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Tracking growth in opossum (*Didelphis Marsupialis*) rehabilitation: a focus on nutritional interventions

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INDEX **PAGE**

FIGURE INDEX **PAGE**

TABLE INDEX **PAGE**

Summary

The primary goal of wildlife rehabilitation is to care for sick, injured, orphaned, and displaced wild animals to return them to their natural habitat. This involves monitoring and acting on various aspects of animal welfare. A fundamental part of the animal's rehabilitation is certainly nutrition. It is therefore crucial to identify the macro- and micronutrients required for the growth and recovery of the animal as well as the methods of feeding. However, as specified above, there are other parameters, both physical, physiological, and behavioural, that must be taken into account in this process.

One of the animal species that is most commonly received at wildlife rehabilitation centres in Central America is the common opossum (*Didelphis marsupialis*). The rehabilitation of this species is not as simple as people may think, as there is little information about it, and consequently, reference is often made to the Virginia opossum species (*Didelphis virginiana*), as it is very similar in terms of nutrition and behavioural characteristics.

During my internship at Alturas Wildlife Sanctuary (Costa Rica), I was assigned a project where I had to monitor the *Didelphis marsupialis* orphans' growth once arrived at the centre. The research was carried out on twelve subjects over a total period of two months. Their growth patterns were studied through morphometric analysis of selected body parts and observations of behavioural and nutritional changes.

The main objectives of the research project were:
1) to collect useful data on the growth of rescued common opossums in order to increase knowledge of their normal growth patterns
2) to contribute to the standardization of morphometric data collection methods
3) to correlate morphometric changes to a correct or incorrect nutrition
4) to correlate morphometric changes to specific pathologies
5) to contribute to the protocol creation for the common opossum rehabilitation

The data collected was then useful to the Sanctuary staff both for monitoring the growth of the animal and for identifying any problems in its rearing. In addition, data were used in the research and development of an age-specific diet.

1. Introduction

1.1. Small introduction to the species: ecology and biology

Of the 270 species of marsupials in the world, approximately 70 species are found in the New World, principally in South America. The only marsupial that we can find in the Americas is the opossum, and Costa Rica is home to at least 9 species. In particular, the common opossum (*Didelphis marsupialis*) inhabits much of Central and South America, going from Mexico to Brazil, and down to Argentina. In addition, they have been introduced to several islands in recent times (Brito, et al., 2008; Cerqueira and Tribe, 2008).

Common opossums are considered habitat generalists as they are very adaptable to many environments and can thrive in human-altered environments. While they avoid very high elevations (below 2000 meters on average) and extremely dry areas, they can be found in mountainous regions in Costa Rica and can survive in areas with different levels of precipitation, indeed they prefer tropical, subtropical, old growth, evergreen, and gallery forests in lowland regions (Vaughan et al., 1999). This specie is known to primarily be active on the ground or in the understory vegetation of canopy forests (Malcolm, 1991; Lambert et al., 2005). However, through trapping studies, spool-and-line-tracking, and direct observations (Charles-Dominique et al., 1981; Rasmussen, 1990), it has been found that they do climb trees for resting, grooming, eating fruit, or visiting nectar-producing flowers.

The common opossum belongs to the family of *Didelphidae* and is a robust marsupial with thick fur that gives it a somewhat unkempt appearance. Its back fur is usually dark, ranging from blackish to greyish, though occasionally it may have a whitish hue. On the other hand, its belly fur is yellow or cream-colored. These animals typically have white fur on their faces, with a dark stripe that extends to the top of their heads and a black ring around their eyes. Their ears are big and completely black. Common opossums have sharp claws, long whiskers, and a mostly bare, prehensile tail that is slightly longer than their body. Their sizes vary having a head and body length ranging between 260-400 mm, a tail of 300-450 mm and weighting between 0.6-2.4 kg (Reid, et al., 2022). The main sexual dimorphisms between the two sexes are the size, the adult male being larger and having longer canines than the females, and the presence of a pouch in the female.

Common opossums have a short lifespan, typically living less than 2 years in the wild. They face high mortality rates before reaching adulthood and while caring for their young (Kajin, et al., 2008; Reid, 2009; Sunquist, et al., 1987). Collisions with cars are a common cause of death for common opossums (Pinowski, 2005).

Common opossums are solitary and nocturnal. Throughout the day, they typically stay in their burrows, which can be found in various locations such as tree cavities, underground, in palm, in the

tree canopy, or in abandoned nests of other species. These animals do not stay in one burrow for very long, with males typically remaining in a den for about 1.5 days on average and females staying for about 5.1 days (Adler, et al., 2012; Julien-Laferriere and Atramentowicz, 1990; Reid, 2009; Sunquist, et al., 1987; Vaughan, et al., 1999). Male common opossums have a significantly larger home range compared to females. On average, females occupy 12-16 hectares, while males have a home range of approximately 123 hectares. Male home ranges often overlap with other males' and females' ones, with an average of one individual per hectare (Brito, et al., 2008; Sunquist, et al., 1987).

This specie can be more accurately described as generalized predator as it has a very broad diet. Their diet is diverse, consisting of invertebrates, vertebrates, leaves, fruits, nectar, and carrion. Common opossums may vary their food intake seasonally, consuming mammals and birds more frequently during the dry season and relying on fruits, snakes, and toads during the wet season (Almeida-Santos, et al., 2000; Cordero and Nicolas, 1987). Invertebrates such as earthworms, beetles, and grasshoppers are always a key component of their diet, regardless of the season.

Common opossums have a polygynous mating system, where males compete for females to reproduce with (in February and July). While these animals are primarily solitary, they temporarily come together for breeding purposes. Didelphids do not engage in courtship behaviours or form lasting pair bonds. Females have two uteri and two vaginas, but the young are born through a third canal that forms only temporary. To adapt to the females' reproductive system, the male possesses a bifurcate penis. Females give birth after 12 ½ to 13 days to approximately 20 larvae that immediately make their way to the mother's teat for lactation (Wainwright, 2007). Unfortunately, only a few survive and once attached to the teat their mouth develops and tighten around it. There they remain for approximately two months once they reach 16 cm and weight 100 g (Reid, et al., 2022). After this period, they exit the pouch and stay usually on the mother's back.

Their role in the ecosystem is fundamental as they are not only carrier of different parasites (Jimenez, et al., 2011), but they are also prey of many different animals such as ocelots, jaguarundis and harpy eagles (Gustavo, et al., 1990). Moreover, they play a crucial role in the seed dispersion as they move some seeds due to ingestion after eating fruits, such as for cecropia (Castillo-Flores and Calvo-Irabien, 2003; Medellin, 1994).

1.2 Captive management of the opossum *Didelphis marsupialis*: Description of structures and management practices in orphanages and rehabilitation centres

The captive management of wildlife includes important aspects such as valuation, handling, nutrition, welfare and maintenance of the animals which are embraced by all environmental entities

or authorities and people who work constantly with wildlife. Therefore, when an animal arrives in a rehabilitation centre or orphanage, there are different practices and parameters that have to be followed. As little references and rehabilitation guidelines on the common opossum management can be found, reference should be made on similar species (*Didelphis virginiana*).

1.2.1 Enclosure design and housing method

According to the age of arrival to the centre, and after the medical evaluation of its health status, the animal has to be housed in an appropriate enclosure. It is always important to keep in mind that common opossums are solitary species (Reid, et al., 2022; Wainwright, 2007) and especially during quarantine or hospitalization, they have to be housed singly. When they are little, the newborns should stay together, but as they grow older, they should be separated. Females can stay together for a while but males should be isolated to avoid aggression (Arcangeli, J. 2014).

Healthy adult opossums should be housed outdoor, however indoor housing is also possible but for a limited period. If the animal is housed indoor, a modified rabbit or ferret cage is often used (Johnson-Delaney, 2014; McRuer, et al., 2009). Juveniles over 350-400g and adults should ideally be housed in large, outdoor cages ranging from 1.2 m X 1.2 m X 2.4 m to 3.0 m X 3.7 m X 2.4 m with branches for climbing (Pollock C, et al., 2018; Gage L.J. 2002; Miller E.A., 2012). Housing for an injured adult opossum should be approximately 0.6 m X 0.6 m X 0.6 m (Gage L.J. 2002). In any case, as they usually live in dens and dark places during the day, the outdoor enclosure should be in a shade area and covered, at least partially, by a dark towel. It is important, however, that they can have direct access to sunlight for the absorption of vitamin D. The substrate of the outdoor enclosure should not be mesh, instead, leaves or straw should be used.

Nursing/Pre-weaned opossum can be housed in sturdy boxes or aquariums/incubators of about 0.9 m X 0.9 m X 0.9 m (Miller E.A., 2012). An aquarium/incubator is preferred, as humidity and ventilation are much easier to control. Always place a heating pad under the container, not in it, and make sure it is only under a portion of the “nest” area or use a warm light source (Gage L.J., 2002; Miller E.A., 2012). As substrate paper strips as well as towels or T-shirts can be used.

Parameters such as temperature, humidity and ventilation are fundamental to control. Drastic changes in them can lead not only to physical problems, but also to the death of the animal. For individuals housed indoor the temperature should be as their body temperature, approximately 36°C, and a humidity similar to the one of the outside environments that can change between seasons (Arcangeli, 2014).

1.2.2 Enrichment

In order to prepare opossums for release back into the wild, it is important to promote natural behaviours through enrichment. Environmental enrichment is mostly used. This can include adding climbing structures like logs or branches in order to enhance the locomotory activity as well as use of the prehensile tail (hanging behaviour). Using leaves as substrate promotes the natural behaviour of grasping them with the tail and moving them into the nest. On the other side, hanging them around the enclosure, will promote the hiding behaviour as well as provide some shelter. Providing a nest made of natural material such as wood or bamboo can reproduce their natural nesting site also in captivity (Miles et al., 1981; Moraes and Chiarello, 2005b; Delciellos et al., 2006; Miller E.A., 2012).

Food enrichment is also a fundamental aspect to consider. Providing them with live prey for hunting practice, encouraging foraging behaviour by the use of scatter feeding, and allowing outdoor exploration to acclimate them to their surroundings is crucial. It is also recommended to keep their shelter in a dimly lit area to support their nocturnal habits. By engaging in these activities, young opossums can extend their active periods and learn to cohabit peacefully with other individuals (Vivas-Serna, C., 2016).

1.2.3 Age determination

Age determination is not so simple in this specie; furthermore, no studies on the common opossum age determination have been done until now. However, it is crucial to understand the age of the animal in order to know how to manage it. Therefore, nowadays many rehabilitation centres use two main ways to determine the age: the use of tables of age determination in *Didelphis virginiana* (Arcangeli, 2014) or the dental formula technique used for *Didelphis albiventris,* which consists of observing the eruption and tooth wear of the upper jaw (Petrides, 1949; Reynolds, 1952).

Table 1. Estimation of the age of a baby opossum in its first 90 days of life based on morphological description (Petrides, 1949; Reynolds, 1952).

DAYS	MORPHOLOGIC DESCRIPTION
1-15	Begin development of the hind legs. Voluntary tail twisting
17	Sex organs are observed
20-25	Ears free from the head. They can move their hind legs
35	Vibrissae (whiskers) and hair begin to grow
50	The length of the body from head to tail is about 10-12 cm. The coat is short and sparse. The back is already pigmented. The eyes and mouth are partially closed.
60	Eyes can be opened. The hair is already dark and covers the body. Eruption of the third premolar occurs.
70	The hair is long and completely covers the body, except for those areas that will be naked. Eruption of the second premolar.
80-90	The length of the body from head to tail is about 20 cm. Incisors, canines and the first premolar appear.

1.2.4 Nutrition and hydration status

Feeding opossums in a rehabilitation centre requires careful attention to their nutritional needs at different stages of their development. First of all, the general guidelines take care of both the dietary diversity and the hydration status of the animal. As the common opossum is a generalist, its diet should include a variety of foods to mimic what they would find in the wild. For very young, orphaned joeys, a formula designed for marsupials, such as puppy milk replacer (cats or dogs), is typically recommended. It is important to find a formula that closely mimic the mother's one and to follow the manufacturer's instructions for mixing and feeding. As they grow, the formula can be mixed with solid foods. They are usually fed from multiple times per day (Gage, 2002; OSUS, 2021; Ruth, 2012). Juvenile opossums can be fed three times a day, transitioning to twice a day as they approach release age. The diet varies with always a source of protein, some mixed fruits and vegetables and a calcium source (Hoppes, 2014; Fowler, et al, 2008). Adult opossums typically eat twice a day. The diet is similar to the juvenile's one with a focus on the variety of protein source administered. Fundamental is to present the animal with natural diet, that they will encounter in the wild, such as native fruits, insects, and small vertebrates. Both for juveniles as well as adults, as they are approaching the release moment, scatter feeding or hiding food in natural enrichment provided in the enclosure is suggested (Gage L.J. 2002; OSUS, 2021; Ruth, I. 2012).

More information about this topic will be analysed in the chapter 1.4.

When administering the food, it is always important to wear the “special” uniforms to avoid imprinting.

Hydration is crucial for the rehabilitation of the common opossum, particularly for those that are young or have been compromised by illness or injury. Fresh water should always be accessible, and for dehydrated opossums, an electrolyte solution like Pedialyte can be administered to restore their fluid balance. In cases where opossums are unable to drink on their own, subcutaneous or oral rehydration methods may be necessary. Monitoring hydration levels is essential, as it plays a significant role in their overall recovery and health. Regularly checking for signs of dehydration, such as skin elasticity and moisture levels in the mucous membranes, ensures that the opossum receives appropriate care (Ruth, 2012).

1.2.5 Hygiene: Cleaning and disinfection

The opossum hosts a wide variety of parasites, with one study identifying up to 46 different internal and external species (Jimenez, Catzeflis, and Gardner 2011). These parasites include various worms such as cestodes, nematodes (e.g., Ancylostoma caninum, Trichinella spiralis, Alaria marcianae, Paragonimus spp.), acanthocephalan, and protozoan parasites (e.g., Leishmania infantum, Trypanosoma cruzi, Toxoplasma gondii) located in their intestines (Tasca et al. 2001; Bezerra-Santos et al. 2021). Additionally, opossums can carry viruses and bacteria that pose zoonotic risks to humans, including yellow fever, rabies, and leptospirosis. Therefore, maintaining a proper hygiene both of the animal, its enclosure, enrichment and feeding instruments is crucial during the rehabilitation process. After feeding, all utensils should be cleaned by soaking them in a mixture of 3 tablespoons (15 ml) of liquid bleach in a dishpan filled with hot, soapy water. Then rinse them thoroughly. For crock bowls, plastic lids, and jars, it can be used a dishwasher. It's best to wash animal dishes separately from other dishes. All bedding should be washed with hot water, detergent, and 1/2 cup (120 ml) of liquid bleach per load. A small amount of unscented liquid fabric softener can also be added. It is important to use a brush to scrub cages and the enrichments with a solution of 1 tablespoon (15 ml) of liquid bleach in 1 cup (240 ml) of hot water. After this process they have to be rinsed well and let them dry in the sun. It is crucial to make sure to clean the cages thoroughly after each litter (Gage L.J. 2002).

If cardboard boxes are used, they should be changed once a week and the paper stripes (substrate) every second day (Vivas-Serna, C., 2016). To prevent the hatchlings from disposing of their waste inside the cardboard box, a latrine can be made by covering a plastic container with newspaper. Each time they feed, the opossums are placed there. As soon as they eliminate their urine and droppings, they are removed and the newspaper is changed (Arcangeli, 2014).

Without a proper cleaning and disinfection process it is very easy to transmit a pathogen both to humans but also between individuals in rehabilitation.

1.2.6 Handling

It is advisable to minimize the handling of young individuals, suggesting that only one person takes care of handling them. This person should gradually reduce the contact with the newborn until handling is limited to feeding and cleaning (Arcangeli, 2014). As a general rule, captive wildlife should be managed in a manner that reduces their interaction with humans to minimize stress and prevent imprinting. This is particularly important when handling offspring, as imprinting on humans can hinder their ability to be relocated effectively.

To safely handle a small opossum that is still regulating its body temperature, it is best to approach from behind and either pick it up by the tail or body. Avoid going over their head, as this can be perceived as threatening and may result in a harmless snap. When handling the opossum, be sure to gather up its legs and tail within your hand or transfer it to a piece of cloth to wrap up. If the opossum's tail is loose, it may whip around looking for purchase, while its feet may grab onto anything in their path. If the opossum's nails grab holds of its bedding or cage, gently use a forward, then up motion to unhook their little feet to prevent injuries. Pay close attention to the positioning of each foot and tail as you lift the opossum from its enclosure to avoid causing harm.

To safely handle an opossum, grasp it by the tip of the tail if it is under 1-3 kg, keeping your hand far from its mouth. If the opossum is facing you, gently push its head away with a rolled-up newspaper (or similar object) to expose the tail for easier handling. Lift the opossum by the tail, support its body with the newspaper (or similar object) between its legs, and keep it at arm's length to prevent it from grabbing onto you. Place the opossum in a carrier nearby to avoid it escaping. For opossums over 1-3 kg, hold them by the base of the tail to prevent injury. Always be cautious and keep the carrier open and close by for secure transport.

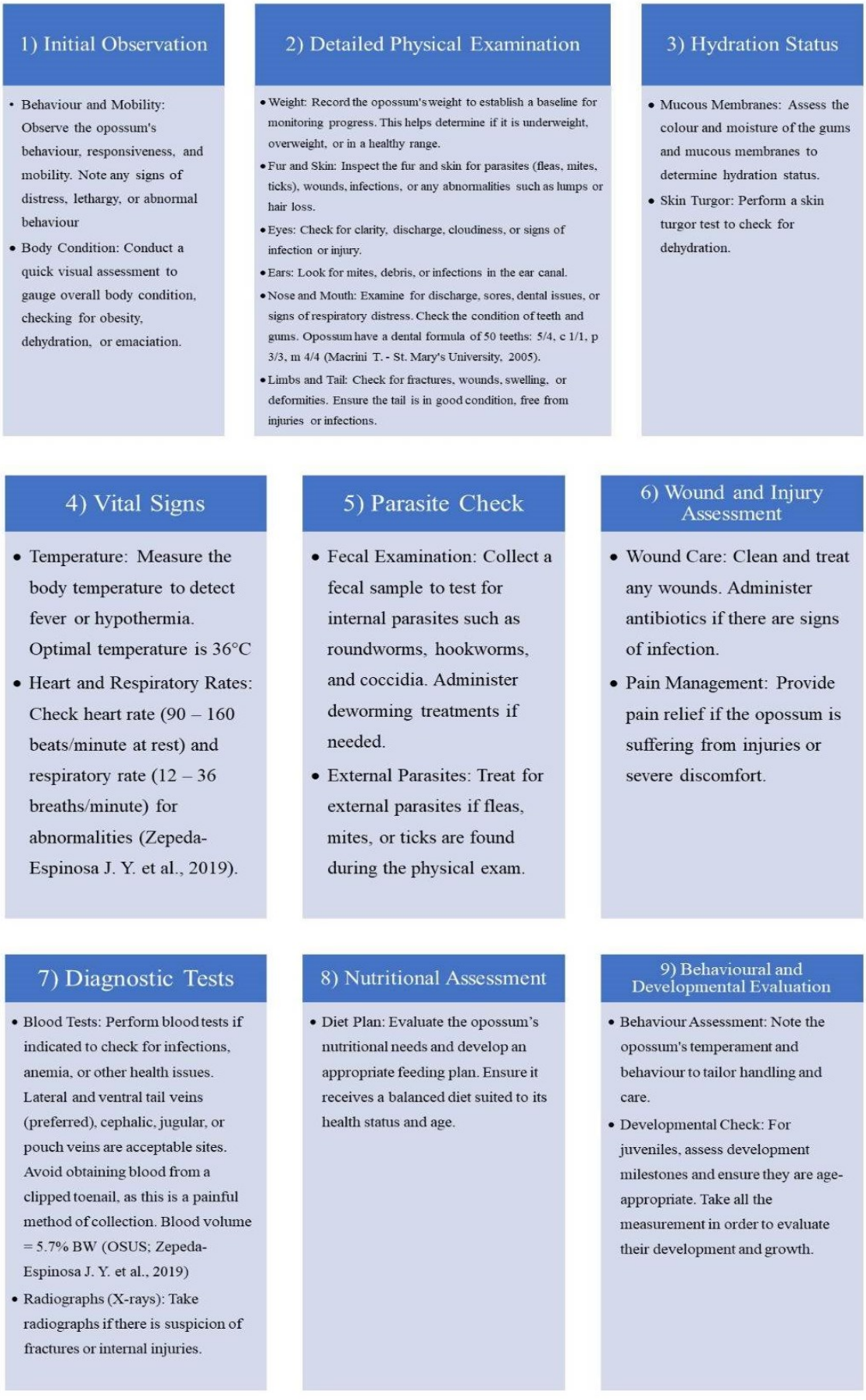
To handle a tail-less opossum, use a rolled-up newspaper (or similar object) to gently push its head away from you to prevent biting. Assess its reaction by lightly touching its posterior part of the body to see if it tries to bite. Continue to push its head away while moving your fingers up its body, eventually pinning its shoulders down with your thumb and forefinger behind its front legs. This prevents the opossum from turning its head to bite. Grasp it by the shoulders and then secure its tail base with your other hand to safely transport it to a carrier (OSUS, 2019).

1.2.7 Health checks

Upon the admission of a common opossum to a rehabilitation centre/orphanage, a thorough initial health assessment is critical to identify any immediate health concerns and to develop an appropriate care plan.

The following are the key health checks typically performed during the admission process (Miller E.A., 2012; Ruth I., 2012):

Figure 1: Health check steps done at the arrival of the patient and routinely



Once all these checks have been carried out, if the animal is at risk or appears to be at risk of transmitting infections or parasites, it must be isolated, quarantined and monitored closely. Furthermore, when an animal arrives at a rehabilitation centre, it is essential to collect information regarding the circumstances of rescue, previous injuries, or known medical conditions.

The health checks to be carried out routinely are most of those mentioned above. In particular, one focuses mainly on observing the animal and its behaviour to detect certain abnormalities or signs of pain. In addition, especially for juveniles, it is important to monitor their growth associated with constant measurements of their weight and body parts. Monitoring the animal allows us to ensure that, in the event of problems during rehabilitation, solutions can be found to improve their well-being (OSUS, 2024).

1.2.8 Techniques to avoid imprinting

Avoiding imprinting in the rehabilitation of common opossums is crucial to ensure they retain their natural behaviours and survival instincts for successful release back into the wild. Firstly, during their management, it is important to minimize human interaction, and therefore limit the handling of the animal as well as the number of caregivers interacting with them. It is crucial to handle opossums as little as possible and only when necessary for medical care or feeding. Use towels or gloves to avoid direct contact.

Secondly, it is fundamental to use species-specific enclosure with naturalistic habitat design as well as enrichment. This, in order to mimic the animal's natural habitat, including features like plants, logs, rocks, and water sources.

Whenever possible, animals of the same species, if it is not dangerous for them, should be housed together. This, not only promotes their health and safety, but it also helps them form proper social bonds and behaviours with their conspecifics (Miller, 2012). In case of introduction of two conspecific, gradual step-by-step introduction should be done. When possible, use adult animals of the same species as role models or surrogates to teach juveniles appropriate behaviours. For some species, mechanical or stuffed surrogates can be used to provide comfort and reduce dependency on human interaction.

Direct feeding should be minimized, avoiding hand-feeding by using automated feeders or place food in the enclosure for the animals to find themselves. In case of very young animals, that require hand-feeding, use specialized puppets to feed them. It is of fundamental importance to encourage the natural foraging behaviour by scatter food or by hiding it in the enclosure.

During the feeding or handling of the animal, special "uniform" to prevent animals from recognizing the caretaker should be used.

They include: a lab coat or coveralls, gloves, rubber boots, a mask.
Finally, placing enclosures away from high-traffic human areas and use barriers to minimize visual and auditory exposure to humans can actually help the animal to reduce stress and prevent imprinting (BCSPCA, 2022; OSUS, 2019).

1.3 Desired Physiological and behavioural conditions to be released

Deciding whether an animal is capable of surviving in the wild for any length of time after release requires careful consideration. Therefore, specific observation and evaluation should be done. As we do not have guidelines related to the release parameters of the common opossum, general ones used for the rehabilitated wildlife should be considered. A brief physical exam should be conducted to ensure the patient is healthy and ready for release. Candidates for release must generally meet the criterias described in table 2 (Miller, 2012).

Table 2: Criteria to be met for the release of a rehabilitated animal

Physical/Physiological consideration	Exhibit full recovery from the original injury or any injuries sustained during care: Recovery from injury or illness involves several key points: 1) Identify and address both primary and secondary problems early on to avoid delaying recovery and release. Do comprehensive examinations using diagnostic tools such as radiographs, faecal analysis, and blood work, along with the assistance of an experienced veterinarian for accurate diagnosis and treatment. 2) Recovery should not be assumed; follow-up testing should be conducted to confirm improvements. 3) Be vigilant for nosocomial illnesses and injuries, which can occur in a rehabilitation setting and must be promptly recognized and treated to prevent spread and ensure the animal is fit for release
	Have normal laboratory values, if tested (e.g., PCV, TS, BUN)
	Possess adequate pelage for their species to survive: A thin layer of fur and fat reserves are crucial for many animals' protection against cold, wind, and water. The condition of this insulation at release is vital for survival. Animals sheltered during rehabilitation must be acclimated to current weather conditions before release, particularly hand-raised orphans and those released in winter
	Possess functional completeness: Demonstrate necessary locomotive skills for survival and have sufficient vision to find and catch food and navigate normally. In case of handicap, specific evaluation should be made in order to understand if the animal, once released, will have a negative impact due to it.
	Be of correct weight for their sex, species, age, and season: Age alone is not a sufficient indicator of release readiness for a hand-raised animal. Suggested release ages in the literature should be regarded as the average time when hand-raised individuals of a species typically reach a suitable stage of physical, psychological, and behavioural development for release. For hand- raised species of *Didelphis virginiana* the proper length to be reached for the release is more or less 30 cm (minimum) and be at least five months old (Diehl, S., 1991).
	No longer require medical treatment
	Be acclimatized to the outside environment: All animals need to be housed in an outside environment for at least two weeks prior to release (unless they have only been in care for a very short time).
Psychological/Behavioural consideration	Exhibit appropriate fight or flight responses
	Display normal species-specific behaviours: An animal's behaviour can be evaluated by comparing it to that of healthy wild counterparts. The six key behavioural areas to consider when developing any rehabilitation program are: capability of acquiring and processing food, avoiding predators, interacting appropriately with conspecifics, finding or constructing nests and shelters, orienting and navigating within a complex environment, and, for territorial species, defining the limits of their territories (Kleiman, 1989).
	Do not display imprinted or tame behaviour: animals should avoid and escape from humans

In addition to the animal's condition, several other considerations are crucial for a successful release. A suitable habitat with sufficient food and water supply, appropriate weather conditions, season, and time of day are essential. Releases must comply with local, state, and federal regulations or laws. Factors such as proximity to busy roadways, presence of natural or introduced predators (e.g., domestic cats), human developments, existing populations of the species, and long-term availability of food sources must all be evaluated to determine the suitability of a release site (Diehl, S., 1991; Miller, E.A. 2012).

1.4 Evaluation of the opossums' natural diet and nutritional requirements

The opossum is a highly adaptable, generalist feeder. Known for its opportunistic omnivorous diet, *Didelphis* species can survive on a wide variety of foods (Lay, 1942; Stieglitz & Klimstra, 1962). Research on *Didelphis marsupialis* in Venezuela indicates that their diet consists of 63.5% animal matter and 22.9% plant matter by volume (Cordero & Nicolas, 1987). Another study found the diet to be more balanced, comprising approximately 50% animal matter and 50% fruit (Julien-Laferriere and Atramentowicz, 1990). Therefore, evaluating the natural diet and nutritional requirements of the opossum *Didelphis marsupialis* is essential to inform rehabilitation feeding practices.

1.4.1 Identification of macro- and micronutrients essential for Opossum health and growth

To evaluate the diet and understand which macro- and micronutrients are essential for the common opossum, the knowledge of the morphometry of the gastrointestinal tract of this species is crucial. Literature found refers only on the structure of the specie *Didelphis marsupialis insularis*. However, most *Didelphid marsupialis* appear to possess the same basic structures (Hume, 1999). The species *D. m. insularis* was found to have a simple, unilocular stomach; with a small intestine making up the majority of the GI tract and a short but wide large intestine with a large caecum. It is characterized by features of the carnivore GI tract as well as features of the hindgut fermenters. The possession of a simple stomach may indicate that its diet includes high proportion of animal protein (Santori et al., 2004). In addition, the lining of the oesophagus mucosa by stratified squamous epithelium (not keratinized), is a feature that is usually found in carnivores and indicates that their diet includes animal tissues (Eurell & Frappier, 2013). On the other, hand, a well-developed large intestine indicates an adaptation to increase water absorption from some fruits (Charles-Dominique et al., 1981; Santori et al., 2004). Moreover, they possess a large caecum that has an important role in the plant fermentation and food vitamin storage (Carceres 2005). Finally, Ascorbic acid (Vitamin C) synthesis occurs in the liver of the opossum, allowing this species to produce its own Vitamin C (Hume 1999).

For what it concerns the diet, the essential macronutrients for the common opossum (*Didelphis marsupialis*) are proteins, lipids and carbohydrates and can be derived by their natural wild diet. However, there is not a clear and specific diet of this species; therefore, a general framework of the diet component will be described.

PROTEINS:

Proteins are crucial for the growth, repair, and maintenance of tissues. In the wild, opossums obtain protein from sources such as insects, small mammals, eggs, and carrion. A protein-rich diet supports their muscle development and overall health. Dietary studies of Didelphids, based on fecal and stomach analyses, show that these animals do not specialize in a single type of invertebrate prey. Commonly consumed invertebrates include beetles, ants, roaches, and crickets. Ants may be overrepresented in these studies due to accidental ingestion with other food items. Millipedes and harvestmen are commonly eaten by ground-foraging opossums, while arboreal species often consume Hemiptera and Lepidoptera. Occasional consumption of spiders, isopods, and centipedes is also noted. Invertebrates provide high protein but low carbohydrates and lipids (Redford and Dorea, 1984). Didelphids also prey on small mammals, birds, lizards, and frogs, with rodents being the most frequently identified mammalian prey (Voss, R. S. et al., 2021), although small Didelphids are sometimes eaten by large opossums (e.g., Monodelphis by Philander; Macedo et al., 2010). Vertebrate tissues, rich in protein and lipids, are highly digestible despite the challenges in capturing such prey. Didelphids have evolved mechanisms to neutralize toxins from defended vertebrates like toads, newts, and venomous snakes, so they become sometimes their preys. In addition, even though reported in just one study done in Panama (Mangan and Adler, 2000), it has been found that fungi may be a cryptic component of many Didelphid diet and therefore be also a potential source of protein.

LIPIDS:

Lipids provide a concentrated source of energy, essential for the high metabolic demands of opossums. They also play a role in the absorption of fat-soluble vitamins (A, D, E, and K) and the maintenance of healthy skin and fur. The essential lipids for this species are Omega-3 and Omega-6 fatty acids. These essential fatty acids are crucial for maintaining healthy cell membranes, supporting brain function, and reducing inflammation. They are found in various animal fats and certain plant oils. As the primary form of stored energy, triglycerides are essential for sustaining the opossum's high metabolic rate. They are obtained from both animal and plant sources in their diet. Phospholipids are important components of cell membranes and are involved in signalling pathways within the body. Phospholipids are found in animal tissues and egg yolks. Cholesterol is necessary for the synthesis of certain hormones, vitamin D, and bile acids, which are critical for digestion.

Cholesterol is naturally present in animal-based foods (Voss, R. S. et al., 2021; Hume, I., 1999; Reynolds, R. J. et al., 1997).

It is important to remember that Didelphids lack of appropriate dental adaptation to eat seed endosperm, therefore other plant-based sources are eaten.

CARBOHYDRATES:

Carbohydrates are an important energy source and are obtained primarily from fruits, vegetables, and other plant materials. Carbohydrates also provide dietary fibre, which aids in digestion. Opossums consume a wide variety of fruits, as shown by studies observing their feeding behaviours and analysing stomach contents in French Guiana. It was documented that they are consuming fruits from canopy trees, understory treelets, lianas, stranglers, and hemiepiphytes (Atramentowicz, 1988). The fruit pulp they eat is rich in sugars and lipids but low in protein. Consumable parts of these fruits (such as the aril, exocarp, epicarp, mesocarp, pericarp, and sarcotesta) are either exposed or lightly protected when ripe. Canopy trees, often producing large, synchronously ripening crops, offer fruits that arboreal opossums eat in situ, while terrestrial species consume fallen fruits (Charles-Dominique et al., 1981). Additionally, opossums derive carbohydrates from nectars and gums. Nectars are easily digestible but lack significant quantities of other nutrients (Nicolson & Thornburg, 2007). Gums, primarily composed of complex polysaccharides, require bacterial fermentation in the gut and may contain useful minerals like calcium (Voss, R. S. et al., 2021).

The common opossum (*Didelphis marsupialis*) requires a variety of micronutrients for optimal health. These micronutrients include vitamins and minerals that play crucial roles in various physiological processes. Here in the table some of the essential micronutrients for opossums are reported:

Table 3: Essential micronutrients in the Didelphis marsupialis' diet (Allen, et al., 1993; Hume, 1999)

MICRONUTRIENTS	FUNCTION/SOURCE
Vitamin A	Essential for vision, immune function, and skin health. Sources: Animal liver from prey, insects, and certain fruits and vegetables such as leafy greens.
Vitamin D	Important for calcium absorption and bone health. Sources: Synthesized through exposure to sunlight, and from consuming fish, eggs, and possibly amphibians.
Vitamin E	Acts as an antioxidant, protecting cells from damage. Sources: Plant oils, nuts, seeds, and green leafy vegetables.
Vitamin K	Necessary for blood clotting and bone health. Sources: Green leafy vegetables, liver from prey, and fermented foods.
Vitamin B (B1, B2, B3, B6, B12, folate, pantothenic acid, biotin)	Essential for energy metabolism, red blood cell formation, and nervous system function. Sources: Insects, small vertebrates, fruits, eggs, and leafy greens.
Calcium	Vital for bone and teeth health, muscle function, and nerve signalling. Sources: Bones of prey, eggshells, and leafy green vegetables.
Phosphorous	Work with calcium to build bones and teeth and is important for energy metabolism. Sources: Bones and tissues of prey, nuts, seeds, and plant materials.
Magnesium	Important for muscle and nerve function, blood sugar control, and bone health. Sources include nuts, seeds, whole grains, and leafy green vegetables.
Iron	Essential for the formation of haemoglobin in red blood cells, which carries oxygen throughout the body. Sources: Meat from prey, insects, and fortified plant materials.
Zinc	Supports immune function, wound healing, and DNA synthesis. Sources: Meat, shellfish, insects, and legumes
Copper	Important for iron metabolism and the formation of red blood cells. Sources: Liver from prey, nuts, seeds, and shellfish.
Manganese	Involved in bone formation, blood clotting, and reducing inflammation. Sources: Nuts, seeds, whole grains, and leafy vegetables.
Selenium	Acts as an antioxidant and supports immune function. Sources: Nuts, seafood, and meat.
Iodine	Necessary for the production of thyroid hormones, which regulate metabolism. Sources: Seafood, eggs, and plant material grown in iodine-rich soil.

To sum up, the availability of certain food items may vary according to the season and also to the habitat in which the animal is living. Moreover, Didelphis spp. exhibit significant dietary and substrate utilization changes as they age. Young opossums primarily consume insects and plants and show a higher degree of arboreality. In contrast, older opossums have a diet richer in vertebrates and carrion and are more terrestrial (Fonseca, et al., 1990; Cordero, et al., 1987).

1.4.2 Nutrition during rehabilitation in the orphanage: Analysis of the feeding practices used in the orphanage for the opossum *Didelphis marsupialis* and evaluation of the adequacy of the diets provided in terms of nutritional composition and energy intake (based on the different life stages).

Feeding practices in orphanages and rehabilitation centres for the common opossum (*Didelphis marsupialis*) need to be carefully analysed to ensure that the diets provided meet the nutritional and energy requirements of opossums at different life stages. The information below is mainly taken from the source: "Hand-Rearing wild and Domestic Mammals" of Gage L.J. 2002 and refers to the species *Didelphis virginiana.* Therefore, when considering the energy requirements consider the difference in the climate, habitat and activity level between the two species.

FEEDING YOUNG OPOSSUM (JOEYS):

For very young, orphaned joeys, a formula designed for marsupials, such as Esbilac puppy milk replacer, is typically recommended. The feeding frequency depends on their age and size. Newborns have to be fed every 2-3 hours, including at night, 2-3 weeks old joeys every 4 hours, 4-5 weeks old joeys every 5 hours and 6-7 weeks old ones every 6 hours, with a longer interval at night (Gage, 2002; OSUS, 2021; Ruth, 2012). Feeding techniques include tube feeding or syringe feeding (using 1-cc to a 3-cc syringe and a Tomcat catheter or IV one without needle), having tube feeding as preferred one as it is faster, more accurate and less stressful for the animal. It is important to obtain an accurate weight before deciding the feeding amount and monitor it the days after to keep a record of the animal's growth. Ensure the amount fed adequately fills the stomach without causing discomfort and that the formula is always warm up before feeding. Overfeeding can lead to diarrhoea or stomach paralysis. Monitor the stomach's fullness closely before, during, and after feeding; it should feel soft and gently rounded, not hard and taut. Occasionally, you might see the milk's whiteness as it enters the stomach.

Once a joey arrives in an orphanage, prior to feeding, the opossum needs to be rehydrated and the feeding formula introduced gradually. Rehydrating solutions are lactated Ringer's solution or Pedyalite (Ross). After administering a minimum of three feedings with rehydration solution (or more if the infant is significantly dehydrated), gradually introduce the formula as follows: 1) Mix ¾

part rehydration solution with ¼ part formula; 2) Then, mix ½ part rehydration solution with ½ part formula; 3) Finally, mix ¼ part rehydration solution with ¾ part formula. This in order to prevent digestive problems.

When infant opossums have their eyes open and are reasonably steady (typically weighing 45 grams or more), they can start learning to lap up formula. Since lapping is not an instinctive feeding behaviour for them, they will need encouragement and guidance. When opossums begin lapping on their own and hand feeding is reduced to once or twice daily or stopped when they weigh 60 grams or more, their formula should be thickened by adding ground or whole Purina Kitten Chow mixed with water, creating a "chow-pudding" (Gage L.J. 2002; OSUS, 2021).

Table 4: Opossum feeding chart in relation to the weight and energy requirement

BODY WEIGHT (g)	DAILY CALORIC REQUIREMENT (Kcals/day)	AMOUNT TO FEED (ml) 6 times/day
20	7,43	1,12
30	10,07	1,51
50	14,77	2,22
70	19	2,86
80	21,01	3,16
90	22,95	3,45
100	24,83	3,73

The feeding amount should be at least twice the basal energy requirement daily, typically spread over a 16- to 18-hour period. For marsupials, this requirement can be estimated using the formula: KCALS required = 2.85 x (49 x body weight in kg^ $^{0.75}$).

FEEDING JUVENLE OPOSSUMS:

When the opossums reach a weight of 80-100 grams, introduce the Modified-Jurgelski Diet, which consists of one part ground, raw beef liver and nine parts Purina Kitten Chow-pudding with a calcium source. It's crucial to adhere to these exact measurements. Any deviation, such as altering the amount of liver or using a different brand of kitten food, can lead to metabolic bone problems. Once the opossum is reliably consuming the Modified-Jurgelski Diet, gradually introduce small amounts of various fresh fruits and vegetables, incorporating one new item at a time. Juvenile opossums can be fed three times a day, transitioning to twice a day as they approach release age. Here below the feeding chart for the Modified-Jurgelski Diet for opossum.

Figure 2: Feeding chart for the Modified-Jurgelski Diet for opossum (Gage L.J. 2002; Figueroa, C., 2009)

INSTRUCTIONS

Diet: Mixture of 1 part ground, raw beef liver and 9 parts Kitten Chow®/water mixture, all by volume (i.e., tsp, tbsp, cup, or parts of any of these). Soak Kitten Chow® in enough water to create a pudding-like consistency. Combine 1 part ground, raw beef liver with 9 parts Kitten Chow®/water mixture. The amount of liver is not discretionary. Pulverize Caltrate® 600 tablets (or use a calcium carbonate powder which specifies on the label that it contains 700 to 800 mg of calcium per tsp), and add 1/4 tsp per cup of Kitten Chow®/water/liver mixture. No other supplemental vitamins or minerals should be used with this diet unless recommended by a veterinarian for a specific medical condition.

	%SOLIDS	%FAT	%PRO	%CARBO	KCALS/TBSP
Diet Composition	33.2%	3.3%	13.9%	11.2%	21.2

FEEDING CHART

BODY WEIGHT		DAILY BASAL ENERGY REQUIREMENT	AMOUNT TO FEED PER DAY 2 TIMES BASAL REQUIREMENT (1 tbsp = 3 tsp)	2-1/2 TIMES BASAL REQUIREMENT (1 tbsp = 3 tsp)
OUNCES	GRAMS	(KCALS/DAY)	(To convert to cups: 4 tbsp = 1/4 cup)	
2.5	70.0	9.5	1 tbsp + 0 tsp	1 tbsp + 0 tsp
2.8	80.0	10.5	1 tbsp + 0 tsp	1 tbsp + 1 tsp
3.2	90.0	11.5	1 tbsp + 0 tsp	1 tbsp + 1 tsp
3.5	100.0	12.5	1 tbsp + 0 tsp	1 tbsp + 2 tsp
3.9	110.0	13.4	1 tbsp + 1 tsp	1 tbsp + 2 tsp
4.2	120.0	14.3	1 tbsp + 1 tsp	1 tbsp + 2 tsp
4.6	130.0	15.2	1 tbsp + 1 tsp	1 tbsp + 2 tsp
4.9	140.0	16.0	1 tbsp + 1 tsp	2 tbsp + 0 tsp
5.3	150.0	16.9	1 tbsp + 1 tsp	2 tbsp + 0 tsp
7.1	200.0	21.0	2 tbsp + 0 tsp	2 tbsp + 1 tsp
7.9	225.0	22.9	2 tbsp + 0 tsp	2 tbsp + 2 tsp
9.7	275.0	26.6	2 tbsp + 1 tsp	3 tbsp + 0 tsp
11.5	325.0	30.2	2 tbsp + 2 tsp	3 tbsp + 2 tsp
13.2	375.0	33.6	3 tbsp + 0 tsp	4 tbsp + 0 tsp
14.1	400.0	35.2	3 tbsp + 1 tsp	4 tbsp + 0 tsp
15.9	450.0	38.5	3 tbsp + 2 tsp	4 tbsp + 2 tsp
17.6	500.0	41.7	4 tbsp + 0 tsp	5 tbsp + 0 tsp
19.4	550.0	44.8	4 tbsp + 0 tsp	5 tbsp + 1 tsp
21.2	600.0	47.8	4 tbsp + 1 tsp	5 tbsp + 2 tsp
22.9	650.0	50.7	4 tbsp + 2 tsp	6 tbsp + 0 tsp
24.7	700.0	53.6	5 tbsp + 0 tsp	6 tbsp + 1 tsp
26.5	750.0	56.5	5 tbsp + 1 tsp	6 tbsp + 2 tsp
28.2	800.0	59.3	5 tbsp + 1 tsp	7 tbsp + 0 tsp
30.0	850.0	62.0	5 tbsp + 2 tsp	7 tbsp + 1 tsp
33.5	950.0	67.4	6 tbsp + 1 tsp	8 tbsp + 0 tsp
37.0	1050.0	72.7	6 tbsp + 2 tsp	8 tbsp + 2 tsp

FEEDING ADULT OPOSSUM:

Adult opossums typically eat twice a day. The three main diet components should always be: protein source, fruits/vegetables and calcium source. Adult feeding techniques can be either scatter feeding in the enclosure, so to encourage the foraging behaviour, or on/in natural enrichment (such

as branches or bamboo feeders). It is crucial to administer a variety of protein source, trying to change it every day. Remember to give the animal also bones or food containing calcium. Vegetables include: yellow, red and green vegetables such as carrots, broccoli and spinach, lettuces, cauliflower, cabbage, bell peppers, squashes, etc. Fruits of the season and in all form could be given. Weekly live preys could be given as well as other plant-based food such as grass or flowers. Adults reaching the release moment should be fed more frequently with natural food (OSUS, 2021).

1.4.3 Diet-related problems

Not only an imbalanced diet, but also wrong feeding practices can lead to health problems during the common opossum rehabilitation.

METABOLIC BONE DISEASE

Metabolic Bone Disease (MBD) is a common problem resulting from imbalanced diets, involving defective bone formation or excessive resorption (Kumar, R. et al., 2018). Defective formation includes osteoporosis and rickets/osteomalacia, while excessive resorption leads to bone breakdown. MBD causes can vary but often involve improper calcium to phosphorus ratios and vitamin A or D imbalances (Chen, et al., 2020). Most mammals require a calcium to phosphorus ratio of 1.0:1.0 to 1.2:1.0, and low calcium triggers parathyroid hormone release, demineralizing bones independently of Vitamin D. Vitamin A deficiency leads to vision, immune, reproductive, and growth problems, while excess vitamin A can cause hypercalcemia and osteoclast activity, leading to bone pain and damage (Farrell, et al., 2015). Vitamin D deficiency causes rickets and osteomalacia, while excess vitamin D can lead to hypercalcemia, with symptoms like anorexia, nausea, and kidney damage. To prevent it feed high quality, balanced diet with sufficient Ca and proper Ca:P ratio.

DENTAL PROBLEMS

Dental problems in common opossums (*Didelphis marsupialis*) often result from an improper diet, leading to significant health issues. High consumption of sugary or sticky foods can cause dental decay and gum disease, while the lack of fibrous foods, which help clean teeth naturally, contributes to plaque build-up and dental deterioration. Insufficient calcium intake further weakens teeth and bones, exacerbating these problems. This can lead to painful conditions like tooth abscesses, difficulty eating, and nutritional deficiencies. Moreover, maintaining a soft diet for a long time will prevent the proper growth of the teeth of this species. Preventing dental problems requires a balanced diet with fibrous foods and adequate calcium, avoiding sugary items, and ensuring regular dental check-ups and proper oral hygiene practices (OSUS, 2021).

INHALATION OF THE FORMULA

Especially during the infant feeding, administering too much formula at once or too quickly can cause the infant to aspirate or expel the fluid through the nose. This poses a risk of the fluid entering the lungs, potentially leading to aspiration pneumonia (Ruth, 2012). The presence of foreign material in the lungs can lead to inflammation and swelling. Some substances, like milk formula, can also promote bacterial growth and infection. In severe cases, the volume of foreign material may be so large that it prevents the animal from inhaling enough air to breathe properly. Such extreme instances are more accurately described as drowning, even though the respiratory issue stemmed from improper feeding. Some signs of aspiration are easily noticeable, while others are subtle, develop gradually, and are harder to detect. Wild animals instinctively hide health issues to avoid appearing vulnerable to predators, making it even more challenging to identify emerging problems. The more familiar a rehabilitator is with the animal's normal behaviours, such as activity, respiration, sleep, appetite, and growth, the better they can recognize the subtle early indicators of health issues (Casey, et al., 2012).

DIARRHEA:

Diarrhoea in common opossums (*Didelphis marsupialis*) frequently results from an improper diet or incorrect feeding methods. Diets rich in sugary or fatty foods can disrupt the opossum's digestive balance, leading to gastrointestinal issues. Moreover, feeding the infant with cow’s milk can lead to such a problem. Such diets can alter gut microbiota, fostering bacterial overgrowth that causes diarrhoea. A lack of dietary fibre also impairs proper digestion, contributing to this condition. Sudden dietary changes can upset the digestive system, as it requires time to adjust to new foods. Overfeeding or irregular feeding practices can further stress the digestive system, exacerbating the problem (Ruth, 2012). Additionally, feeding spoiled or contaminated food introduces harmful pathogens into the gut, leading to infections and diarrhoea. In order to prevent these issues, it is crucial to provide a balanced, species-appropriate diet, introduce new foods gradually, and maintain proper feeding schedules and hygiene practices (OSUS, 2021).

BLOAT

Bloat, colic, or gaseous distension typically affects neonate or very young wild infants and can be caused by factors such as hypothermia with food in the gastrointestinal tract, overfeeding, inappropriate milk replacer, internal parasites, diet changes, constipation, or internal abnormalities. To diagnose, attempt to identify the gas entrapment location, whether in the proventriculus/crop, stomach, or lower intestines. Radiography is the best diagnostic tool to determine enteral impaction or gaseous distension. If gastrointestinal stasis is suspected, place the infant in warm water to cover the abdomen and gently massage the area for about five minutes. Keep the infant warm and dry it.

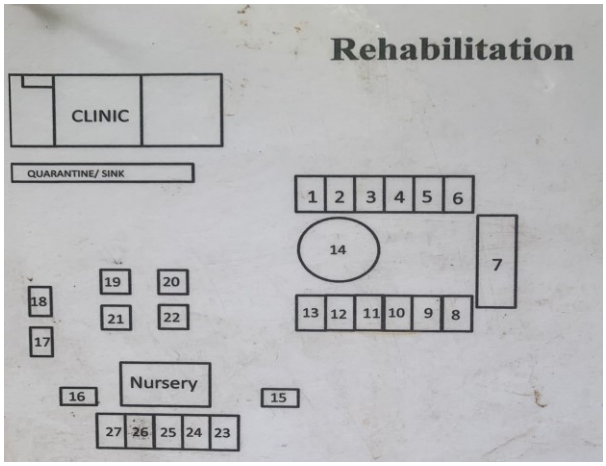
Repeat the process every half hour until the issue is resolved. Administering a carefully measured dose of infant Simethicone (65 to 130 mg total dose, orally, three times a day) may alleviate some of the pain and distress caused by gas. If the animal appears to be in severe pain and discomfort, a pain reliever may be beneficial (Ruth, 2012).

2. MATERIALS AND METHODS

2.1. Environment and subjects

The study was carried out at the Alturas Wildlife Sanctuary in Costa Rica from the 03/03/2024 to 05/05/2024. More specifically, the study was carried out in the rehabilitation. The latter was divided into: Nursery, Rehab (outer part of the nursery) and Clinic.

Figure 3: Area where the study was conducted



The subjects of my study were twelve *Didelphis marsupialis* (Table 5). They were all rescued from the wild by the staff of the Sanctuary during the period of the study. To be included in the study, the animals had to be in good health status or at least without evidence of injuries or pathologies that could get worse with handling. Usually, animals that could potentially become too habituated to humans by handling and, therefore, that could risk becoming unreleasable, were excluded from the study. But this was not the case of the common opossum as they get not so easily imprinted with humans. General information of the individuals can be found in the following table.

Table 5: General information on each individual of common opossum, collected at their time of arrival at the rescue centre.

I.D. Name	Sex	Category	Date of capture	Locality	Reason for rescue	Weight at the arrival
Marcia	F	Juvenil	5/03/2024	San José	/	73 g
Jojo	F	Juvenil	5/03/2024	San José	/	74 g
Jakob	M	Juvenil	5/03/2024	San José	/	64 g
Guarumo	M	Newborn	13/03/2024	San Isidro	Mom was killed by a dog	52 g
Bamboo	M	Newborn	13/03/2024	San Isidro	Mom was killed by a dog	45 g
Paloma	F	Newborn	13/03/2024	San Isidro	Mom was killed by a dog	50 g
Buck	M	Newborn	13/03/2024	San Isidro	Mom was killed by a dog	51 g
Pink	F	Newborn	5/03/2024	Puntarenas	Mom was killed by a dog	36 g
Green	F	Newborn	5/03/2024	Puntarenas	Mom was killed by a dog	35 g
Yellow	F	Newborn	5/03/2024	Puntarenas	Mom was killed by a dog	35 g
Blue	F	Newborn	5/03/2024	Puntarenas	Mom was killed by a dog	35 g
None	F	Newborn	5/03/2024	Puntarenas	Mom was killed by a dog	36 g

At their arrival, all the individuals went through a veterinary health check following all the steps mentioned in the chapter 1.2.7 “Health checks”, and eventually some drugs were administered. Even though some of the subjects were in a good hydration status, some Pedialyte (hydrating solution) was administered to all of them. They were all marked on the tail with non-permanent marking colour. After that, according to their weight and age determination, a specific diet was opportunely formulated. They were then housed in the nursery.

2.2. Housing methods

The subjects of the study were housed in five incubators (Figure 4) as follows: Incubator 1 (Marcia, Jojo, Jakob), Incubator 2 (Green, Yellow, Blue), Incubator 3 (Pink and None), Incubator 4 (Guarumo, Bamboo, Paloma) and Incubator 5 (Buck). This subdivision was according to the date of arrival and related to the litter to which each subject belonged. However, Pink and None also came from the same litter as Green, Blue and Yellow, but were separated from their sisters as the initial intention was to feed them different milks. Buck was sibling to Guarumo, Bamboo and Paloma, but upon his arrival he was diagnosed with neurological problems and was consequently separated. The source of heat was either the heating mat or direct. The direct light was shut down at 5 p.m. every day and a hot water bag was used as replacement. Temperature was ranging between 30-35°C with

a humidity of more than 50%. Each towel/T-shirt/blanket and pouch was changed every morning and the incubator disinfected. Most of the captive management practices used were similar to the ones in the chapter 1.2. When the ears start to show black pigments and weight between 70-100 g, they are moved to the box. When the ears are fully black, they are housed in the outdoor enclosure (Figure 5). When they are more 200 g or more, according to their health status and behaviour, they get usually released.

Figure 4: Incubator. Size: 50 cm X 30 cm X 30 cm

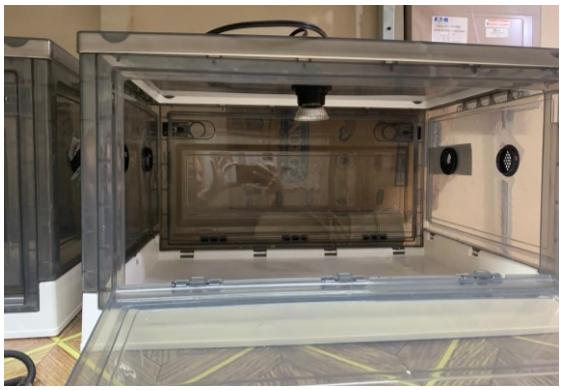


Figure 5: Outside enclosures. Size: 143 cm X 155 cm X 155 cm

2.3. Diet

The type of diet was created according to the weight of the animal and its presumed age of arrival, so the observations made in the study must then take both variables into account. Each monday the animals were weighed and according to the change in weight and their behaviour the quantities, the type of diet and its presentation were changed. As explained in chapters 1.2.4 and 1.4.2, as the

individuals grew, the frequencies were modified too. The feeding was organised into: day feeding and night feeding. Day feeding had the following shifts: 7 am, 11 am, 2 pm; while night feeding was divided into: 5 pm, 8 pm, 11 pm, 2 am shift and only infrared light was used so as not to disturb the animals and alter the circadian cycle. No method to avoid imprinting was used while feeding these animals.

Diet 1:

The diet consists of the ingredients in the following table (Table 6).

Table 6: Ingredients of the opossum diet used in the Sanctuary based on the days of the week

MONDAY, TUESDAY, WEDNESDAY		THURSDAY, FRIDAY, SATURDAY, SUNNDAY	
Ingredients	**Grams (g) x 1**	**Ingredients**	**Grams (g) x 1**
Papaya	20	Watermelon	15
Apple (without seeds)	10	Carrot	15
Broccoli	15	Green beans	20
Cucumber	15	Corn (no husk or core)	5
Guava	10	Mango	15
Egg (cooked)	30	Chicken (no bones)	30
Total	100	**Total**	100

Once the solid part was weighted for the feeding, the milk part was added. The milk used is the same of the other two diet. 'Powdered Whole Goat Milk, 12oz.' of Meyenberg. The formula was diluted with water respectively: 2 spoons of milk powder with 1 cup of water.

Figure 6: Nutritional composition of 'Powdered Whole Goat Milk, 12oz.'

The individuals fed with this diet were Marcia, Jakob, Jojo, Guarumo; Bamboo, Buck and Paloma. The frequency, amount changes and the period of administration can be reported in the table below (Table 7).

Table 7: Administration period, amounts and frequency of Diet 1

Dates inteval	Marcia, Jojo, Jakob			Guarumo, Bamboo, Buck, Paloma		
	Amount of milk	Amount of solid	Frequency	Amount of milk	Amount of solid	Frequency
05/03/24-13/03/24	1,5 ml	3 g	6 times/day	X	X	X
14/03/24-24/03/24	1,5 ml	5 g	6 times/day	X	X	X
25/03/24-07/04/24	1 ml	10 g	4 times/day	X	X	X
08/04/24-14/04/24	X	X	X	1 ml	5 g	5 times/day

Diet 2:

The diet consists of the same ingredients as in Diet 1, except for the banana, and the same quantities as shown in Table 6. Once the ingredients were combined, they were blended to form a kind of paste; then the banana was added to increase palatability. Once the paste was created, the same type of milk as in Diet 1 was added and the mixture mixed. The paste was then administered on a plate.

The individuals fed with this diet were Guarumo, Bamboo, Paloma and Buck. The frequency, amount changes and the period of administration are reported in the table below (Table 8).

Table 8: Administration period, amounts and frequency of Diet 2

Dates interval	Amount of milk	Amount of paste	Frequency
19/03/24-03/04/24	2 ml	2 g	6 times/ day
04/04/24-07/04/24	1 ml	3 g	6 times/ day

Diet 3: This diet consisted of milk and egg white firstly, then was changed to cat-canned food with milk. At the beginning, when the first formulation of the diet was used, once the part of milk was taken, the correct proportion of egg-white was added. The animal was fed with a 1 ml syringe with a nipple. Milk is the main source of fat, carbohydrates and minerals, while egg-white is the protein one. As we changed and started using the cat-canned food together with the milk, the proportions of the two food items were calculated, then they were mixed and passed through a strainer. This last passage was done to eliminate any granulose part that the subjects refused to eat. The preparation was administered on a plate.

The individuals fed with this diet are Pink, None, Green, Yellow and Blue; the younger ones. The frequency, amount changes and the period of administration can be reported in the chart below (Table 9).

Table 9: Administration period, amounts and frequency of Diet 3

Dates interval	Amount of milk	Food+amount	Frequency
16/03/24-25/03/24	1,8 ml	Egg-white: 0,2 ml	6 times/day
26/03/24-03/04/24	2 ml	Egg-white: 0,25 ml	6 times/day
04/04/24-07/04/24	2 ml	Canned-cat food: 2 g	6 times/day
07/04/24-13/04/24	2 ml	Canned-cat food: 3 g	6 times/day

2.4. Pharmacological treatments

During the study some individuals were treated with specific drugs. Here below a table (Table 10) explaining their use, posology and the treatment period. All the drugs were administered following the indications of the veterinarian.

Table 10: Drugs administered during the study, posology and treatment period

Use	Drug	Posology	Treatment period
Anticoccidial drug	Cocci-1 al 5% (Toltrazuril)	20 mg/kg bw/day	Between 3-5 days, depending on the severity of the infection (follow vet indications)
Probiotic (supporting digestive health)	Bene-Bac plus	A pinch per feeding (Usually once a day)	1-2 weeks (or as long as needed to support digestive health during and after antibiotic treatment or gastrointestinal upset)
Internal Antiparasitic drug	Prafentel-3 (Praziquantel)	0,5 ml/kg bw/day	3 days (if necessary, a follow-up dose after 2 weeks)
Topical Antiparasitic drug	Xelamec (Ivermectin)	6 mg/kg bw	Single dose (repeat the treatment if necessary, after 2 weeks)
Antifungal drug	Clotrimidazole	A thing layer on the affected area	2-3 times/day for 7-14 days (or until the infection disappears)
NSAID	Meloxicam	0,1-0,2 mg/kg bw/day	3-7 days (follow the vet instructions)
Antibiotic	Amoxiclav	For dogs: 12,5-25 mg/kg bw every 12 hours For cats: 10 - 20 mg/kg bw every 12 hours	7-10 days (can be extended depending on the severity of the infection, follow the vet instructions)
Antibiotic and Antiprotozoal agent for gastrointestinal infections	Metronidazole	For rats: 10 mg/kg bw every 12-24 hours	5-7 days (may be extended to 10-14 days according to the infection severity)
Vitamin supplement	Farvital	0,05 ml/day	1-2 weeks (can be continued if needed for nutritional support)
Antihistamines	Histaminex and Dextamethasone	Follow the vet-specific instructions	As prescribed
Antifoaming agent	Simethicone	50-100 mg/kg every 8-12 hours	2-3 days (or until the bloating and gas symptoms disappear)
Antiemetic agent	Metoclopramide	0.2 to 0,5 mg/kg bw 2-3 times/day	3-5 days (can be extended, follow vet instruction)
Intestinal lubricant	Mineral oil	A few drops to 1 ml as needed (depending on the size and condition of the animal)	A single dose (or for few days until the situation recovers, follow the vet instructions)
Euthanasia agents	Isoflurane and Euthanax	Depends on the size and animal species	A single dose

Other treatments related to animal health were enemas and massages of the abdomen in warm water to facilitate the expulsion of excreta. While for the reduction of rectal prolapse, treatments with saline and sugar were made.

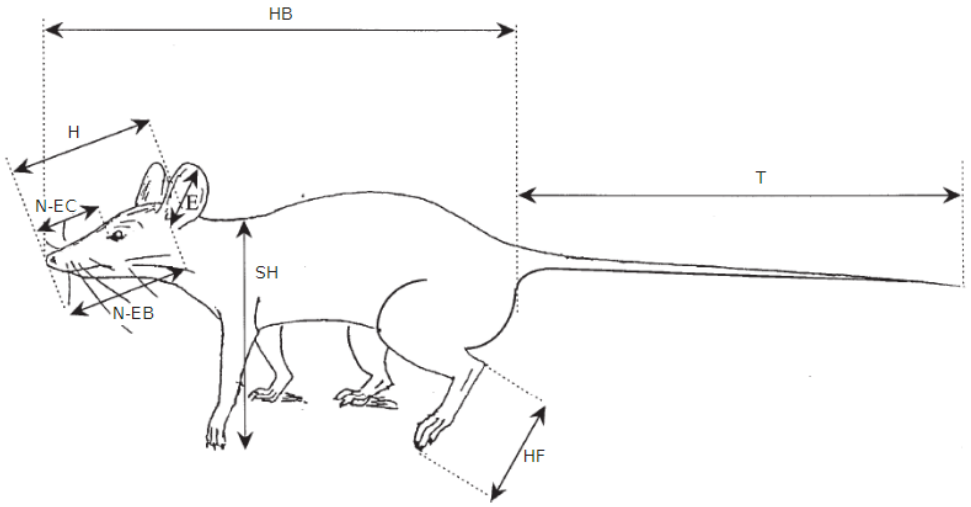
2.5. Body Measurements and observations

Morphometric data were collected from each subject twice a week (on Wednesday and Sunday), always at 7 a.m. The procedure data collection took on average 4-5 minutes per individual. The body weight was collected before the first morning feeding session. The morphometric measurements were collected on a total of 7 variables: Body Weight, Total Body Length, Body Length, Tail Length, Ear Length, Hindfoot Length and Axillary Girth. Each measurement was taken just one time per session. The materials used for the measurements were an electronic scale, a meter, a ruler and a bowl.

Table 11: Morphometric measurement done on the subjects of the study

Weight (W)	• Measured by applying the animal in a bowl on the top of the electronic scale
Total Body Length (TBL)	• Defined as the distance from the tip of the nose or snout to the end of the fleshy part of the tail (caudal vertebra) • Measured with the «over the curve method: measuring along the contours of the body in the mid-line, from the end of the nose, then over the head, neck and back to the tail.
Body Length (BL)	• Measured by the subtraction of the tail length from the total body length
Tail Length (TL)	• Defined as the distance from its point of junction with the body to the tip of the last caudal vertebra • Measuring the upper side of the tail, holding it at right angle to the back to identify the root and stretching it on the ruler
Ear Length (EL)	• Defined as the distance from the base of the notch to the furthest point on the margin of the pinna • Measuring always the left ear by stretching it on the ruler
Hindfoot Length (HFL)	• Defined as the distance from the back of the heel to the end of the fleshy part of the longest toe • Measured by pressing the sole of the hindfoot gently against the flat side of a ruler so to straighten the toes
Axillary Girth (AG)	• Defined as the widest point of the thorax • Measured immediately behind the forelegs by using the meter

Figure 7: Schematic representation of the morphometric variables measured (Richard-Hansen et al., 1999)



The other information found during the study mainly concerned the observation of the animal. In particular, the colour and presentation of the coat, the colour of the mucous membranes, the shape and structure of the teeth (crucial for the diet), posture and behaviour were observed. There are no graphs linked to the latter data, but abnormalities were noted.

2.6. Data analysis

The young opossums were classified into three groups according with their weight at the arrival in the centre and the consequent nutritional regimens they were subjected to. Animals in Group 1 (n=3) were the heavier subjects and received the diet 1. Animals in Group 2 (n=4) were characterised by an intermediate weight at the arrival and received the diet 2. Animals in Group 3 (n=5) were the lighter subjects and received the diet 3. Morphometric traits were collected in an Excel file in table from the subjects' arrival in the sanctuary. As a first descriptive analysis, the morphometric traits are shown as the means (± s.e.m.) of each group from the first walk. To compare the joint effect of the age of arrival and the nutritional regimen on the growth of the animals, morphometric traits were analysed by ANOVA, considering the variations observed after the subject reached a body weight greater than 80 g for seven subsequent measurements. The Group, Sample and Group*Sample were the fixed effects, and the Animal within Diet was considered the random effect (option UNIANOVA, SPSS 29.0). Correlations among the morphometric traits were finally analysed by Pearson correlation test (SPSS 29.0).

3. RESULTS

The individuals used in the study arrived at the Sanctuary on two different dates, 05/03/24 and 13/03/24 respectively. They were then divided according to the litter to which they belonged, their weight upon their arrival and physical condition. The table below (Table 12) presents the initial weight measurements of animals observed across three distinct groups, along with overall statistics. Group 1 consists of 3 animals with a mean weight of 90.67 units, Group 2 includes 4 animals with a mean weight of 53.50 units, and Group 3 comprises 5 animals with a mean weight of 34.20 units. The overall mean weight across all groups is 54.75 units. The standard error, standard deviation, median, minimum, and maximum weights are also reported for each group and overall. These descriptive statistics provide a comprehensive overview of the weight distribution at the beginning of the observations, highlighting variability within and between the groups.

Table 12: Initial weight measurements of animals across three groups

Weight at the beginning of the observations				
	Group 1	Group 2	Group 3	Overall
N. Animals	3	4	5	12
Mean	90,67	53,50	34,20	54,75
Standard error	2,91	0,65	0,73	6,77
Median	90,00	53,50	35,00	52,50
Standard deviation	5,03	1,29	1,64	23,46
Min	86	52	32	32
Max	96	55	36	96

Throughout this study, the subjects were undergoing some pharmacological treatments (Table 13) to address parasitic infections and other medical issues that arose during their rehabilitation. These treatments were crucial for managing the health and recovery of the subjects, ensuring their well-being and the integrity of the study. The table below summarizes the treatments, providing a baseline for evaluating the effects of the medical interventions on their overall health and growth during the rehabilitation period.

Table 13: Pharmacological treatments of the subjects during the study

Pathology	Group of animals affected	Pharmacological treatment	Treatment period
Ectoparasite infestation	Group 2	Xelamec (Ivermectin)	First application: 13/03/24 Second application: 04/04/24
	Group 3		21/03/24
Suspected endoparasite infestation and diarrhoea	Group 1	Cocci-1 al 5% (Toltrazuril)	First treatment: 19/03/24-21/03/24 Second treatment: 27/03/24-29/03/24
Restoring gut microflora and diarrhoea	Group 1	Bene-Bac plus	19/03/24-1/04/24
	Group 3	Bene-Bac plus	08/04/24-14/04/24
Skin problems	Group 3	Coconut oil	25/03/24-29/03/24
Suspected endoparasite infestation and diarrhoea	Group 1, 2, 3	Prafentel -3 (Praziquantel)	28/03/24-30/03/24
Suspected Zinc and mineral deficiency due to skin problems	Group 3	Farvital	30/03/24-13/04/24
Suspected allergic reaction to the new feed formula (symptoms: swollen eyes and skin)	Group 3	Histaminex	05/04/24
Suspected fungus affecting the skin of the animals (symptoms: irritated and itchy skin)	Group 3	Clotrimidazole	08/04/24-14/04/24
	Group 2 (only one individual)		10/04/24-19/04/24
Gas in the abdomen and difficulties in defecating (suspected protozoa infection)	Group 3	Simethicone, Metronidazole, Bene-Bac plus	08/04/24-14/04/24
Anal prolapse (suspected perineal hernia)	Group 2 (only one individual)	Meloxicam	14/04/24-17/04/24
		Amoxiclav	14/04/24-26/04/24
Difficulties in defecating together with anal prolapse (suspected perineal hernia)	Group 3	Meloxicam	14/04/24-17/04/24
		Metoclopramide	15/04/24-18/04/24
		Mineral oil	First administration: 23/04/24-24/04/24 Second administration: 30/04/24-03/05/24
		Simethicone	23/04/24-24/04/24
Metabolic bone disease	Group 3 (only one individual)	Isoflurane and Euthanax	24/05/24

Other practices performed during the study period were mainly related to the animal's motility and the health of the gastrointestinal tract (Table 14).

Table 14: Non-pharmacological practices performed on the subjects during the study period

Practices performed	Group of animals affected	Treatment period
Walk outside (to move, climb, defecate/urinate, exposition to sunlight)	Group 2, 3	From the 30/03/24 1-2 times/day
Enema	Group 2 (only to three individuals), 3 (only to one individual)	Timeframe between 14/04/24-30/04/24
Abdominal massages (helping for defecating/urinating)	Group 3	3-4/04/24
Anal prolapse reduction (with saline and sugar)	Group 2 (only to three individuals), 3 (only to one individual)	Timeframe between 30/04/24 -03/05/24

With regard to the variation of morphometric parameters measured during the study period, some peculiarities can be noted. If we observe the Weight variation over time (Figure 8, Graph B), we can notice that all subjects gained weight over the observation period, but the rate and extent of weight gain vary by group. Group 1 results in the most substantial and rapid weight gain, starting with the highest initial weight and consistently increasing to the highest final weight. Group 2 starts with a moderate initial weight and shows a steady, moderate increase, ending with a middle-range weight. Group 3 begins with the lowest initial weight and exhibits the slowest and most gradual increase. It has however a more pronounced increase from day 28th until the end and ends up reaching a higher weight compared to Group 2 in the late phase of the sample period (day 52-58).

Similarly, to the Weight, also for the Total Body Length (Figure 9, Graph B) all three diets show an increase in parameter measured, indicating growth for the subjects and following more or less the same trend as the ones of the body weight. What we can observe for the Group 1 is a slight fluctuation where the growth rate seems to slow down slightly and shows a dip between day 20 to day 38. Then it seems to reach a sort of plateau trend. For Group 2 there is a noticeable dip around day 24 and day 32 where the total body length decreases slightly before continuing to increase. The

growth rate in Group 3 is nearly flat with very minimal increase at the beginning (until day 10), having a slow and gradual increase from afterwards.

The Body Length trends (Figure 10, Graph B) of all the three diets have remarkable fluctuations but with an overall upward trend. There are several noticeable fluctuations of Group 1 trend throughout the time period, particularly around days 8, 25, and 45, where the body length temporarily decreases before continuing to increase. Group 2 exhibits a relatively steady and consistent upward trend with minor fluctuations, showing a more stable pattern compared to Group 1. Group 3 shows more variability, especially in the initial phase, with fluctuations around days 5, 28, and 38. The error bars in all three groups are slightly large, indicating more variability in the data.

As far as the Tail Length is concerned (Figure 11, Graph B), a rapid increase during the initial 10-15 days can be seen, reaching around 20 cm in Group 1. After 15 days, the growth rate slows but continues to increase, stabilizing around 23 cm by day 60. In Group 2 the Tail Length increases steadily, but the rate of growth is slower compared to Group 1 and by day 40 stabilizing around 20 cm. In Group 3 there is a starting point of the Tail Length of approximately 12 cm. The Tail Length increases gradually but at a slower rate compared to Groups 1 and 2.

The Ear Length (Figure 12, Graph B) in Group 1 and 2 start more or less with the same length value, having then in the Group 1 a rapid increase between the first phase (day 0-10), then a decrease and matching again with Group 2 value on day 20 (approximately 3,15 cm). There is then another point in which the two Groups cross each other that is around day 41 and ranging between 3,6 cm. If we look to the trend of Group 2, it shows a slight decrease at the beginning, increasing then with a more or less gradual trend and overtaking the values of Group 1. Related to Group 3 trend, we can notice a gradual and slow increase until day 22 and then a steeper trend reaching similar values to the other two diets.

The graph regarding the Hindfoot Length (Figure 13, Graph B) trend shows a more or less distinct trend of the three diets with a gradual but not completely linear increase. There are dips in all three diets' trends. In particular, fluctuations in Group 1 are visible from day 14 onwards, and the error bars are quite wide in the final phase, indicating a lot of variation in the values. Also in Group 2, apart from several fluctuations between days 28 to around 45 and two dips (on days 3 and 42 respectively), the error bars indicate that there is variability in the data. Group 3, on the other hand, shows a linear trend; however, between days 35 and 40 there is a dip, thus a minimum decrease.

With regard to the last parameter measured, Axillary Girth (Figure 14, Graph B), there appears to be a more or less linear and gradual trend in all three diets (except in Group 1 which shows a dip on day 3) up to day 24. From that day onwards, different fluctuations can be seen and with wider error bars in Group 1 and 2. Group 1, once it reaches a peak of about 15 cm on day 34, has a downward

trend. Whereas Group 2 shows a trend with an almost vertical slope from about day 42 until the end of the measurements. Group 3, after some fluctuations between days 35 and 42, seems to stabilise.

Figure 8: Graphs of the Weight variation under three different groups.
(A) The graph shows the average weight (in grams) of subjects on three different groups (Group 1, Group 2, Group 3) at six sampling points. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. Error bars represent the standard error of the mean (SEM).
(B) The graph shows the average weight over 60 days from the first walk, with Group 1 showing the highest weight gain, followed by Group 2, and Group 3 with the least gain. Error bars represent the standard error of the mean (SEM).

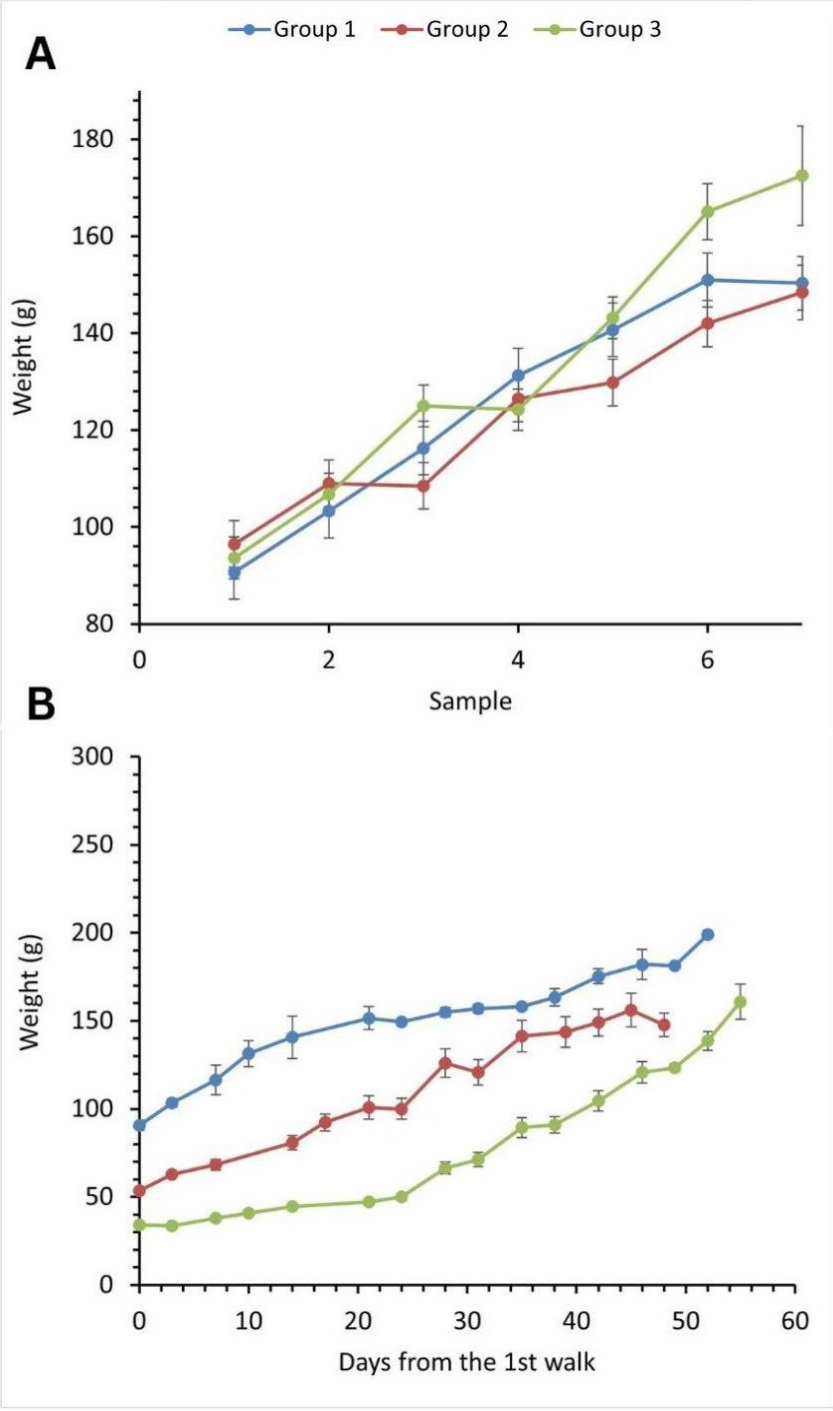


Figure 9: Graph of Total Body Length variation under three different groups.
(A) The graph shows the average total body length (in cm) for Group 1, Group 2, Group 3. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. The data points represent mean values, with error bars indicating the standard error of the mean (SEM). Asterisks (*) denote the time points where a significant difference ($p < 0.05$) among the groups was detected.
(B) This graph illustrates the average total body length (cm) of subjects on the same three diets over a period of 60 days, starting from the first measurement. Measurements were taken periodically to observe growth trends. Again, the data points represent mean values, with error bars showing the SEM.

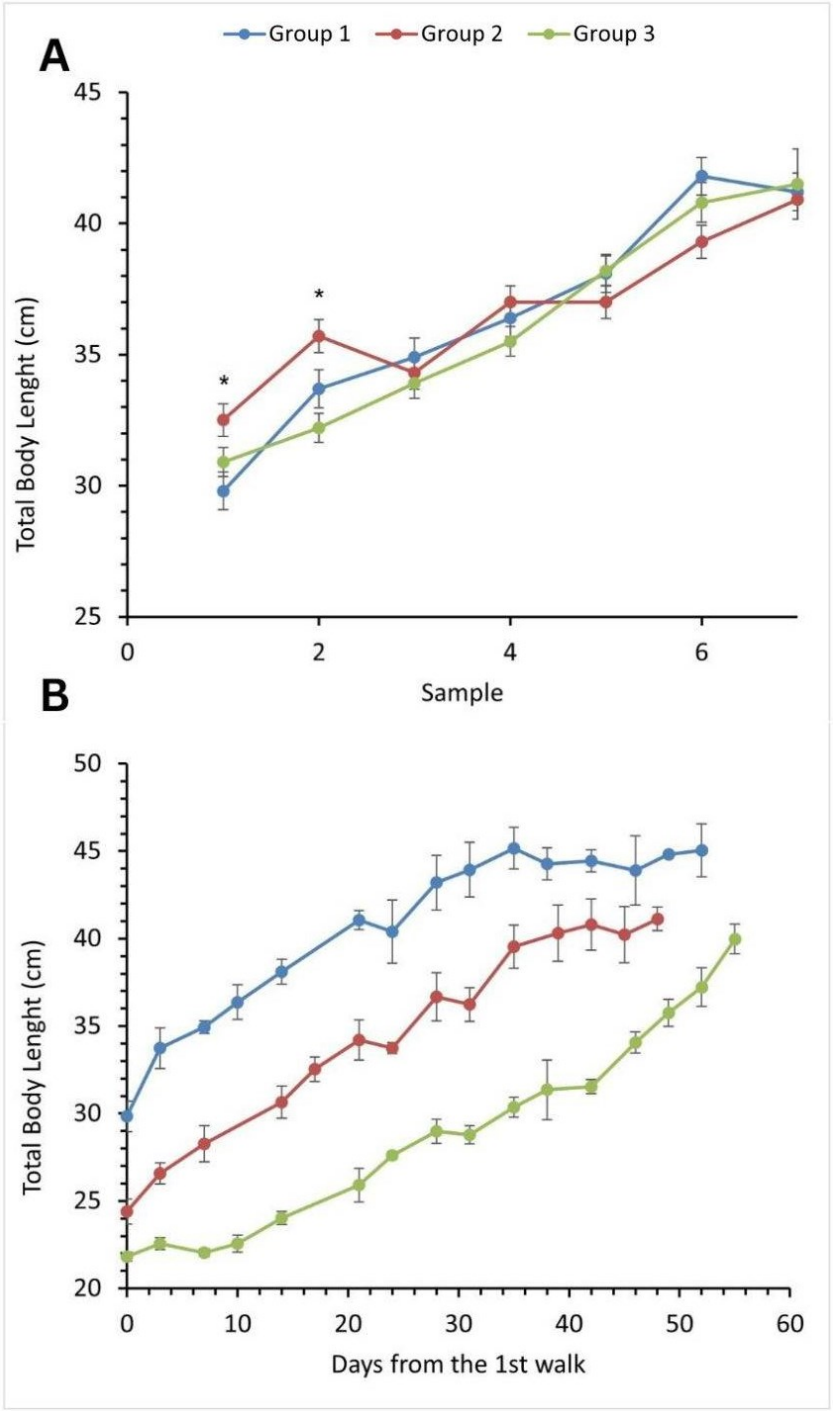


Figure 10: Graph of Body Length variation under three different groups.
(A) This graph presents the mean body length (in cm) of subjects on three different groups (Group 1, Group 2, Group 3) measured at six distinct sampling points. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. Each data point reflects the average body length, with error bars representing the standard error of the mean (SEM).
(B) This graph depicts the mean body length (cm) of subjects on the same three groups, monitored over a span of 60 days from the initial measurement. The body lengths were recorded periodically to capture growth trends. Each data point represents the mean body length, with error bars denoting the SEM.

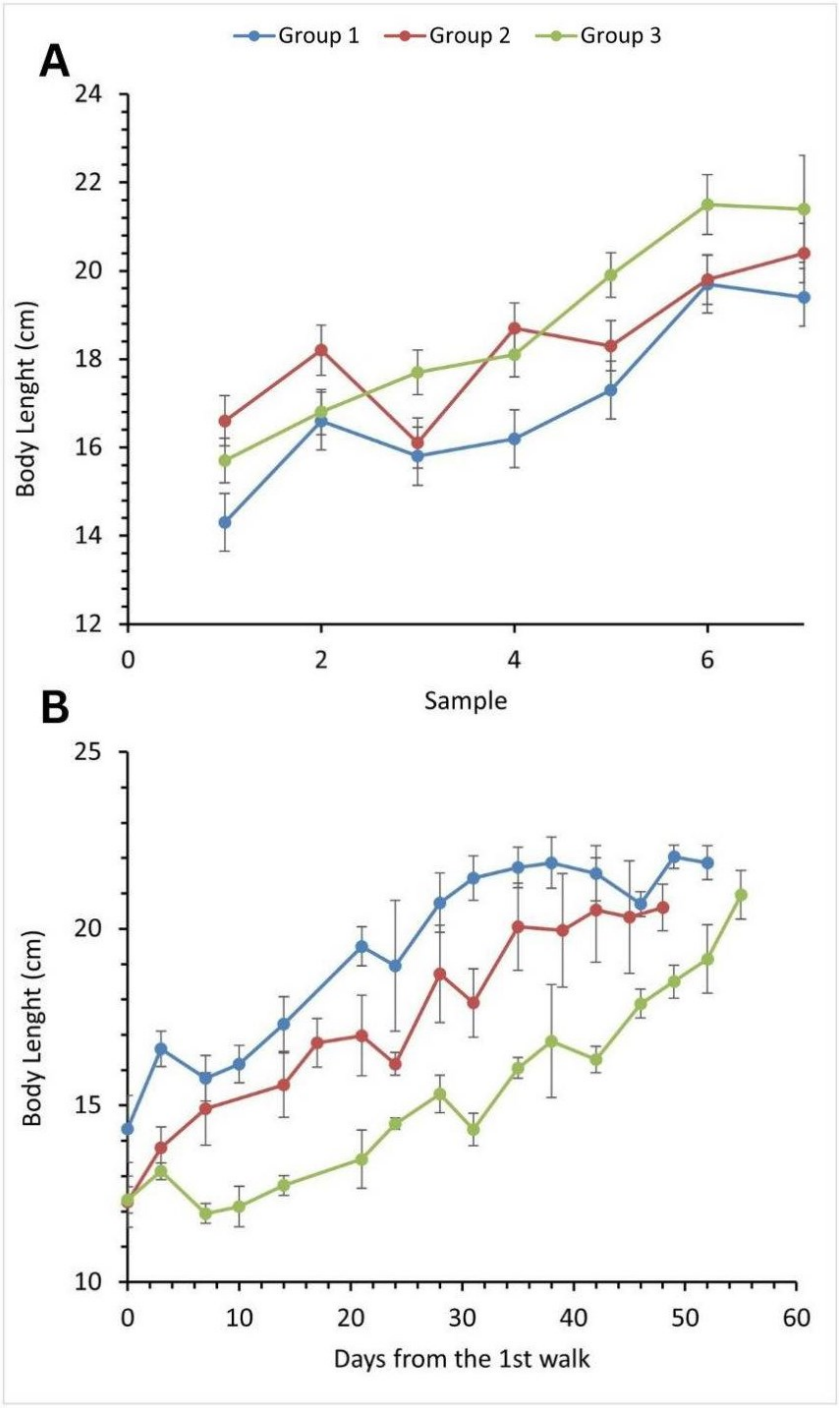


Figure 11: Graph of Tail Length variation under three different groups.
(A) The graph shows the average tail length (in cm) for Group 1, Group 2, Group 3. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. The data points represent mean values, with error bars indicating the standard error of the mean (SEM). Asterisks (*) denote the time points where a significant difference ($p < 0.05$) among the groups was detected.
(B) This graph illustrates the average tail length (cm) of subjects on the same three groups over a period of 60 days, starting from the first measurement. Measurements were taken periodically to observe growth trends. Again, the data points represent mean values, with error bars showing the SEM.

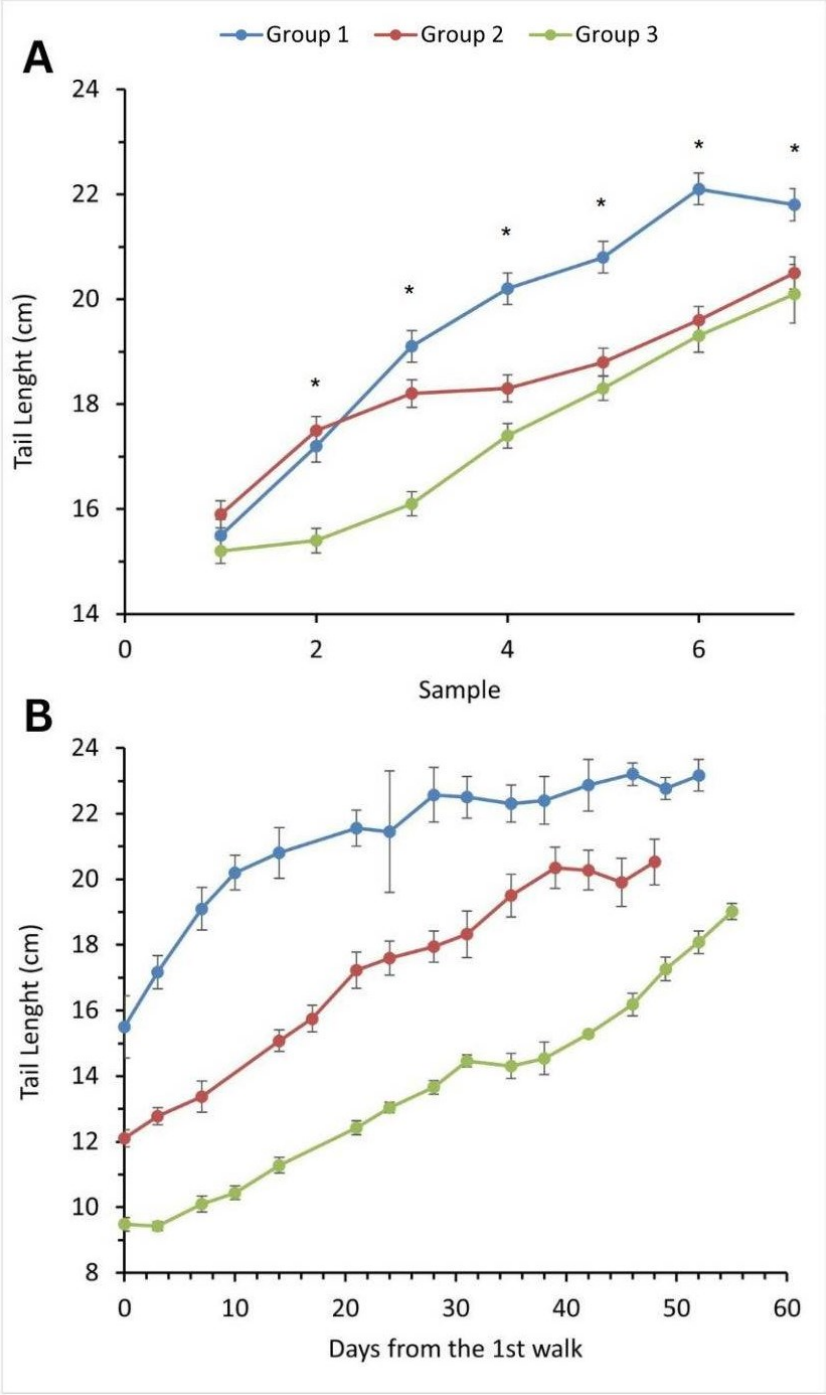


Figure 12: Graph of Ear Length variation under three different groups.
(A) The graph shows the average ear length (in cm) for Group 1, Group 2, Group 3. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. The data points represent mean values, with error bars indicating the standard error of the mean (SEM). Asterisks (*) denote the time points where a significant difference ($p < 0.05$) among the groups was detected.
(B) This graph illustrates the average ear length (cm) of subjects on the same three groups over a period of 60 days, starting from the first measurement. Measurements were taken periodically to observe growth trends. Again, the data points represent mean values, with error bars showing the SEM.

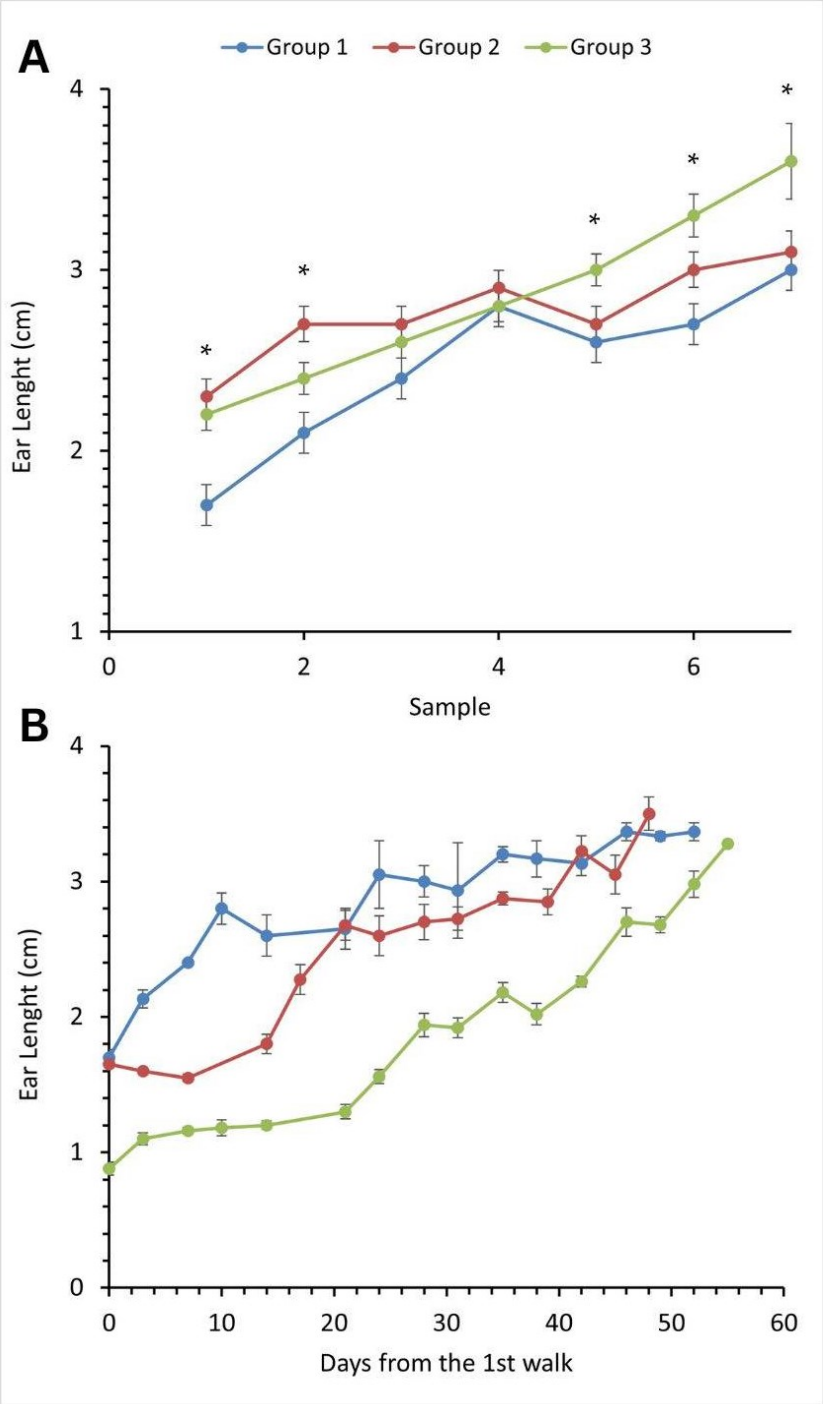


Figure 13: Graph of the Hindfoot Length variation under different groups.
(A) This graph presents the mean hindfoot length (in cm) of subjects on three different groups (Group 1, Group 2, Group 3) measured at six distinct sampling points. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. Each data point reflects the average body length, with error bars representing the standard error of the mean (SEM).
(B) This graph depicts the mean hindfoot length (cm) of subjects on the same three groups, monitored over a span of 60 days from the initial measurement. The body lengths were recorded periodically to capture growth trends. Each data point represents the mean body length, with error bars denoting the SEM.

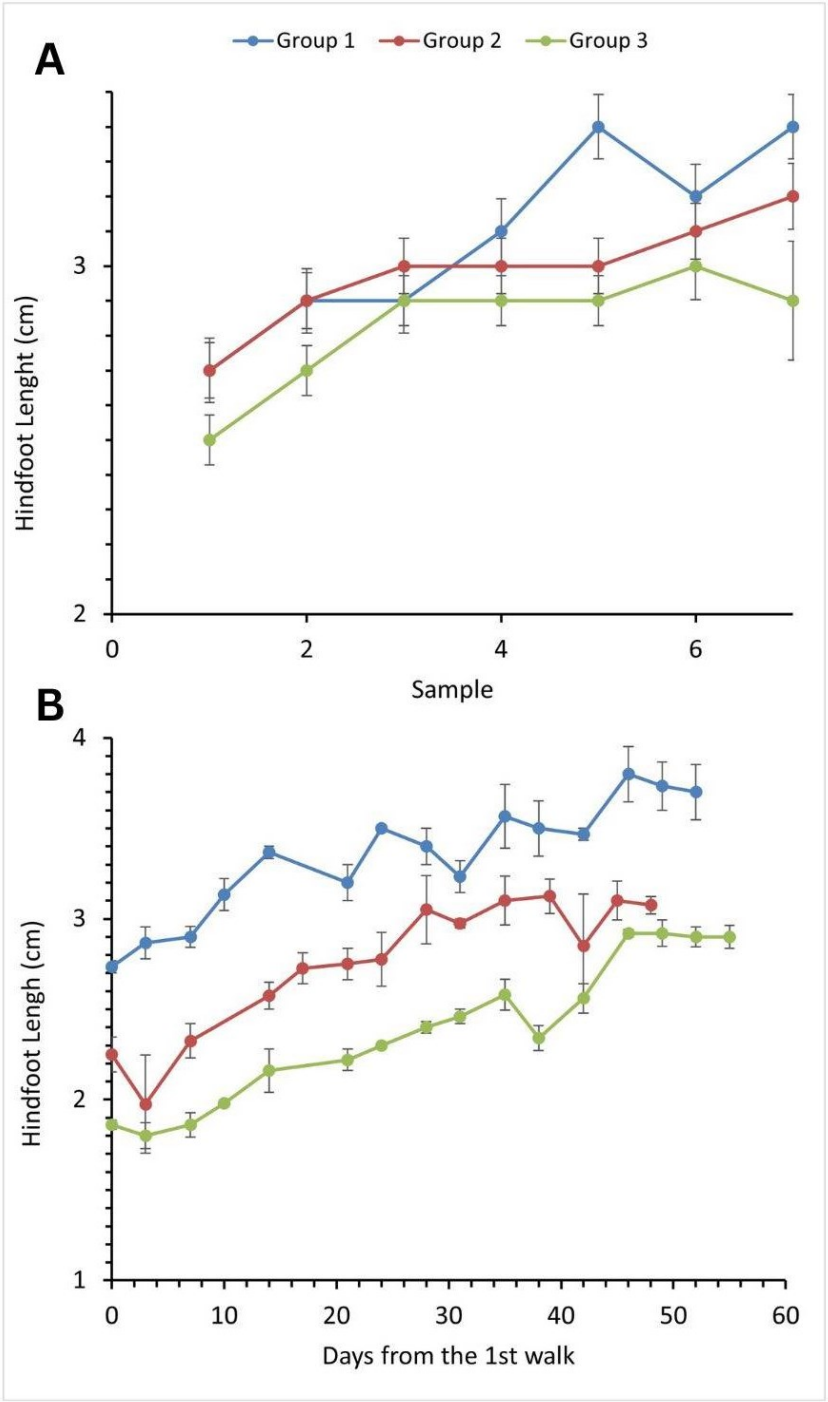
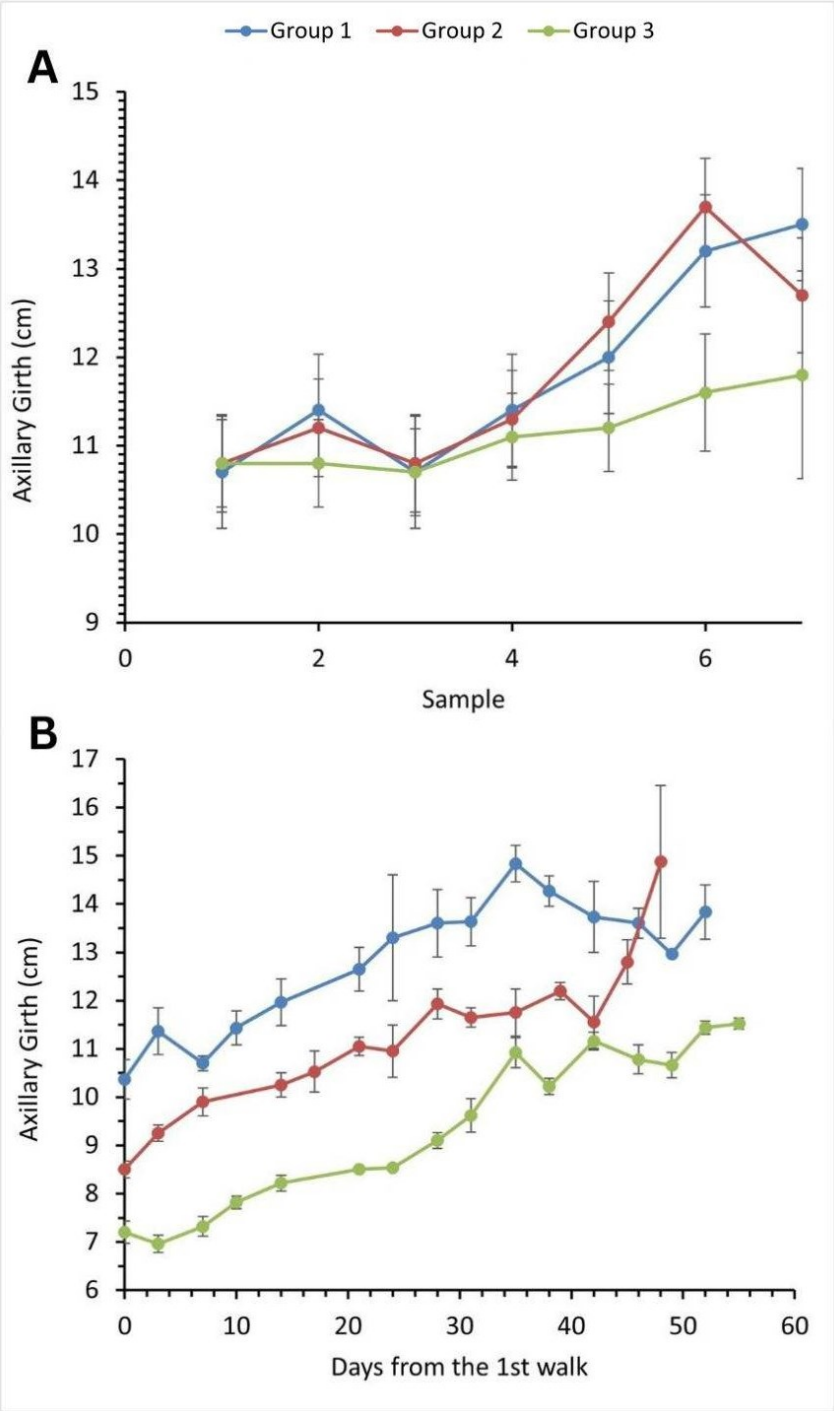


Figure 14: Graph of the Axillary Girth variation under three different groups.
(A) This graph presents the mean axillary girth (in cm) of subjects on three different groups (Group 1, Group 2, Group 3) measured at six distinct sampling points. Group 1 is indicated by blue circles, Group 2 by red circles, and Group 3 by green circles. Each data point reflects the average axillary girth, with error bars representing the standard error of the mean (SEM).
(B) This graph depicts the mean axillary girth (cm) of subjects on the same three groups, monitored over a span of 60 days from the initial measurement. The axillary girth was recorded periodically to capture growth trends. Each data point represents the mean body length, with error bars denoting the SEM.

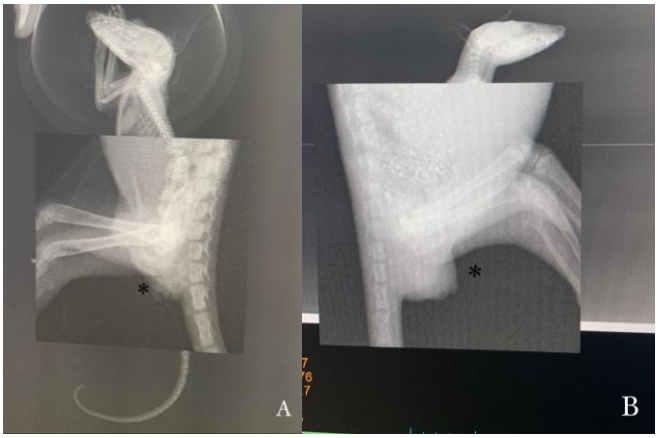


In the course of the study, it is possible to identify particular individual and/or management aspects that may have influenced and compromised the growth of one or more individuals within a group. Genetic factors that may have influenced growth were difficult to identify, this due to the fact that some of the subjects were siblings, their number was small to be able to identify genetic factors and genetic analyses were not carried out in the sanctuary. One of these, however, could be a predisposition for Metabolic Bone Disease (MBD), which is due to one or more genetic mutations. However, this disease can also occur due to improper nutrition (see Chapter 1.4.3). In one of the subjects belonging to Group 3 the disease was diagnosed by x-rays and animal was consequently euthanized.

Figure 15: X-ray of one of the subjects of the Group 3 for the detection of the Metabolic Bone Disease. (*) Asterisk indicating the location of the affected bone: femur of the left hindleg

Another situation that occurred during the growth of the study subjects and that may have both a genetic and nutritional origin is the formation of perineal hernias. This hypothesis was not confirmed as we did not have the contrast fluid to do proper tests. This condition was originally identified in one subject in Group 2 and then in all subjects in Group 3.

Figure 16: Comparison of a healthy anal region of a Didelphis marsupialis (A) with a non-healthy one of the same species (B). Figure B represents the x-ray done to the subject of Group 2 of the study representing a possible perineal hernia. (*) Asterisk indicating the location of the affected area: Anal and perianal area.



Health problems can also influence an individual's growth. In particular, as can be seen in Tables 10, 13 and 14, the animals were subjected to various pharmaceutical and non-pharmaceutical treatments that may have influenced their development.

In addition, housing conditions may also have influenced growth. One of these could be keeping them together in the same incubator/box for some time. This could, consequently, have had an impact on nutritional intake and the formation of social hierarchies (hence competition for resources).

One cannot overlook environmental stressors, such as moving the animal from a more controlled environment (incubator) to one with more exposure to variation (outdoor enclosure).

The history of the animal before reaching the sanctuary may also have an influence in the growth of the individuals. As we can see for all the subject of the study (see Table 5), the death of the mother was due to a dog attack and by this, some of the babies could have been affected. This specifically happened to one of the subjects of the Group 2, Buck. As he got in the sanctuary, he was showing neurological signs such as balance problems and kept turning in on himself. Therefore, he was isolated from the other siblings.

Finally, two other interrelated factors that are fundamental in forming the basis of good growth are: birth weight/early development and maternal care. The individuals that arrived at the sanctuary were not all the same weight or age. Initial conditions at birth, such as birth weight and early development stages, can set the trajectory for future growth. Even the level of maternal care could not be detected but the quality of maternal care received during the early stages of life can significantly affect growth.

Table 15: Representation of the ANOVA test results

		W		TBL		BL		TL		EL		HFL		AG	
Variables		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Intercept		3784,793	0,000	17383,468	0,000	17735,998	0,000	7574,535	0,000	1958,844	0,000	7301,152	0,000	4234,262	0,000
Group	Fix effect	1,955	0,195	0,313	0,739	13,278	0,001	7,588	0,011	3,307	0,083	4,526	0,042	1,451	0,281
Sample	Fix effect	54,265	0,000	77,809	0,000	23,231	0,000	122,379	0,000	34,784	0,000	14,403	0,000	5,763	0,000
Group*Sample	Fix effect	1,565	0,135	2,838	0,005	1,712	0,094	6,055	0,000	2,305	0,021	1,103	0,380	0,617	0,817
Animal (Group)	Random effect	3,250	0,004	3,428	0,003	0,924	0,513	11,728	0,000	6,952	0,000	3,261	0,004	1,787	0,096

The ANOVA revealed that all the morphometric traits significantly increased throughout the observation period ($P<0.001$) in all Groups. Statistically significant differences among Groups were found for Body Length ($P<0.001$), Tail Length and Hind Foot Length ($P<0.05$). On average, animals of Group 1 displayed shorter Body Length, but longer Tail Length and Hind Food Length. Statistically significant effects of the Group*Sample were found for Total Body Length ($P<0.01$), Tail Length ($P<0.001$) and Ear Length ($P<0.05$). In figures 9, 11 and 12, the asterisks indicate the samples where a significant difference among groups has been detected. The correlations between the morphometric traits are shown in Table 16. The Pearson correlation coefficient among variables was always very high. However, it is interesting to observe that the Axillary Girth showed the lower degree of correlation with the other traits.

Table 16. Pearson correlation coefficients (r) between the morphometric traits (n=179 observations).
W: Weight; TBL: Total Body Lenght; BL: Body Lenght; TL:Tail Lenght; EL: Ear Length; HFL: Hindfoot Length.

	W	**TBL**	**BL**	**TL**	**EL**	**HFL**
TBL	0.960^{**}					
BL	0.903^{**}	0.958^{**}				
TL	0.950^{**}	0.973^{**}	0.868^{**}			
EL	0.928^{**}	0.914^{**}	0.859^{**}	0.906^{**}		
HFL	0.894^{**}	0.890^{**}	0.805^{**}	0.905^{**}	0.863^{**}	
AG	0.877^{**}	0.899^{**}	0.830^{**}	0.899^{**}	0.844^{**}	0.832^{**}

4. DISCUSSION

As an initial and baseline point, it must be emphasised that the study subjects belong to three different litters and have different ages. The precise age has not been estimated; however, it was possible to assume that they belong to three different age groups from the morphometric variables but also from the behaviour. The subjects in Group 1 are the oldest, followed by those in Group 2 and finally Group 3.

Weight (W) is under the influence of several factors, both nutritional and medical. Visualising the data in the presented case study, the growth curves of all three Groups (see Figure 8) appear to follow a gradual and more or less homogenous upward trend, with individuals in Group 3 overtaking individuals in Group 2. However, the representation may be misleading. This is because, as can be seen in the trend for Group 3, initially there is a slow but more or less linear increase in the weight of the individuals (who were taking Diet 3: milk with egg white), but then the slope increases somewhat from day 07 April. From that date onwards, the weight values begin to increase more rapidly. However, when observing the animals, it was noted that there was an increase in the volume of the abdomen, which was soft to the touch. The hypothesis leads us to think that the change in diet from milk with egg white to cat-canned food with milk carried out on 04 April (see Table 9), induced the formation of gas in the abdomen of the individuals of Group 3 who, despite being stimulated to defecate and taken out for a walk, still failed to eliminate all the gas. Indeed, fluctuations in the trend due to this problem can be seen from this point onwards. This persistent condition has also created problems to defecation and, consequently, to the occurrence of the anal prolapse and suspected perineal hernia, due to the pressure of the gas on the abdominal wall and intestines. During this period, the drugs administered are those listed in Table 10 and other practices done are listed in Table 14. As a result, the measured Weight (W) was likely overestimated due to the presence of gas and material within the subjects' intestines.

A similar situation also happened to some subjects in Group 2. In particular, from 14 April some of them started to show symptoms of bloating, except for Buck. From that day onwards, fluctuations in weight can also be seen in this Group. However, it must be specified that the swelling of the abdomen was minimal and lasted only a few days. The subject most affected was Paloma, the only female in Group 2. She was the first to show anal prolapse and the suspected perineal hernia (see Figure 16 B). Again, the hypothesis was that this condition was due to an effect of an unbalanced diet. More specifically, it was thought that it was the prolonged intake of bananas within the diet that created constipation in the individuals. Consequently, all affected subjects were given only hydrating fruits (such as Papaya, Pineapple, Watermelon, etc.) to aid in defecation. This however, did not helped all the subjects affected in eliminating the escreta, still showing abdominal bloating

for the next weeks. What did, however, have an effect in helping the animals to defecate were the mineral oil administered on the days shown in Table 13 and the abdominal massages carried out after the mineral oil treatment (see Table 14).

However, when analysing the situation, not having the same diets in both groups studied, two hypotheses can be considered:

1) The individuals in the two Groups were still in that age range where changes in diet must be gradual and consequently a rapid change can lead to digestive problems and allergic reactions (Ruth, 2012).

2) As females are the ones affected, it could be related to a genetic predisposition for perineal hernias. Obviously, nutrition has encouraged its development. However, bibliographies only reported case studies of perineal hernias in dogs, showing that the most affected where males (Burrows et al., 1973).

Another observation that can be made when looking at the Weight (W) graphs is the presence of some dips. Some of them are related to the situation explained above, while others, as for Group 1 on 31 March and Group 2 on 22 April and 04 May, are related to the movement of individuals from the nursery boxes to the outdoor enclosures. This change also led to alterations in the weight of the animals. This could be due either to a separation from the other individuals with whom they were housed; in fact Buck, being housed alone from the beginning, did not experience weight fluctuations; or from the fact that they are in a new environment with also different environmental stressors. These situations make us realise that Weight (W), taken as the only variable under analysis, cannot fully represent an indicator of age as it is too variable. However, by comparing weight with other variables such as dentition (see Table 1), size (body proportions), physical features (fur/skin), bone density and growth plates, sexual maturity, behaviour and so on, it is possible to estimate the age of an animal (Fowler, et al, 2008; Richardson, 1992).

When measuring the other variables, it's crucial to recognize that the subjects are live animals, which are constantly moving. This movement is especially pronounced during handling and restraint, which are stressful for the animals. As a result, these procedures must be performed as quickly and accurately as possible. However, because these are manual measurements on live, moving animals, there is an inherent margin of error. Additionally, since the animals are very small, the error can be on the scale of millimetres, making handling particularly challenging. Therefore, it is essential to consider both the repeatability of the measurements and the precision with which they can be taken.

As we can observe in the Figure 9 of the Total Body Length (TBL) Graph A, the variable measured appears to be longer in Group 2 rather than Group 1, that were supposed to be the older ones and

therefore bigger. This can be explained by the fact that in the Graph A we are considering two different starting sample point. More specifically, since the age of all subjects was not estimated at the beginning of the study, in order to do the analysis as correctly as possible, I opted for a "Weight-estimated age" (WEA), thus analysing the data from when the weight exceeds 80 g. Accordingly, the data reported for Group 1 starts from their arrival at the sanctuary (10 March), while those for Group 2 start approximately one month after their arrival (approximately 14 April). In fact, the subjects in Group 2 were already older and therefore bigger. TBL in particular is not only difficult to measure because of the animal's movement, but also due to the animal's posture, which is usually crouched. A better representation can be seen in Graph B of the same figure, where the data start from the subjects' arrival at the sanctuary. In this graph, we can see how the Total Body Length increases rapidly until reaching 37-42 cm where it increases but more gradually, almost like a plateau.

The variables Body Length (BL) and Tail Length (TL) are closely related. Consequently, as one varies, the other varies. More precisely, the BL is measured as TBL-TL=BL; therefore, as we can observe in Figure 10, there are many fluctuations in both graphs. In the trend of Group 3 both for the TBL and for the BL the measurement was easier to take and therefore more accurate as they were younger and easier to manipulate.

Among the three measurements, the easiest to take in the most accurate way is the Tail Length. Even in graphic representations, trends are more distinct between the three Groups (see Figure 11). Analysing the Graph B, we can see a very rapid increase in the length of the tail of Group 1, which remains shorter than the BL. This situation, however, changes once reached the 20-22 cm of tail. This can be noticed in a clearer way in the trend of Group 2, in which once reached the 20 cm of length of the tail and the 20 cm of BL, the growth trend seems to stabilize and consequently the two variables seem to grow proportionately and in a parallel way. This does not happen in Group 3 subjects, as they are smaller. As a result, once the Body Length (BL) and Tail Length (TL) equalize, the Tail Length becomes a less reliable age indicator in adults, whereas can be used as a growth parameter in juveniles.

In juvenile opossums, the ears tend to be proportionally larger than the head size compared to adults. This is because the growth of different parts of the body can occur at different rates. Very young opossums will have relatively large ears, which may appear more prominent due to their smaller overall body size. It is noticeable in Figure 12 that Ear growth (EL) was very rapid in Group 3 compared to the other two Groups. In addition, the other two, show different fluctuations in the trend as well as different values in common. Fluctuations can be due to the fact that animals move during measurements and that this species usually has folded ears (Voss et al., 2021) that have to be

stretched in order to measure their length properly. Whereas, the fact that at some point in the trend there are values in common between the two groups, indicates that the Ear Lenght could be an age indicator, for younger individuals, but then it becomes less and less reliable. This because once the animal becomes an adult, opossums typically have a more balanced appearance with ears in proportion to their larger heads and bodies. Ear Length might be affected by the nutrition and external factors, but may be less affected than the Tail Length and therefore be more independent from the two variables mentioned before. However, no reference is found on the age related to the ear size.

Analysing the graphs shown for the variable Hindfoot Length (HL, see Figure 13), there are several fluctuations especially in the trend of Group 1 and minor but frequent in Group 2 as well. This could be mainly due to measurement error, again because the animal was moving but also due to the error of the ruler used in the study (1mm). In addition, it should be specified that this species does not possess a completely flat sole of the foot, consequently due to the conformation and from the fact that they often half-closed their toes under stress, it made measurement difficult and very variable.

As can be seen from the graphs in Figure 14, the Axillary Girth (AG) is highly variable. The hypothesis related to this variability could be related mainly to the animal's posture. During the measurement it was noted that this species, when under stress, tends to show the so-called 'opossum posture', i.e. contracting the body muscles by assuming a huddled position, as a result of which it is very difficult to lay the animal down in order to make an accurate measurement. This hypothesis is reinforced by a study done on Brown fur seals (*Arctocephalus pusillus*) showing how Axillary Girth measurement varies depending on the animal's position, nutrition and hydration (Allan et al., 2019). In addition, the fluctuations seen in the trend of Group 3 from 10 May onwards could be due to the presence of gas in the abdomen and consequently the AG was overestimated. According to some research, Axillary Girth (AG) is also used as a measurement associated with weight to estimate the growth and body condition score of an animal (Cook et al., 2003; Kolenosky et al., 1989). However, in this species (*Didelphis marsupialis*) it does not appear to be a reliable measurement for estimating the age of an animal and assessing its proper growth.

By combining all the graphs presented, it is possible to have a general picture of the situation and growth of the subjects involved in the study. In particular, it can be observed that some measurements are simpler and more accurate to measure on younger individuals and therefore more representative of their growth. On the other hand, if only the measurements are taken into account and not each individual as a whole, these measurements can be overestimated or underestimated. Considering the different situations of the three Groups together with the measurements done, it

seems that, the animals arrived at the sanctuary with a greater age respond better to care and are fitter than to the individuals arriving with a younger age. This could also be related to the longer maternal care they had. In addition, they respond better to the change of nutrition, which apparently did not happen with smaller subjects. Having a total vision of the subjects in fact, it was possible to diagnose the Metabolic Bone Disease in one of the subjects of Group 3, noticing a lameness in his walk, but not using only the variables analysed (see Figure 15).

Moreover, it is not possible to visualize it from the graphs as there is no analysis of the single animal but of the Group, it has been possible to notice how separating the individuals, from a certain moment of their rehabilitation or from immediately (based on their age upon arrival), lead to a rapid and more homogeneous growth. This could be due to more space in the enclosure, better access to resources, and consequently better control of the individual animal by the Caretaker. This shows how environmental factors have an impact on an animal.

5. CONCLUSIONS

The aim of this study was to enhance the understanding of the growth patterns of the rescued common opossum and to provide a base for their effective rehabilitation. The key findings were aligned to the research objective and include:

- Growth data collection. Through regular measurements it was possible to collect many data on the growth of this species enhancing therefore the knowledge on the growth patterns. This information may be a useful reference for wildlife rehabilitators and researchers, offering insights into expected growth trajectories and potential deviations that may signal health issues.
- Standardization of morphometric data collection. Through this research there has been an attempt of development and validation of consistent protocols for morphometric data collection. This contributed to a possible standardization. This standardization is vital for ensuring reliable and comparable data across different studies and rehabilitation centres.
- Correlation with nutrition. The analysis identified significant correlations between morphometric changes and nutritional status. Proper nutrition was found to be critical for the healthy growth and development of common opossums, while improper nutrition led to noticeable deviations in growth patterns. These findings highlight the importance of providing species-appropriate diets in rehabilitation settings.
- Correlation with pathologies. Through the analysis it was possible to discover specific morphometric changes associated with particular pathologies, enabling early detection and treatment of health issues based on measurable physical parameters. This improves the overall care and recovery outcomes for rescued opossums.
- Protocol development for rehabilitation. The research contributed to the creation of a rehabilitation protocol for this species. The protocol incorporates standardized morphometric data collection methods and nutritional guidelines derived from our findings, aiming to optimize the rehabilitation process and increase the survival and release rates of these animals.

By critically assessing this work, it is possible to notice that the weight of the animals may not be the most accurate indicator of age. Various factors, such as nutritional status and health conditions, can significantly affect an opossum's weight, leading to inaccurate age estimations. While weight trends can be a simple and useful method for monitoring the health status of opossums, relying solely on weight to estimate age can be misleading. Therefore, it is possible to suggest that a combination of morphometric parameters should be used to more accurately assess the age and

health of opossums. These can include the parameters measured in the study as well as dentition measurement, body proportions, physical features and behaviour observations.

Several limitations should be acknowledged to provide a balanced perspective and guide future research efforts such as:

- Sample size and representativeness. The data were predominantly collected from a specific region, which may not fully represent the diversity of environments and conditions that common opossums encounter in the wild or across different rehabilitation facilities. Also, the number on of individuals used in the study may not be fully representative of the whole species growth.
- Measuring live animals. The measurement taken may not fully represent the real situation and body growth of the animal. There will be always an error as the animals were awake (no anaesthesia was used) and moved, leading to an over- or underestimation of the parameters.
- Controlled experimental conditions. The study predominantly used observational data from rescued opossums undergoing rehabilitation, where environmental and nutritional variables were variable and not consistently regulated. While this method mirrors real-world conditions, conducting controlled experimental studies in more controlled environments could offer greater insights into precise cause-and-effect relationships among nutrition, health, and growth.
- Short term study. The study's focused on morphometric measurements and their correlations with nutrition and pathologies provided valuable insights into short-term growth trends. Long term studies are needed to fully understand and observe the growth pattern of the animals and how health indicators evolve throughout the lifespan of common opossums.
- Lack of species- specific information. The study contains many information of the species *Didelphis virginiana* as few information of *Didelphis marsupialis* are available.
- Small sized animals. The individuals involved in the study were small, so taking a blood sample was impossible. This limited the study as different parameters and blood values would have helped in the research and understanding of the growth patterns of this species.

This research has implications that reach beyond immediate application, providing a crucial foundation for wildlife rehabilitators to effectively monitor and promote the healthy development of common opossums. The correlations identified between morphometric changes and nutritional or pathological conditions offer practical insights that can significantly improve early intervention strategies and overall care protocols. Looking ahead, future research should build upon these findings by investigating the long-term outcomes of reintroducing opossums into their natural habitats. Furthermore, expanding the scope to include various species of opossums and similar

small mammals will contribute to a more comprehensive framework for advancing wildlife rehabilitation practices globally. These efforts aim to refine rehabilitation techniques and enhance the successful reintegration of wildlife back into their natural environments.

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