, 1989). This extended period of dependency is crucial for their survival in the wild, where they learn to forage and navigate their environment.

In clinical settings, the care of Derby's woolly opossums requires specialized knowledge and understanding of their unique physiology and behavior. Veterinarians and caretakers must be aware of their nutritional needs, as a diet primarily composed of fruits and insects is essential for maintaining health. Malnutrition can lead to significant health issues, including metabolic bone disease and dental problems (Fowler, 2008). Monitoring weight and growth is crucial, particularly for juvenile opossums, to ensure that they are developing appropriately.

In addition to nutritional considerations, Derby's woolly opossums are susceptible to various health issues, including parasitic infections and respiratory diseases. Regular veterinary check-ups are essential for early detection and treatment of any health concerns (Gardner & Hildreth, 1992). Furthermore, handling should be minimized during medical examinations to reduce stress, as these animals are particularly sensitive to environmental changes and disturbances (Kreeger & Arnemo, 2012).

In cases of rescued or rehabilitated Derby's woolly opossums, providing a suitable environment that mimics their natural habitat is critical for their recovery and eventual release into the wild. Enclosures should offer ample climbing opportunities and hiding spaces to reduce stress and promote natural behaviors (Kreeger & Arnemo, 2012).

Materials and Methods

Six (n=6) neonatal opossums, comprising five males and one female from two distinct species: five (n=5; f=1; m=4) CO (Didelphis marsupialis) and one (n=1; m=1) DWO (Caluromys derbianus) have been included in this study. These animals were rescued from the wild for different reasons, stated in the next paragraphs, by the Alturas Wildlife Sanctuary staff (Costa Rica) between January and April 2024. Measurements were taken between February 19th and April 4th of 2024.

To evaluate the impact of dietary protein type on growth, the six neonatal opossums were divided into two subgroups based on protein source. Group A was provided with a diet where the primary protein source was chicken eggs, while Group B received raw chicken meat as their protein source. Both diets were supplemented with a base of fruits and vegetables (e.g., mango, papaya, broccoli, and corn) to mimic the natural dietary components the animals might encounter in the wild. The subdivision and introduction of the differentiated diets began on February 20th, 2024. Measurements were initially taken with all animals receiving the same diet for the first measurement. Following the diet adjustment, every other measurement of this study was conducted with the animals divided into their respective dietary subgroups. The weekly adjustments to the amount of protein and overall feed were made to ensure that all individuals received adequate nutrition proportional to their weight gain. This approach ensured that any differences in growth between the groups could be attributed to the protein type rather than caloric deficiencies or imbalances.

For organization and evaluation of the collected data, Google® Sheets has been used.

Inclusion criteria required the animals to be in good health, with no signs of injuries or conditions that could be exacerbated by handling at the beginning of measurements. Species prone to excessive habituation through handling, which could jeopardize their release back into the wild, were excluded from the study.

Further species-specific information is provided in the subsequent sections.

Data Collection Schedule and Methodology

The measurements used in this study were selected based on protocols from the Hand-Rearing Resource Center and relevant literature, with each measurement clearly defined and the data acquisition process rigorously documented (e.g., Ansell, 1965; Whitaker & Hamilton, 1998; Lundrigan, 2010; Schmidly & Bradley, 2016).

Morphometric data were collected from each animal twice a week, specifically on Mondays and Thursdays. The data collection sessions were conducted consistently at 7 a.m. to minimize variability in measurement conditions. Each session lasted an average of 5 minutes per animal.

Prior to the morning feeding session, body weight was measured for all animals using the same electronic scale (AMIR® brifit) with a precision of 0.1 g.

Morphometric measurements were collected with a flexible measuring tape. Each measurement was taken only once per session to avoid increased stress on the animals. The measurements were recorded to the nearest 0.1 mm, ensuring a high level of precision in the data collection. All measurements have been performed by the same operator, with two helper operators turning to help restrain the animals.

As noted by W. F. H. Ansell (1965), key measurements for mammals include total length (ToL), head and body length (HBL), tail length (TL), hindfoot length (HFL), and ear length (EL). corroborates this by listing

Similar standard measurements have been described in the literature (J. O. Whitaker and W. J. Hamilton (1998), B. Lundrigan (2010), and D. J. Schmidly and R. D. Bradley (2016)). The measurement of head and body length are frequently omitted in the literature as these parameters can be calculated from total length and tail length.

1. Total Length (ToL)

Total length is defined as the measurement from the tip of the nose or snout to the end of the fleshy portion of the tail, specifically the terminal part of the last caudal vertebra, excluding the tuft of hair found at the tail's end.

There are two recognized methods for measuring total length in mammals, as outlined by Ansell (1965) and Hoffmann et al. (2010). The first is known as the “point to point” or “between pegs” method, which involves placing the animal in a relaxed position in either dorsal or lateral recumbency, with the tail extended in alignment with the spine. Pins or pegs are then positioned at both ends, and the distance between these points is measured to obtain

ToL. An alternative approach is to use rods positioned perpendicularly to the body's long axis. A modified version of this method entails laying the animal flat on a ruler, with a stop at the ruler's end for direct measurement. Although Ansell (1965) deems this method preferable, it is not suitable for awake animals or those unable to maintain a relaxed position. Therefore, in this study, measurement of ToL of neonates was obtained using the "over the curves" technique, as described by Ansell. In this method, the animal is measured along the midline contours starting from the end of the nose, over the head and neck, and down to the tail, utilizing a flexible measuring tape. This approach significantly minimized stress for the newborns during handling.

2. Tail Length (TL)

Tail length is conventionally defined as the distance from the junction of the tail with the body to the tip of the last caudal vertebra. The base of the tail, at its attachment to the body, typically marks the division between the body and tail (from first to last caudal vertebrae). However, this point can be challenging to pinpoint in practice (P. A. Jewell and P. J. Fullagar, 1966). To address this, three methods for measuring tail length have been established, all considered equally reliable by Ansell (1965). For this study, tail length was measured using the traditional method (as described by Ansell, 1965) which involves measuring along the upper side of the tail with a ruler while holding it perpendicular to the back to identify the base. Other methods have been described yet have been considered not suitable for the investigated species. Tail length can be significant in behavioral studies, as it may impact balance and mobility.

3. Head and Body Length (HBL)

Head and body length is calculated as the total length minus the tail length. This parameter provides insight into the overall growth and development of the animals over time, giving thereby an indication on health and genetic factors and may correlate with age and maturity.

4. Hindfoot Length (HFL)

Hindfoot length is defined as the distance from the back of the heel to the tip of the longest toe (s.u. = sine unguis) or the largest claw (c.u. = cum unguis) (A. Hoffmann et al., 2010). In this study, it was measured from the toe, with the sole of the hindfoot gently pressed against a flat ruler to straighten the toes, as recommended by Devra G. Kleiman et al. (2010).

Measurements were consistently taken from the left hind foot in all individuals, which is regarded as standard practice according to A. Barnett and J. Dutton (1995). Foot size can affect locomotion and foraging efficiency, making it a relevant variable in assessing habitat use and behavioral ecology.

5. Ear Length (EL)

Ear length is defined as the distance from the base of the notch (located below the ear opening) to the furthest edge of the pinna, excluding any fur. This measurement was consistently taken from the left ear using a ruler. Prior to measuring, the ear was briefly stretched and then released, following the guidance of A. Hoffmann et al. (2010). This measurement can be important for identifying species-specific characteristics and potential adaptability to environmental changes.

6. Chest Girth or Axillary Girth (AG)

Chest girth is defined as the widest point of the thorax, measured immediately behind the forelegs. This measurement should only be taken from live animals or fresh carcasses, as the body may become distended shortly after death (Devra G. Kleiman et al., 2010). Chest girth serves as a valuable metric for assessing body weight and health in various domestic animals (Jones et al., 1980; Heinrichs et al., 1992) and is widely recognized for its applicability in wildlife studies as well (e.g., Quirke et al., 2005). It is considered the most accurate predictor of scale weight for brown bears (*Ursus arctos*) (Reynolds et al., 1987), while a notable correlation between body weight and chest girth has been observed in mountain lions (*Puma concolor*) (Jansen & Jenks, 2012) and white-tailed deer (*Odocoileus virginianus*). For elk younger than one year, age significantly influenced the relationship between girth circumference and body mass (R. C. Cook et al., 2003). Including chest girth measurements could be beneficial for studies aiming to extend the relationship between chest girth and body mass to other species. Chest girth is important for assessing body condition and fat reserves, making it particularly relevant for understanding an animal's health and nutritional status.

Data collection on Common Opossums

In this research project morphometric data was collected from five (n=5) CO , consisting of four (n=4) males and one (n=1) female (Table 1). The female opossum (Esperanza) was housed on her own while the four males were housed in couples (Mazapan and Tamal;

Lemon and Lime). To facilitate individual identification of the opossums housed together, the four male opossums were marked with non-permanent markers on their tail at the start of the study. Lemon and Lime, Mazapan and Tamal were full siblings, respectively. No information on other family relations were available.

General information on each *Didelphis marsupialis* was recorded upon their arrival at the rescue center (table 2).

ID	sex	age class	weight	date of capture	locality of capture	reason for rescue
Esperanza	F	neonate	66g	January 6th, 2024	OSA (Puntaerenas)	orphaned, was found on the street
Mazapan	M	neonate	48g	December 26th, 2023	OSA (Puntaerenas)	orphaned, mum was run over
Tamal	M	neonate	45g	December 26th, 2023	OSA (Puntaerenas)	orphaned, mum was run over
Lemon	M	neonate	51g	January 23rd, 2024	Perez Zeldeon (San José)	orphaned, mom was killed by a dog
Lime	M	neonate	55g	January 23rd, 2024	Perez Zeldeon (San José)	orphaned, mom was killed by a dog

Table 1. General information on each individual of CO, collected at their time of arrival at the rescue center; m=male; f= female.

At the beginning of the study, the age of all individuals was estimated to be 70 days, based on the fact that the weaning age for *Didelphis marsupialis* is approximately 90 days (Didier Julien-Laferriere & Martine Atramentowicz, 1990; George A. Petrides, 1949). The animals were therefore considered neonates until complete weaning by March 4th, 2024.

Data collection on Derby's Woolly Opossum

Morphometric data on one (n=1) individual of DWO (*Caluromys Derbianus*) during the whole study period was collected.

General information on the individual of DWO, collected at its time of arrival at the rescue center may be seen in Table 2.. It was housed in an incubator at stable temperature for the first few weeks, and then transferred in an external cage.

ID	sex	age class	weight	date of capture	locality of capture	reason for rescue
Woolly	M	neonate	48g	February 8th, 2024	OSA (Puntarenas)	orphaned, as found on the street

Table 2. General information on the individual of DWO, collected at its time of arrival at the rescue center; m=male.

It was not possible to estimate the age in days of the studied individual due to a lack of information on this species in literature. However, differences in body size and weight allowed us to distinguish the rescued one from an adult specimen.

Effect of differences in diet

The dietary intake of juvenile opossums plays a crucial role in their growth and development, influencing key morphometric parameters such as weight, body length, and other physical measurements. This analysis compares the effects of two distinct versions of the same diet—Egg and Chicken protein on a base of fibers and starches—on the growth trajectories of the opossums over time. The feed is prepared so that the nutritional value of the protein intake stays the same even though the type of protein is different, with adjustments on the amount made weekly based on the weight gain of each individual specimen to optimize their dietary intake.

For each group a dietary plan was developed ensuring correct nutritional values although the origin of protein may be considered different. Both diets consisted of a mixture of fruits and vegetables that would later be possibly found by the animals in nature, such as: mango, papaya, broccoli, corn and other similar components.

The protein source for Group A consisted of chicken eggs, while the protein source for Group B consisted of raw chicken meat. These diets were fed from the day February 20th onwards.

Results

The results of the present study will be described separately for each measurement. Initially a general description will be provided on the growth-curves and fluctuations for each respective individual. Subsequently results and differences between the two subgroups with differing diets will be described.

Before delving into the analysis of the growth and morphological data presented in the following graphs, it is important to consider some key background information regarding the conditions and events that influenced the opossums' development throughout the study. Several environmental and health-related factors, as well as changes in their care regimen, played a significant role in shaping their growth patterns.

Between the second and third measurement dates, several opossums (Esperanza, Lemon and Woolly) that had been housed in incubators were moved to larger, individual outdoor enclosures. However, Lemon was returned to an incubator after the third measurement date due to hypothermia caused by exposure to heavy rain, and was administered Pedialyte® for rehydration. Around the fourth measurement date, Lime experienced a bout of diarrhea, resulting in temporary weight loss. By the fifth measurement, all the opossums except Woolly had been weaned, and Tamal and Mazapan were also moved to outdoor enclosures. Notably, all opossums moved outdoors had their feeding schedules adjusted by reducing the frequency of feedings from five to three per day, to accommodate their natural nocturnal feeding behaviors, though the total amount of food remained the same. At the seventh date, Lime was also transitioned to an outdoor enclosure. Around the ninth measurement date, scatter feeding was introduced to stimulate foraging and hunting behaviors, which may have contributed to slight weight loss in subsequent measurements. By the eleventh date, Esperanza had lost weight, prompting a veterinary check-up, but no health issues were identified. Additionally, Tamal reached the target release weight of 200 grams and was deemed ready for release. In the twelfth measurement, Tamal was released, while both Esperanza and Lemon were taken to the clinic for a three-day course of anticestodal treatment (administering Prafentel orally) subsequent to parasitic presence detection in their feces. After this, all other opossums underwent the same treatment as a preventive measure under the veterinarians instructions (figure 3 and 4). Lemon managed to escape from the clinic, effectively releasing himself. By the thirteenth measurement, both Esperanza and Lime had reached their target weight and were subsequently released.


Figure 3 and 4: pictures taken during Prafentel oral administration on Tamal and Woolly.

Understanding these contextual elements is crucial for interpreting the fluctuations observed in the data, as they provide insight into the external factors that may have impacted the opossums' physical development and the accuracy of the measurements taken. With this foundational context in mind, the subsequent analysis will examine the trends and variations in the opossums' morphometric data over the course of the study.

- **Weight**

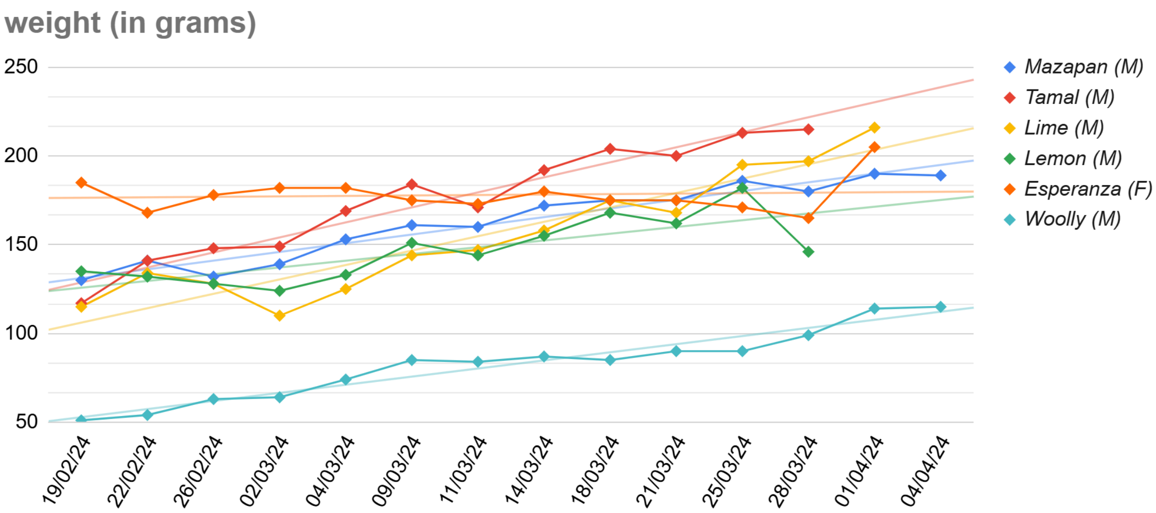

Figure 5. Graph comparing the weight of every individual over the study period.

Weight increase and fluctuations may be seen in Figure 5. In all individuals weight loss compared to the weight of the previous measurement has been observed at different measurement dates and in different degrees. In the case of Lemon, fluctuations have been very obvious with the final weight being lower than the weight at the beginning of measurements.

Weight loss compared to the day before was observed in 3 CO's and the DWO at the seventh measurement. After initial weight loss between the first and second measurement, and slight increase in the period of measurements three to five the only female subject has shown gradual but continuous weight loss until the second to last measurement. Although the last measurement of this subject has shown a rapid increase, the final weight was only slightly higher than the weight at the beginning of measurements.

Between the second and third measurement dates, Esperanza, Lemon, and Woolly transitioned from indoor incubators to larger, individual outdoor enclosures. This environmental change is reflected in Lemon's marked weight loss, which necessitated a return to the incubator and rehydration with Pedialyte® due to hypothermia after prolonged exposure to rain. Conversely, Woolly and Esperanza maintained relatively stable weight trajectories during this period, suggesting individual variability in adaptation to external environmental stressors.

By the fourth measurement, Lime exhibited weight loss attributed to diarrhea, an acute gastrointestinal issue likely impacting nutrient absorption and energy reserves. Around the fifth measurement, a major developmental transition occurred as all opossums except Woolly underwent weaning. Simultaneously, Tamal and Mazapan were moved to outdoor enclosures, which, alongside a reduction in feeding frequency from five to three meals per day (with caloric intake unchanged), represented a substantial alteration in their nutritional schedule. This adjustment was intended to align with their nocturnal feeding behaviors but appears to have temporarily disrupted growth, evidenced by minor fluctuations in their weight trajectories.

Notably, the move to outdoor enclosures and subsequent feeding modifications highlight the challenges of balancing physiological adaptation with natural behavioral stimulation in a controlled developmental context. Further significant fluctuations are observed later in the timeline. Lime's move to an outdoor enclosure by the seventh measurement was followed by a steady weight increase, indicating successful adaptation to his new environment. However, scatter feeding was introduced around the ninth measurement to stimulate foraging behaviors, resulting in a slight weight reduction across individuals as they adjusted to the increased energetic demands of this feeding strategy.

By the eleventh measurement, Tamal had reached the target release weight of 200g, marking a successful culmination of growth and readiness for release into the wild. However, Esperanza experienced an unexplained weight loss during this period, prompting veterinary evaluation, which did not reveal any underlying pathology, hence further examinations were executed to assess parasitic presence in the feces.

Toward the twelfth measurement, environmental and health-related factors once again disrupted weight trends. Both Esperanza and Lemon were treated with oral antiparasitic medication (Prafentel®) at the clinic, introducing additional stress. Lemon's subsequent escape and self-release precluded further data collection, but his prior weight trajectory suggested potential readiness for release.

By the thirteenth measurement, both Esperanza and Lime achieved the target weight of 200g, completing their developmental benchmarks and signaling readiness for release into their natural habitat.

- **Total Body length**

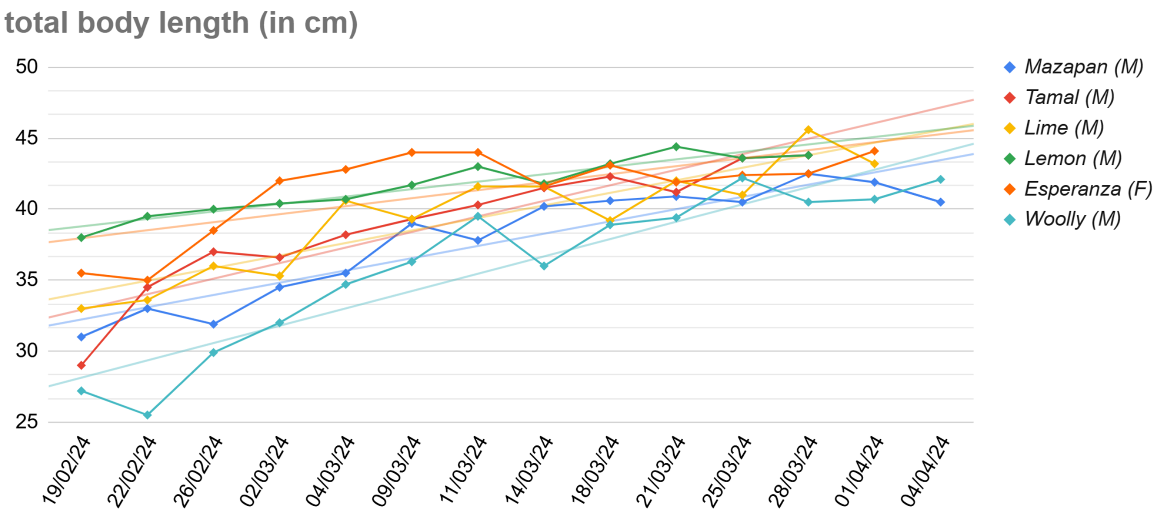

Figure 6. Graph comparing the values of the total body length of every individual over the study period.

The total body length of the juvenile opossums showed consistent growth over the study period (Figure 6), with occasional fluctuations likely caused by measurement error or environmental and health-related factors.

Esperanza exhibited a remarkable increase in TBL, from 35 cm on the first measurement to 42 cm by the third, which coincided with her transition to an outdoor enclosure. The larger environment may have facilitated greater movement and muscle development, reflecting not just growth in length but also physical adaptation to her new environment. However, her subsequent decline in TBL after the ninth measurement is improbable biologically and likely attributable to handling variability during her antiparasitic treatment. Lemon, who transitioned outdoors during the same period, showed consistent growth early on but plateaued at 44.4 cm by the tenth measurement. This stabilization, along with his subsequent self-release, suggests that he had reached a developmental endpoint before the study concluded.

Lime displayed significant variability, with a peak at 45.6 cm by the eleventh measurement, reflecting recovery and growth after his diarrhea episode. Woolly's TBL rose steadily from 27.2 cm to 42.1 cm by the study's conclusion, despite his aggressive behavior, which may have introduced measurement inconsistencies. Mazapan and Tamal followed similarly stable trajectories, peaking at 42.5 cm and 43.8 cm, respectively, though both exhibited minor fluctuations likely tied to handling variability and developmental transitions, such as weaning.

TBL trends also reflect the interaction between individual health and environmental conditions. For instance, Lime's significant recovery in growth after his diarrhea episode highlights the resilience of juvenile opossums when provided with proper care. The fluctuations observed for Esperanza during her antiparasitic treatment may indicate the physical stress associated with these interventions, which could momentarily influence growth metrics. Additionally, Lemon's plateau in TBL before his self-release might suggest a stabilization in skeletal growth as the animals approached their developmental milestones. The consistent growth observed in Woolly, despite his smaller initial size and more aggressive temperament, underscores the importance of individualized care in rehabilitation programs.

- **Head and Body length**

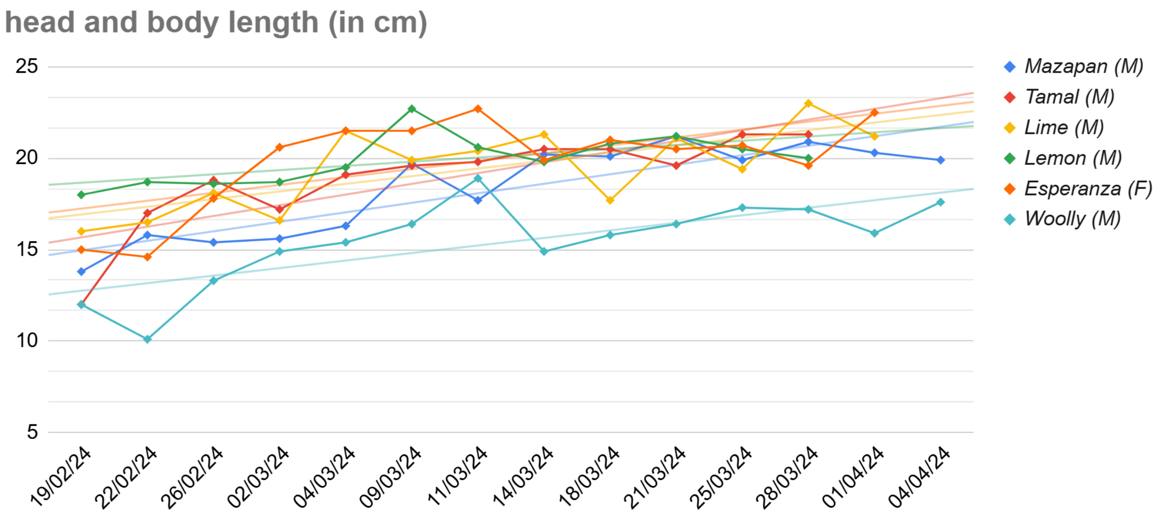

Figure 7. Graph comparing the values of the head and body length of every individual over the study period.

Head and body length measurements demonstrated steady overall increases (Figure 7), with individual variability influenced by health challenges, environmental changes, and possible measurement inconsistencies.

Mazapan displayed moderate but steady growth, reaching 20.9 cm by the twelfth measurement before stabilizing at 20.3 cm. His minor fluctuations may reflect handling differences, as his overall health remained stable throughout the study. Tamal showed consistent increases, peaking at 21.3 cm before his release, indicating steady growth without significant disruptions. Lime's increase to 21.5 cm by the fifth measurement followed by a drop to 17.7 cm at the ninth aligns with his bout of diarrhea but is also likely influenced by measurement inconsistencies. A decrease of this magnitude is physiologically improbable and may reflect Lime's physical condition (e.g., posture or stress) during the measurement process. Lemon's HBL peaked at 22.7 cm by the sixth measurement before plateauing, with subsequent small declines likely tied to stress from his hypothermia and clinic treatment. Esperanza showed consistent growth, reaching 22.7 cm before her treatment and displaying resilience by stabilizing her HBL post-treatment. Woolly, despite being the smallest and most aggressive, demonstrated steady increases, culminating in 17.6 cm by the study's conclusion.

In addition to general growth trends, HBL measurements offered insight into how external stressors impacted development. Lime's significant drop during his illness illustrates how acute health events can momentarily halt growth, requiring careful management to ensure recovery. Similarly, minor declines in Lemon's HBL during hypothermia treatment highlight the potential trade-offs between immediate survival strategies and long-term growth. For Esperanza, the stabilization of her HBL after treatment reflects her resilience and adaptation to care interventions. Woolly's steady yet slower growth trend likely aligns with his smaller starting size and unique developmental trajectory as a less common species in the study.

- **Tail length**

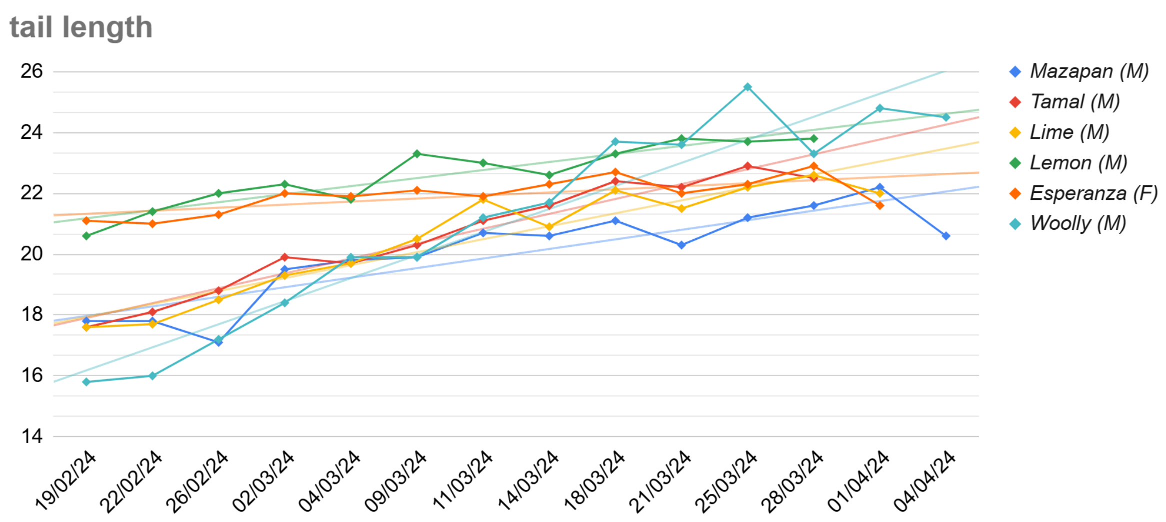

Figure 8. Graph comparing the values of the tail length of every individual over the study period.

Tail length growth appeared stable across individuals (Figure 8), but occasional fluctuations suggest interactions between environmental changes, health stressors, and potential measurement inconsistencies.

Mazapan showed steady increases in TL, peaking at 22.2 cm by the twelfth measurement before a minor decline, likely reflecting handling variability. Tamal demonstrated similar consistency, reaching 22.9 cm by the tenth measurement before his release. Lime exhibited steady growth in TL throughout, culminating in 22.6 cm by the twelfth measurement, reflecting his recovery following earlier health challenges. Lemon's TL plateaued at 23.8 cm, coinciding with his self-release, suggesting that his skeletal development had reached a stable endpoint. Esperanza displayed consistent increases early on but fluctuated slightly post-treatment, stabilizing at 22.9 cm by her final measurement. Woolly's TL increased dramatically, from 15.8 cm to 24.8 cm, with his aggressive behavior potentially contributing to minor outliers.

TL growth provides additional insight into the physical development of the opossums. Notably, Mazapan and Tamal's consistent increases suggest robust skeletal health and an absence of significant stressors. In contrast, Lime's delayed but eventual stabilization demonstrates the capacity for compensatory growth following a setback.

The plateau observed in Lemon's TL suggests that he may have reached a developmental endpoint earlier than the others, potentially influenced by environmental factors such as his premature self-release. Esperanza's consistent tail growth, even after her antiparasitic treatment, highlights the importance of maintaining health during critical growth stages. Woolly's dramatic proportional increase, despite measurement variability, illustrates how a smaller initial size does not necessarily limit growth potential when adequate care is provided.

- **Hindfoot length**

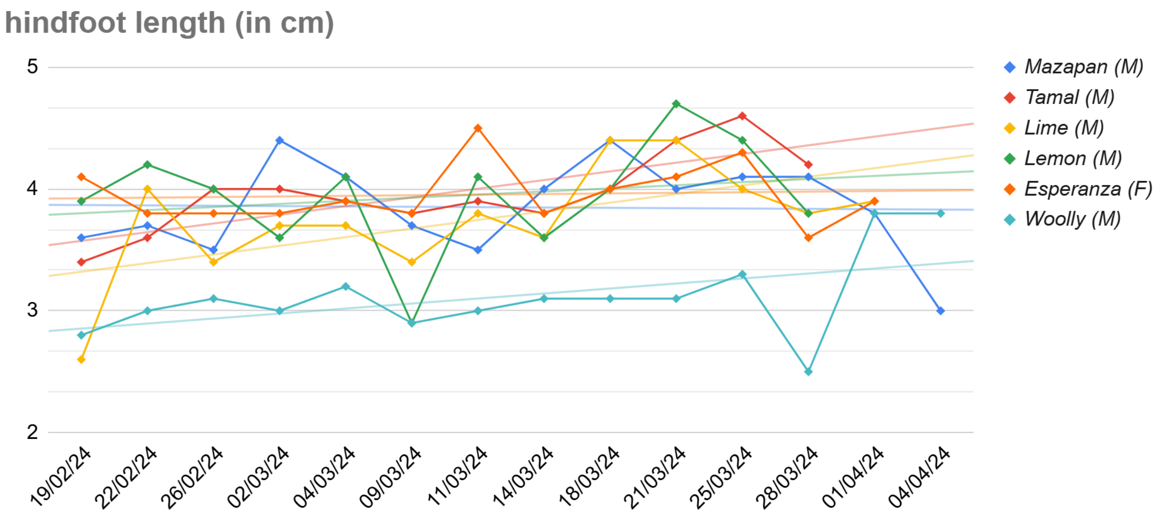

Figure 9. Graph comparing the values of the hindfoot length of every individual over the study period.

Hind foot length (HFL) measurements exhibited minor fluctuations (Figure 9), often reflecting environmental and health stressors or measurement variability.

Mazapan displayed steady growth early on, peaking at 4.4 cm, though his values declined slightly near the study's conclusion. Tamal reached a maximum HFL of 4.6 cm, aligning with his target release weight, reflecting robust skeletal development. Lime's stabilization at 3.9 cm by the twelfth measurement demonstrates recovery and growth following his earlier gastrointestinal issues. Lemon's irregular trends, including a peak at 4.7 cm by the tenth measurement followed by a slight decline, align with his health-related stressors during hypothermia and subsequent clinic treatment. Such fluctuations are unlikely to reflect true changes in bone or tissue structure, pointing instead to handling variability. Esperanza displayed consistent growth, stabilizing at 4.3 cm, with minor fluctuations likely linked to handling challenges during her treatment period. Woolly, though the smallest and most aggressive, showed gradual increases, peaking at 3.8 cm. These findings suggest that while HFL is a reliable indicator of skeletal growth, handling difficulties and stress can introduce variability into the measurements. Woolly's aggressive behavior may have contributed to some of the inconsistencies in his earlier values, though his overall trajectory indicates steady progress.

HFL trends further emphasized the resilience of the opossums under differing circumstances. For example, Mazapan and Tamal's steady growth aligned with their overall physical readiness for release, reflecting the role of skeletal measurements in assessing developmental milestones. Lime's recovery after gastrointestinal illness underscores the impact of health on locomotion-related growth, as hindfoot development is critical for mobility and survival in the wild. Lemon's slight declines during hypothermia treatment suggest that energy may have been diverted from growth to immediate survival needs. Esperanza's ability to maintain consistent hindfoot growth after her treatment highlights her resilience, while Woolly's gradual improvement reflects the adaptive care required for smaller, more challenging individuals.

- **Ear length**

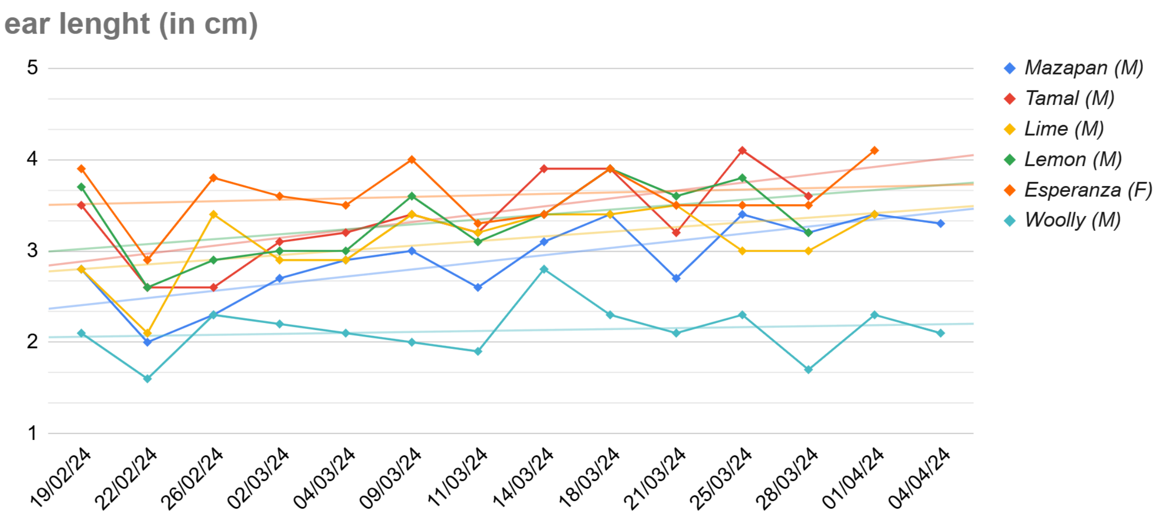

Figure 10. Graph comparing the values of the ear length of every individual over the study period.

Ear length (EL) displayed steady increases in most individuals (Figure 10), with occasional variability reflecting, once again, health challenges, environmental changes, or handling-related errors.

Mazapan demonstrated consistent growth, with EL stabilizing at 3.4 cm by the twelfth measurement, reflecting steady development despite minor fluctuations. Tamal showed a similar trajectory, peaking at 4.1 cm by the eleventh measurement before his release. Lime's decline from 3.4 cm at the sixth measurement to 3 cm at the tenth could reflect the influence of scatter feeding, which increased energy expenditure and likely shifted growth priorities. Lemon's EL also fluctuated, peaking at 3.8 cm before his release, with his hypothermia and clinic treatment possibly contributing to variability. Esperanza displayed resilience, with her EL increasing steadily to 4.1 cm by her final measurement despite undergoing antiparasitic treatment. Woolly's EL remained the smallest throughout the study, reflecting his physiologically slower developmental trajectory, though his steady improvement to 2.3 cm indicates gradual progress.

The trends in EL provide another perspective on the opossums' development, particularly under stress. Mazapan and Tamal's consistent growth highlights the stability of this parameter in animals without major disruptions. Lime's temporary declines during scatter feeding could suggest that increased energy expenditure temporarily diverted resources from growth, though his eventual recovery indicates adaptability. Lemon's fluctuations, particularly during clinic treatments, may reflect the physiological impact of stress and environmental changes on peripheral growth. For Esperanza, the steady increases in EL, even during antiparasitic treatment, underscore her capacity to adapt to care interventions. Woolly's incremental improvements align with his overall slower developmental trajectory and highlight the importance of tailored rehabilitation strategies.

- **Axillary Girth**

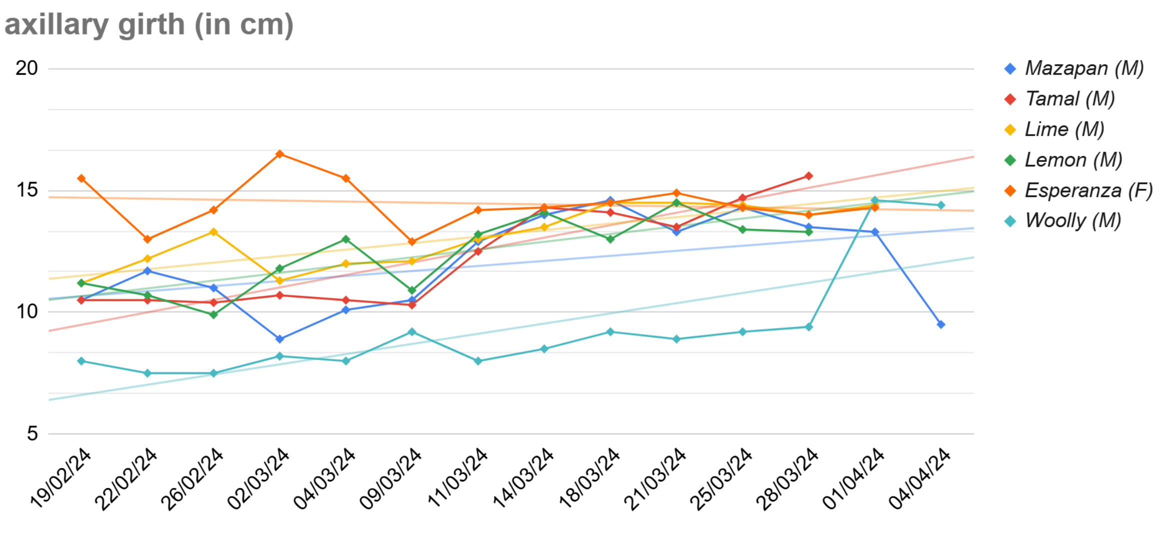

Figure 11. Graph comparing the values of the axillary girth of every individual over the study period.

Axillary girth exhibited the greatest variability among the measured parameters (Figure 11), likely influenced by feeding transitions, stress, and measurement challenges (e.g., thick fur on the specimens).

Mazapan showed a sharp decline from 14.6 cm to 9.5 cm by the final measurement, an unlikely physiological change that suggests handling variability or temporary stress during measurement. Tamal demonstrated consistent increases, peaking at 15.6 cm before his release, aligning with his achievement of target weight. Lime showed relatively stable growth, reaching 14.4 cm by his final measurement, reflecting recovery and adaptation to his outdoor enclosure. Lemon's AG fluctuated significantly, peaking at 14.5 cm before declining during his clinic treatment, possibly reflecting the combined effects of stress and handling challenges. Esperanza displayed stable AG values throughout most of the study, peaking at 14.9 cm before her final release, underscoring her resilience despite health interventions. Woolly demonstrated the most dramatic increase, from 9.2 cm to 14.6 cm, reflecting robust physiological development despite his aggression, which may have contributed to variability in earlier measurements.

AG patterns revealed a greater sensitivity to external and physiological factors compared to other morphometric parameters. For instance, Mazapan's sharp decline near the study's end likely reflects handling variability or temporary stress rather than true physiological changes. Tamal's consistent increases and eventual stabilization at release emphasize the utility of AG as a growth indicator when data collection is stable. Lime's steady recovery, even after earlier setbacks, mirrors his broader developmental trajectory, suggesting that AG can serve as a proxy for overall health. Lemon's marked fluctuations during treatment and subsequent self-release highlight the importance of minimizing stress during critical health interventions. For Esperanza and Woolly, the steady growth in AG despite challenges highlights their resilience and underscores the importance of maintaining consistent care in rehabilitation settings.

Effect of differences in diet

By calculating and examining the average values for each diet across multiple measurement dates, this section aims to highlight differences in growth patterns, evaluate the nutritional efficacy of each diet, and identify potential trends or disparities in developmental outcomes. Understanding these differences is essential for optimizing dietary protocols in neonatal opossum care and ensuring the healthiest growth trajectories possible.

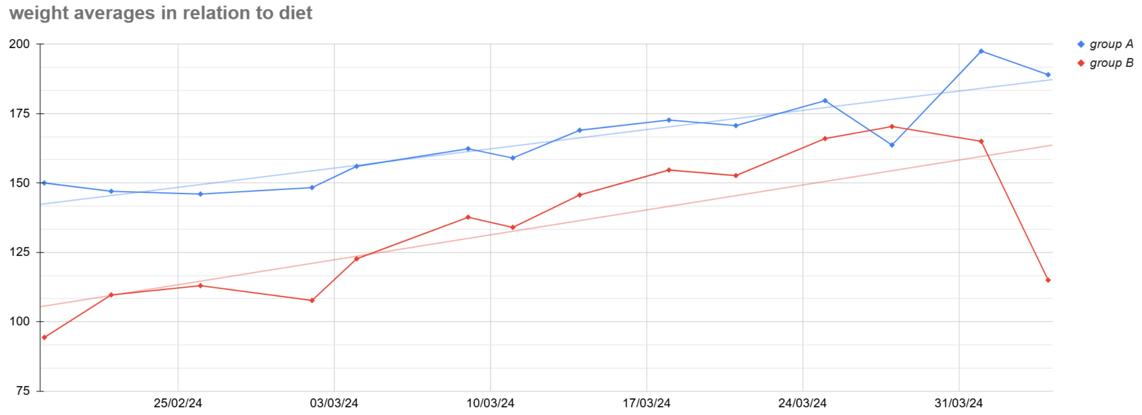

Figure 11. Graph comparing the average weight values in grams of the specimens divided in two dietary subgroups, over the study period.

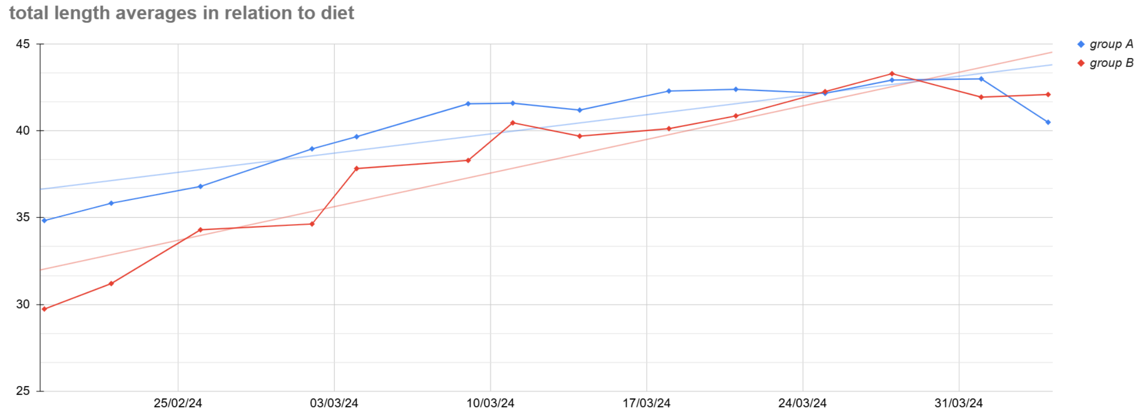

Figure 12. Graph comparing the average total body length values in centimeters of the specimens divided in two dietary subgroups, over the study period.

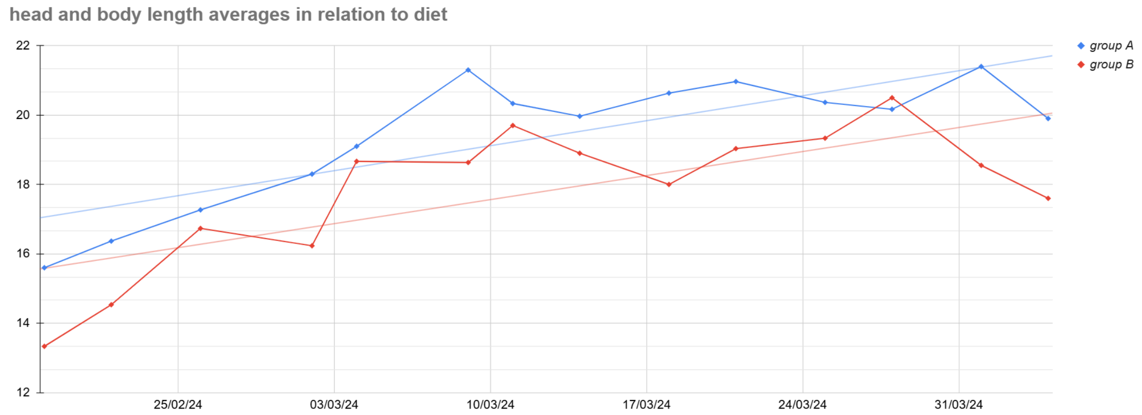

Figure 13. Graph comparing the average head and body length values in centimeters of the specimens divided in two dietary subgroups, over the study period.

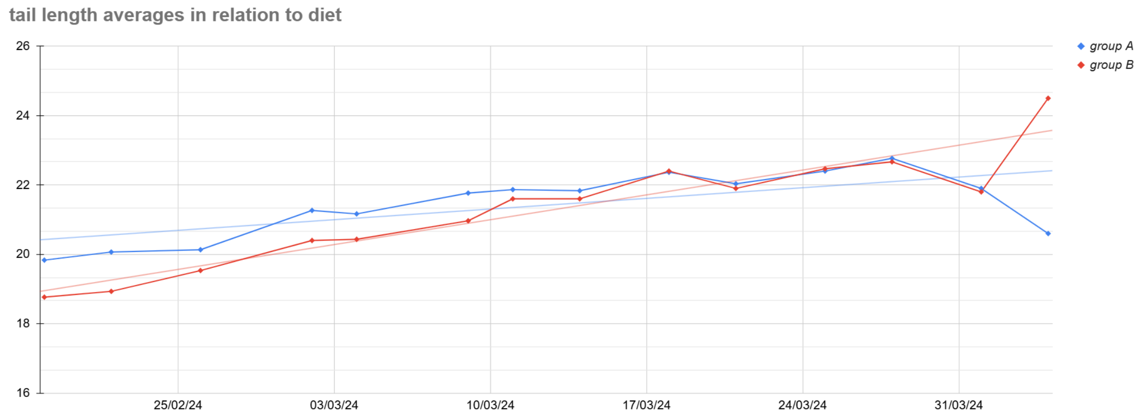

Figure 14. Graph comparing the average tail length values in centimeters of the specimens divided in two dietary subgroups, over the study period.

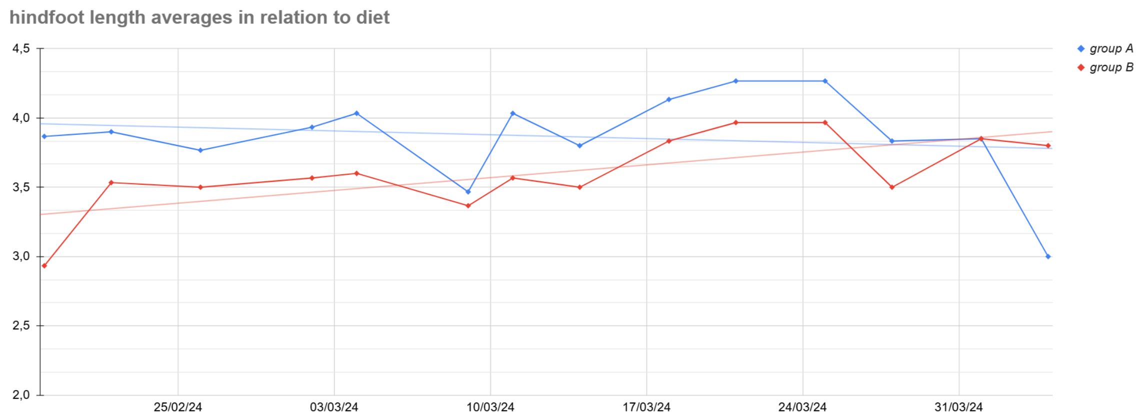

Figure 15. Graph comparing the average hindfoot length values in centimeters of the specimens divided in two dietary subgroups, over the study period.

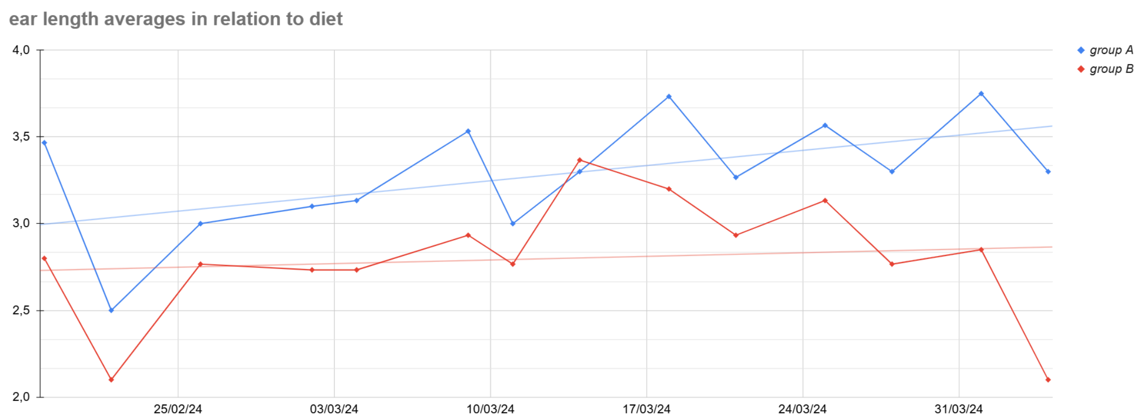

Figure 16. Graph comparing the average ear length values in centimeters of the specimens divided in two dietary subgroups, over the study period.

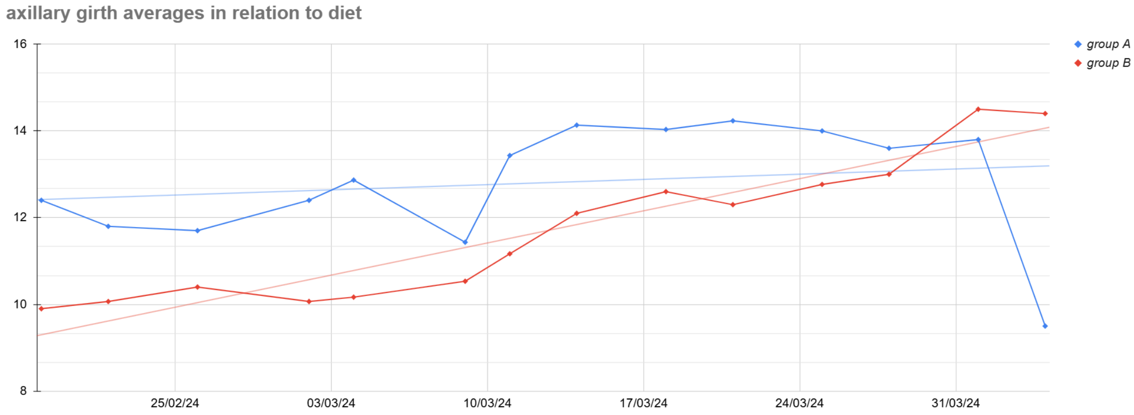

Figure 17. Graph comparing the average axillary girth values in centimeters of the specimens divided in two dietary subgroups, over the study period.

Figures 11 to 17 show growth based on the type of protein in the diet (egg and chicken). When comparing weight (Figure 11), TbL (Figure 12), TL (Figure 14), HFfL (Figure 15), and AG (Figure 17) the tendency lines of the chicken fed opossums seems to have a steeper slope, suggesting an accelerated growth. However, results regarding HbL (Figure 13) and EL (Figure 16), seem to contradict this tendency.

Discussion and Limitations

In this study some measurements were occasionally affected by error, resulting in underestimation of body part lengths. These discrepancies were noticed during a revision of the collected data and likely arose from random variations in the measuring process, such as animal movement, aggressive behavior that modified the handling (e.g., biting) or postural changes (e.g., curling). Other elements that might have caused error include the use of a cloth to prevent animals movement during the weighting process, that was previously weighted on its own and then subtracted from the final measurement. Additionally, during the study period, it became evident that accurately measuring girth in both opossum species was challenging: the substantial fur thickness interfered with consistent tensioning of the measuring tape around the chest, and care was taken to avoid excessive tightening to prevent potential discomfort to the animals.

- **Weight**

The weight fluctuations observed among the six juvenile opossums demonstrate the interplay between environmental modifications, physiological responses, and developmental milestones.

Weight is arguably one of the most crucial morphometric parameters in neonatology, as it directly reflects the overall health, growth, and development of juveniles. In the early stages of life, weight gain is a key indicator of nutritional status and physiological well-being, as it signifies the ability to metabolize food and sustain bodily functions. Monitoring weight provides critical insights into growth trajectories and helps identify potential health issues, such as undernutrition or disease, which can have lasting impacts on an individual's development.

These findings underscore the complex interrelationship between environmental, dietary, and health factors on weight progression in juvenile opossums, emphasizing the importance of closely monitoring individual responses to ensure successful growth and adaptation.

- **Total body length, Head and Body length**

These observations highlight the interplay between environmental factors, individual resilience, and methodological precision in tracking growth, as well as the importance of considering health, environmental changes, and handling conditions when interpreting HBL growth patterns.

- **Tail length**

While TL is generally less variable than other parameters, The trends seen highlight its potential sensitivity to health-related disruptions and environmental adaptation, as well as the influence of handling conditions during measurements.

- **Hindfoot length**

The relatively minor variability across most individuals highlights the robustness of HFL as a growth metric, though care must be taken to account for measurement error when interpreting small fluctuations.

- **Ear length**

The observed patterns suggest that EL, while generally stable, may be sensitive to stressors and methodological inconsistencies, requiring careful interpretation in conjunction with other growth parameters.

- **Axillary girth**

The observations made in the results highlight AG as a dynamic growth indicator sensitive to environmental changes and stress, necessitating careful monitoring to distinguish between biological trends and measurement inconsistencies.

- **Effect of differences in diet**

One possible explanation for the apparent lack of variation between subgroups may be the limited sample size. Additionally, the possible bias in data collection mentioned before, may have introduced variability that further obscured potential differences. Thus, while the study aimed to assess dietary protein effects, these limitations suggest caution in interpreting the results and underscore the need for larger, more controlled studies to draw more definitive conclusions. While the current study provides valuable insights into the effects of dietary protein on opossum growth, the limitations in sample size and data variability emphasize the complexity of interpreting morphometric data.

Moving forward, more robust research is needed to fully understand the underlying factors influencing growth patterns, which will ultimately inform better care practices and more precise benchmarks for animal development in both rehabilitation and breeding programs.

Monitoring animal growth to detect potential morphological abnormalities is essential for both breeding and rehabilitation programs, as consistent tracking of body weight and morphometric measurements can offer valuable insights into an animal's health trajectory and developmental progress, including the ability to assess health status, detect early signs of malnutrition or disease, and evaluate whether the animal is achieving normal developmental milestones. For instance, consistent weight gain and proportional body growth suggest that an animal is receiving adequate nutrition and responding well to its environment, while stagnation or abnormal growth patterns may signal underlying health issues or stress factors.

In rehabilitation or hand-rearing contexts, such data can help caregivers adjust diets, improve environmental conditions, and tailor care to optimize growth and survival chances. Additionally, growth data can reveal how different factors—like dietary composition or social interactions—affect development, offering evidence-based guidance for improving care practices and helping to set realistic benchmarks for species-specific growth under managed care. This is particularly important in hand-rearing programs, where close monitoring helps ensure that young animals meet critical growth milestones. Nevertheless, morphometric data frequently exhibit considerable variability, even among individuals and populations of the same species. Such variability can stem from a range of factors, including environmental conditions, dietary differences, and social structures, all of which influence growth patterns and body condition. Studies have documented these variations across adult and juvenile animals alike, highlighting the challenge of applying standardized, laboratory-derived morphometric data to wild populations, where conditions are inherently more variable and less controlled (Gay & Best, 1996; James, 1970; Fuentes & Jaksic, 1979; Delany & Monro, 1985).

As noted, accurately collecting morphometric measurements is challenging, particularly when animals move, resist handling, or change posture, which can introduce variability. For rescued animals, additional stress or health issues may further complicate the process, making it harder to capture true, consistent measurements. Environmental factors like temperature or humidity can also affect the physical state of the animal or the tools used, adding yet another layer of potential inaccuracy. Another major challenge is sample size. In many morphometric studies, small sample sizes limit the ability to detect subtle differences or patterns, especially when animals must be measured repeatedly over time. This limitation is compounded in rescue and rehabilitation contexts, where resources, staffing, and available animals may be constrained.

Beyond data collection, interpreting morphometric data poses challenges as well. Individual variability within a species can be significant due to genetic, environmental, and developmental factors, making it difficult to establish standardized baselines for "normal" growth and morphology. Additionally, applying findings from captive or managed populations to wild populations is problematic, as different diets, behaviors, and stressors can lead to different growth outcomes. Finally, ethical considerations are crucial, as capturing precise measurements must be balanced with minimizing animal distress and ensuring welfare. Together, these challenges underscore the need for careful methodological design and transparent reporting when using morphometric data in animal studies.

To overcome some of these challenges, the main focus of future research should be on standardizing data collection methods to allow correct comparison of results from different studies. Efforts like the Hand-Rearing Resource Center (www.handrearingresourcecenter.com), initiated by the Association of Zoos and Aquariums (AZA) Nutrition Advisory Group in 2014, aim to create shared tools and databases for hand-rearing and growth monitoring. This web-based resource provides hand-rearing summaries, taxonomic resources, and standardized forms for data collection, promoting collaboration among zoos, wildlife rehabilitation centers, and academic institutions (Hedberg et al., 2015). These forms can be completed by caretakers, submitted for review, and, once approved by the advisory board, made publicly available. This initiative represents a valuable effort to develop an internationally shared resource aimed at enhancing knowledge of animal growth and improving the success rates of hand-rearing programs.

Another important topic to discuss is that of live animal measurements opposed to museum specimen measurements. Comparing live measurements to the known limitations of museum measurements provides insight into the sources and impacts of variability: in morphometric studies there are key differences between museum and live animal measurements that impact the accuracy, accessibility, and relevance of data. Museum specimens, preserved and sometimes altered during the preservation process, offer the advantage of accessibility and consistency over time; they allow researchers to take repeated measurements without stressing or impacting live animals. However, museum specimens often exhibit shrinkage, distortions, or other post-mortem changes that can bias measurements, especially soft-tissue structures crucial in studies on growth and juvenile morphology. In contrast, live animal measurements provide a more accurate reflection of current morphology, including dynamic aspects like muscle tone and flexibility, which are essential for assessing growth patterns in juvenile opossums. Yet, live measurements can be challenging, as they often require specialized handling, may be subjected to variability based on the animal's position or behavior, and can potentially stress the animals, affecting welfare and ethical considerations. For a morphometric study on juvenile opossums, using live measurements could yield more biologically relevant data, though it requires balancing accuracy and animal welfare, whereas museum specimens offer a non-intrusive reference for historical comparisons despite possible alterations due to preservation.

Conclusions

This study highlights the potential of morphometric analysis as a reliable tool for monitoring of growth and development of juvenile opossums in rehabilitation settings. Despite the limitation in the assessment of differences between the two dietary subgroups, the findings underscore the intricate interplay of environmental, nutritional, and health factors in shaping growth trajectories. Measurement variability and a limited sample size likely contributed to the inability to detect subtle dietary effects, emphasizing the importance of larger, more controlled studies in future research. Nevertheless, data collected were valuable in informing care practices, guiding dietary adjustments, and supporting the successful release of rehabilitated individuals. By combining rigorous monitoring with a welfare-centered approach, this research contributes to establishing baseline growth data for rescued wildlife and advancing best practices in hand-rearing programs. These findings reaffirm the critical role of morphometric data in improving animal care and fostering species conservation efforts.

Bibliography

Ansell, W. F. H. (1965). Mammals of the Order Marsupialia. In J. A. McCulloch (Ed.), *The Handbook of Mammals* (pp. 1-14). Oxford University Press.

Atramentowicz, M. (1988). Feeding habits of Didelphis marsupialis in French Guiana. *Biotropica, 20*(2), 136-141.

Barnett, A. A., & Dutton, J. (1995). Measuring body size and shape in mammals: A case study of the Australian marsupial. *Journal of Mammalogy, 76*(1), 211-219.

Bolter, D. R., & Zihlman, A. L. (2003). Morphometric analysis of growth and development in wild-collected Ugandan Cercopithecus aethiops. *Journal of Human Evolution, 44*(3), 283-312.

Ceballos, G., & Miranda, A. (1986). The effects of forest fragmentation on small mammal communities in Costa Rica. *Conservation Biology, 2*(4), 340-347.

Charles-Dominique, P., Atramentowicz, M., Charles-Dominique, M., & Hladik, C. M. (1981). Ecology of the opossum in Neotropical forests. *Journal of Mammalogy, 62*(1), 61-67.

Cole, T. J. (2012). The development of growth references and growth charts. *Annals of Human Biology, 39*(5), 382-394.

Cook, R. C., Roberson, T. M., & Iversen, D. A. (2003). Age influences on the relationship of chest girth circumference to body mass in Rocky Mountain elk (Cervus elaphus nelsoni). *Journal of Wildlife Management, 67*(3), 520-528.

Delany, M. J., & Monro, R. H. (1985). Comparison of natural and laboratory growth in wild and captive Nile rats. *Journal of Zoology, 206*(3), 381-394.

Eisenberg, J. F. (1989). *Mammals of the Neotropics: The Northern Neotropics* (Vol. 1). University of Chicago Press.

Emmons, L. H., & Feer, F. (1997). *Neotropical Rainforest Mammals: A Field Guide*. University of Chicago Press.

Fleagle, J. G., & Samonds, K. (1975). Growth and development of Cebus monkeys (Cebus albifrons): Implications for growth disturbance. *American Journal of Physical Anthropology, 42*(1), 1-15.

Fowler, M. E. (2008). *Zoo and Wild Animal Medicine*. Elsevier Health Sciences.

Fuentes, E. R., & Jaksic, F. M. (1979). Environmental, dietary, and social factors affecting size variation in adult animals. *Journal of Animal Ecology, 48*(3), 559-572.

Gardner, S. L., & Hildreth, M. B. (1992). Parasitic infections in Didelphis marsupialis: A survey of gastrointestinal parasites. *Journal of Wildlife Diseases, 28*(3), 456-461.

Garshelis, D. L. (1984). Age estimation of sea otters. *Marine Mammal Science, 1*(2), 161-171.

Gay, S. W., & Best, T. L. (1996). Variations in size among animal populations and individuals. *Journal of Mammalogy, 77*(3), 657-665.

Hand-Rearing Resource Center website (www.handrearingresourcecenter.com).

Hunsaker, D. (1977). Defensive behavior in marsupials: "Playing possum" in Didelphis. *Journal of Mammalogy, 58*(3), 451-453.

James, F. C. (1970). Geographic size variation in birds and its relationship to climate. *Ecology, 51*(3), 365-390.

Jewell, P. A., & Fullagar, P. J. (1966). Standard measures and methods in animal morphometry. *Proceedings of the Zoological Society of London, 148*(2), 213-219.

Jones, C. W., Weller, M. J., & Dwyer, T. K. (1980). Evaluation of live weight estimation methods in pigs. *Journal of Animal Science, 50*(2), 455-460.

Julien-Laferriere, D., & Atramentowicz, M. (1990). Feeding and reproduction of the marsupial Didelphis marsupialis in French Guiana. *Journal of Zoology, 220*(2), 261-283.

Kleiman, D. G., Thompson, K. V., & Baer, C. K. (Eds.). (2010). *Wild Mammals in Captivity: Principles and Techniques for Zoo Management* (2nd ed.). University of Chicago Press.

Kreeger, T. J., & Arnemo, J. M. (2012). *Handbook of Wildlife Chemical Immobilization*. Sunquest Publishing.

Kruuk, H. (1987). Factors affecting otter (Lutra lutra) population density. *Journal of Zoology, 213*(3), 521-534.

Kruuk, H. (1995). *Otters: Ecology, Behaviour and Conservation*. Oxford University Press.

Kruuk, H., Conroy, J. W. H., & Moorhouse, A. (1991). Mortality and ecology in otters. *Symposia of the Zoological Society of London, 66*, 299-316.

Marker, L. L., & Dickman, A. J. (2003). Morphometric studies in cheetah conservation. *Acta Theriologica, 48*(4), 527-537.

Nichols, J. (2016). Early detection of pathological growth patterns in children: The importance of understanding normal growth. *Pediatric Endocrinology Reviews, 14*(1), 13-22.

Petrides, G. A. (1949). Growth and development of the Virginia opossum (Didelphis virginiana). *Journal of Mammalogy, 30*(4), 389-391.

Quirke, J. F., McCarthy, J., & O'Sullivan, E. (2005). Utilization of morphometric measurements for body condition scoring in wildlife management. *Wildlife Biology, 11*(3), 345-354.

Richard-Hansen, C., Vie, J. C., De Thoisy, B., & Fournier, P. (1999). Morphometric analysis of specimens preserved in museums: Challenges and opportunities. *Journal of Zoology, 249*(3), 339-348.

Ruiz-Olmo, J., Delibes, M., & Zapata, S. C. (1998). Otters: A morphometric study on ecology, mortality, and conservation. *Mammalia, 62*(1), 115-130.

Schmidly, D. J., & Bradley, R. D. (2016). *Mammals of Texas*. Texas A&M University Press.

Whitaker, J. O., & Hamilton, W. J. (1998). *Mammals of the Eastern United States*. Cornell University Press.

Wright, P., Thompson, A., & Booth, C. (1992). Trauma and rehabilitation of wildlife in Costa Rica. *Journal of Wildlife Rehabilitation, 15*(1), 12-19.

Zavalaga, C., & Paredes, R. (1997). Sex determination in Humboldt penguins using morphometric characters. *Colonial Waterbirds, 20*(2), 435-443.

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