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RESEARCH ARTICLE

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Morphometric Analysis on Captive-bred Pig-tailed macaque (*Macaca nemestrina* Linnaeus, 1766) Growth

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Abstract
The pig-tailed macaque has a high vulnerability to human infectious disease pathogens as well as nonhuman primate viruses, making it a good animal model for biomedical research. The objectives of this study are to gain a better understanding of the morphometric and growth rate differences between male and female pig-tailed macaques living in tropical captivity as their natural climate. It will be beneficial in creating research approaches as well as changing and developing equipment and instruments. The research was conducted on 70 captive-bred pig-tailed macaques (32 males and 38 females). The characteristics are separated into four categories: head size, torso, upper limbs, and lower limbs. There was no visible difference in body and extremity sizes between male and female animals between the ages of 1 and 4 years. Males' head proportions appear to expand consistently in height and width after they are beyond 2 years old, in contrast to females. The growth trend of females tends to decrease from one year of age until sexual maturity, in contrast to males which show a growth spurt at the ages of 1 and 4 years. Differences in size and growth rate indicating sexual dimorphism are visible after the female reaches sexual maturity

Key words: captivity, growth rate, morphometric, pig-tailed macaque, sexual dimorphism

1. Introduction.

Nonhuman primates (NHPs) play a crucial role in biomedical research as animal models because they share genetics, anatomy, physiology, and behavior with humans (NASEM 2023). NHP used in biomedical research aids in the discovery of therapies for cancer, AIDS, Alzheimer's, Parkinson's, obesity/diabetes, and a variety of other human disorders (Ator *et al.* 2017). Long-tailed (crab-eating or cynomolgus) macaques, pig-tailed macaques, Rhesus macaques, squirrel monkeys, owl monkeys, marmosets, baboons, spider monkeys, capuchin monkeys, and other primates are frequently used as study models.

Pig-tailed macaque (*Macaca nemestrina* Linnaeus 1766), known as Beruk in Indonesian (Ruppert *et al.* 2022), is a primate in the Cercopithecidae family, generally buff-brown in color with a darker back and lighter lower parts of the body. These macaques are sexually dimorphic, with adult males measuring 495 to 564 mm (1.62 to 1.85 ft) and weighing 6.2 to 14.5 kg (13.7 to 32.0 lb), while adult females measure 467 to 564 mm (1.53 to 1.85 ft) and weigh between 4.7 and 10.9 kg (10.4 and 24.0 lb) in the wild. They move quadrupedally on the ground and through trees (Lang, 2009). Primarily inhabiting islands in Indonesia (distributed in Central Kalimantan, East Kalimantan, South Kalimantan, Lampung, Bengkulu, South Sumatra, Jambi, West Sumatra, North Sumatra, Aceh, and Bangka Island), and also distributed in Malaysia, Brunei, and southern peninsular Thailand (Supriatna and Wahyono 2000; Groves 2001; Roos *et al.* 2014).

Demonstrate a high susceptibility to human infectious disease pathogens as well as nonhuman primate viruses, making the pig-tailed macaque suitable animal models in biomedical research such as HIV research (Garcia-Tellez *et al.* 2016), non-AIDS infectious disease research (Shen *et al.* 2004; Gardner *et al.* 2008; Jegaskanda *et al.* 2013; Patton *et al.*

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2014), and also neuroscience research (Willoughby *et al.* 2012; Walton *et al.* 2017). Knowing basic information such as the morphometrics of the animal models, helped collect the preliminary data for the research (Huber *et al.* 2020). Pig-tailed macaques are often bred in ex-situ captive breeding facilities. This study aims to understand the morphometric and growth rate variations between male and female pig-tailed macaques living in tropical captivity as their natural climate. The findings of this study are critical in creating research procedures as well as modifying and developing equipment and instruments.

2. Materials and Methods

2.1 Ethical Note

The animals were maintained in the Research Facility Lodaya, Primate Research Center of IPB University, accredited by The Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996) and were approved by the PRC-IPB Animal Care and Use Committee. Environmental enrichment such as barrels, perch board, swinging devices, toys and food modifications are provided to support animal welfare programs.

2.2 Study Subjects

The observations were conducted on pig-tailed macaques captive born and raised at the breeding facility of RAF-L PRC-IPB. This facility used two breeding cages with coral-type animal housing (127.5 m^2/each) and gang cages of various sizes. Every cage provided with Environmental Enrichment (EE) such as barrels, perches with a various height level, chains as swinging device, and other toys, also food modification. The animals were provided with water ad libitum and fed with commercial monkey chow (Charoen Pokphand-Thailand) twice daily, with additional monkey chow biscuits produced by PT. Citra Ina Feedmill and some fruits such as bananas, guavas, and other seasonal fruits or vegetables.

2.3 Data Collection and Morphometric Analysis

Body parameters were measured from 2016 to 2023 as part of a routine health evaluation program. Examinations were performed every three to six months covering the animals' pre-adulthood period (<50 months). Animals were sedated using Ketamine Hydrochloride (7-15 mg/kg body weight) for overall physical examination and tuberculin testing. Body weight was measured using a digital weighing scale, and the measurement of body parameters was done using a digital caliper, based on the method previously described (Suzuki *et al.* 2003). The parameters were divided into four regions: head size, the torso, upper limb, and lower limb regions (Table 1).

2.4 Data Analysis

Data were analyzed descriptively; results were summarized and presented as mean and standard deviation (S.D.) in tabular and graphical form using Microsoft Excel for Mac software, version 16.16.27.

3. Results

A total of 70 individuals, consisting of 32 males and 38 females were measured during the study period. Repetition of measurements at different time points according to body development varies for each individual depending on the animal's status at that period (Figure 1).

The ages analyzed for comparison of male and female sizes were 5, 8, 12, 15, 19, 24, 27, 31, 36, 39, 45 and 50 months. There is no visible difference in the body length of males and females at that age (1 to 4 years), although in the 4th year, males appear slightly longer (Figure 2).

Table 1. Description of physical parameters (Suzuki *et al.* 2003)

	Parameters	Definition
Head	Head Height (HH)	Highest frontal bone - mandible joint (symphysis)
	Head Width (HW)	Right zygomatic bone to left, upper ear
Body	Body Length (BL)	Clavicle sternum joint to pubical bone crest
	Chest Width (CW)	Rib axilla (Right-Left), front of nipple
	Chest Thickness (CT)	Chest (nipple base) – posterior scapula
	Waist Width (WW)	Hip front of navel
Upper Extremity	Upper arm Length (UAL)	Proximal humerus to elbow joint
	Forearm Length (FL)	Proximal to distal bones of the radius
Lower Extremity	Thigh Length (TL)	Proximal femoral bone up to the patella
	Calf Length (CL)	Proximal of tibial bone to ankle joint

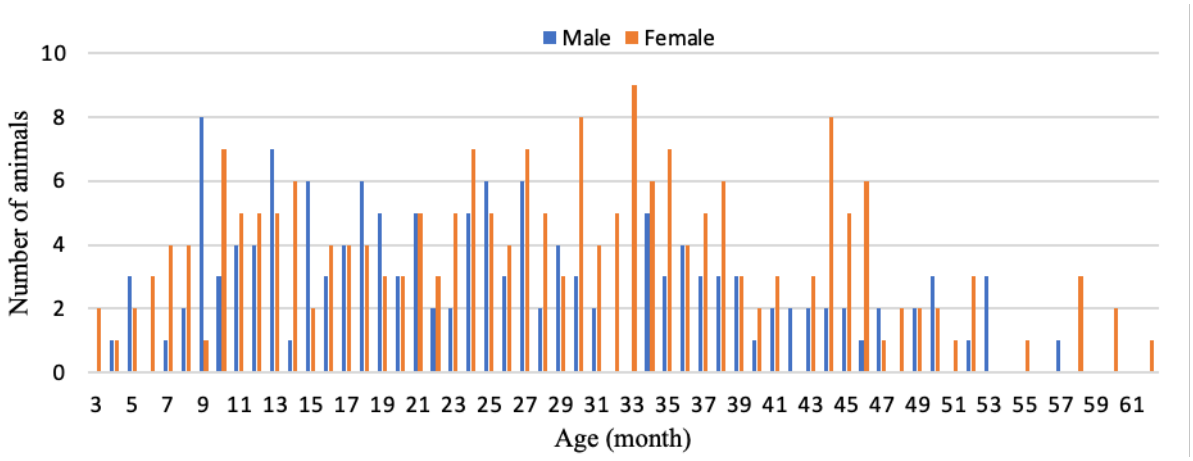


Figure 1. Sample distribution on each measurement time points

In general, there is no difference in head size between males and females in the first to third years, but differences are visible in the fourth year. This also can be seen in the growth rate of male head height, which appears to increase in the fourth year, while the development of head width does not show any difference until the fourth year (Figure 3).

Comparisons of the length of the extremities of both hands and feet of male and female animals showed no difference between the ages of five months and 45 months. Differences begin to appear in animals aged 50 months or over four years (Figure 4).

The growth rate of the body and extremities of female animals is higher than males at the age of one to one and a half years, with a decreasing trend until the age of four years. Meanwhile, male animals have an increasing trend in the age of one to two years, then decrease in the age of two to three years, and increase again in the age of three to four years. The head size growth tends to appear with no difference between males and females, flat at one to two years of age, then decreasing at two to three years and then increasing at three to four years (Figure 5A). In general, the average growth rate of body, head, and extremities in female animals shows a decreasing trend from one to four years of age, while male animals appear to fluctuate with the lowest rate at one and three years of age and the highest at two and four years of age (Figure 5B).

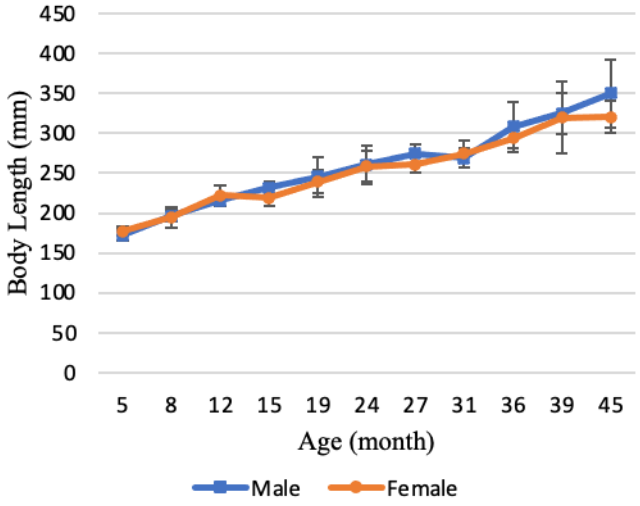


Figure 2. Body Length size of males and females on stage of ages

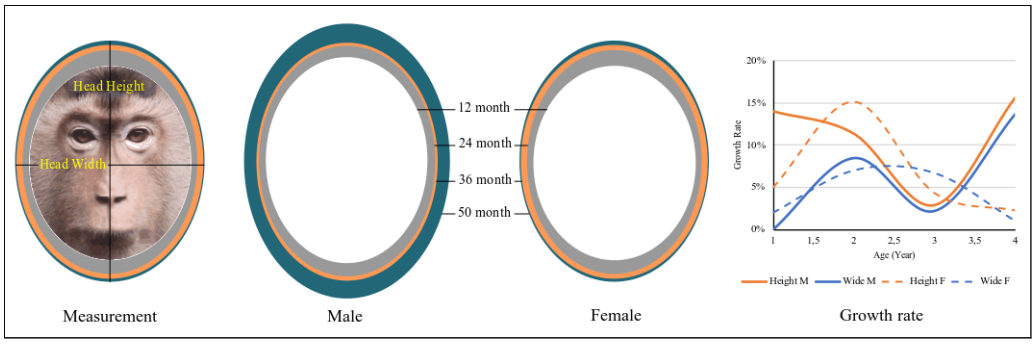


Figure 3. Head size illustration

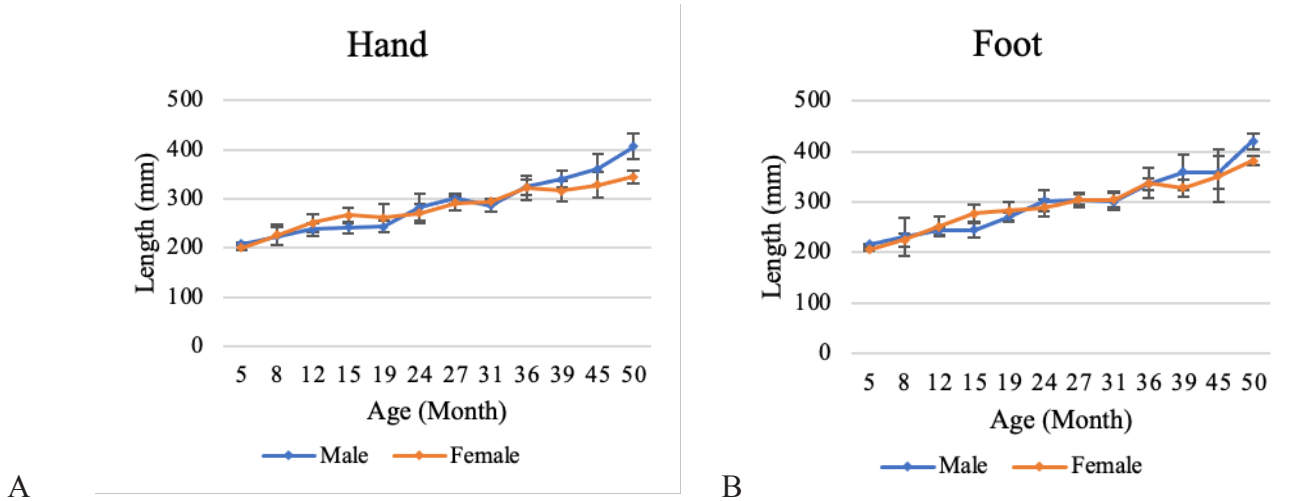
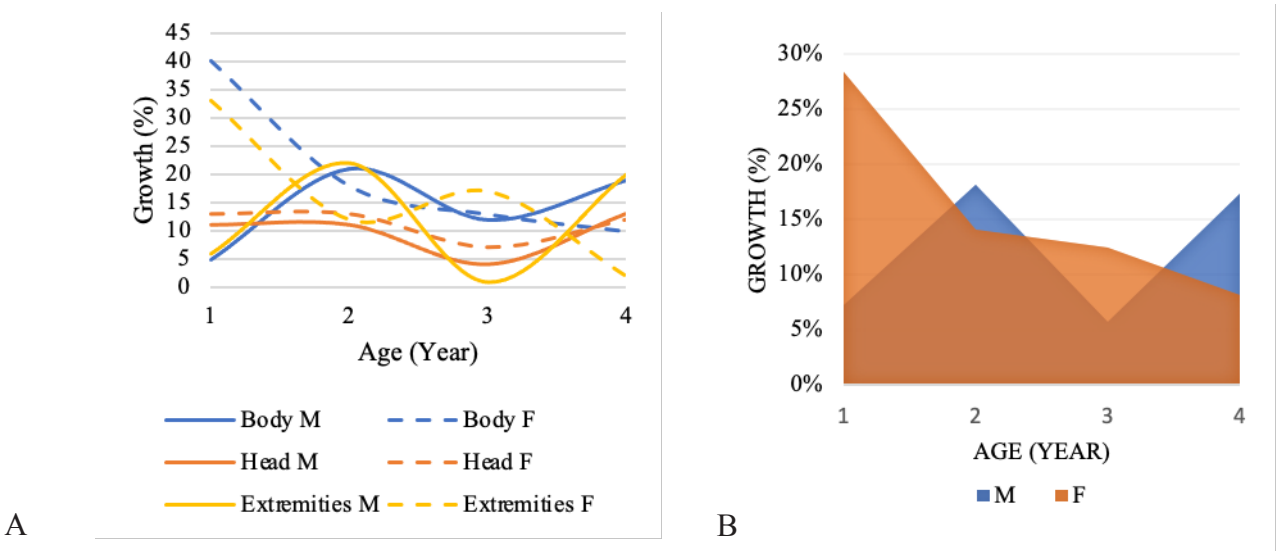


Figure 4. Length comparison of hand and foot between male and female macaque



A B

Figure 5. A. Growth rate for each parameters (%) B. Totals average of growth rate (%)

4. Discussions

Pig-tailed macaques are primates that have clear sexual dimorphism between males and females (German *et al.* 1994). Based on the morphometry results of the body size and extremities of males and females, no differences were seen at ages under 45 months or four years (Figures 2 and 4). However, from the results of this study the body size at birth cannot be ascertained because measurements were not taken to avoid disrupting the relationship between mother and baby. The larger size of males than females begins to appear at the age of over 45 months; however, it cannot be ascertained whether this trend continues after the age of 50 months due to limited data. Morphometric data over the age of 50 months were not analyzed because the number of samples was small and uneven, so they could not be compared, and bias due to variation between individuals could occur (Figure 1). This difference could possibly be caused by the influence of reproductive hormones, which develop earlier in female animals than in male animals. Female sexual maturity occurs at the age range of 30-46 months (Hadidian and Bernstein 1979) or 3-3,5 years, while male puberty occurs at the age range of 4-4,5 years (Lang 2009; Nowak 1999). Puberty and female reproductive maturity can be influenced by seasonal and environmental changes and will slow down body growth and development (Stephens and Wallen 2013).

The same trend can also be seen in head size; the difference between males and females is very visible in animals aged 50 months. In contrast to females, the growth of head dimensions in males appears to be consistent in height and width after they are over two years old (Figure 3). Differences in growth rate patterns between males and females were not visible in the analysis using more age time points (Figure 5A). This can be caused by higher sample variations and closer measurement distances, which will affect the results of the average growth rate at each time point. High individual variability regarding physical growth and pubertal measures was also found in rhesus monkeys (Kovac *et al.* 2023). Primates are animals that have the largest proportion of brain to body size compared to other animals (DeCasien *et al.* 2017). The larger male head size in line with the increased rate of tooth growth, likely plays an important role in the strategy of maintaining internal group and inter-group dominance for colony survival. Physical growth, pubertal measures (testicular volume, blood testosterone concentrations), and brain

size increase sharply from pre- to peri-puberty in male Rhesus macaques (Kovacs *et al.* 2023). However, it is necessary to carry out more in-depth studies to see the correlation between head (brain) size and intelligence (cognitive) abilities between male and female animals because the relationship between brain size and cognitive abilities cannot be generalized (Fichtel *et al.* 2020).

The growth rate showed fluctuations in both male and female animals (Figure 5AB). The growth rate trend of female animals tends to slow down considerably at the age of 1-2 years, although it increases slightly again until the age of 3 years when seen at half to 1 year of age, which is much higher even compared to male animals. The bond between parents and female offspring is stronger than with male offspring, and changes in social development occur around the age of 2-3 years (Amici *et al.* 2019). In contrast to male animals, which starts at a low rate, then increases at the age range of 1-2 years, then slows down slightly and increases again at the age range of 3–4 years, The majority of these chicks are raised by their mothers and weaned at around six months of age when they can eat fruit and monkey biscuits. Environmental adaptation, the digestive system's ability to absorb nutrients, and complex social structures influence the growth of sexual dimorphism (Li *et al.* 2023). Growth hormone Growth hormone (GH)-insulin-like growth factor (IGF) is consistently associated with growth measures (Bernstein 2017).

Differences in size and growth rate indicating sexual dimorphism are visible after the female reaches sexual maturity. The finding of differences in body size and type of growth rate between male and female needs to be considered in order to optimize captive management programs. Apart from that, this data can be used as a reference in using these animals as model animals or experimental animals in the biomedical field, especially in making or modifying tools and instruments. This study needs to be continued and refined to determine the trend in growth rates from pre-adolescence to adulthood and its correlation with other internal and external factors.

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